



University of Novi Sad - Univerzitet u Novom
Sadu



Faculty of Agriculture - Poljoprivredni fakultet

CONTEMPORARY AGRICULTURE SAVREMENA POLJOPRIVREDA

The Serbian Journal of Agricultural Sciences
Srpski časopis za poljoprivredne nauke

5th CASEE Conference

*"Healthy Food Production and Environmental
Preservation – The Role of Agriculture, Forestry and
Applied Biology"*

May 25 - 27, 2014

University of Novi Sad, Faculty of Agriculture, Serbia

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Preface

Life sciences play a major role in healthy food production and environmental preservation in the present global situation. Although the principal and fundamental role of life sciences is providing food, it is necessary to consider other ecosystem services provided by agriculture and to explore new frontiers for the future. The aim of the 5th CASEE conference is to meet the mentioned needs in order to find tools to deal with environmental problems coupled with the increasing demand for food.

The proposed Conference program has been divided in four sessions:

- Session I: Agronomic techniques for preserving ecosystem services
- Session II: Plant – soil – water interaction
- Session III: Applied biology
- Session IV: Ecosystem resilience to mitigate climate change effects

During these four sessions of the 5th CASEE conference, the latest scientific results were introduced in order to identify new scientific trends and to put forward recommendations to political decision-makers, to promote scientific collaboration and improve cooperation activities, to facilitate the integration of researchers from central and eastern European countries into the ICA.

Prof. Dr. Branko Ćupina

THE BEHAVIOR OF A COMPLEX FORAGE MIXTURE UNDER THE INFLUENCE OF MINERAL FERTILIZATION

ANAMARIA MALINAS, IOANROTAR, ROXANA VIDICAN, FLORIN
PACURAR¹

SUMMARY: *Grass-legume mixtures have a great economic importance mainly due to the fact that these cultures can storage huge quantities of N without any negative effects on production, being also able to determine an increase in forage nutritional value. This study aims to evaluate the behavior of the mixture consisting in Medicago sativa, Lolium hybridum, Festuca arundinacea and Dactylis glomerata under the influence of mineral fertilization, in the soil-climatic condition specific to Transylvania Plateau, Romania. The experience was established in the spring of 2012, according to the subdivided parcels method, in 8 variants with 4 replications. The mixture was seeded on two different distances between roads, namely 12.5 cm and 25 cm. Four treatments (doses) with mineral fertilization were performed: V₁- control variant, unfertilized, V₂- N₅₀P₆₀K₈₀, V₃- N₇₅P₆₀K₈₀, V₄- N₁₀₀P₆₀K₈₀. In order to accomplish the objective proposed which is to provide a deep and comprehensive analysis of the forage mixture studied, data related to green mass and DM production and floristic composition are given. This paper summarizes the results registered on 2013. The seeded forage species had a good response to this specific mixture and to the climatic conditions. The results showed that mineral fertilization with N has a significant influence both on species green mass and DM production and on their floristic composition.*

Key words: *forage, mixture, behavior, fertilization, production.*

INTRODUCTION

One of the most important tasks ahead for agriculture worldwide is to secure sufficient food for a growing population without further straining our environmental resources. The challenge is to produce more food with less external input (ÁslaugHelgadóttiret *al.*, 2013). The economic and ecologic importance of grassland is outstanding; these huge green surfaces are priceless connected to human life and environment conservation (Rotar, 2010). Nowadays a higher increasingly importance is given to the establishment of seeded grasslands which represent in fact a valuable forage source both from quantitative and qualitative point of view.

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Mixtures of grass and legume species in forage production can contribute to higher yields and reduced needs for fertilizers (Sturludottiret *al.*, 2013). Forage legumes play an invaluable role in the nitrogen balance and in sustainability grassland agriculture encompassing cold to warm climes (Søgaardet *al.*, 2007). Grasses have higher carbohydrate contents and can benefit from the nitrogen fixed by legumes when they are grown together. Compared with grasses, forage legumes generally have higher content of protein, pectin, lignin, carotene and vitamins (Frame, 2005), and therefore growing grasses and legumes in mixtures can improve herbage nutritive value compared with grass monocultures (Miriam Kizekováet *al.*, 2013). In choosing the grasses and leguminous species in order to form a mixture one should take into account the soil-climate and nutritional demands of each species.

MATERIAL AND METHOD

The present study aims to follow the behavior of a complex forage mixture (formed by *Medicago sativa* (40%), *Lolium hybridum* (15%), *Festuca arundinacea* (15%) and *Dactylis glomerata* (30%)) under the influence of mineral fertilization, in soil-climatic condition specific to Transylvania Plateau, Romania. The experience was installed in the spring of 2012 in the field of Agricultural Research-Development Station, Turda, Romania. The experimental site is characterized by an annual medium temperature (2013) of 10.4°C and annual precipitations of 523.2 mm. The soil type is phaeozemargiloiluvialvertic.

The experience was installed according to the subdivided parcels method: in 8 variants with 4 replications, each plot having 50 m² (10l X 5 L). The species were sown on two different distances between roads, namely 12.5 cm and 25 cm. The mixture was fertilized in the beginning of March month of every year with NPK complex and Ammonium nitrate (33.3%) in 4 different doses: V₁-control variant, unfertilized, V₂- N₅₀P₆₀K₈₀, V₃- N₇₅P₆₀K₈₀, V₄- N₁₀₀P₆₀K₈₀. Plots were mowed three times/year. Statistical interpretation of data was performed using Polifact program. The present study comprises data registered in 2013, data related to green mass and dry matter (DM) production and floristic composition of the mixture studied.

RESULTS

The complex forage mixture formed by alfalfa (*Medicago sativa* (40%)), hybrid ryegrass (*Lolium hybridum* (15%)), tall fescue (*Festuca arundinacea* (15%)) and cocksfoot (*Dactylis glomerata* (30%)) has a good response to mineral fertilizers. The yield increase registered (Table 1) is higher on variant V₃, fertilized with N₇₅P₆₀K₈₀, where we have a difference in yield of 9.37 t green mass/ha. An increase of fertilizer doses up to N₁₀₀P₆₀K₈₀ is not justified since caused a decrease in green mass production from 35.50 t green mass/ha (V₃) to 27.89 green mass/ha (V₄).

Dry matter production follows, as expected, the green mass production. The third fertilization graduation, V₃, registered the highest DM harvest of 13.62 t DM/ha.

Increasing the dose up to $N_{100}P_{60}K_{80}$ leads to a decrease in DM harvest up to 10 t DM/ha (on V_4).

The variants sown on 25 cm distance between roads had different reaction to the same dose of fertilizer when compared to the variants sown on 12.5 cm distance between roads (Table 2). On V_2 - $N_{50}P_{60}K_{80}$ green mass harvest difference is 4.04 t green mass/ha while on maximum dose of fertilizer this parameter reaches up to 11.82 t green mass/ha. Increasing fertilizer dose up to $N_{100}P_{60}K_{80}$ caused increases both on green mass and DM production.

The analyses of green mass and DM production (Table 3) highlights that the variants sown on 25 cm distance between roads are superior to those sown on 12.5 cm distance between roads. The highest green mass yield increase (8.95 t/ha) was registered on the variant V_4 , fertilized with maximum dose of $N_{100}P_{60}K_{80}$ and is very significant statistically ensured when compared with the average of the two sown distances. Same reaction is observed also on DM harvest where on V_2 was registered a yield difference of 1.32 t DM/ha and 2.32 on V_4 .

Analyses of the complex mixture formed by alfalfa, hybrid ryegrass, tall fescue and cocksfoot highlighted variations in species composition as a result of fertilizers doses applied. On 12.5 cm distance between roads the highest percentage was occupied by alfalfa on all 4 doses of fertilizer, which registered values between 58.06% on V_4 and 72.40% on V_1 (Figure 1). The smallest results were registered by tall fescue (10 % average/4 fertilization graduation), which participation percentage varies between 5.82% on V_2 and 15.21% on V_3 .

The influence of mineral fertilizer is felt also on the variants sown on 25 cm distance between roads (Figure 2). When plant feeding space is changed also the percentage in species composition of the mixture changes.

The only species whose percentage of participation decreases is alfalfa (from 65%- the average/4 fertilization graduation of the variants sown on 12.5 cm distance between roads to 53% on those sown on 25 cm) while all the other species met a small increase. Cocksfoot showed a good response to fertilization with $N_{50}P_{60}K_{80}$ on both distances between roads.

DISCUSSION

Starting from the installation capacity of the species taken into mixture, the evolution of floristic composition shows a different behavior to the technological input used in our study. A relevant example in this direction is the behavior of *Medicago sativa*. Alfalfa's high capacity of competition is more than obvious (aspect highlighted also by Deak, 2012). Alfalfa occupies the highest percentage of participation both on variants sown on 12.5 cm distance between roads and on variants sown on 25 cm distance between roads. Mineral fertilization leads to a decrease in the percentage of participation of alfalfa. This could be explained by the fact that the input of fertilizer stimulated competition between species, which met an

increase in the percentage of participation in mixture (hybrid ryegrass increased its share from 10.83% on V_1 to 17.84% on V_4). The behavior of tall fescue must be noted as well. Tall fescue according to scientific literature (Peeters, 2004; Deak, 2012) has small installation capacity and competitiveness.

While on the variants sown on 12.5 cm distance between roads tall fescue occupies only 8.67% (V_1), once with the grow of plants feeding space (variants sown on 25 cm distance between roads) the percentage of tall fescue grows as well to 29.69% (V_1). Variations on tall fescue percentage of participation in mixture were produced also as a result of mineral fertilization. Under the influence of mineral fertilizers this species percentage of participation grows from 8.67% (V_1) to 15.21% (V_3). The intensification of the system stimulates species competitiveness which leads to a decrease of tall fescue percentage of participation. *Dactylis glomerata* has a good response to fertilization with $N_{50}P_{60}K_{80}$ (V_2) which stimulates this species competitiveness. The percentage of this species grows on V_2 (both on the variants sown on 12.5 cm and 25 cm distances between roads) in the detriment of hybrid ryegrass and tall fescue which are dominated by this species. On the variants sown on 12.5 cm between roads tall fescue showed more competitiveness on cocksfoot than hybrid ryegrass, while on the variants sown on 25 cm between roads the proportions are reversed (hybrid ryegrass registered the highest percentage from all other experimental variants).

The stress exercised by mineral fertilization on alfalfa is notable also on green mass and DM production. From the variants sown on 12.5 cm between roads the highest yields are registered on the variants fertilized with $N_{100}P_{60}K_{80}$. An increase of fertilizer dose is not justified because this caused decreases on both green mass and DM production. Similar results were reported also by previous researchers (Talpanet al., 2007). This dose of fertilizer had a positive influence on hybrid ryegrass and tall fescue whose percentage of participation in mixture grows (according to floristic composition analysis). Hybrid ryegrass showed a behavior in accordance with the scientific literature (Peeters, 2004) where is highlighted the fact that this species has high demands on fertilization input. So hybrid ryegrass expresses its full potential on the variants fertilized with the highest doses. From the variants sown on 25 cm distance between roads the highest green mass and DM production were registered on the variants fertilized with $N_{100}P_{60}K_{80}$ (V_4). This treatment had a positive influence on cocksfoot and hybrid ryegrass. It was noticed that hybrid ryegrass registered high percentage of participation in the variants which registered the highest yields (both on the variants sown on 12.5 cm and 25 cm distance between roads). *Dactylis glomerata* was more competitive than tall fescue and is the species responsible for the superiority of the variants sown on 25 cm distance between roads.

CONCLUSION

The results showed that fertilization had a high influence on the mixture studied (both on species productivity and floristic composition).

Floristic composition analysis showed that under the influence of mineral fertilization the species with high demands in mineral nutrients are favored. The species studied had a behavior in accordance with previous researches. From all the species taken in study *Medicago sativa* was the most competitive and dominated the other species.

Green mass and dry matter production analysis showed that the variants sown on 25 cm distance between roads is superior on all fertilization doses to the variants sown on 12.5 cm distance between roads.

From the variants sown on 12.5 cm distance between roads the highest yields were registered on the variants fertilized with $N_{75}P_{60}K_{80}$ while from the variants sown on 25 cm between roads the highest yields were registered on the variants fertilized with $N_{100}P_{60}K_{80}$.

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Tables and figures

Table 1. The influence of mineral fertilization on green mass and DM production on the variants sown on 12.5 cm distance between rows

Fertilization doses	Production [t/ha]		Percent		Difference		Significance	
	Green mass [*]	DM ^{**}	Green mass [*]	DM ^{**}	Green mass [*]	DM ^{**}	Green mass [*]	DM ^{**}
V ₁ -0 kg/ha	26,12	9.86	100,0	100,0	0,00	0,00	Mt	Mt
V ₂ - N ₅₀ P ₆₀ K ₈₀	25,57	9.92	97,9	100.7	-0,56	0.07	000	000
V ₃ - N ₇₅ P ₆₀ K ₈₀	35,50	13.62	135,9	138.2	9,37	3.76	***	***
V ₄ - N ₁₀₀ P ₆₀ K ₈₀	27,89	10.70	106,8	108.6	1,76	0.85	***	***

^{*}DL (p 5%)0,08

DL (p 1%)0,12 DL (p1%)0,18

^{**}DL (p 5%) 0.32

DL (p 1%)0.47

DL (p 0.1%)0.69

Table 2. The influence of mineral fertilization on green mass and DM production on the variants sown on 25 cm distance between rows

Fertilization doses	Production [t/ha]		Percent		Difference		Significance	
	Green mass [*]	DM ^{**}	Green mass [*]	DM ^{**}	Green mass [*]	DM ^{**}	Green mass [*]	DM ^{**}
V ₁ -0 kg/ha	33,97	10.25	100,0	100,0	0,00	0,00	Mt.	Mt.
V ₂ - N ₅₀ P ₆₀ K ₈₀	38,01	12.55	111,9	122.5	4,04	2.30	***	***
V ₃ - N ₇₅ P ₆₀ K ₈₀	36,09	13.18	106,2	128.5	2,12	2.92	***	***
V ₄ - N ₁₀₀ P ₆₀ K ₈₀	45,79	15.46	134,8	150.8	11,82	5.21	***	***

^{*}DL (p 5%) 0,00

DL (p 1%)0,00

DL (p 0.1%)0,00

^{**}DL (p 5%) 0,08

DL (p 1%) 0,12

DL (p 0.1%) 0,7

Table 3. Interactions distance between roads-fertilization doses

Symbol		Variant		%		Difference		Significance	
Fertilization doses	Distance between rows [cm]	Green mass*	DM**	Green mass*	DM**	Green mass*	DM**	Green mass*	DM**
V ₁ -0 kg/ha	D0-average	30,05	10,05	100,0	100,0	0,00	0,00	Mt.	Mt.
	12.5	26,13	9,86	98,0	98,0	-3,92	-0,20	000	-
	25	33,97	10,25	102,0	102,0	3,92	0,20	***	-
V ₂ - N ₅₀ P ₆₀ K ₈₀	D0-average	36,76	11,24	100,0		0,00	0,00	Mt.	Mt.
	12.5	35,50	9,92	88,3	100,0	-1,26	-1,32	000	0
	25	38,01	12,55	111,7	88,3	1,26	1,32	***	*
V ₃ - N ₇₅ P ₆₀ K ₈₀	D0-average	30,83	13,40	100,0	111,7	0,00	0,00	Mt.	Mt.
	12.5	25,57	13,62	101,7		-5,26	0,22	000	-
	25	36,09	13,18	98,3	100,0	5,26	-0,22	***	-
V ₄ - N ₁₀₀ P ₆₀ K ₈₀	D0-average	36,84	13,08	100,0	101,7	0,00	0,00	Mt.	Mt.
	12.5	27,89	10,70	81,8	98,3	-8,95	-2,38	000	00
	25	45,79	15,46	118,2		8,95	2,38	***	**

*DL (p 5%) 0,10

DL (p 1%) 0,16

DL (p 0.1%) 0,32

**DL (p 5%) 1,01

DL (p 1%) 1,82

DL (p 0.1%) 3,98

Figure 1. The influence of mineral fertilization on floristic composition of the variants sown on 12.5 cm distance between rows

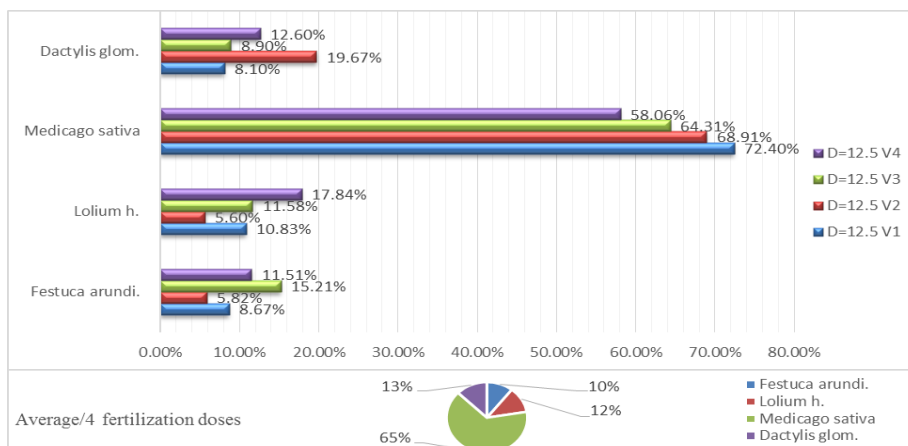
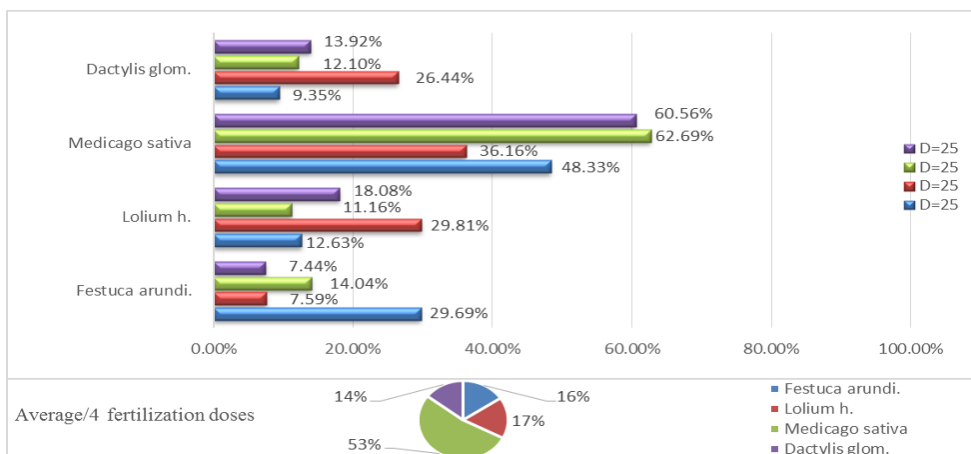


Figure 2. The influence of mineral fertilization on floristic composition of the variants sown on 25 cm distance between rows



COMPARATIVE STUDY CONCERNING THE EFFICACY OF CONVENTIONAL AND UNCONVENTIONAL TREATMENTS AGAINST LATE BLIGHT IN POTATO CULTURE FROM TRANSYLVANIA

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SUMMARY: *Our study aims to identify best combinations between treatments and fertilization type in fight against late blight in specific climatic conditions. The trial was developed according to a two factorial experiment, with N₅₀P₆₀K₈₀ and compost as fertilizers, and conventional and unconventional treatments. Bordeaux mixture and mineral fertilization led to lowest attack degree. According to cluster analyze, the combination unconventional products - mineral fertilization, had the highest efficacy.*

Key words: *basic statistics, mineral fertilization, compost, multiregression analyze*

INTRODUCTION

The potato cultures had huge importance worldwide due to multiple use as food and feeding. It is also one of the most valuable food industry component. For this reason, lot of preoccupation is focused on fight against the most harmful pathogens of this culture. Among these pathogens, *Phytophthora infestans* Mont. de Bary and *Alternaria solani* Sorauer are of most importance, because of damages they can produce in potato crops (Coakley et al., 1999, Deahl et al., 2001, Oroian et al., 2006, Sonoda, 1988), and also due to the influence that climatic factors have on their development. But, all over the world, the most important threat against potato remains the late blight produced by the pathogen *Phytophthora infestans* Mont. de Bary (Oroian et al., 2006). Lots of studies emphasized the importance of rainfall regimen and temperature upon the diseases produced by the above mentioned mycosis in potato (Mendelsohn et al., 2001, Rosenzweig et al., 1994, Rosenzweig and Parry, 1994). Because potato culture is a basic one in Transylvania, Romania, managing healthy cultures is a continuous challenge for farmers. Thus, the importance of controlling the disease produced by the above mentioned mushroom arise from the great influence of

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climate conditions (which cannot be controlled) on the extent of the late blight attack degree.

Due to specific climatic conditions of Romania, in early spring and early fall the attack degree of, late blight is low because in specific climate average temperature is not more than 10 °C, and usually the rainfall supply is reduced; it increases in late spring and summer when average temperatures are around 20 °C and rainfall average more than 65 mm/month (Oroian et al., 2006, Puia, 2005). Our study aims to identify the most suitable combinations of conventional and unconventional treatments in combination with different types of fertilization in fight against *Phytophthora infestans* Mont. de Bary (late blight pathogen agent) in climatic conditions of Transylvania region.

MATERIAL AND METHOD

Our trial was implemented on a private vegetal farm of 2,000 m² located in Top village, county of Cluj, Romania. Potato is the single vegetal culture practiced in the farm. Two experimental plots, 250 m² each, were organized. One was fertilized with N₅₀P₆₀K₈₀ mineral complex, while the other with compost. Resdec potato variety was cultivated on both plots, on argic chernozem soil. Conventional (Infinito 687.5 SC from Bayern) and unconventional (Bordeaux mixture prepared on farm, and Mimoten + Zytron mixture from Holland Farming Agro) were used. Each plot was treated according to the same treatment pattern. The following variants were obtained V1 – N₅₀P₆₀K₈₀ fertilized and treated with Bordeaux mixture (unconventional treatment), V2 – N₅₀P₆₀K₈₀ fertilized and treated with Infinito 687.5 SC (conventional treatment), V3 – N₅₀P₆₀K₈₀ fertilized and treated with Mimoten + Zytron mixture, V4 – N₅₀P₆₀K₈₀ fertilized and not treated, V5 – compost fertilized and treated with Bordeaux mixture (unconventional treatment), V6 - compost fertilized and treated with Infinito 687.5 SC from Bayern (conventional treatment), V7 - compost fertilized and treated with Mimoten + Zytron mixture (unconventional treatment), and V8 - fertilized with compost and not treated. Observations on the field were performed from April up to September 2013.

The *Phytophthora infestans* Mont. de Bar attack intensity (%) and frequency (%) were recorded three times a week, and function of them, the attack degree (AD%) was calculated (Oroian et al., 2006). The climatic factors (temperature and rainfall regimen) were also taken into account. They were recorded with a meteorological station placed on the experimental field. STATISTICA 7.0 v. programme was used for statistical data processing (averages, dispersion parameters, significance of differences, multiregression and cluster analysis).

RESULTS

Table 1 emphasize the results of the basic statistics. The lowest *Phytophthora infestans* Mont. de Bary attack degrees were recorded in V1 (N₅₀P₆₀K₈₀ fertilized and unconventionally treated with Bordeaux mixture) and V3 N₅₀P₆₀K₈₀ fertilized and unconventionally treated with Mimoten + Zytron mixture). As table 1 and Box-plot diagram (fig. 1) emphasize, the biggest attack degree was recorded in V7 (fertilized with compost and treated with Mimoten + Zytron mixture). Similar average attack degrees were reported for treatment with Infinito 687.5 SC and in no treated plot,

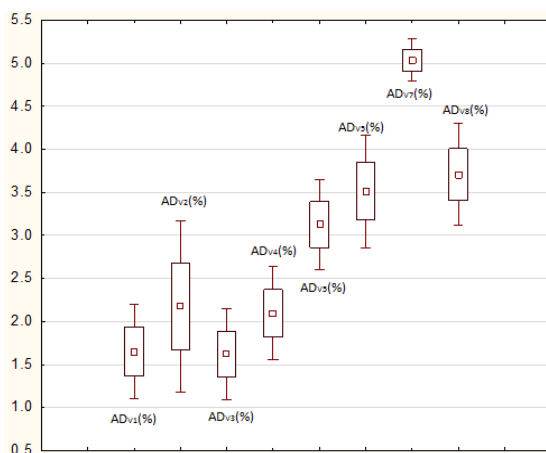


Figure 1. The Box-plot diagram of *Phytophthora infestans* Mont. de Bary average attack degrees (%) recorded in all experimental variants, V1 – V8

when both N₅₀P₆₀K₈₀ (2.17% and 2.09%, respectively) and compost (3.51% and 3.70%, respectively) were administered as fertilizers (table 1). The maximum value for *Phytophthora infestans* Mont. de Bary attack degree (AD%) was recorded in variant V7, fertilized with compost and unconventionally treated with Mimoten + Zytron mixture (5.25%), and minimum *Phytophthora infestans* Mont. de Bary attack degree in variants V1 and V3 (1.32%) fertilized with N₅₀P₆₀K₈₀ and unconventionally treated with Bordeaux mixture, and Mimoten + Zytron mixture (table 1).

Table 1. Basic statistics for *Phytophthora infestans* de Bary, attack degree (AD%) and meteorological data (precipitation regimen – Pp, mm, and temperature – t⁰C) in all experimental variants (different treatments applied to mineral and organic fertilized potato cultures)

Issue	n	Mean	Minimum	Maximum	Standard deviation	Coefficient of variability	Standard error of mean	ANOVA p
AD _{V1} (%)	75	1.620	1.323	1.990	0.269	16.618	0.031	
AD _{V2} (%)	75	2.176	1.400	2.640	0.508	23.346	0.058	
AD _{V3} (%)	75	1.652	1.320	1.990	0.281	17.002	0.032	
AD _{V4} (%)	75	2.096	1.880	2.567	0.276	13.150	0.032	
AD _{V5} (%)	75	3.125	2.733	3.420	0.266	8.508	0.030	- 1.505***
AD _{V6} (%)	75	3.512	3.010	3.810	0.336	9.554	0.038	- 1.336**
AD _{V7} (%)	75	5.036	4.920	5.250	0.126	2.494	0.014	- 3.411***
AD _{V8} (%)	75	3.706	3.277	3.990	0.302	8.153	0.034	- 1.610***
t ⁰ C	3527	18.044	17.290	18.440	0.465	2.578	0.008	
Pp, mm	3527	96.496	89.130	100.120	4.356	4.514	0.073	

*** - p < 0.001; ** - p < 0.01

The statistic analyze is representative for all sample (coefficient of variability < 30% in all cases), with greater variation in variant V2, N₅₀P₆₀K₈₀ fertilized and treated with Infinito 687.5 SC (23.34%), and lowest in variant V7 fertilized with compost and treated with Mimoten + Zytron mixture (2.49%). Average temperature (18.04 °C) and average precipitation regimen (96.49 mm) by analyzed period (April - September 2013) framed within multiannual averages, by 100 years recorded in Romania.

The multiregression analyze (table 2) conducted in order to emphasize the way meteorological conditions influence the *Phytophthora infestans* Mont. de Bary attack degree (%) in different conditions of fertilizing and treatment, demonstrates different interactions. Very strong and strong multiple correlations emphasized by the correlation coefficients were reported for variant fertilized with N₅₀P₆₀K₈₀ and treated with Mimoten + Zytron mixture (R= 93.90%), and Infinito 687.5 SC (R=73.61%), and also in variants fertilized with compost and treated with Bordeaux mixture (R= 83.40%), Infinito 687.5 SC (R=70.60%).

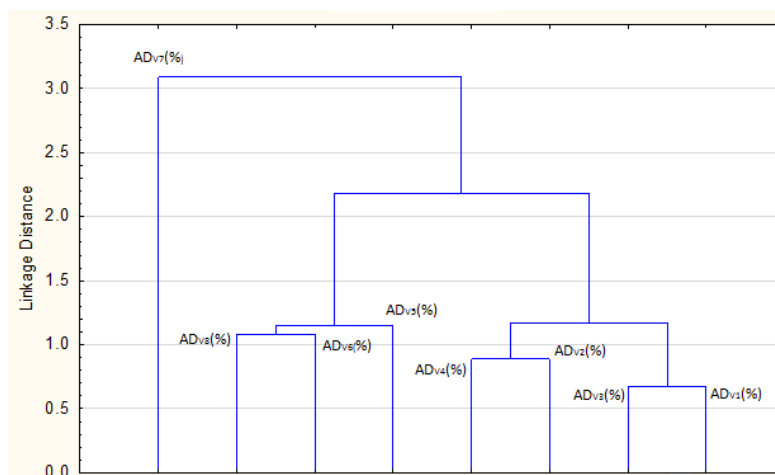


Figure 2. The cluster analysis applied to *Phytophthora infestans* Mont. de Bary attack degrees (%)

Table 2. The multiregression analyze applied in experimental variants for emphasizing the multiple correlations between *Phytophthora infestans* de Bary, attack degree (%) in potato cultures and climatic conditions data (precipitation regimen – mm, and temperature – °C)

Issue	R	R ²	Regression line
AD _{V1} (%) - t ⁰ C - Pp, mm	0.374	0.140	Y = 0.006 - 0.107X1 + 0.461X2
AD _{V2} (%) - t ⁰ C - Pp, mm	0.731	0.533	Y = 10.005 + 1.346X1 - 1.275X2
AD _{V3} (%) - t ⁰ C - Pp, mm	0.939	0.882	Y = 6.483 - 1.448X1 + 1.761X2
AD _{V4} (%) - t ⁰ C - Pp, mm	0.438	0.192	Y = 1.387 + 0.154X1 + 0.301X2
AD _{V5} (%) - t ⁰ C - Pp, mm	0.834	0.696	Y = 3.203 - 1.483X1 + 1.523X2
AD _{V6} (%) - t ⁰ C - Pp, mm	0.706	0.498	Y = 0.586 + 0.968X1 - 1.301X2
AD _{V7} (%) - t ⁰ C - Pp, mm	0.491	0.241	Y = 5.304 - 0.545X1 + 0.858X2
AD _{V8} (%) - t ⁰ C - Pp, mm	0.214	0.046	Y = 1.268 + 0.401X1 + 0.339X2

Moderate correlations were reported for the interaction *Phytophthora infestans* Mont. de Bary attack degree - temperature - precipitation regimen for the variants fertilized with $N_{50}P_{60}K_{80}$ not treated ($R=43.80\%$) treated with Bordeaux mixture ($R=37.40\%$), and fertilized with compost and treated with Mimoten + Zytron mixture ($R=49.10\%$). The weakest correlation was reported in variant ($R=21.40\%$) fertilized with compost and not treated (table 2).

The regression lines emphasize that the increase of the precipitation quantities determine the decrease of the *Phytophthora infestans* Mont. de Bary attack degree (AD%) in variants conventionally treated with Infinito 687.5 SC in both fertilization variants, mineral and compost, V2 and V6, respectively (table 2). The increase of the temperature negatively affects the *Phytophthora infestans* Mont. de Bary attack degree in variants unconventionally treated, with Bordeaux mixture and Mimoten + Zytron mixture in both fertilizing practices, with mineral fertilizer and compost, respectively.

DISCUSSION

The analyze of basic statistics shows differences between the *Phytophthora infestans* Mont. de Bary attack degrees reported in variants fertilized with $N_{50}P_{60}K_{80}$ mineral fertilizer and compost for all treated and also untreated variants. In all cases they are bigger in compost fertilized variants, differences being statistically distinct significant ($p<0.01$) and very significant ($p<0.001$), as shown in table 1. We note the biggest difference reported between variants unconventionally treated with Mimoten + Zytron mixture $N_{50}P_{60}K_{80}$ mineral fertilized and fertilized with compost (V3 and V7), and smallest between variants conventionally treated with Infinito 687.5 SC (variants V2 and V6). These results, all characterized by normal distribution (fig. 1), demonstrate that choosing NPK mineral fertilizing option, one obtains *Phytophthora infestans* Mont. de Bary lower attack degree on potato culture, whatever treatment, conventional, unconventional, and even in no treatment conditions.

The cluster analysis (fig. 2) implemented in order to emphasize the hierarchy of the results obtained as consequence of two pest fight managerial choices, confirm the findings above mentioned. As first option, the results of the fertilizing and treatments managerial methodology, may be divided in two groups. First, the biggest *Phytophthora infestans* Mont. de Bary attack degree recorded in potato culture fertilized with compost and unconventionally treated with Mimoten + Zytron mixture – AD_{V7} (%), and second with much lower attack degrees. This group can also be divided in two groups. One is represented by the lowest *Phytophthora infestans* Mont. de Bary attack degrees recorded in cultures $N_{50}P_{60}K_{80}$ fertilized, with two branches, one unconventionally treated with Bordeaux mixture and Mimoten + Zytron mixture – AD_{V1} (%) and AD_{V3} (%), respectively, and the other, conventionally treated with Infinito 687.5 SC and not treated – AD_{V4} (%) and AD_{V2} (%), respectively (fig. 2). The other is represented by average *Phytophthora infestans* Mont. de Bary attack degrees recorded in variants fertilized with compost, and treated with Infinito 687.5 SC, Bordeaux mixture, and not treated.

The multiregression analyze also emphasize that meteorological conditions affecting the *Phytophthora infestans* Mont. de Bary attack degree have the biggest influence on untreated variants, both $N_{50}P_{60}K_{80}$ fertilized and with compost– V4 and V8, in conditions of weak correlation (21.40%), almost not representative between

interrelated factors (table 2). The interrelation between *Phytophthora infestans* Mont. de Bary attack degree, temperature and precipitation regimen are balanced in direction of enhanced influence of precipitation input in majority of variants. It contributes to increase of the *Phytophthora infestans* Mont. de Bary attack degree value in potato culture variants unconventionally treated in both fertilization practices, N₅₀P₆₀K₈₀ and with compost. The biggest contribution may be noticed in variant N₅₀P₆₀K₈₀ fertilized and treated with Mimoten + Zytron mixture –V3. In variant fertilized with compost and conventionally treated with Infinito 687.5 SC, balanced is also in advantage of precipitation regimen, but it contributes in a major manner to decrease of the *Phytophthora infestans* Mont. de Bary attack degree in potato (coefficient 1.301, compared to 0.968 for temperature), V6 (table 2). Temperature has bigger influence compared to precipitation regimen only in variant N₅₀P₆₀K₈₀ fertilized and conventionally treated with Infinito 687.5 SC, V2, respectively.

CONCLUSION

The comparative analyze of the influence of conventional and unconventional treatments applied in two different fertilization conditions upon the intensity of *Phytophthora infestans* Mont. de Bary attack degree in Resdec potato cultures demonstrates specific particularities. The N₅₀P₆₀K₈₀ mineral fertilization has better influence on potato culture resistance against the pathogen attack, compared to compost fertilization. The conventional and unconventional treatments have different efficacy, function of the type of fertilization. Unconventional treatments have enhanced efficacy in conditions of mineral fertilization. Three categories of *Phytophthora infestans* Mont. de Bary attack degree intensities are emphasized in our study: high (V7 – fertilized with compost and unconventionally treated with Mimoten + Zytron mixture), medium (all variants fertilized with compost - V8 untreated, V6 conventionally treated with Infinito 687.5 SC and V5 unconventionally treated with Bordeaux mixture), and low (all variants fertilized with mineral fertilizer – V4 not treated, V2 conventionally treated with Infinito 687.5 SC, V1 and V3 unconventionally treated with Bordeaux and Mimoten + Zytron mixtures, respectively).

Precipitation regimen (mm) is the most important climatic factor affecting the amplitude of the *Phytophthora infestans* Mont. de Bary attack degree in potato. According to our study we can state that best combinations of conventional and unconventional treatments reported to different types of fertilization in fight against (late blight in climatic conditions of Transylvania region, are represented by N₅₀P₆₀K₈₀ mineral fertilization and unconventional treatments, Bordeaux mixture and Mimoten + Zytron mixture, respectively, when, in our study lowest *Phytophthora infestans* Mont. de Bary attack degrees were obtained, 1.62% and 1.65%, respectively.

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DETERMINATION OF FATTY ACID PROFILE AND EFFECT OF MIXOTROPHIC GROWTH IN SELECTED CYANOBACTERIAL STRAINS*

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IVAN MILOVANOVIĆ, ALEKSANDRA MIŠAN²

SUMMARY: *Microalgae are photosynthetic microorganisms used in the nutrition of humans and animals (especially in aquaculture) due to the production of vitamins, proteins, polyunsaturated fatty acids, antioxidants, etc. Among microalgae, cyanobacteria (blue-green algae) are particularly recognized as the producers of different nutritive and biotechnologically valuable compounds. Although fatty acids of the microalgal origin are today available in the form of dietary supplements and are incorporated into various food products, microalgae are still an insufficiently explored source of these nutrients. In the present study, fatty acid compositions of four cyanobacterial strains belonging to the Nostoc and Anabaena genera have been analysed. Furthermore, since in photoautotrophic cultures, the biomass production of microalgae is mostly low, the present study reviews the effect of the mixotrophic mode of nutrition (besides CO₂ cells use organic matters as a carbon source) on the biomass production of the selected cyanobacteria.*

The analyses of fatty acid methyl esters (FAMES) were carried out by gas chromatography coupled with flame ionization detection (GC-FID) after 42 days of the cyanobacterial cultivation. The results showed that the most significant constituents of these strains are 16 carbon (16:0 and 16:1 types) chain fatty acids, linoleic acid (18:2n6c) and α-linolenic acid (18:3n3). Oleic acid (18:1n9c) was also present in significant amounts, while myristic acid (14:0) and stearic acid (18:0) were present in small amounts. Erucic acid (22:1n9) was present in traces only in the strain Nostoc 2S₁, while caproic acid (6:0) and myristoleic acid (14:1) were present only in the strains Nostoc S₈ and Nostoc 2S₁.

For the purpose of determining the mixotrophic growth, the strains were cultured at different glucose concentrations (1.5 and 3 g/l) for 42 days, and the biomass

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production was determined periodically by spectrophotometrically measuring of the chlorophyll a concentration. The only strain which grew better in the autotrophic culture was Anabaena LC₁B, with the maximum production of only 0.1 g/l. In all the other strains, the biomass production of mixotrophic cultures surpassed 1g/l, at the lower concentration of glucose (1.5 g/l). The most productive strain was Nostoc 2S₃B, which, after 28 days with 1.43 g/l, had 18 times higher production in comparison with the autotrophic culture. The strains Nostoc S₈ and Nostoc 2S₁ were the most productive after 42 days, with 1.05 and 1.48 g/l, which was 1.32 and 4.84 times higher production than in the autotrophic cultures respectively.

Taking into account the aspects of biomass production in mixotrophic cultures with glucose and fatty acid composition, among the tested cyanobacterial strains, Nostoc 2S₃B has the greatest biotechnological potential.

Key words: *Cyanobacteria, fatty acids, mixotrophic growth*

INTRODUCTION

Microalgae are photosynthetic microorganisms with the vast potential in the production of food, feed and high-value metabolites. Although they have already been found as a source of number biologically active compounds such as carotenoids, phycobiliproteins, fatty acids, polysaccharides, vitamins and sterols (Singh et al., 2005; Thajuddin and Subramanian, 2005; Spolaore et al., 2006; Plaza et al., 2009), microalgae are proposed as almost unlimited source in search of novel natural functional ingredients (Plaza et al., 2009). Among microalgae, cyanobacteria (blue-green algae) are particularly recognized as health food, as well as producers of different bioactive compounds such as antiviral, anti-tumor, antibacterial, etc. (Singh et al., 2005).

Fatty acids of the microalgae origin are nowadays available in the form of dietary supplements, and incorporated into various food products. In each major algal group, fatty acids are generally recognized as characteristic of that group (Behrens and Kyle, 1996). The major saturated fatty acids within algae are 12, 14, 16 and 18 carbon fatty acids, while unsaturated fatty acids range from 16 to 22 carbons, containing from 1 to 6 double bonds in the cis-configuration (Behrens and Kyle, 1996). Polyunsaturated fatty acids (PUFAs) play a key role in cellular and tissue metabolism (Cardozo et al., 2007). They are especially important as constituents of the membranes phospholipids, where they appear to confer distinctive properties, in particular by decreasing their rigidity. Among PUFAs ω -3 and ω -6 fatty acids belong to the essential fatty acids, and as they cannot be synthesized in human body, they have to be administered by diet. Algae are thought to be the principal producers of some PUFAs in the biosphere (Behrens and Kyle, 1996). Although marine fish is the principal dietary source of some PUFAs, they are actually synthesized by microalgae and fish receive them via food chain. In addition, using fish as a source is connected with problems such as pollutants accumulation, unpleasant odor, control of the fatty acids proportion, and also they are not suitable for vegetarians (Spolaore et al., 2006; Cannon, 2009). Moreover, since microalgae cells are rich in antioxidant carotenoids and vitamins, and lipids are bioencapsulated by the cell wall, microalgae may have superior lipid stability in comparison with traditional PUFAs (Patil et al., 2007). Among most important PUFAs, with numerous nutraceutical and pharmaceutical

applications are eicosapentaenoic acid (EPA, 20:5n3), docosahexaenoic acid (DHA, 22:6n3) and arachidonic acid (AA, 20:4n6). Different food products with incorporated oil of microalgae origin rich in these fatty acids, including formulations for infants, can be found on the market.

Today, microalgae are usually cultivated in photoautotrophic cultures and their commercial application is limited by the low biomass yield and high production costs. Using mixotrophic mode of nutrition biomass production, as well as target metabolites can be increased several times since organic matters are used as the second carbon and energy source. In mixotrophy within the cell, simultaneously occur photosynthesis and aerobic respiration, and during the night culture may continue to grow through heterotrophic nutrition. Moreover, production costs can be reduced because lower light intensity is required (Yu et al., 2009), cells can start to grow at a lower average light intensity (Yu et al., 2011) and there is less biomass loss during the dark phase (Andrade and Costa, 2007). It is showed that in some microalgae mixotrophy increased the level of lipids (Ceron Garcia et al., 2006; Wan et al., 2011; Kong et al., 2013), as well as fatty acids, such as EPA (Ceron Garcia et al., 2006). It is expected that mixotrophic cultivation is especially suitable for the production of high value bioactive compounds, fine chemicals and pharmaceuticals (Yu et al., 2011).

Filamentous, nitrogen-fixing cyanobacteria can be an excellent biotechnological source, because they do not need nitrogen in the culture medium, which results in reduction of production costs, while filamentous nature facilitates the process of biomass harvesting. Among them, some species of the *Anabaena* and *Nostoc* genera are used in human nutrition in Chile, Mexico, Peru and Philippines (Thajuddin and Subramanian, 2005). It is shown that *Anabaena* sp. PCC 7120 can be cultured for production of variety of fine chemicals and bioactive compounds, such as sulfolipids, pigments and exopolysaccharides, and it is very promising for production of high value transgenic proteins (cited in Yu et al., 2011). *Nostoc flagelliforme* is species with high economic value, used in nutrition for more than 2000 years, with reported anti-tumor and anti-viral activity (Yu et al., 2009). With high amount of fibre, species *Nostoc commune* is a potential dietary fibre source (Thajuddin and Subramanian, 2005).

The aim of this study was to investigate the fatty acid profile and effect of mixotrophic nutrition with glucose in selected filamentous, nitrogen-fixing cyanobacterial strains, belonging to the *Nostoc* and *Anabaena* genera.

MATERIALS AND METHODS

In the present study, four filamentous, nitrogen-fixing cyanobacterial strains were tested: *Anabaena* LC₁B, *Nostoc* S₈, *Nostoc* 2S₃B and *Nostoc* 2S₁. All strains were isolated from different soil types of Vojvodina region, then purified and cultured in BG-11 medium without nitrogen for 42 days, at room temperature.

Fatty acid methyl esters were prepared from the extracted lipids by transesterification using 14% boron (III)-fluoride in methanol (Karlović and Andrić, 1996). The obtained samples were analyzed by a GC Agilent 7890A system with flame-ionization detector (FID), autoinjection module for liquid samples, equipped with fused silica capillary column (DB-WAX 30 m, 0.25 mm, 0.50 µm). Helium was used as a carrier gas (purity > 99.9997 vol %, flow rate = 1.26 ml/min). The fatty acids peaks were identified by comparing retention times with retention times of

standards from Supelco 37 component fatty acid methyl ester mix (Sigma-Aldrich, EU) and with data from internal data library, which are based on previous experiments. Results were expressed as mass of fatty acid or fatty acid group (g) in 100 g of fatty acids.

Biomass production in mixotrophic conditions was determined at two concentrations of glucose (1.5 and 3 g/l). It was determined periodically, every seven days, by spectrophotometrically measuring of the chlorophyll a concentration, and it was calculated using an indirect method (Mckinney, 1941). Each sample was filtered (10 ml) and each filter paper was placed in a test tube in which was added 5 ml of methanol for extraction. This process lasted 24 hours in the dark at temperature 4°C. Samples were then sonicated 10 minutes in cycles of 30 seconds and centrifuged. Supernatants were collected and used for spectrophotometrical analysis of absorbance at 663 nm. The concentration of chlorophyll a was calculated using the following formula:

$$cc\ Chl\ a = \frac{A_{663} \times 12,64 \times V_1}{V_2} \left[\frac{\mu g}{ml} \right]$$

Where: ccChl a – chlorophyll a concentration [$\mu g/ml$]; A_{663} – absorbance at 663 nm; 12.64 – conversion factor; V_1 – sample volume [ml]; V_2 – methanol volume [ml]. Biomass production was calculated using the following formula:

$$B = ccChl\ a \times 67 \left[\frac{mg}{ml} \right]$$

Where 67 is conversion factor.

RESULTS AND DISCUSSION

The results of fatty acid profile of tested cyanobacterial strains are shown in Table 1. The most significant constituents of tested cyanobacterial strains are 16 carbon (16:0 and 16:1 types) chain fatty acids, linoleic acid (18:2n6) and α -linolenic acid (18:3n3). Oleic acid (18:1n9) was also present in significant amounts, while myristic acid (14:0) and stearic acid (18:0) were present in small amounts. Erucic acid (22:1n9) was present in traces only in the strain *Nostoc* 2S₁, while caproic acid (6:0) and myristoleic acid (14:1) were present only in the strains *Nostoc* S₈ and *Nostoc* 2S₁. The results also showed that strain *Anabaena* LC₁B is the most suitable for production of 18:1n9c and 18:2n6c, strains *Nostoc* S₈ and *Nostoc* 2S₁ for 16:1, and strain *Nostoc* 2S₃B for 18:2n6c and 18:3n3. Finally, considering proportion of PUFAs in total fatty acids, strain *Nostoc* 2S₃B is the most promising.

In the present study, most significant constituents of tested strains are 16 carbon (16:0 and 16:1 types) chain fatty acids. It has been recently found that in animal tissues, palmitoleic acid (16:1n7) have a distinctive function in mice as a lipid hormone lipokine, while sapienic acid (16:1n10) is the single most abundant component in human sebum lipids. Among most significant fatty acids of tested strains are also linoleic acid (18:2n6) and α -linolenic acid (18:3n3), essential dietary components which in animal and plant tissues serve for synthesis of the remaining members of ω -6 and ω -3 family of fatty acids. Oleic acid (18:1n9) which was also

present in significant amounts, has number of important biological properties, and it is the biosynthetic precursor of a family of fatty acids with *n*-9 terminal structure and with chain lengths of 20 to 24, or more. Myristic acid (14:0), which is essential for the function of protein components in certain proteolipids was present in small amounts. This acid is a ubiquitous component of lipids in most living organisms, but usually at levels of only 1-2%. Milovanovic et al. (2012) investigated fatty acid profile in 3 filamentous, nitrogen-fixing cyanobacterial strains and as well as in our study, 16 carbon and 18 carbon chain fatty acids represented the most significant constituents, with the highest content of 16:1. Their strains as well had a significant amount of α -linolenic acid (18:3n3).

Table 1: Fatty acid profile of tested cyanobacterial strains

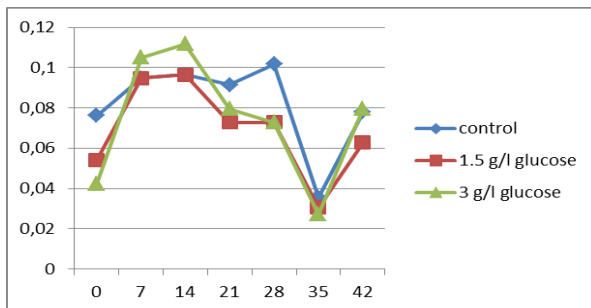
Fatty acid	<i>Anabaena</i> LC ₁ B	<i>Nostoc</i> S ₈	<i>Nostoc</i> 2S ₃ B	<i>Nostoc</i> 2S ₁
	%	%	%	%
6:0	0,00	20,75	0,00	2,21
14:0	0,54	0,66	0,66	0,68
14:1	0,00	0,49	0,00	0,47
16:0	21,57	12,23	15,94	18,29
16:1	26,45	31,63	24,83	36,75
18:0	0,80	0,47	3,11	0,70
18:1n9c	13,22	3,91	11,26	4,60
18:2n6c	19,59	16,00	20,09	15,64
18:3n3	17,82	13,85	24,10	20,13
22:1n9	0,00	0,00	0,00	0,53
SFA	22,90	34,11	19,72	21,88
MUFA	39,68	36,04	36,09	42,35
PUFA	37,42	29,86	44,19	35,77
UFA	77,10	65,89	80,28	78,12

SFA - saturated fatty acids, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acids, UFA - unsaturated fatty acids

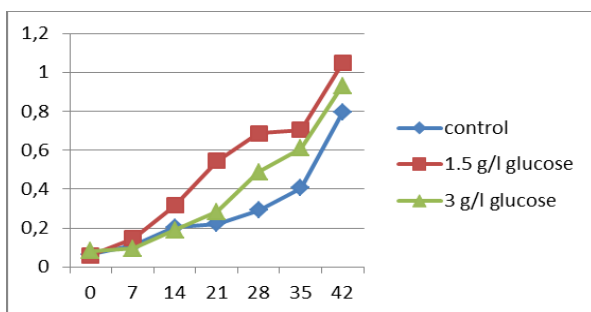
Furthermore, they also detected undecanoic acid (11:0), relatively rare γ -linoleic acid (GLA, 18:3n6), arachidic acid (20:0) and cis-13, 16-docosadienoic acid (22:2), which were not detected in our strains. Patil et al. (2007) tested fatty acid composition of 12 microalgae including two cyanobacterial strains, *Chroococcus* spp. and *Synechococcus* spp. *Chroococcus* spp. had 16:0 and 18:2n6 as the dominating fatty acids, with lower amounts of 16:1 and 18:3n3, while in *Synechococcus* spp. the most abundant were 14:0, 16:0 and 16:1. Viso and Marty (1993) reported the fatty acid composition of 28 marine microalgae species. Among tested cyanobacteria, *Anacystis quadruplicatum* was characterized by a high proportion of PUFA 18:3n3 and 16:2n6,

while *A. marina* and *Synechocystis* sp. were rich in monounsaturated 16:1n9. In the study of Milovanovic et al. (2012), among tested cyanobacterial strains *Spirulina*, strains had the highest content of GLA (18:3n6) and significant amount of cis-11-eicosenoic acid (20:1). Interestingly, species *Spirulina platensis* represents a source of many fatty acids which are potential functional ingredients, i.e. oleic acid (antioxidant activity), linolenic acid (antimicrobial activity), and palmitoleic acid and DHA (reduce risk of certain heart diseases) (cited in Plaza et al., 2009). In this species, DHA (22:6n3) can account up to 9.1% of the total fatty acids content (Yukino et al., 2005). The study of Patil et al. (2007) revealed that in all the tested 12 microalgae, major saturated fatty acid was 16:0, while major monosaturated fatty acids were 16:1 or 18:1. According to them, marine algae are major producers of the ω 3-PUFAs (EPA and DHA), while in the freshwater algae dominate saturated or monosaturated fatty acids. In cyanobacteria major fatty acids are 16:0 and 16:1 (Behrens and Kyle, 1996). Furthermore, the fatty acid compositions (particularly their content of monounsaturated and PUFAs) shown to be different for unicellular and filamentous algae (Kenyon and Stanier, 1970). In addition, while some algae produce large quantities of lipids in form of large droplets of triacylglycerol in the cytoplasm (Boswell et al., 1992), cyanobacteria generally do not produce oils as a storage material, and virtually all fatty acids are present as complex lipids used for cells photosynthetic membrane system (Behrens and Kyle, 1996).

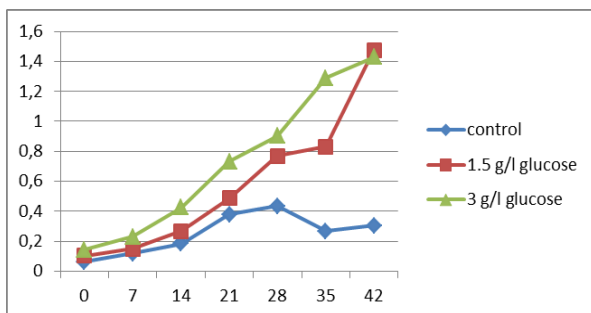
The results of biomass production in mixotrophic cultures are presented in Graph. 1-4. The only strain which grew better in autotrophic culture was *Anabaena* LC₁B, with maximum production of only 0.1 g/l. In all other strains, biomass production of mixotrophic cultures surpassed 1g/l, at the lower concentration of glucose (1.5 g/l). The most productive strain was *Nostoc* 2S₃B, which, after 28 days with 1.43 g/l, had 18 times higher production in comparison with autotrophic culture. Strains *Nostoc* S₈ and *Nostoc* 2S₁ were most productive after 42 days, with 1.32 and 4.84 times higher production than in autotrophic cultures respectively. Furthermore, strains *Nostoc* S₈ and *Nostoc* 2S₃B were more productive at 1.5 g/l of glucose, in relation to *Nostoc* 2S₁ which showed higher biomass production at 3 g/l of glucose. Gradual increase of biomass during time in strains *Nostoc* S₈ and *Nostoc* 2S₁ could indicate adaptation of strains to mixotrophic mode of nutrition, since in laboratory they have been routinely maintained in phototrophic cultures. The most productive strain *Nostoc* 2S₃B in mixotrophic cultures showed decrease of biomass, due the exhaustion of glucose.



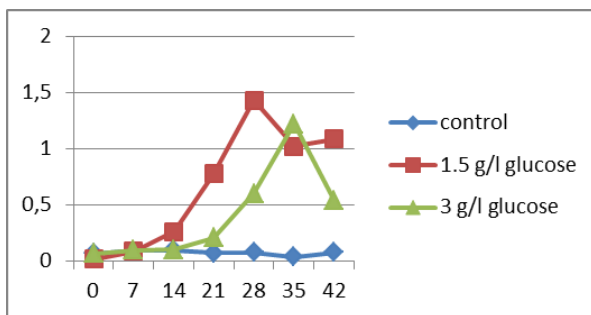
Graph. 1. Biomass production in strain *Anabaena* LC₁B



Graph. 2. Biomass production in strain *Nostoc S₈*



Graph. 3. Biomass production in strain *Nostoc 2S₁*



Graph. 4. Biomass production in strain *Nostoc 2S₃B*

There are many studies which showed that mixotrophic cultivation can significantly increase biomass production. In *Nostoc flagelliforme* biomass production in mixotrophic culture was 4.98 times higher than in autotrophic culture (Yu et al., 2009). In the study of Bhatnagar et al. (2011), mixotrophic growth of 3 microalgae was 3-10 times higher relative to phototrophic growth. It is considered that under mixotrophic conditions exist synergistic effects of heterotrophy and phototrophy (Bhatnagar et al., 2011). As the final metabolite of respiratory metabolism, CO₂ can be recycled for photoautotrophic growth (Cheirsilp and Torpee, 2012). In mixotrophic culture of *Anabaena* PCC 7120, increased activities of glucokinase and glucose-6-phosphate dehydrogenase indicated ability of cellular metabolism regulation of exogenous glucose at least at enzymatic levels (Yu et al., 2011). However, the degree

of utilization of different carbon sources is species specific phenomenon affected by the presence of specific permeases (Bhatnagar et al., 2011).

CONCLUSION

Cyanobacteria are microorganisms with enormous biotechnological potential. It is expected that their commercial application will grow with the development of technology and that will increase biomass production and decrease production costs. Therefore mixotrophic cultivation can be considered as very promising. Cyanobacteria are certainly important source of fatty acids. Strains which were tested in the present study showed ability to produce important fatty acids, as well as significant biomass increase in mixotrophic cultivation conditions. Taking into account the highest content of polyunsaturated fatty acids, as well as the highest biomass production, strain *Nostoc* 2S₃B has the greatest biotechnological potential.

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IMPLICATION OF CELLULAR TRAITS IN THE RESPONSE OF TOMATO UNDER WATER DEFICIT*

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SUMMARY: The effects of reduced irrigation treatments (PRD or DI) on the reduction of pericarp cell size are the most expressed during the fruit expansion phase. At maturity, PRD and DI have differ in effects on the outer layers: under PRD cells in most cell layers reached a similar final size as in FI treatment, while in DI cell grew slower and reached smaller cell size than in FI.

Key words: pericarp, cell size, drought

INTRODUCTION

The ability to transport and accumulate water and assimilates in the economically important organs such as fruit is very important for the productivity of crops and depends on the resistance and adaptation of plants to water reduction. Final tomato fruit size depends on fruit growth, especially on the pericarp cells growth. According to Ho and Hewitt (1986), the development of pericarp tissue determines more than two-thirds of the potential fruit weight. Tomato fruit development consists of three phases, including cell division, cell expansion, and ripening (Gillaspy et al., 1993). Both division and expansion activities in pericarp tissue, as determinants of tomato growth, depend on some environmental conditions, such as temperature and light (Bertin, 2005, Fanwoua et al., 2012), and water supply (Granier et al., 2000). It is well known that water deficit affects differentiation of reproductive tissue in citrus fruit (Syvertsen, 1990) through cell size effect (Đaković and Jovanović, 2003). It is well

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known that DI restricts fruit growth rate and final tomato fruit size (Pulupol et al., 1996, Davies et al., 2000, Savić et al., 2008, Pervez et al., 2009). However, the effect of PRD on cell division and cell expansion of tomato fruit size or tomato pericarp are not clearly understood. Some studies have demonstrated beneficial effects of PRD on tomato fruit yield, without reduction in fresh weight (Zegbe et al., 2006). The objective of the present study was to determine the effect of different irrigation treatments (FI, PRD and DI) on tomato fruit histology during fruit development in tomato wild type.

MATERIAL AND METHOD

Tomato plant (*Solanum lycopersicum* L.), cv. Ailsa Craig was grown from seed and at the fifth leaf stage repotted into pots (one plant per pot) filled with 11 kg of commercial compost (Potground H, Klasmann-Deilmann, Germany) and grown in a chamber (photoperiod 14h; light intensity at plant level $300 \mu\text{mol m}^{-2} \text{s}^{-1}$, day/night temperature 25/18°C and relative humidity 70%) at the Faculty of Agriculture, University of Belgrade. Pots (height 65 cm, diameter 20 cm, volume 20 dm^3) were specially designed for PRD experiments in such a way that they were vertically separated into two equally sized compartments (Fig. 1A and B). The root of each fifth leaf old plant was divided into approximate halves and repotted into these two hydraulically separated pot compartments. Compartments were classified as PRD-L (left side) and PRD-R (right side) (Fig. 1B). Ten days after repotting, 15 plants per genotype were subjected to the three following irrigation treatments: full irrigation (FI), in which the whole root system was irrigated daily to reach field capacity around 35%; partial root-zone drying (PRD), where the amount of water in one half was kept to 35% (wet side) while the other half was allowed to dry (dry side); the irrigation from wet to dry side was shifted when volumetric soil water content of the dry side had decreased to 15%-20%, and so alternating until the end of the experiment, and deficit irrigation (DI), in which water was evenly applied to the whole root system to reach 15-20% soil water content. The volumetric soil water content of both compartments of each pot was measured daily using TDR probes (time domain reflectometer, TRASE, Soil Moisture Equipment Corp., USA) at 20 cm depth. In total PRD and DI, plants received about 30-40% of the water that was applied for irrigation of the FI plants, respectively for PRD and DI.

For histological analyses, the first five fruits of the third truss were used. Tomato fruits were harvested at 12 daa (indicated on late cell division) and 44daa (indicated on cell expansion). Pericarp was divided into two equatorial halves, and on one half was used for histological analysis. Slides for light microscopy were made according to a standard paraffin procedure (Ruzin, 1999). Pericarp sections were observed with an Eclipse E800, Nikon stereomicroscope and images were acquired by a Photometric cool snap HQ² digital camera.

Mean and individual cell size measurements were estimated using the “analyze particles” tool of Image J (Rasband, 1997-2009, <http://rsbweb.nih.gov/ij/>), after manual adjustment of the segmentation threshold. Cell size was estimated on three to four independent sections devoid vascular bundles. Coloration on pericarp cross section was obtained by the ROI color coder plug-in of Image J software using sixteen color classes.



Figure 1. Tomato plants under climatic chamber conditions (A) and tomato plant in irrigation treatment (B).

For the analysis of the effects of PRD and DI treatments on cell growth kinetics within the pericarp, we identified the different cell layers on cross sections in relation to the position of vascular bundles. The cell layers located between the vascular bundles and the exterior pericarp were named: E1 (outer epidermis), E2 (outer sub-epidermis), E3 and E4 and the cell layers distributed between vascular bundles and internal pericarp were named: I1 (inner epidermis), I2 (inner sub-epidermis) and I3. During cell division new cell layers generated by E2 and I2 were named E2a, E2b, E2c, etc. and I2a, I2b, I2c, etc. respectively as illustrated on Fig. 2.

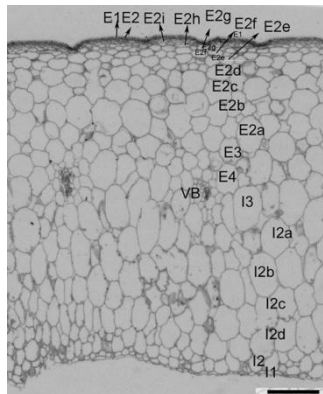


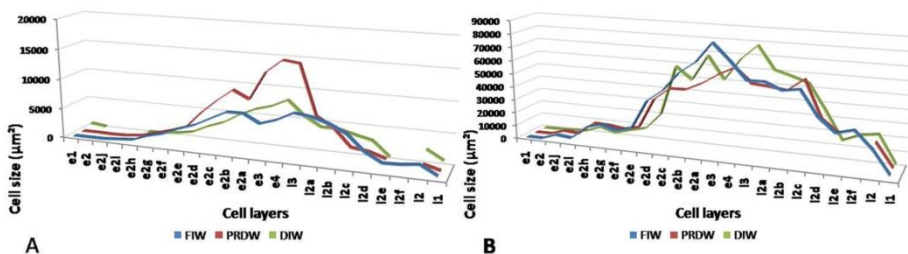
Figure 2. Tomato pericarp cross section with marked cell layers at 44 daa. The scale bars represent 500 μ m. VB: vascular bundle.

To get more precise insight on effects on cell area, differences between treatments observed at 12 daa and 44 daa, were analyzed separately by Unequal HSD test. The distribution of cell sizes was calculated with the mean and standard deviation of at least 1000 measurements per treatment. Modeled cell size distribution was plotted

using square root transformed data. The statistical analysis was carried out with the statistical package STATISTICA (Statsoft, USA).

RESULTS

The analysis of cell size combined with the identification of cell layers allowed us to visualize the spatial distribution of cell sizes (mean cell size per cell layer) according to their position in the pericarp at 12 daa to 44 daa, indicated late cell division and cell expansion phases, respectively. At 12 daa we identified about 23 layers for FI, four of them in the external part: E1 (outer epidermis), E2, E3, E4, and three of them in the inner part: I1 (inner epidermis), I2, I3, relative to the position of vascular, and cell layers were generated, mainly from periclinal divisions of the outer sub-epidermal layer (E2 successively giving E2a, E2b, E2c, etc.) and to a lesser extent from periclinal cell divisions of the inner sub-epidermis (I2 generating I2a, I2b, etc). In the course of pericarp development these newly formed layers were therefore pushed away towards the central part of the mesocarp. At 12 daa in wild type (Fig. 3A), significant differences were observed among treatments: fewer cell layers were generated in PRD conditions (21) compared to FI (23) and even fewer in DI conditions (19). These differences were maintained, to a lesser extent, until the 44 daa (Fig. 3B).



noted in E1 layer, with significant differences ($p < 0.001$) between PRD and DI on cell size, value from $229 \mu\text{m}^2$ (DI) to $339 \mu\text{m}^2$ (FI). The largest contribution to whole pericarp size at 44 daa was provided by the central pre-existing cell layers (generated before anthesis), e.g. for FI, E3 cell layer (value $83165 \mu\text{m}^2$), for PRD E4 cell layer (value $64000 \mu\text{m}^2$) and for DI I2a cell layers (value $78256 \mu\text{m}^2$). In WT, the negative effects of reduced irrigation (PRD or DI) on cell size appeared late in the development and were maximum for most outer cell layers (e.g. E3, E2a, E2b, E2c), whereas the inner layers generated after anthesis (I2a, I2b, I2c) were weakly affected or not affected at all. Under PRD at 44 daa, cells reached the size similar to those in FI treatment in most cell layers. In DI, all cell layers grew slower and reached slightly smaller cell size than in FI. Differences between treatments were significant ($p < 0.001$) at 44 daa.

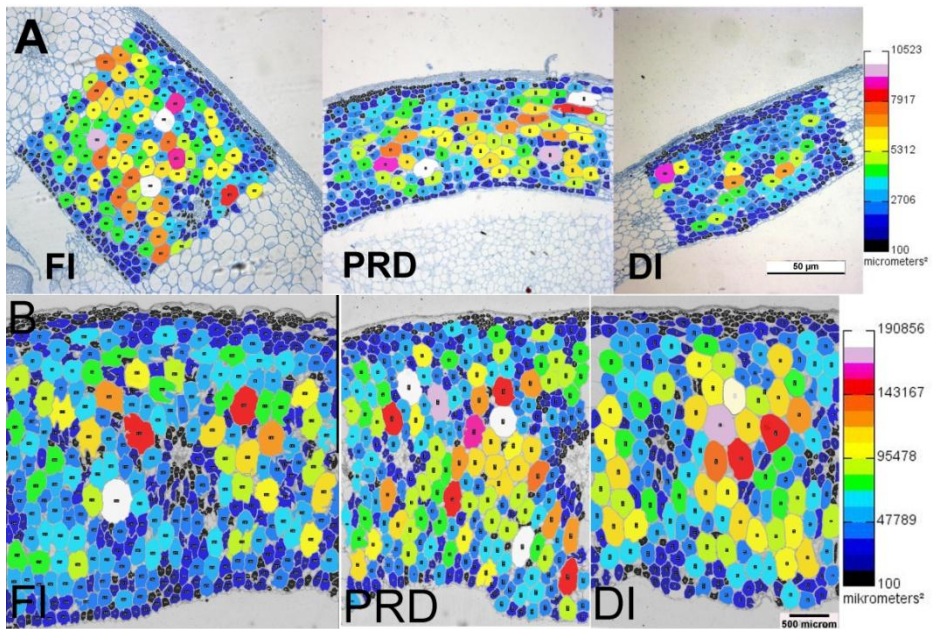


Figure 4. Microscopic characterization of PRD and DI effects on WT tomato fruit pericarp at 12 daa (A) and 44 daa (B). Identification of cell layers refer to Figure 2. Cells have been colored according to their class of section area, except for outer and inner epidermis, and first layers of very small cells below the outer epidermis, and vascular bundles. The scale bars represent 50 μm (A) and 500 μm (B).

Fig. 4. illustrates the effects of the irrigation treatments on the spatial distribution of cell sizes (colored according to their class of section area) in cross section of a wild type fruit pericarp at 12 daa and 44 daa. At 12 daa in PRD treatment cell size was smaller than in FI in most of the cell layers, especially in the outer pericarp. The figure clearly shows the strong negative effect of DI on number of cell layers and on cell size in outer and inner pericarp parts. At 44 daa, according to cell size colouring, under PRD similar cell sizes were recorded inside cell layers compared with FI, as well as a negative impact on cell size, under DI treatment, in most of cell layers which gives great impact on whole pericarp size, e.g. cell layers I2a, E2b, I3.

Figure 5 shows the distribution of cell areas measured on isolated cells at 12 daa and 44 daa. At 12 daa, the range of cell areas was similar for PRD and DI (no significant difference between treatments) comparing with FI. At 44 daa, comparing with 12 daa, differences between three irrigation treatments were clearly visible, and cell sizes were larger and more heterogeneous at 44 daa than at 12 daa.

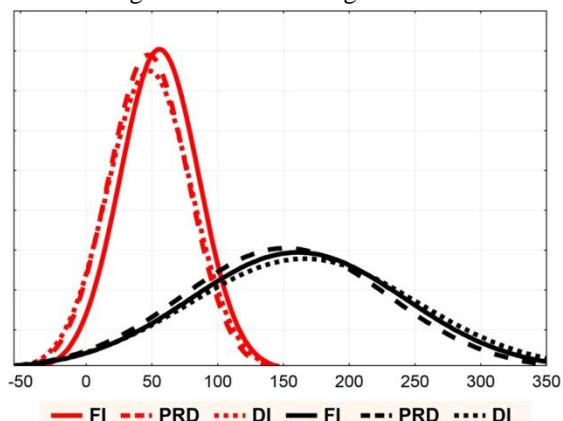


Figure 5. Frequency distributions of cell areas at 12 daa (red lines) and 44 daa (black lines), measured from anatomical sections. Each curve represents one irrigation treatment: FI (full line), PRD (dotted lines) or DI (dashed line).

Treatment effects varied during fruit development. At 12 daa, wild type pericarp cells were smaller and less heterogeneous in PRD and DI than in FI conditions, and differences were significant between FI and PRD, DI ($p < 0.01$) (Table 1). Cell areas measured at 12 daa were significantly lower ($p < 0.01$) in PRD and DI than in FI and the ranges were very narrow, indicating small and homogeneous cell size in the whole pericarp under DI or PRD. At 44 daa, cell size distributions were very similar in all treatments in wild type fruits, without treatment effects (Table 1).

Table 1. Unequal HSD test for pericarp cell size distribution at 12 and 44 daa under different irrigation treatments.

		12 daa			44 daa	
treatment	mean	Unequal HSD ($p < 0.01$)	Unequal HSD ($p < 0.05$)	mean	Unequal HSD ($p < 0.01$)	Unequal HSD ($p < 0.05$)
FI	3988,21	a	a	32508,83	ab	a
PRD	3155,09	b	b	29126,88	b	b
DI	3329,24	b	b	34931,23	a	a

DISCUSSION

The lower number of cell layers and reduction of cell size under DI show that this method of irrigation affected both the process of cell division and the process of cell expansion. In most outer pericarp cell layers, the negative impact of DI was mainly at 44 daa, where the cell expansion probably slowed down earlier than in FI, suggesting that at this late stage, water deficit represented a limiting factor for cell expansion. Water deficit in the inner pericarp affected cell expansion only indirectly by slowing down the rate of cell division, delaying the generation of new cells and therefore their expansion. Ojeda et al. (2001) indicated that pericarp cells in grapevines could reduce after early DI application, and that this change is irreversible. This result supported the hypothesis (Boyer, 1988) that early water deficit leads to cell wall structural changes due to cellulose sensitivity to the water deficit (Iraq and al., 1989), reducing the cell growth during cell expansion (Ojeda et al., 2001). Cell size reduction could be also explained by cell competition inside tomato pericarp, because pericarp cells are considered as competitive sinks in peach fruit (Quilot i Genard, 2008) or in tomato fruit (Bertin, 2005) probably as consequence of source limitation or limitation of phloem transport towards fruit under DI treatment (Rančić, 2011). In this study, we recognised PRD treatment as an irrigation method without a negative effect on cell division and cell expansion, which is indicated through the absence of reduction in pericarp cell size during final fruit development.

CONCLUSION

This study showed that tomato plants under PRD (70% of FI) could produce fruit with similarly sized pericarp cells as in control plants' fruits at 44 daa, eg. the phase near mature fruit stage. However, DI (60% of FI) negatively affected the whole pericarp. The study of water deficit effects on fruit histology and cytology are in progress and could bring new data which could help understanding the effects of water deficit on fruit pericarp in different tomato genotypes. Future investigations are needed to analyze phases of fruit development precisely and help us understand the effects of PRD not only on fruit growth, but also on fruit quality.

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IMPACT OF AMMONIA-LOADED ZEOLITE ON ITALIAN RYEGRASS GROWTH AND YIELD*

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SUMMARY: *The structure of natural zeolite of clinoptilolite type is ideal for sorption and ion exchange processes. In this study the influence of zeolite and ammonia-loaded zeolite on growth and yield of Italian ryegrass (*Lolium multiflorum* Lam.) was investigated on two types of soil: Planosol and Dystric Cambisol. Pot experiment carried out in greenhouse included four different treatments: a) soil (control); b) soil+zeolite; c) soil+ammonia-loaded zeolite; d) nitrogen application by mineral fertilizer; all in 4 replications. Our results show that there is a significant difference in yields obtained on the two types of soil, but the difference between treatments was not significant up to 3rd cut when the optimal yield was obtained in pots containing ammonia-loaded zeolite. The results are yet to be tested in field conditions.*

Key words: *zeolite, ammonia-loaded zeolite, Italian ryegrass, fertilizing.*

INTRODUCTION

Italian ryegrass (*Lolium italicum* L. syn *L. multiflorum* L.) is an important short duration grass in Serbia. High palatability and digestibility make this species highly valued for forage/livestock systems from early spring to late summer. It is used in many environments where fast cover or quick feed is required. Italian ryegrass is well-adapted to high rainfall, but can be grown where a minimum of about 500 mm rainfall occurs during the growing season (Evers et al., 1997). In Serbia, it is a dense-growing winter crop characterized by fast growth that secures quick tillering, high yield potential, fitness for reduced cultivation and good adaptability to heavy and moist soils. There are reports of ryegrass preference for ammonium nutrition over nitrate (Griffith and Streeter, 1994).

Original scientific paper / *Originalni naučni rad*

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Zeolite is used traditionally in agriculture as soil conditioner and as nitrogen retaining medium for nitrogen fertilizing improvements (Polat et al., 2004). The structure of natural clinoptilolite is ideal for sorption and ion exchange processes. Due to its structure and properties this natural, inert and non-toxic material can be used as a slowly releasing carrier of fertilizer, it can improve physical properties of soils and it can be used for treatment of contaminated soils (Reháková et al., 2004). Our experiment was conducted with a natural zeolitic tuff (from “Zlatokop” mine in south Serbia, containing ~70wt. % of clinoptilolite) and ammonia-loaded zeolite (formed by binding of ammonia ions from aqueous solution). The results of studies implicitly suggest that plants may have a good response if clinoptilolite is used as a fertilizer carrier while such method of fertilization is ecologically advantageous since the active compounds and nutrients are washed out into the soil slowly and gradually (Malekian et al., 2011). Clinoptilolite can also influence bioavailability of other plant nutrients and there are some indications that it contributes to releasing phosphorus in the first 24h for about 60%, while modified NH_4^+ -zeolite increases the release of phosphorus for additional 150% when added to the rock phosphate (Lopičić et al., 2013). Furthermore it has been reported that zeolites, with their specific selectivity for ammonium, can take up this specific cation from either farmyard manure, composts, or ammonium-bearing fertilizers, thereby reducing losses of nitrogen to the environment (Sparks et al., 2011). Based on the results of research, natural zeolite can be recommended for agricultural purposes in terms of sustainable fertilizing and improving system cattle farm - manure - organic fertilizer for forage crops (Simić et al., 2013).

MATERIAL AND METHODS

The soils used for the experiment were Pseudogley (*Planosol*) collected from the site in Varna (West Serbia) and Dystric brown soil (*Dystric Cambisol*) collected from the site in Vlasina (South-east Serbia). Soil samples collected at the sites were pooled and homogenized whereas stones and roots were removed. The pots were filled with 2 kg of air-dried soil and the ryegrass seed was sown on 12 December 2013. at a rate of 50 seeds per pot.

The experiments carried out in greenhouse included four different treatments: a) soil (control); b) soil+zeolite CLI (10 g kg^{-1}); c) soil+ammonia-loaded zeolite NH_4^+ -CLI (10 g kg^{-1} equivalent to nitrogen application of 100 kg ha^{-1} N); d) nitrogen application by mineral fertilizer Calcium ammonium nitrate CAN (100 kg ha^{-1} N, CAN contains 27% nitrogen); all in 4 replications.

The pots were placed in an unheated greenhouse and were thoroughly watered, while the growth of the plants was monitored. The temperature in greenhouse was above 10°C during the experiment and it can be considered as optimal for Italian ryegrass since it is physiological active when temperature rises above 0°C (Griffith and Chastain 1997). Plants were cut back three times in all the individual experimental pots, about 2-3 cm above soil level, on 34th, 61st and 84th day after planting when the experiment was terminated. The foliage was collected for determination of fresh and dry weight per cut and the harvested material was left to dry at room temperature for a few days before it was dried in an oven at 60°C until constant mass. Plant tissue was examined for changes in weight and the data were

analysed by analysis of variance (ANOVA). The treatment effect was determined according to Fischer's least significant difference procedure.

The main chemical characteristics of the soils were determined: pH (in water and in aqueous solution of CaCl_2) and the contents of P_2O_5 , K_2O , total C and total N. For pH measurement distilled water and 0.01 M CaCl_2 were used; the pH is measured in suspension by Orion pH meter connected to a Ross combined pH electrode. Loss on ignition is calculated in percent of soil dried at 105°C. The extractions of elements were done in ammonium lactate (AL) solutions; the ammonium-lactate extraction is performed according Egner et al. (1960) and the elements are measured by use of ICP-OES. For determination of elements the inductively coupled plasma optical emission spectrometer (ICP-OES), model Perkin-Elmer Optima 5300 DV, was used. The contents of sand, silt and clay were measured and the textural classes were determined according to USDA Soil Survey manual.

The ammonia-enriched zeolitic tuff (grain size in the range 0.063-0.1 mm) was prepared as described in details by Milovanović et al. (2013).

RESULTS AND DISCUSSION

The soils selected for the experiment are traditionally used for extensive forage production since their physical and chemical properties (Table 1) could be restrictive for an intensive agricultural production. Planosol is marked as conditionally productive soil whereas Dystric Cambisol is marked to serious restrictions (Protic et al., 2003).

The soils have low nitrogen contents. This could considerably reduce yields since the leaves of plants grown with a limiting nitrogen supply are smaller, compared with these of plants grown with an optimum nutrient supply (Griffith and Streeter, 1994). Yield quality could also be affected since nitrogen stress decreases the concentration of protein and enhances that of (hemi) cellulose and lignin (Lambers et al., 2008). Italian ryegrass reacts rapidly to increased N application doubling the yield of proteins in relation to control (Simić et al., 2009).

Table 1. Soil properties

Soil type / <i>Tip zemljišta</i>	Textural class / <i>Teksturna klasa</i>	Chemical properties / <i>Hemijske osobine</i>					
		pH		AL- P_2O_5 mg kg ⁻¹	AL- K_2O mg kg ⁻¹	Total C %	Total N %
		in H ₂ O	in CaCl_2				
Planosol / <i>Pseudoglej</i>	Sandy loam / <i>Peskovita ilovača</i>	5.73	5.07	19.8	115.1	1.37	0.16
Dystric Cambisol / <i>Distrično smeđe zemljište</i>	Clay loam / <i>Glinovita ilovača</i>	5.10	4.18	6.7	63.0	1.10	0.096

Number of seedlings and plant height were measured before the first cut (Table 2). There was no significant effect of treatment or soil type on germination rate.

Table 2. Number of seedlings and plant height (Standard Deviations appear in parentheses)

	Control	CLI	NH ₄ ⁺ – CLI	CAN
Seedlings (Number pot ⁻¹) / <i>Klijanci (Broj po posudi)</i>				
Planosol	44.5 ^a (1.12)	43.5 ^a (1.80)	44.5 ^a (1.50)	44.8 ^a (2.86)
Dystric Cambisol	41.2 ^a (2.86)	46.8 ^a (2.05)	43.0 ^a (1.22)	44.5 ^a (1.11)
Plant height (cm) / <i>Visina biljaka (cm)</i>				
Planosol	24.33 ^a (0.78)	24.82 ^a (2.68)	24.45 ^a (0.35)	24.10 ^a (1.02)
Dystric Cambisol	22.60 ^b (1.35)	22.2 ^b (1.30)	22.13 ^b (0.62)	22.31 ^b (0.62)

* Means with differing superscripts are significantly different (P<0,05).

* *Vrednosti sa različitim superskriptama se statistički značajno razlikuju (P<0,05).*

Average germination rate of 88 % corresponds with the literature (Jovanović and Tešić-Jovanović, 1972) and therefore it can be concluded that addition of zeolite or ammonia-loaded zeolite doesn't have a negative effect on the germination of Italian ryegrass seeds. Soil type did have a significant influence on plant height. In relation to the plants grown on Dystric cambisol the plants grown on Planosol were 8.3 % higher in average. Soil acidity of Dystric cambisol could have an inhibitory effect on plant growth and leaf elongation. Plant height was not affected by nitrogen-source, as well as nitrogen content.

Although Italian ryegrass is tolerant of low soil pH the yield decreases when the pH is below 5.5 (Hart and Mellbye, 2009). Our results show that there is a significant difference in yields obtained on the two types of soil in each cut (Table 3). Acidity is a major limitation to soil productivity and soil pH values below 5.0 to 5.5 warn that soluble levels of certain metals, particularly Al₃⁺ and Mn₂⁺, may be high enough to be biologically toxic (McBride, 1994).

Table 3. Dry matter yield, in grams (Standard Deviations appear in parentheses)

	Control	CLI	NH ₄ ⁺ – CLI	CAN
I cut / <i>I otkos</i>				
Planosol	0.2649 ^a (0.0197)	0.2423 ^a (0.0295)	0.2847 ^a (0.0082)	0.2652 ^a (0.0153)
Dystric Cambisol	0.1835 ^b (0.0087)	0.1888 ^b (0.0209)	0.1455 ^b (0.0259)	0.1571 ^b (0.0026)
II cut / <i>II otkos</i>				
Planosol	0.4628 ^a (0.0121)	0.4166 ^a (0.0482)	0.4948 ^a (0.0518)	0.4565 ^a (0.0664)
Dystric Cambisol	0.2381 ^b (0.0211)	0.2677 ^b (0.0269)	0.2504 ^b (0.0155)	0.2321 ^b (0.0248)
III cut / <i>III otkos</i>				
Planosol	0.5071 ^b (0.1143)	0.3748 ^b (0.0444)	0.7684 ^a (0.1006)	0.6841 ^a (0.0773)
Dystric Cambisol	0.3827 ^c (0.0438)	0.4103 ^c (0.0198)	0.4247 ^b (0.0265)	0.5053 ^b (0.0615)

* Means with differing superscripts are significantly different (P<0,05).

In the first two cuts there were no significant differences in yields between the treatments. The addition of nitrogen was proven to have effect on yield in third cut. A marked increase in the dry matter yield was seen for NH₄-CLI additive, compared to control, while CLI additive did not have a favourable effect on it. As expected, commercial fertilizer CAN significantly increased Italian ryegrass yield compared to control.

The delayed effect of the treatments could be explained by the limiting effect of irradiance. Both the total level of irradiance and the photoperiod, spectral composition and direction of the light affect plant development (Lambers et al., 2008). Furthermore, low red/far red ratio might preclude morphogenic responses in Italian ryegrass even before an important depletion in energy availability takes place (Casal et al., 1987).

CONCLUSION

The results implicitly suggest that plants may have a good response if clinoptilolite is used as a nitrogen fertilizer carrier. Such method of fertilization is ecologically advantageous since the active compounds and nutrients are washed out into the soil slowly and gradually. On the basis of results obtained in pot experiments on the yield of Italian ryegrass under the influence of different N application, it can be concluded that this grass species reacts to N supply increasing the yield of dry weight in relation to control. Italian ryegrass dry matter quality could be good indicator of ammonia intake and is yet to be analysed. Why the yield of Italian ryegrass was not significantly different until the third cut was not determined. Further research is need for testing the proposed hypothesis. The next stage of our research will be testing the results in field conditions.

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ENERGY USE AND ENERGY EFFICIENCY OF CORN PRODUCTION IN DIFFERENT FERTILIZATION STRATEGIES

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SUMMARY: *Due to increasing energy prices, the efficient use of technical energy in cropping systems become more important. In long term trials (2007-2012) at two experimental sites ("Wagna" and "Wagendorf") in the Austrian province of South Styria, the direct energy use (fuel, heating oil, electricity) and indirect energy use (energy for the production of farm machinery, herbicide, fertilizer and seed) as well as energy efficiency in corn production were analysed. The influence of different mineral nitrogen fertilization rates (0, 90, 115, 145, 175, 210 kg N/ha as calcium ammonium nitrate) and a liquid pig manure treatment were compared. The calculated energy efficiency indicators (energy intensity, energy output/energy input-ratio, netto-energy output) showed, that the soil fertility had a big influence on energy efficiency. The energy efficiency indicators at the experimental site Wagendorf with very good soil conditions were better than at Wagna. Within the mineral nitrogen treatments in Wagna, the nitrogen fertilization rate of 90 and 115 kg N/ha reached the highest energy efficiency, whereas at Wagendorf it was at the 0 kg N/ha. Independently of the experimental site, the liquid pig manure treatment had the highest energy efficiency. The largest components of energy input in corn production with mineral fertilizer were the energy for drying (between 20.3 and 48.3 %) and energy for fertilizer production (between 20.7 and 37.2 %). For the mitigation of fossil energy input in corn production, a site specific nitrogen fertilization - at efficient with organic manure - and the application of regenerative energy carrier in corn drying are derived.*

Key words: *energy consumption, energy efficiency, corn, nitrogen-fertilization, drying*

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INTRODUCTION

A goal in sustainable agriculture is to use fossil fuel energy more efficiently in crop production. Due to increasing energy prices, the efficient use of technical energy in cropping become more important. The intensity of agricultural production processes can be evaluated with the direct and indirect energy use. The energy input in plant cropping can be categorised in direct energy (fuel for machinery, heating oil and electricity for drying processes or conveyors) and indirect energy (process energy for the production of fertilizers, pesticides, seeds and farm machinery), Hülshberger (2008).

For reducing the fuel consumption, there are many short-term measurements (e.g. driving with reduced engine speed, machine maintenance; adapted tire inflation pressure and optimised implement adjustment for slip reduction,...) and long-term measurements (e.g. machinery selection with reduced power requirement, change the tillage system to conservation tillage, improvement of the field shape,...), which are mentioned in Moitzl and Boxberger (2009).

Mineral nitrogen fertilizers are energy-intensive in their production and are responsible for increasing cropping yields. Many studies (Hoepfner et al. 2005, Moitzl et al. 2009, Moitzl et al. 2010, Szalay et al. 2009, Schüller et al. 2011) show, that the indirect energy input with mineral nitrogen has the largest energy contribution in conventional crop production systems.

In Austria, corn production has an importance for animal feeding and industrial processing. In the year 2012 27 % (=219702 ha) of arable land (=811509 ha) were cropped with corn, with an average yield of 10,7 t/ha (BMLFUW, 2013). This paper deals with the influence of different nitrogen fertilization strategies (mineral nitrogen: 0 kg N/ha to 210 kg N/ha and organic fertilizer with pig slurry) on energy use and energy efficiency on corn production (from seeding to drying harvested corn). The yield and management data were taken from two long term trials in South Austria.

MATERIAL AND METHODS

The two long term fertilization trials are located in the Austrian province of South Styria, with a good climate for corn cropping (Table 1). The distance between the location "Wagna" from the location "Wagendorf" is about 5 km.

"**Wagna**" is above a gravel terrace of the groundwater body "Westliches Leibnitzer Feld". The fertilization field trial with 12 different fertilization treatment was started in the year 2007. The block design consists of 6 replicates.

"**Wagendorf**" lies on a deep "Wagendorfer" terrace and has a very high soil fertility ("soil number" near 100). Since 2008 the influence of 13 different fertilization strategies on corn yield are studied in a block design with 4 replications.

Table 1. Yearly mean temperature and precipitation at the site Wagna-Leibnitz (ZAMG)

	2007	2008	2009	2010	2011	2012
Yearly mean temperature (° C)	10,4	10,4	10,2	9,6	11,0	11,2
Yearly precipitation (mm)	883	902	1312	1016	724	998

The soil (55 % sand, 33 % silt and 12 % clay; 2,4 % humus) at the location
The soil (9 % sand, 72 % silt and 19 % clay; 2,4 % humus) at the location
Table 2 shows the N-fertilization variants at location "Wagna" (period: 2007-2012)
and "Wagendorf" (period: 2008-2012).

Table 2. Nitrogen fertilization rates at "Wagna" and "Wagendorf"

N-fertilization rate	„Wagna“ 2007-2012	„Wagendorf“ 2008-2012
0 kg N		
90 kg N	45 kg N + 45 kg N as CAN ¹⁾	45 kg N + 45 kg N as CAN
115 kg N	55 kg N + 60 kg N as CAN	55 kg N + 60 kg N as CAN
Pig slurry 1 Application: surface broadcast 2. Application: band spreading with trailing hoses	2007: 146 kg N _{ff} ²⁾ 2008: 164 kg N _{ff} 2009: 117 kg N _{ff} 2010: 142 kg N _{ff} 2011: 115 kg N _{ff} 2012: 124 kg N _{ff} Ø: 135 kg N/ha	2008: 121 kg N _{ff} 2009: 115 kg N _{ff} 2010: 96 kg N _{ff} 2011: 115 kg N _{ff} 2012: 94 kg N _{ff} Ø: 108 kg N/ha
145 kg N	55 kg N + 90 kg N as CAN	55 kg N + 90 kg N as CAN
175 kg N	55 kg N + 60 kg N + 60 kg N as CAN	55 kg N + 60 kg N + 60 kg N as CAN
210 kg N		70 kg N + 70 kg N + 70 kg N as CAN

¹⁾ CAN: Calcium Ammonium Nitrate

²⁾ N_{ff}= 87 % from N_{total}

³⁾ Slurry amount between 23 and 45 m³/ha

⁴⁾ Slurry amount between 29 and 58 m³/ha

The ripeness number of the corn variety was at Wagna 320 and at Wagendorf 400. Fuel consumption was calculated with the fuel calculator from KTBL (Association for Technology and Structures in Agriculture, www.ktbl.de) for a used mechanisation with tractors of 90 and 120 Hp. Energy consumption for drying of the harvested corn was calculated with the basic data from Rossrucker (1977). Heat value of corn: 18,6 MJ/kg DM (Hülsbergen, 2008).
The used energy-equivalents are shown in table 3.

Table 3. Energy-equivalents

	Farm facilities	Energy-equivalent	Source
Direct energy	Fuel, Heating oil	47,8 MJ/l	CIGR, 1999
	Electricity	12 MJ/kWh	CIGR 1999
Indirect energy	Mineral N-fertilizer	60 MJ/kg N	CIGR, 1999 angepasst
	Mineral P-fertilizer	17,4 MJ/kg P ₂ O ₅	CIGR, 1999
	Mineral K-fertilizer	13,1 MJ/kg P ₂ O ₅	CIGR, 1999
	Synth. Herbicide	242 MJ/kg	Hülsbergen 2008
	Seed	100 MJ/kg	Hülsbergen 2008, CIGR, 1999
	Machinery	1956 MJ/ha	Biedermann 2009

The energy efficiency was evaluated with three indicators:

$$\text{Energy intensity (MJ/kg)} = \frac{\text{Energy input (MJ/ha)}}{\text{Corn yield}_{(14\% \text{ w.b.})} \text{ (kg/ha)}}$$

$$\text{Energy output/Energy input-rauo} = \frac{\text{Energy output (MJ/ha)}}{\text{Energy input (MJ/ha)}}$$

$$\text{Netto-energy output (GJ/ha)} = \text{Energy output}_{\text{Corn (14\% w.b.)}} \text{ (GJ/ha)} - \text{Energy input (GJ/ha)}$$

RESULTS AND DISCUSSION

Corn yield

The location in Wagendorf with the fertile soil reached an average yield of 13.651 kg/ha and was 4.534 kg (= 50 %) higher than in Wagna (Table 4). The average moisture content of the harvested corn was in Wagna 22 % w.b. and in Wagendorf 24 % w.b.

Table 4. Mean corn yields (kg/ha at 14 % w.b.); Different letters indicate significant differences (Student-Newman - Keuls Test, $\alpha = 0,05$) between nitrogen fertilization rates

	0 kg N	90 kg N	115 kg N	Pig slurry	145 kg N	175 kg N	210 kg N	Mean
Wagna 2007-2012	5.033 ^a ±1.624	8.962 ^b ±1.313	9.848 ^b ±1.875	8.897 ^b ±1.966	10.797 ^c ±2.380	11.163 ^c ±1.803		9.117 ±2.726
Wagendorf 2008-2012	10.662 ^A ±1.993	13.818 ^{BC} ±1.014	14.223 ^C ±884	13.337 ^B ±1020	14.485 ^C ±976	14.634 ^C ±831	14.402 ^C ±908	13.651 ±1.715

Energy input

The energy input-data in MJ/ha are shown in figure 1 and 2. The energy of drying increases with the corn yield. The heating oil consumption for drying of the harvested corn to a moisture content of 14 % w.b. ranged from 57 to 124 l/ha at Wagna and from 152 to 205 l/ha at Wagendorf.

The energy input increased with increasing mineral N fertilization from 22,2 GJ/ha to 28,7 GJ/ha (Figure 1). In the pig manure fertilization treatment, the total energy input decreased to 15,5 GJ/ha and was around 1 GJ/ha higher than the zero treatment. The surface-related energy input in the pig manure fertilization treatment at the site Wagna was lower by 37 % and 41% in comparison to the mineral nitrogen rates of 115 kg N/ha and 145 kg N/ha. At the site Wagendorf 27 % and 32 % less energy were used in the pig manure fertilization treatment. A Canadian study showed that the substitution of mineral nitrogen fertilization by organic fertilization can save 36-52 % of energy in the production system of maize grains (McLaughlin et al., 2000). Here in the mineral nitrogen fertilization rates (60-164 kg N/ha) energy-input from 19,1 to 22,3 GJ/ha with corn yields of 6,8 to 8,8 t/ha were calculated.

By fertilization with pig slurry the energy-input was reduced to 11,9-12,9 GJ/ha (McLaughlin et al., 2000). In Italian investigations, it could be demonstrated

that organically produced corn needed 58 % lower energy than the conventional production system with mineral fertilizers (Sartori et al. 2003).

In the N fertilization rate (90 kg N/ha) the drying energy was on the similar level as the energy required for the production of mineral N fertilizer (figure 1). The percentage of energy use by the mineral nitrogen fertilizer was at 90 kg N/ha 24,3 %, at 115 kg N/ha 28,5 %, at 145 kg N/ha 32,7 % and at 175 kg N/ha 36,6%. For comparison, McLaughlin et al. (2000) calculated on heavy soil at 60 kg and 77 kg mineral nitrogen rate an energy share of 35 %, on medium soils at 164 kg and 144 kg mineral nitrogen rate an energy share of 54 % and on light soils with a mineral nitrogen rate of 118 kg/ha an energy share of 33 %.

The amount of drying energy in total energy use was at the mineral N-fertilization treatments between 20,3% and 23,6 % (Figure 1). With increasing N fertilization the percentage of fuel energy of 21,1 decreased to 16.5%.

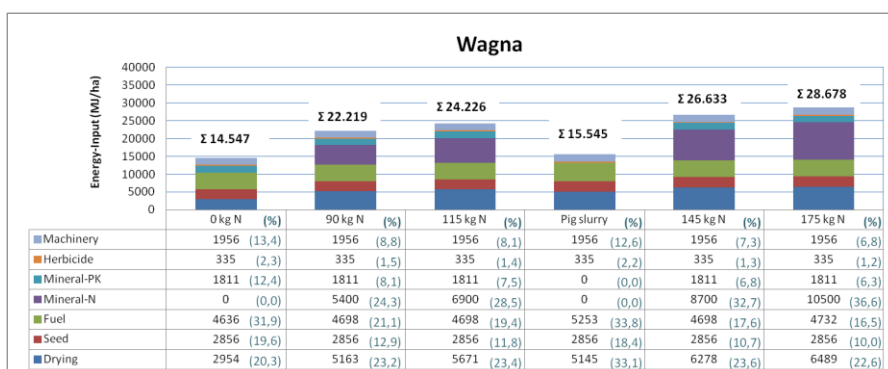


Figure 1: Mean energy input per hectare in different N-fertilization variants at the experimental site Wagna.

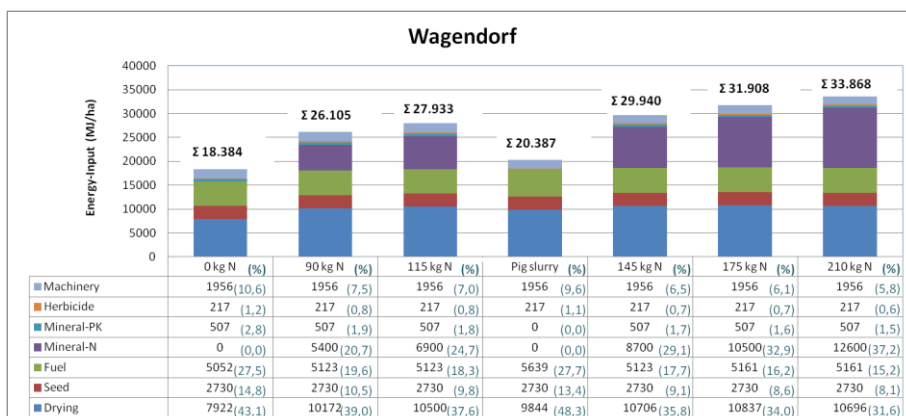


Figure 2: Mean energy input per hectare in different N-fertilization variants at the experimental site Wagendorf.

Energy efficiency

The lowest energy intensity at the highest energy output/input-ratio was reached with 110 kg N/ha at the site Wagna (Figure 3). The highest net energy output with 179 GJ/ha was achieved at a nitrogen rate of 175 kg/ha. The curve of the ratio of net energy output corresponds to the law of diminishing returns by E. A. Mitscherlich (1874-1956). The organic nitrogen fertilization treatment (pig manure with mean 135 kg N/ha), showed at an average netto-energy output of 150 GJ/ha, the lowest energy intensity of 1,75 MJ /kg of maize grain and the highest energy output/energy input-ratio of 10,6:1.

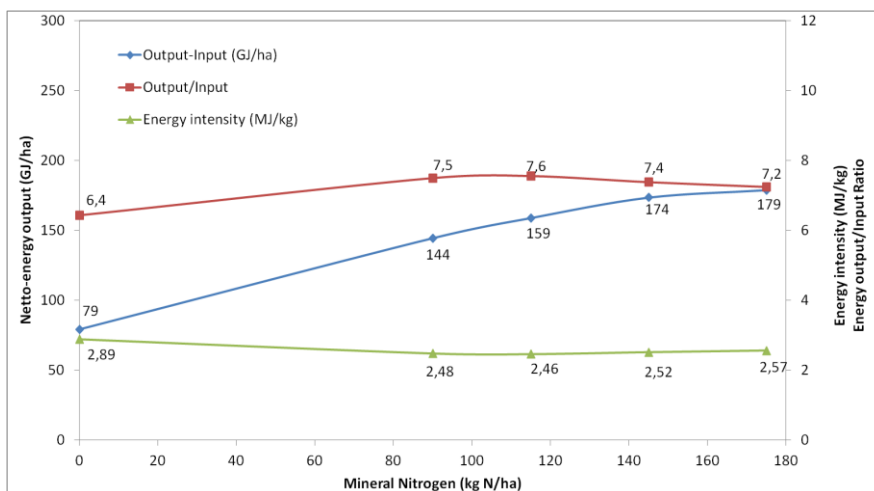


Figure 3: Energy efficiency at mineral N-fertilization variants at the experimental site Wagna

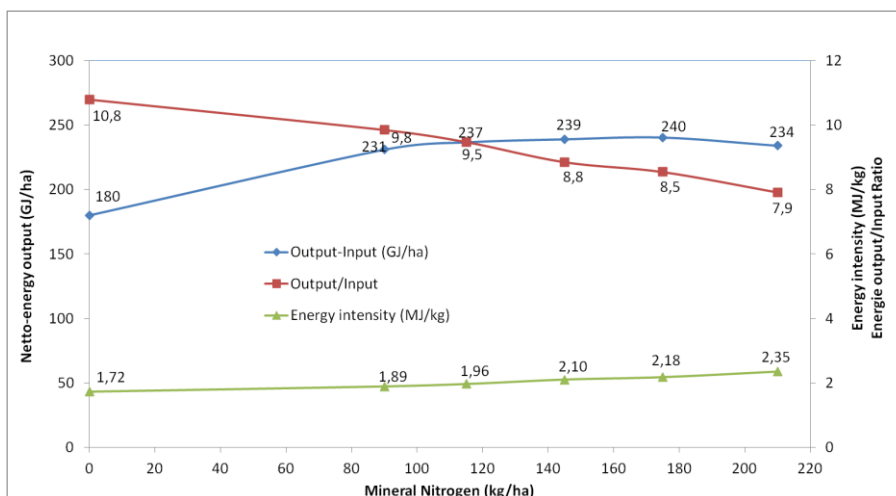


Figure 4: Energy efficiency at mineral N-fertilization variants at the experimental site Wagendorf

With the exception of the net energy output, the curves of the indicators energy intensity and energy output/energy input-ratio for the mineral N-fertilization treatments differed at the site Wagendorf (Figure 4). Compared with all mineral N-fertilization treatments showed the zero treatment (=0 kg N/ha), the lowest energy intensity of 1,72 MJ / kg with the highest energy output/energy input-ratio of 10,8:1. With increasing mineral fertilization the energy indicators (energy intensity and energy-output/energy-input ratio) were worse.

The fertilization variant with pig slurry reached at a net energy output of 228 GJ/ha , the lowest energy intensity of 1,53 MJ/kg and the highest energy-output/energy-input ratio of 12,2:1. The liquid organic manure is a valuable fertilizer in agriculture and provides with environmentally-friendly spreading technology (band spreading with trailing hoses) good yields with high energy efficiency.

CONCLUSION

The site with its characteristic soil and climate conditions had a large influence on energy efficiency in corn production. The nitrogen mineralisation for the organic matter at the very fertile site Wagendorf caused high corn yields. An additional soil organic matter and nitrogen balance can bring further insight, with which mineral N fertilization rate the humus-content can be sustainably stabilized. In comparison to the mineral nitrogen fertilization variants, the liquid organic manure variant reached the highest energy efficiency. Within the mineral nitrogen fertilization treatment at the site Wagna, the highest energy efficiency was achieved at 90 and 115 kg N/ha, while at the site Wagendorf it was in the zero treatment (0 kg N/ha). The following measures for the reduction of fossil energy use in grain maize can be derived: Location adapted nitrogen fertilizer - preferably with organic manure - and the use of renewable energy sources (heat from biomass district heating supply systems or biogas plants) in the maize grain drying.

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STINGING NETTLE CULTIVATION IN FLOATING HYDROPON*

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SUMMARY: *Stinging nettle (Urtica dioica L.) is a perennial plant which has been widely used in medicine, food, textile and cosmetic industries as well as in organic production. Whereas it is quality control of wild plant material difficult and expensive there is a need to introduce nettle in to agricultural production. Application of modern cultivation technology (floating hydropon) can eliminate mentioned problems. Higher yield and increased number of harvests in the period when the nettle is already dormant in the open field can be achieved. The experiment was laid out according to the randomized complete block scheme with three replications in unheated greenhouse. The effect of three sowing densities (0.2, 0.5 and 0.9 g m⁻²) and two substrates (perlite, vermiculite) on the stinging nettle yield and number of harvest were tested. Sowing was conducted in the first decade of September in 2012. During the nettle cultivation five harvests were realized: two in autumn (October 23, November 29) and three in spring period (March 15, April 16 and May 6). In autumn season plants grown in perlite had significantly more leaves and the nodes compared to those grown on vermiculite. Higher sowing density resulted in higher yield in autumn growing period (0.57 kg m⁻²) while in the spring higher yield was recorded in the lowest sowing density (1.01 kg m⁻²). Regardless sowing density an equal yield of stinging nettle was achieved in vermiculite (0.93 kg m⁻²) during autumn and in perlite (1.08 kg m⁻²) during spring period. All tested parameters were significantly affected by sowing density×substrate interaction during the nettle cultivation. In autumn period the combination of vermiculite×0.5 g m⁻² resulted with significantly highest yield (0.96 kg m⁻²) while in spring the highest yield (1.41 kg m⁻²) was achieved by combination of perlite×0.2 g m⁻².*

Key words: *Urtica dioica L., floating hydropon, multyharvest, sowing density, yield*

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INTRODUCTION

Stinging nettle (*Urtica dioica* L.) is a perennial herb widely distributed throughout the temperate regions of the world (Bacci et al., 2009). This valuable medicinal plant is perhaps best known as an abundant and perennial weed (Weiß, 1993; Harwood and Edom, 2012). Nettle has a long history of usage in alternative medicine as an herbal remedy (Bisht et al., 2012) for the treatment of many diseases (Grevsen et al., 2008) and is currently receiving attention as a source of fiber (Harwood and Edom, 2012; Rutto et al., 2013). Despite great medicinal values stinging nettle is undervalued by almost all of us (Bisht et al., 2012). Fresh nettle leaves are rich in chlorophyll, vitamin C, vitamin K, panthotene acid, carotenoids, B group vitamins (B1 and B2), tannins, essential oil, proteins and minerals such as iron, copper, manganese, nickel (Kukrić et al., 2012) and calcium. Processed nettle can supply 90 – 100 % of vitamin A (including vitamin A as β -carotene) and is good source of protein (Rutto et al., 2013). According to Guil-Guerrero et al. (2003) stinging nettle could be used by humans as healthy food because of essential fatty acids and carotenoids which contain in high amounts. Because of great nutritional values the young leaves are the most appropriate for human consumption (Guil-Guerrero et al., 2003). In recent years the scientific studies are increased due to the positive impact of nettle on coronary heart disease, high blood pressure, diabetes, cancer, inflammative, viral and parasitic disease, psychotic disorders (Otles and Yalcin, 2012) and expressed antioxidant and antimicrobial activities (Kukrić et al., 2012; Gülçin et al., 2004).

The largest percentage of *Urtica dioica* L is wild-harvested (Upton, 2013), grown in rich soils in forest clearings, old fields and wasted places (Bisht et al., 2012). Nettle prefers to grow in soil that is nitrogen rich in areas high in inorganic nitrates in heavy metals. Collection should be done in selected areas, avoiding locations where agribusiness and industrial runoffs occur (Upton, 2013). When nettles are gathered from natural habitat the control of quality standards is rather difficult and cost extensive (Weiß, 1993). Considering that in the last few years the quality standards for medicinal plants have been tightened (Weiß, 1993) it is necessary to introduce nettle in to agricultural production. Cultivation enables to control some environmental factors and so enhance the quality of the product. According to Weiß (1993) main quality factors are high leaf yield of herbaceous medicinal plants, homogeneity of plant material, a low nitrate content and low contaminations such as residues of pesticides or heavy metals. Stinging nettle as nitrophilous plant should be fertilized at the beginning of cultivation with 150 kg N ha⁻¹ and with 30 – 40 kg N ha⁻¹ after each harvest (Stepanović et al., 2009). According to Biesiada et al. (2009) the highest yield of raw material was provided by nitrogen fertilization in the dose of 150 kg N ha⁻¹. However, in order to achieve higher yield producers often apply to large amounts of nitrogen fertilizer which can result in accumulation of nitrate, reducing the quality of plant material (Fabek et al., 2012). Application of appropriate modern cultivation technology can eliminate problems of growing nettle in the open field, i.e. higher yield and increased number of harvest in the period when the nettle is already dormant. Higher yield in hydropon is based on the efficient use of nutrients and water, resulting in rapid growth, an early harvest, a larger number of production cycles and biomass production per unit time and area (Osvald and Kogoj-Osvald, 2005; Toth et al., 2008; Toth et al., 2012). Soilless systems, and especially hydroponics (water culture), allow a direct control of plant nutrient supply (Gonnella et al., 2004).

Hydroponic production of medicinal crops in controlled environments provides opportunities for improving quality, purity, consistency, bioactivity, and biomass production on a commercial scale (Hayden, 2006). The floating system is rather new, inexpensive and easy hydroponic technique. It can be used to produce ready-to-use vegetables (D'Anna et al., 2003). In this production system trays continuously float on a water bed or nutrient solution (Pimpini and Enzo, 1997; Nicola et al., 2007). According to Nicola et al. (2007) floating system is suitable to produce vegetables both with short cultural cycle and with high plant density.

Because of the capability of the apical plant parts regeneration it is possible to achieve several harvests at intervals what depends on the purpose and the period of cultivation. During the first harvest the plants should be cut 0.5 cm above cotyledons to avoid damage to plant growing point and ensure rapid and abundant regeneration (Toth et al., 2012).

Since stinging nettle is usually collected from natural habitats very little information about its cultivation has been published. There are no results about the stinging nettle cultivation in floating hydropon. Therefore, the aim of research was to examine the possibility of growing nettle in floating hydropon and to determinate the effect of sowing densities and different substrates on morphological characteristics and nettle yield.

MATERIAL AND METHODS

The two factorial trials were conducted according to the randomized complete block scheme with three replications in unheated greenhouses. The effect of three sowing densities (0.2, 0.5 and 0.9 g m⁻²) and two substrates (perlite, vermiculite) on the stinging nettle yield and number of harvest were tested. Sowing was made manually in polystyrene boards in the first decade of September in 2012. Nutrient solution adjusted for leafy vegetables was prepared according to Tesi (2002). Nettle cultivation in floating hydropon is shown in Picture 1.



Picture 1. Cultivation of stinging nettle in floating hydropon

During the nettle cultivation five harvests were realized: two in autumn (October 23, November 29) and three in spring period (March 15, April 16 and May 6). In autumn growing period the production cycle from sowing to the first harvest lasted 47 days, and the second harvest was conducted after 38 days. After winter dormancy which lasted 106 days, in spring growing period the production cycle were shorter and lasted 32 and 20 days, respectively. The cutting was at the height approximately 5 centimeters and the plants were weighted just after cutting.

The abiotic parameters of nutrient solution (temperature, pH- and the EC-values) and air (relative humidity, minimum, maximum and mean temperature) were measured daily.

The effects of sowing density and substrate were determined using analysis of variance (ANOVA) and average values were tested by the least significant difference (LSD) test at the significance level $p \leq 0.05$ and $P \leq 0.01$.

RESULTS AND DISCUSSION

If nettles is grown as a leafy vegetable, harvest should be done just before flowering (Upton, 2013) which occurs in summer months when the temperatures increase. According to Stubljär et al. (2013) in the first year of stinging nettle cultivation plant growth is slow due to the undeveloped rhizomes so a significant contribution to the green herb cannot be expected. However, cultivation in unheated greenhouses can result with an earlier harvest and higher yields even in the first year of cultivation.

Optimal temperatures for nettle growth are between 15 and 25 °C while at temperatures higher than 30 °C may appear early flowering causing lower yield (personal experience). In unheated greenhouses optimal temperatures for nettle growth can be achieved much earlier than in the open field which is the reason an earlier beginning of harvest. Abiotic parameters of air (minimum, maximum and mean temperature, relative humidity) and nutrient solution (pH- and EC-values) during the nettle cultivation in floating system are shown in Fig. 1. In autumn growing period pH of nutrient solution was in range from 6.38 to 7.02, 6.76 in average. Average pH value in spring season was slightly higher (6.82), however smaller variations between the decades were recorded (from 6.66 to 6.96). Similar to the pH value average value of nutrient solution EC was higher (2.38 dS/m) in spring compare to autumn growing period (2.34 dS/m). D'Anna et al. (2003) had proven in their research that different EC levels have not affected the plant height and slightly influenced the yield of rocket.

The first harvest was in autumn and it was realized 47 days after sowing (October 23) while second harvest was after 38 days (November 29). After winter dormancy beginning of nettle harvest was on March 15 which is almost three months earlier comparing to first harvest achieved by Grevsen et al. (2008) in the open field. Second harvest was after 32 days (April 16) and third after 20 days (May 6). Higher temperatures in spring growing season resulted in shorter production cycles. Although the average mean temperatures were similar in both growing period (16.79 in autumn and 16.31 °C in spring) in spring season have been recorded more days with maximum temperatures above 25 °C resulting shorter period between harvests. Weiß (1993) claims that in spring and mainly in early summer due to photoperiodic reaction plant senescence is accelerated. Relative humidity in growing period was between 54 and 81 % which is within the recommended values for leafy vegetables.

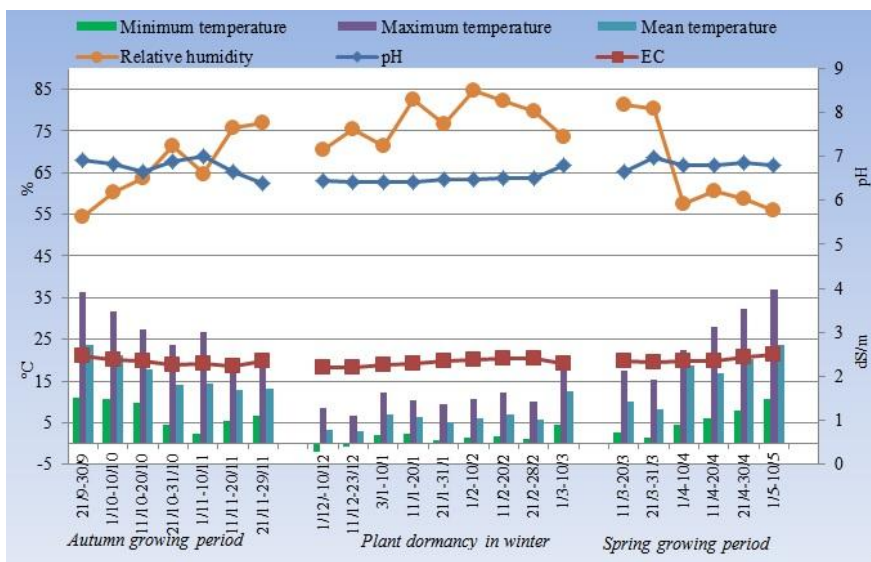


Fig. 1. Abiotic parameters of air (minimum, maximum and mean temperature, relative humidity) and pH- and EC-values of nutrient solution

In the autumn growing period all measured morphometric parameters (number of leaves, length and width of leaves, number of nodes, plant height and plant mass) were significantly affected by substrate×sowing density interaction (Table 1 and 2). Regardless sowing density, plants grown in perlite had significantly more leaves and the nodes compared to those grown on vermiculite. Lower sowing density in perlite in first and in vermiculite in second harvest resulted with more leaves (9.0 and 13.5) comparing to higher sowing density. According to Žnidarčič and Kacjan-Maršič (2008) in higher sowing density plants produce less leaves due to increased competition. The highest leaf length was measured in combination vermiculite×0.2 g m⁻² (42.75 mm) which is contrary to the literature. According to Zanin et al. (2009) leaf length increased along with plant densities. In the same combination of substrate and sowing density, significantly higher plants (190.00 mm) with greater mass (1.02 g) were recorded. In the second harvest this was achieved with combination perlite×0.2 g m⁻² (121.26 mm; 3.29 g).

Table 1. Analysis of variance for morphometric parameters of stinging nettle in autumn growing period

Source of variance	Plant mass, g	Number of leaves	Length of leaves, mm	Width of leaves, mm	Number of nodes	Plant height, mm
<i>First harvest</i>						
Substrate (S)	*	*	*	**	*	**
Sowing density (D)	**	*	n.s.	**	*	**
S×D	*	*	**	**	**	**
<i>Second harvest</i>						
Substrate (S)	*	*	*	**	**	*

Sowing density (D)	**	**	**	**	**	**
S×D	*	**	**	**	*	**

*significant at $p \leq 0.05$, ** $P \leq 0.01$, n.s.=not significant

In the spring growing period significantly influence substrate×sowing density interaction was recorded. Plants grown in perlite were higher and had more leaves which were shorter compared to vermiculite. The highest values all measured morphometric parameters (except leaf length) were recorded at the lowest sowing density (data not shown).

Table 2. Effect of substrate×sowing density on stinging nettle morphometric parameters in autumn growing period

Substrate	Sowing density / g m^{-2}	Plant mass / g	Number of leaves	Length of leaves / mm	Width of leaves / mm	Number of nodes	Plant height / mm
<i>First harvest</i>							
Perlite	0.2	0.85 bc	9.00 a	30.25 C	52.50 A	4.75 A	48.00 E
	0.5	0.55 d	8.00 b	35.00 B	39.50 B	4.00 B	124.50 C
	0.9	0.72 c	8.33 b	32.25 BC	41.75 B	4.25 B	77.50 D
Vermiculite	0.2	1.02 a	8.00 b	42.75 A	34.75 C	4.00 B	190.00 A
	0.5	0.84 bc	8.00 b	35.50 B	27.50 D	4.25 B	183.25 A
	0.9	0.94 ab	8.00 b	41.25 A	30.50 D	4.00 B	163.00 B
<i>Second harvest</i>							
Perlite	0.2	3.29 a	11.00 B	32.50 C	46.26 A	5.50 a	121.26 A
	0.5	1.91 bc	13.50 A	29.26 D	36.00 B	4.50 b	68.75 D
	0.9	1.55 cd	13.33 A	34.50 B	37.00 B	3.50 c	72.50 D
Vermiculite	0.2	2.18 b	13.50 A	35.00 AB	34.00 C	4.76 b	80.00 C
	0.5	1.08 d	10.00 C	28.26 D	26.26 E	2.76 d	90.00 B
	0.9	1.72 bc	12.00 B	36.76 A	30.50 D	2.50 d	80.00 C

*Mean values followed by the same letter within each column do not differ significantly at $p \leq 0.05$ and $P \leq 0.01$ according to the LSD test

Significant influence of substrate, sowing density and substrate×sowing density interaction in the autumn and spring growing period on stinging nettle yield was recorded (Table 3). Greater sowing density in autumn growing season resulted in higher yield (0.74 and 0.70 kg m^{-2} at 0.5 and 0.9 g m^{-2}) while in spring higher yield was recorded in the lowest sowing density (1.31 kg m^{-2} at 0.2 g m^{-2}). This indicates that in spring growing period greater sowing density had negative impact on yield, contrary to the plants grown in autumn. Similar results have been achieved with cultivation nettle in controlled environment agriculture aeroponic and soil-less medium system (Pagliarulo et al., 2004). It is contrary to Toth et al. (2012) which have achieved an equal average total yield in lowest and in greater sowing density in spring growing period. Regardless the sowing density an equal yield of stinging nettle was achieved in vermiculite (0.93 kg m^{-2}) during autumn and in perlite (1.09 kg m^{-2}) during spring period.

The highest yield was achieved by combination perlite \times 0.2 g m⁻² (1.41 kg m⁻²) and vermiculite \times 0.2 g m⁻² (1.22 kg m⁻²) what was reached in the last harvest in spring growing period. This is contrary to Grevsen et al. (2008) which claim that first harvest and second re-harvest (cut) gave the highest yield whereas the third re-harvest is not profitable. These results confirm that the floating system gave a continuous water flow and supply from the roots to the shoots, which was an advantage for leaf mass production (Nicola et al., 2007).

Table 3. Effect of substrate and sowing density on stinging nettle yield

Treatment	Yield (kg m ⁻²)				
	Autumn period		Spring period		
	First harvest	Second harvest	First harvest	Second harvest	Third harvest
<i>Substrate</i>					
Perlite (P)	0.36 B	0.37	0.71 b	0.68	1.09 a
Vermiculite (V)	0.93 A	0.43	0.83 a	0.60	0.97 b
<i>Sowing density</i>					
0.2 g m ⁻²	0.50 b	0.42	0.91 A	0.80 A	1.31 A
0.5 g m ⁻²	0.74 a	0.39	0.65 B	0.56 B	0.90 B
0.9 g m ⁻²	0.70 a	0.39	0.74 AB	0.56 B	0.87 B
<i>Substrate \times Sowing density</i>					
P \times 0.2 g m ⁻²	0.09 c	0.38 ab	0.86 ab	0.92 a	1.41 a
P \times 0.5 g m ⁻²	0.52 b	0.35 b	0.64 c	0.62 b	0.93 b
P \times 0.9 g m ⁻²	0.47 b	0.38 ab	0.62 c	0.49 b	0.93 b
V \times 0.2 g m ⁻²	0.90 a	0.45 a	0.96 a	0.69 b	1.22 a
V \times 0.5 g m ⁻²	0.96 a	0.43 ab	0.67 bc	0.49 b	0.87 b
V \times 0.9 g m ⁻²	0.94 a	0.41 ab	0.86 ab	0.62 b	0.82 b

*Mean values followed by the same letter within each column do not differ significantly at $p \leq 0.05$ and $P \leq 0.01$ according to the LSD test

The cumulative yield of two growing periods (autumn and spring) is shown in Fig 2. The highest accumulated yield after five harvest (two in autumn and three in spring) was recorded in combination vermiculite \times 0.2 g m⁻² (4.22 kg m⁻²). The lowest accumulated yield (2.89 kg m⁻²) was achieved by sowing in perlite in the greatest sowing density.

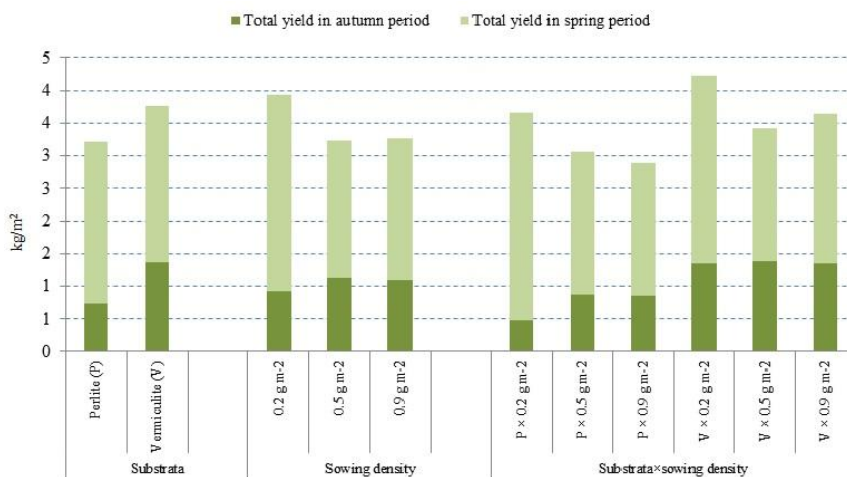


Fig 2. Effect of substrate and sowing density on stinging nettle total (after autumn and after spring harvests) and cumulative (autumn+spring harvests) yield during nettle cultivation

CONCLUSION

Stinging nettle showed good suitability to soilless cultivation by floating system achieving satisfactory yield per harvest already in the first year of cultivation. High yield and increased number of harvests have been achieved in the months when the nettle is dormant in the open field. The highest yield were recorded in spring growing period in combination perlite \times 0.2 g m⁻² (1.41 kg m⁻²) and vermiculite \times 0.2 g m⁻² (1.22 kg m⁻²).

Lower plant density has to be preferred because it gives higher yield and it allows reducing the leaf length enhancing quality especially in spring growing period. Due to economic cost effectiveness combination of perlite \times 0.2 g m⁻² can be recommended for nettle cultivation in floating hydropon.

Further investigations are required to research nutritional and chemical values of stinging nettle grown in floating system at different nutrient solutions in order to produce high quality of plant material for nutritional and medicinal purpose.

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ANTIMICROBIAL ACTIVITY OF GARLIC (*A. SATIVUM* L.) AGAINST *SALMONELLA ENTERITIDIS* AND *STAPHYLOCOCCUS* *AUREUS**

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SUMMARY: *Worldwide emergence of antimicrobial resistance among zoonotic pathogens imposes a need for an alternative approach to antibacterial therapy. The aim of this study was to evaluate activity of raw garlic extract against locally isolated serotypes of Salmonella Enteritidis and Staphylococcus aureus. For this purpose, standard antibiogram method was used. Results showed high efficacy of full garlic concentration, except in the case of one isolate of S.aureus, and varying efficacy of half extract doses, which was higher for S. aureus than for S. Enteritidis.*

Key words: *Allium sativum*, antimicrobial activity, *Salmonella Enteritidis*, *Staphylococcus aureus*

INTRODUCTION

Bacteria from genus *Salmonella* represent an important zoonotic pathogen of economic significance in both humans and animals. *Salmonella Enteritidis* (*S. Enteritidis*) and *Salmonella Typhimurium* are the serovars most frequently associated with human illness. Human cases of *S. Enteritidis* infection are most commonly associated with the consumption of contaminated eggs and poultry meat, and usually

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result in gastrointestinal illness of varying severity (EFSA and ECDC, 2013b). Invasive forms of the disease, such as bacteremia and meningitis may occur in a smaller subset of patients, while the state of asymptomatic carrier (convalescent or chronic) may be frequently present in both humans and animals (D'Aoust, 1991; EFSA and ECDC, 2013b).

Staphylococcus aureus (*S. aureus*) is an ubiquitous organism that has been recognized as one of the leading causes of foodborne bacterial intoxications worldwide. It is also responsible for a diverse spectrum of human and animal diseases ranging from minor skin infections to life threatening conditions, such as pneumonia and meningitis (Normanno et al., 2007; Sasidharan et al., 2011).

Although mild and self-limiting in many cases, infections caused by these two pathogens may also turn dubious, or even fatal, in the absence of efficient antibiotic therapy. Worldwide, number of reports on antimicrobial resistance in both human and animal bacteria is increasing, making it an important public health concern (Normanno et al., 2007). The resistance develops as a consequence of extensive prophylactic and therapeutic usage of antimicrobial drugs, often in subtherapeutic concentrations, and due to their administration as growth promoters in food animal production, where large number of animals is being exposed irrespectively of their health (De Oliveira et al., 2005; Yildirim et al., 2011). Consequently, antimicrobial-resistant bacteria in food animals may threaten the efficacy of human drugs if antimicrobial-resistant bacteria or antimicrobial-resistance genes become incorporated into human bacterial populations (De Oliveira et al., 2005).

Results of studies on complementary and alternative medicine practices suggest that introduction of plant extracts, such as garlic extract, in antimicrobial therapy may significantly decrease this emerging burden of drug resistance (Ćirković et al., 2012).

Garlic (*Allium sativum* L.) is a hardy annual monocotyledon plant, and one of the oldest cultivated vegetable crops. The earliest record of the medical properties of garlic and directions for its use come from historian Herodotus and Hippocrates, „the father of western medicine“ (Jones and Mann, 1963; Rabinowich and Brewster, 1990). It is a well known fact that garlic has been used to treat infections for centuries (Onyeagba et al., 2006). Today, garlic's antidiabetic, antibiotic and perhaps anticancer effects are receiving a worldwide recognition due to the abundance of scientific literature supporting these effects. It is also believed that garlic has hepatoprotective, and antioxidant effect (Lalošević i sar., 2013).

The aim of this study was to evaluate antimicrobial activity of two crude garlic extract concentrations against locally isolated serotypes of *S. Enteritidis* and *S. aureus*.

MATERIAL AND METHOD

In the experimental work of the study selected strains of *S. aureus* and *S. Enteritidis*, originating from human swab, were included. All strains were isolated and identified in the Center for Microbiology, of the Institute of Public Health of Vojvodina in Novi Sad. In this scientific work, 9 strains of *S. aureus* and 13 strains of *S. Enteritidis* were used.

The sensitivity of strains to crude garlic extract was tested by the disk diffusion technique according to Kirby-Bauer on Muller-Hinton agar (pH 7.3 ± 0.1 at 25°C). After autoclaving, the prepared substrate was poured into Petri dishes thickness 4 mm. An overnight culture of the bacterial suspension was prepared in physiological saline, concentration of 6×10^8 cfu ml⁻¹ (the 2th scale per McFarland (Klement et al, 1990.)). Inoculation of bacterial strains on Muller-Hinton agar was performed with sterile swab. Thirty minutes after application of the bacterial suspension, filter disks size 6 mm, immersed in a crude extract of garlic and distilled water, were placed on the surface of the substrate at regular intervals. The crude extract of winter garlic (*Allium sativum* var. *vulgare*) was obtained by the mechanical pressing of cloves, after previous immersion in 75% alcohol and rinse with water for surface disinfection. Concentrated extract, and 50% solution of the extract, were used for testing, while sterile distilled water was used as control.

The incubation period was 20 hours at $37 \pm 1^{\circ}\text{C}$. After that, measuring of the inhibition zones, in mm, was carried out. Performing the procedure by disk-diffusion method and reading the results was performed according to the recommendation and guidance made by Clinical and Laboratory Standards Institute (CLSI 2010). The experiment was set up in three replication.

Statistical analysis was performed using software package Statistica 10. Interspecies differences in susceptibility to garlic were investigated with t-test, for level of significance of 95% ($p < 0.05$ was considered significant). Differences in mean inhibition zone values were compared using Duncan test.

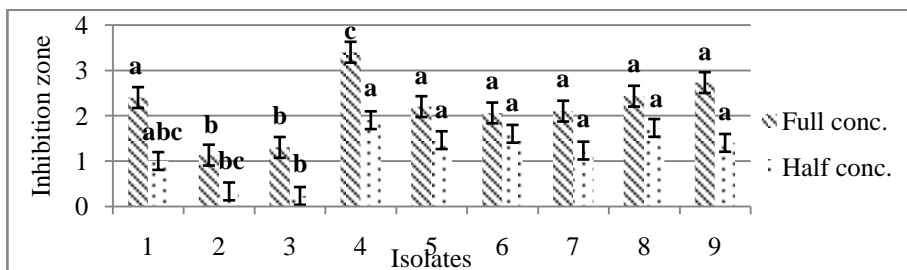
RESULTS

Antibacterial activity of crude garlic extract was observed in all clinical isolates of *S. aureus* and *S. Enteritidis* from the study. For both 100% and 50% extract concentrations, highest average inhibition zone was found in *S. aureus* than in *S. Enteritidis* (Table 1), but the observed difference could not be considered statistically significant (p -value was well above 0,05). However, significant differences were observed when sensitivity to crude garlic extract was compared between isolates of the same species/serotype (Graph 1 and Graph 2) which appeared more prominent in the case *S. aureus*.

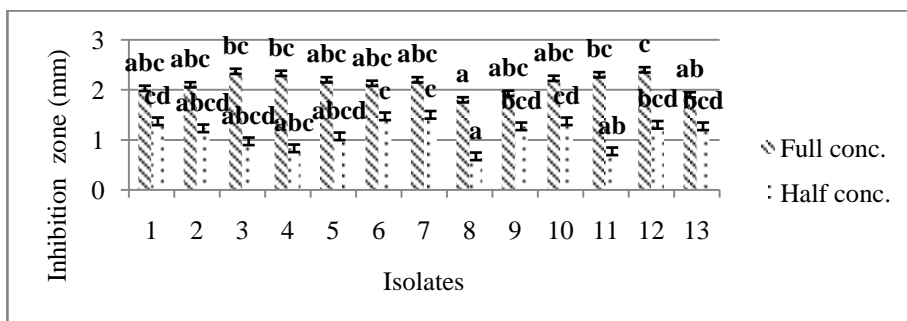
Table 1: Average inhibition zones, given for the 100% and 50% concentration of garlic extract

	Extract concentration	Inhibition zone of bacteria (mm)		p-Value
		<i>S. aureus</i>	<i>S. Enteritidis</i>	
Average	100%	21.6	21.4	0.940
	50%	12.1	11.6	0.815

Graph 1: Comparison of susceptibility of isolates within the *S. aureus* species



Graph 2: Comparison of susceptibility of isolates within the *S. Enteritidis* serotype



DISCUSSION

According to the European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) (2013a), a statistically significant decrease in the number of human salmonellosis cases was observed in the European Union (EU) over the period 2008-2011. On the other hand, studies around the world report an increase in antimicrobial resistance of this pathogen, with resistance prevalence varying between and within countries as well as between *Salmonella* serotypes (De Oliveira et al., 2005). Serotype with the lowest antimicrobial resistance seems to be *S. Enteritidis*, despite the fact that it is being most frequently isolated from samples. Along with the prevalence, varies the pattern of antimicrobial resistance. Fortunately, resistance to the critically important antimicrobials for human

medicine, cefotaxime (a third-generation cephalosporin) and ciprofloxacin (a fluoroquinolone) is relatively low (EFSA and ECDC, 2013b). In Serbia, resistant strains of *S. Enteritidis* have been confirmed in both human and animal samples (Stošić et al., 2006; Petrović et al., 2008; Kozoderović et al., 2012).

In staphylococci, the methicillin-resistance is considered to be the most significant form of antimicrobial resistance, because it is conferred by the presence of *mecA* gene, which encodes for production of an altered penicillin binding protein that has a low affinity for all beta-lactam antimicrobials (penicillins, cephalosporins, carbapenems), making methicillin-resistant bacteria insensitive to this broad range of antibiotics (Weese and van Duijkeren, 2010).

Until recently, human isolates of methicillin-resistant *S. aureus* (MRSA) were usually associated with hospital-acquired infections; currently, it became evident that MRSA may also represent a challenge as both community-acquired infections and colonizing zoonotic agent in companion and food producing animals (Normanno et al., 2007; Weese and van Duijkeren, 2010). Although recent data showed decreasing or stabilizing MRSA percentages in most European countries, they exceeded 25 % in eight states (EFSA and ECDC, 2013b) making MRSA a lingering human public health priority and a perfect candidate for alternative medical approaches.

Garlic has been described as one of the most significant plants of complementary and alternative medicine. It holds up the highest concentration of sulfur compounds such as allicin and other biologically active components. These are responsible not only for its pungent smell but also for its medicinal value (Kemper, 2000). The thiosulfate allicin has confirmed anti-microbial properties, but other non-sulphur constituents, like proteins, saponins and phenolic compounds, may also contribute to its anti-microbial activity (Corzo-Martinez et al., 2007). The mechanism of antibacterial activity of garlic has not been fully elucidated. According to Feldberg et al. (1988) allicin from garlic extract, directly or indirectly blocks the synthesis of bacterial RNA, without significant effect on DNA and protein synthesis. Therefore, it is considered that the primary tool of allicin is the blocking of RNA formation.

In our previous work, antimicrobial activity of garlic extract was established against different species of zoonotic pathogenic bacteria, such as: *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium*, *S. Enteritidis*, *S. bovis morbificans* and *S. aureus* (Vlajić et al., 2013). The experiment, however, included only one isolate of both *S. Enteritidis* and *S. aureus*, in contrast to the present study, where 9 isolates of *S. aureus* and 13 isolates of *S. Enteritidis* were examined. Current results showed a slightly higher sensitivity of *S. aureus* isolates in comparison to *S. Enteritidis* isolates; furthermore, variations in sensitivity between isolates were distinctively higher within *S. aureus* than within *S. Enteritidis* group. Higher sensitivity of *S. aureus* was also observed by other Serbian authors (Ćirković et al., 2012), although their research was of the quantitative nature. Antimicrobial efficacy of garlic against *Salmonella* and *Staphylococcus*, including the resistant strains, has

been observed by authors around the world, in both *in vitro* (Shokrzadeh et al., 2006; Daka and Awole, 2009; Belguith et al., 2010) and *in vivo* (Tsao et al., 2003; Bakri and Douglas, 2005; Uzodike and Igwe, 2005; Adebolu et al., 2011) studies. All these studies reported a concentration/dose-dependent antimicrobial efficacy of garlic compounds, which is in concordance with our results.

Synergistic action of garlic, which was observed in combination with some antibiotics (Tsao et al., 2003; Ćirković et al., 2012), and suggestion (Ankri and Mirelman, 1999) that development of resistance to beta-lactam antibiotics is 1000-fold easier than development of resistance to allicin from garlic, additionally contribute to existing antibacterial potential of garlic.

CONCLUSION

Both *S. aureus* and *S. Enteritidis* human isolates from our study proved susceptible to the crude garlic extract indicating its therapeutic potential. Average inhibition zones were slightly higher for *S. aureus* isolates, which also exhibited reciprocal differences in susceptibility levels. Correlation between crude garlic extract concentrations and diameter of inhibition zones was observed.

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ANNUAL DRAINAGE VOLUMES AND NITRATE-NITROGEN LEACHING UNDER SILAGE MAIZE IN WET AND DRY YEAR

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SUMMARY: The quantity of nitrate in groundwater is affected by diffuse agricultural pollution and hence, it is strongly linked to the amount of applied nitrogen (in surplus) on agricultural land. In order to determine nitrate-nitrogen leaching in diverse precipitation conditions, lysimetric field experiment was set in 2011 in Varaždin County, Croatia. Leachate from three zero-tension pan lysimeters on agricultural plot (maize) was collected twice a month or after abundant rainfall. Research results show that 25% of total precipitation leached in 2012 and 13% in 2013. ANOVA results confirm there is a statistically significant difference in $\text{NO}_3\text{-N}$ concentrations between hydrological dry and wet years. In dry years $\text{NO}_3\text{-N}$ concentrations vary from 36 to 56 mg/l and in 2013 from 8 to 40 mg/l.

Key words: nitrate-nitrogen, leachate, hydrological conditions, lysimeter, maize

INTRODUCTION

Intensive loss of nitrate or high nitrate level in different water bodies has been increasing over recent decades. Highly soluble NO_3^- , if not taken up by crops or denitrified, can be moved below the root zone and has the potential to affect the quality of groundwater. Nitrate leaching to groundwater, as one of the major concern of intensive agricultural production depends not only on the amount of nitrate in the soil profile but also on the amount of water in soil. Diverse climatic condition (precipitation seasonality) in interaction with inherent site factors, such as micro relief attributes, agricultural management practice, hydraulic soil properties and the soil biological activity cause year-to-year differences in percolation at given location. High nitrate concentration in water is one of the main reasons for drinking water quality decrease and eutrophication of surface waters and can cause serious health problems as well. Biogeochemical exchange between terrestrial ecosystems and groundwater is predominantly unidirectional in most landscapes, where downward water transport from ecosystems to aquifers (recharge) is the only flux connecting them and the primary pathway of nitrogen inputs to groundwater (Galloway et al., 2003). In these situations soil nutrients can be leached and laterally delivered to lower

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positions of the landscape via subsurface water flow (Portela et al., 2009). Understanding N exchange between crop ecosystems and groundwater becomes a crucial ecological issue, especially in intensive agricultural area. To mitigate these growing problems different action programs were designed to reduce nitrate concentration below Water Directive (80/778/EEC) limit of 50 mg l⁻¹ for nitrate (11,3 mg l⁻¹ for nitrate-nitrogen). The EU legislation had adapted The Nitrate Directive (91/676/ECC) with main goal to prevent advanced soil and water degradation caused by nitrate due to diffuse losses from agriculture. The Nitrate Directive demands that EU Member States define the catchments affected by nitrate pollution where agriculture is a significant source of nitrate pollution as Nitrate Vulnerable Zones (NVZs).

In Croatia five regions were classified as NVZs. Designation of NVZs is based on data derived from GIS database of water bodies and catchments boundaries, hydro geological map, map of natural groundwater vulnerability, available land use databases and ground, surface, transitional and coastal waters national monitoring results. Northern part of Varaždin County, in the northern Croatia, is one the delineated zones. Varaždin area represents one of the most intensive agricultural areas in Croatia; 59% of total county area is used as agricultural land. The most common agricultural production there is typical crop production (Vincek and Ernoić, 2009). There is highly developed intensive vegetable production on small family plots with an average size of 0,23 ha, which is twice as smaller than average plot size in Croatia (Statistical Yearbook of the Republic of Croatia, 2003). Also, there is a significant number of cattle and chicken farm. The stated indicates that agricultural pressures in Varaždin area are very high.

On the other hand, hydro geological conditions in this area are also very specific. The whole area is under influence of the large rivers Drava and Mura on its border, so the aquifer in this part is composed of gravel and sand with variable portions of silt (Urumović et al., 1990). The most common soil type is alluvial soil with shallow active profile mainly on gravel base. This means that the covering layer of aquifer is not continuously developed; in some parts it exceeds 2 meters and in some it completely disappears (Lavra and Marković, 2007). That is considered as favorable for aquifer recharge, but on the other hand this makes aquifer very subject to different pollutions.

There are few methods for direct nutrient and pollutant measurement and lysimeter installation is one of commonly used methods in terrestrial and aquatic ecosystems (Romić et al., 1997; Aronsson and Bergström, 2000; Romić et al., 2003; Matlou and Haynes, 2006; Bubalo et al., 2013). Field lysimeters study is set in Varaždin County on plot used for crop production (silage maize). There are some factors affecting nitrate leaching that cannot be controlled, such as precipitation and natural soil properties. However, knowledge about their impact on leaching rate and levels can optimize land management and diminish negative agricultural effects on resources pollution. So the objective of this research was to quantify and investigate annual and seasonal differences in leachate amount and N-leaching losses over a 3-year period under conventional silage maize production in northern Croatia.

MATERIAL AND METHOD

In April 2011 triplicate of zero-tension pan lysimeters (Fig. 1) was installed on rain fed silage maize plot (46°17'32"N, 16°06'31"E – according to WGS84 system) in Varaždin County, in a hilly part of the county. Since the plot has a noticeable incline, lysimeters were installed in a flatter part of the plot.

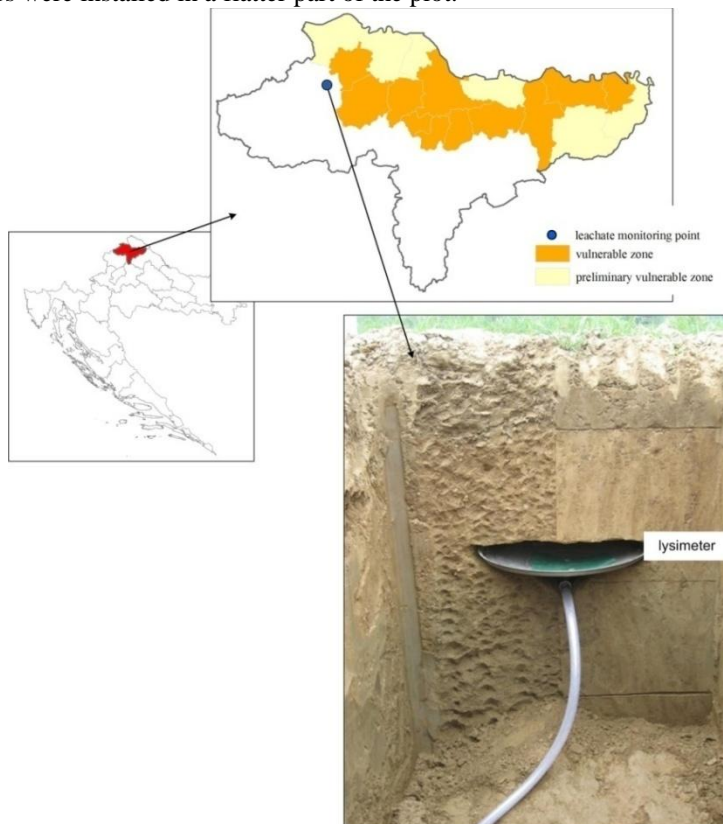


Figure 1. Location of lysimetric monitoring station in Varaždin County (Croatia)

The first step in lysimeters installation was opening the soil profiles and determining the depth of less permeable Cg horizon. The soil profile was additionally excavated at that depth so the soil layer above lysimeter remains undisturbed. PVC reinforced hose of suitable diameter is connected to lysimeter drain and is set with sufficient slope to ensure the flow of leachate. Leachate is collected in a plastic container in which the PVC pipe is built. In the same container the stiff plastic PVC pipe is vertically integrated and it has perforations along the wall to allow the pumping of the collected water. Vertical stiff PVC pipe is sealed with a plastic cap to prevent collection of rainwater and potential sources of contamination in the tank, more precisely to preserve the leachate samples. In the final stage, open pedological profiles were filled with excavated soil and additionally compacted to prevent the peripheral flow of groundwater towards the opened profile. This also enabled the unobstructed sowing, planting and technology of culture processing.

The plot was used in three consecutive years for production of silage maize. Sowing was done in third decade of April and harvest was done in second decade of September each year. Fertilization was done twice a year: manure fertilization after harvest (total N input=123 kg ha⁻¹) and additional mineral fertilization before sowing (total N input=48 kg ha⁻¹).

Soil samples, both bulk and cylinders, were collected after opening pedological profile. In cylinder samples from the genetic horizons of the profile were done the soil density test, soil capacity for water and air, and vertical water permeability of the soil (Laboratory of The Department of Amelioration, Faculty of Agriculture, Zagreb, own method). Bulk soil samples were analyzed for NO₃-N using a continuous-flow auto analyzer (Skalar San++ Analyzer, Breda, Netherlands) according to HRN ISO 14255:2004 to determine initial NO₃-N status.

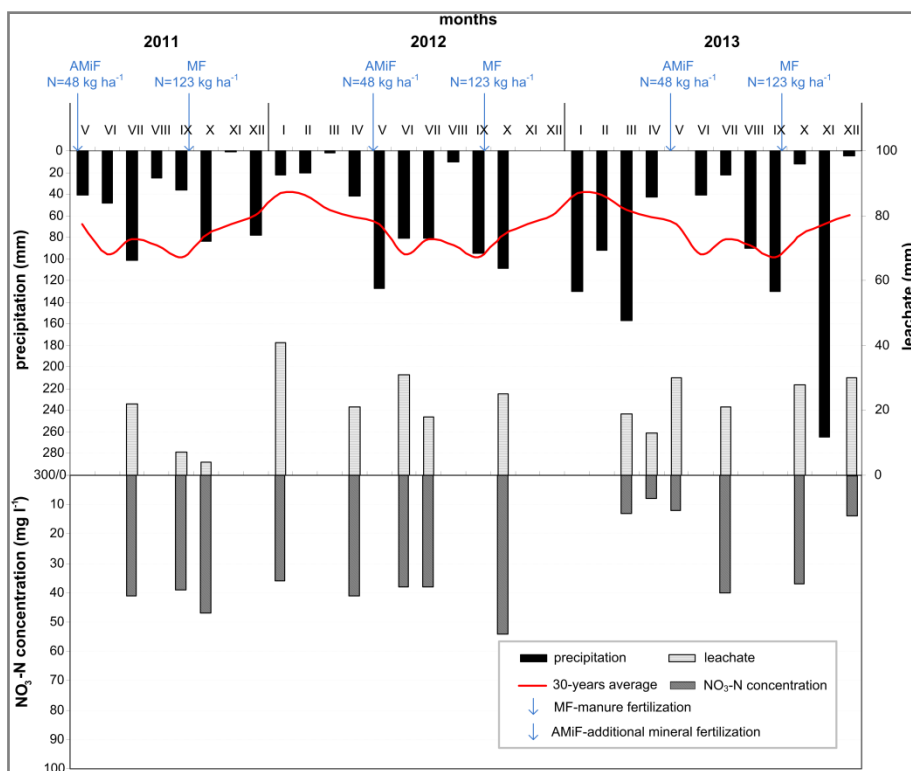
Leachate samples are collected by pumping in period May 2011-December 2013 according to determined dynamics: or after abundant rainfall or twice a month. Leachate samples are collected 14 times in that period. Samples are taken and transported to the laboratory under low temperature condition and kept under that condition until the analysis. Leachate samples were analyzed for NO₃-N using a continuous-flow auto analyzer (Skalar San++ Analyzer, Breda, Netherlands). In the paper the data for leachate amount and NO₃-N concentration in leachate will be expressed as an average value from collected samples in each sampling campaign. Analysis results were analyzed by analysis of variance (ANOVA) using the SAS statistical software package (SAS Institute, 2007).

RESULTS AND DISCUSSION

Results of cylinder samples analysis showed that the soil have high porosity and high permeability (the average value of permeability coefficient K at depth of 10-15 cm is $4,1 \times 10^{-3} \text{ cm s}^{-1}$) which enables fast nutrient leakage to deeper horizons. NO₃-N content in top layer (0-35 cm) was 36 mg kg⁻¹ and there is noticeable decreasing trend with increasing depth; NO₃-N content at depth 95-130 cm was 2,0 mg kg⁻¹.

Graph 1 displays precipitation variation in research period, 30-years average annual precipitation, leachate amount and measured NO₃-N concentrations in leachate.

Total annual precipitation at meteorological station Varaždin has a 30-years average of 832 mm. In research period both hydrological situations occurred: years 2011 and 2012 were dry and year 2013 was wet, compared to 30-years average. Total annual precipitation was 414, 589 and 1103 mm, respectively. Leachate amount analysis on annual level did not include year 2011 because research was set in April, so the whole hydrological year could not be covered. Leachate amount in research period varies from 4 to 43 mm with an average of 21 mm. Minimum amount was recorded in September and October 2011 and maximum was recorded in January 2012.



Graph 1. Nitrogen-nitrate leaching (amount, mm and concentration, mg l⁻¹) and precipitation (mm)

High precipitation and plant's inability to uptake all available nitrate results in surplus nitrate leaching (Romić et al., 2003). Researches indicate that in Croatia in current climatic conditions the risk of nitrogen leaching from the topsoil is highest during the autumn and winter (Šimunić et al., 1997; Romić and Borošić, 1998). Research results show that 25% of total precipitation leached in 2012 and 13% in 2013. Leachate amount in hydrological significant precipitation period for study area - autumn (11% in October 2011, 12% in October 2012, 12% in October 2013) corresponds to the leachate amount on annual level in wet conditions (2013). Due to high precipitation in 2013, especially in autumn and winter, i.e. outside the vegetation period, the samples are collected more frequently. Maximum leachate of 30 mm in 2013 was collected in two occasions, in May and December. In that year, 4 leachate samples were larger than the average and less volumes samples were collected in spring. In hydrological dry conditions (2011 and 2012), maximum leachate amount was recorded in January 2012, which consists with the statement that the risk of nitrogen leaching from the topsoil is highest during the autumn and winter. On the other hand, in the same period two leachate samples in summer (July 2011 and June 2012) were 23 and 33 mm, larger than the average leachate sample.

Average NO₃-N concentration in research period is 34 mg l⁻¹, which is approximately 3 times higher than maximum allowed concentration (MAC) of 11,3

mg l⁻¹. Maximum NO₃-N concentration of 56 mg l⁻¹ was recorded in October 2011 and minimum (8 mg l⁻¹) in April 2013. It is noticeable from graph 1. that fertilization (time and amount) affects nitrate-nitrogen leaching. Manure fertilization (MF) in September (input N=123 kg ha⁻¹) causes increase in NO₃-N concentration. This increase is more stressed in a hydrological dry years and in 2013 there is even slight decrease in NO₃-N concentration after manure fertilization, which is caused by more intense leaching due to high precipitation amount. Additional mineral fertilization (AMiF) does not such impact on NO₃-N concentrations because the total N-input (48 kg ha⁻¹) is 3 times lower and NO₃-N concentration in the period after AMiF varies from 38 to 41 mg l⁻¹. Overall, NO₃-N concentration exceeds MAC in 85% samples, which indicates that fertilizer amount is abundant in given soil and precipitation conditions, especially in hydrological dry year and fertilization management, especially fertilization outside vegetation period, should be adapted to those conditions. ANOVA results confirm there is a statistically significant difference ($F(2,12)=5,77, p=0.018$) in NO₃-N concentrations between hydrological dry and wet years. In dry years NO₃-N concentrations vary from 36 to 56 mg l⁻¹ and in 2013 from 8 to 40 mg l⁻¹. A Tukey post-hoc test revealed that NO₃-N concentrations were statistically significantly lower in wet conditions in 2013 (22 ± 15.2) comparing to dry conditions in 2011 (42.4 ± 7.7 mg/l, $p = .027$) and in 2012 (41.4 ± 7.3 mg/l, $p = .035$).

CONCLUSION

Field lysimetric experiment set on maize plot in northern Croatia covered both precipitation events, hydrological dry (2011 and 2012) and wet (2013) year. In given precipitation and soil conditions, leaching rate on annual level is twice as higher in dry then in wet conditions. Leachate amount in hydrological significant precipitation period for study area (autumn) corresponds to the leachate amount on annual level in wet conditions. Average NO₃-N concentration in research period was approximately 3 times higher than MAC of 11,3 mg l⁻¹. Maximum NO₃-N concentration was recorded in dry year and minimum in wet year. Overall, statistical analysis confirms that fertilizer amount is abundant in given soil and precipitation conditions, especially in hydrological dry year and fertilization management should be adapted to those conditions.

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EFFECT OF DIFFERENT IRRIGATION REGIMENS ON WATER-YIELD RELATIONSHIPS OF POTATO

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BOŠKO GAJIĆ³

SUMMARY: Yield response to irrigation of different crops is of major importance in production planning. The study aim of the experiment was to determine the effect of different irrigation regimens on water-yield relationships of potato. The study was carried out at the experimental field of the Institute of Field and Vegetable Crops in Novi Sad in the period 2002-2003. The trial included three irrigation variants (pre-irrigation soil moisture of 60, 70 and 80% of field water capacity – FWC, or irrigation was performed when 70%, 50% and 30% of available soil water, respectively, was consumed), and the non-irrigated, control variant. The trial was established in a system of random blocks and adapted to technical specifications of the sprinkling irrigation. Water used on evapotranspiration of potato was determined by balancing the amounts of water taken up from the soil layer of 2 m depth, from natural rainfall and irrigation. To assess the effectiveness of irrigation IWUE and ETWUE coefficients were used. Effects of irrigation on potato yield were positive and highly significant. The highest yield of potato was obtained on the irrigation variant of 70% of FWC (43158 kg ha⁻¹). The amounts of water used on evapotranspiration under irrigation (ET_m) and non-irrigation conditions (ET_a) ranged from 451.4 to 501.4 mm, and 373.4 to 381.2 mm, respectively. The values of IWUE and ETWUE varied from 71.4-112.5 kg ha⁻¹ mm⁻¹ and 67.0 do 91.9 kg ha⁻¹ mm⁻¹ respectively. As the highest values of yield, IWUE and ETWUE coefficients of potato were obtained on the irrigation variant of 70% of FWC that level of soil moisture represents the time when to start with irrigation if potato is grown in a soil with medium soil texture, under climatic conditions of the Vojvodina region.

Key words: potato, irrigation, water-yield relationships

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INTRODUCTION

Production of potato (*Solanum tuberosum* L.) takes a very important place in world agriculture, with a production potential of about 368 million t harvested and 19.3 million ha planted area with an average yield of 19.1 t ha⁻¹ (FAO, 2012). In Vojvodina the northern part of Serbia potato is grown at about 18.000 ha with an average yield of 15.3 t ha⁻¹ (Statistical Yearbook of the Republic of Serbia, 2012). The yield in the region is three times lower than this achieved in the leading potato-growing countries (Germany 45 t ha⁻¹, France 45 t ha⁻¹, Belgium 44 t ha⁻¹, Swiss 43 t ha⁻¹, FAO, 2012). The low yields are the consequence of inadequate management practices, insufficient amount and unfavorable arrangement of precipitation in the growing season and inappropriate irrigation scheduling applied.

Irrigation scheduling is one of the most important tools for developing best management practices for irrigated areas (Vučić, 1976, Yuan et al., 2003, Onder et al., 2005, Halim et al., 2006, Kiziloglu et al., 2006, Milić 2010). If shortage of readily available water in the soil is eliminated by irrigation it is possible to achieve high and stable yields of potatoes, at the level of 40-50 t ha⁻¹ or higher. Bošnjak and Pejić 1997, Milić et al., 2010 found out that the lower limit of optimum soil moisture for potatoes is 70% of field water capacity (FWC) when this crop is grown in a soil with medium texture. Thonthon and Siecka 1980, Costa et al., 1997, claimed that the lower limit of optimum soil moisture for potatoes is reached when 50% of available water is consumed.

A preliminary step to a more intensive exploitation of the available agroecological conditions or to the development of irrigation schedules for any crop implies a study of crop requirements for water, that is, the evapotranspiration (ET) for any particular crop. To fully utilize the genetic yield potentials of potato, to achieve high and stable yields, it is necessary to gain knowledge of the crop's capabilities under conditions of dry farming and irrigation. Wright and Stark (1990), Bošnjak and Pejić (1997) stressed the influence of local conditions on seasonal water use of potato.

Dwindling water resources and increasing food requirements require greater efficiency in water use, both in rainfed and in irrigated agriculture (Smith et al., 2002). The irrigation water use efficiency (IWUE) provides a more realistic assessment of the irrigation effectiveness, but the evapotranspiration water use efficiency (ETWUE) establishes whether the growing period is favorable for plant production or not.

The objective of this study were (1) to examine the effects of different pre-irrigation soil moisture on potato yield, water used on seasonal evapotranspiration, evapotranspiration and irrigation water use efficiency (2) to determine the best pre-irrigation soil moisture that would contribute to potato irrigation scheduling, particularly to assist in developing strategies for improved production technology of potato in the Vojvodina and similar regions.

MATERIALS AND METHODS

The field trial was conducted at Rimski Šančevi experiment field of the Institute of Field and Vegetable Crops in Novi Sad, Serbia (19° 51' E, 45° 20' N, 84 m above sea level), on the calcareous chernozem soil of the loss terrace. Sprinkling irrigation was applied. The trial was conducted in two years (2002/2003). There were three irrigation variants, with pre-irrigation soil moisture of 60% FWC, 70% FWC and 80%

FWC or irrigation was performed when 70%, 50% and 30% of available soil water, respectively, was consumed. To establish the irrigation schedule, soil moisture dynamics was monitored in 10 – 20 cm layers to the depth of 60 cm. Soil samples were taken at 7 day intervals or at shorter intervals when necessary. Moisture was determined by the thermogravimetric method, in the dryer at 105 – 110 °C. Evapotranspiration was calculated by the water balance method (Doorenbos and Kassam, 1986):

$$ET = P + I - D \pm \Delta W$$

where P is the precipitation (mm), I is the irrigation water applied (mm), D is the deep percolation (mm), and ΔW is the change in water storage of the soil profile to 2 m depth (mm).

Evapotranspiration water use efficiency (ETWUE, $\text{kg ha}^{-1} \text{mm}^{-1}$) for each treatment was calculated as tuber yield divided by seasonal evapotranspiration (ET) (Hassan et al., 2002). Irrigation water use efficiency (IWUE, $\text{kg ha}^{-1} \text{mm}^{-1}$) was determined as (Zhang et al., 1999):

$$IWUE = Y_i - Y_d / I$$

Y_i is the tuber yield of irrigated treatments (kg ha^{-1}), Y_d is the tuber yield of non-irrigated treatment (kg ha^{-1}), ET_m is the evapotranspiration of irrigated treatments (mm), ET_a is the evapotranspiration of non-irrigated treatment (mm), and I is the amount of irrigation water applied (mm).

The experimental potato plots (20 m^2) received conventional growing technology adjusted to the conditions of irrigation. Potato was harvested at technological maturity and yield (Y) was calculated by kg ha^{-1} . Data on effects of different pre-irrigation soil moisture on potato yield was done by the analysis of variance (ANOVA) and testing the obtained results by the Fisher's LSD test ($P < 0.05$, $P < 0.01$ levels between the means).

RESULTS AND DISCUSSION

Effects of irrigation on potato yield were positive and highly significant (Table 1). On average, the yields were increased by 89 to 164 %. In a dry 2003 year, on the irrigation variant of 70% of FWC, increase in yield of potato was 185%. Results are in agreement with those of Borza et al. (2010) who found out that irrigation increased the yield of potato in average of 137.2%, but in years with severe drought an increase in yield was 210%. Bošnjak and Pejić 1997 also reported that in extremely dry years irrigation could increase the yield of potato more than two times.

The potato yield of 43158 kg ha^{-1} obtained on the irrigation variant of 70% of FWC (50% of available water) was statistically higher than those obtained on 80% of FWC, 36926 kg ha^{-1} (30% of available water) and 60% of FWC, 30887 kg ha^{-1} (70% of available water), (Table 1). van Loon (1981) and Doorenbos and Kassam (1986), Kiziloglu et al. (2006), Erdem et al. (2006) also reported that to optimize yields of potato, the total available soil water should not be depleted by more than 30–50%. Wilson et al. (2001), Kang et al. (2004) found that good yields of potatoes can only be achieved at high values of soil moisture, at -25 and -35 kPa.

Table 1 Potato yield (Y), precipitation (P), amount of irrigation water applied (I), evapotranspiration on irrigated treatments (ET_m), evapotranspiration of non-irrigated treatment (ET_a), irrigation (IWUE) and evapotranspiration (ETWUE) water use efficiency at different irrigation treatment

Year	Variant	Yield	P	I	ET_m/ET_a	IWUE	ETWUE
2002	80 % FWC	36808	212.7	240	484.2	83.5	76
	70 % FWC	40860	212.7	225	471.8	107.1	86.6
	60 % FWC	32468	212.7	180	469.7	87.3	69.1
	Ø	16759	212.7	0	381.2	-	-
2003	80 % FWC	37043	156.1	270	501.4	78.1	73.9
	70 % FWC	45456	156.1	250	467.3	118	97.3
	60 % FWC	29306	156.1	240	451.5	55.6	64.9
	Ø	15966	156.1	0	373.4	-	-
2002/2003	80 % FWC	36926	184.4	255	492.8	80.8	75
	70 % FWC	43158	184.4	237.5	469.6	112.5	91.9
	60 % FWC	30887	184.4	210	460.6	71.4	67
	Ø	16362	184.4	0	377.3	-	-

LSD	A	B	AB
0.05	3.149	2.226	4.453
0.01	4.287	3.031	6.062

In the study period, evapotranspiration rate in irrigation variants (ET_m) ranged from 451.5 to 501.4 mm, and in rainfed conditions (ET_a) in the range from 373.4 to 381.2 mm (Table 1). As the highest yields of potato were obtained in the irrigation variant of 70% of FWC (50% of available water) that pre-irrigation soil moisture should be recommended to schedule irrigation when potato is grown in the soil with medium soil texture. The water used on evapotranspiration in that irrigation variant of 470 mm, on the average, (Table 1) may be considered as the water requirement of potato grown under climatic conditions of the Vojvodina region. According to FAO (2002) data, due to climatic conditions, evapotranspiration changed from 500 to 700 mm for potato to get high yield at 120-150 days vegetation period. Obtained results are in accordance with those of Kiziloglu et al. (2006) that seasonal evapotranspiration of potato was 475.2 mm under semiarid conditions in the east Turkey. Erdem et al. (2006) reported that seasonal evapotranspiration of potato, drip irrigated, at the level of 50% of the available water, varied from 473 mm to 524 mm under semiarid conditions of Trakya region in Turkey. Onder et al., (2005) stressed that crop water requirements are a function of climatic factors, methods of irrigation and the length of the growing period.

The best methods to describe the effectiveness that irrigation has in agriculture production are coefficients of irrigation (IWUE) and evapotranspiration (ETWUE) water use efficiency. Many researchers have evaluated water use efficiency in

different ways (Viets, 1962; Begg and Turner, 1976; Howell, 2001). Consequently, care should be taken when comparing WUE values. The highest values of IWUE ($112.5 \text{ kg ha}^{-1} \text{ mm}^{-1}$) and ETWUE ($91.9 \text{ kg ha}^{-1} \text{ mm}^{-1}$), on average, were obtained in the irrigation variant of 70% of FWC (50% of available water). The results are in line with those reported earlier by Kang et al. (2004), Onder et al. (2005), and Erdem et al. (2006). Kang et al. (2004) reported that the highest IWUE values were achieved with a soil matric potential threshold of -25 kPa ($114.8 \text{ kg ha}^{-1} \text{ mm}^{-1}$). Onder et al. (2005) found out IWUE values of $102.5 \text{ kg ha}^{-1} \text{ mm}^{-1}$ and $99.1 \text{ kg ha}^{-1} \text{ mm}^{-1}$ for surface drip and subsurface drip irrigation methods respectively. Erdem et al. (2006) reported that the highest WUE values were generally obtained from application of irrigation when 30% of the available water was consumed. Drip irrigation method yielded higher values of IWUE ($137.1 \text{ kg ha}^{-1} \text{ mm}^{-1}$) and WUE ($91.0 \text{ kg ha}^{-1} \text{ mm}^{-1}$), since drip irrigation consumed less water than furrow irrigation.

CONCLUSION

Irrigation significantly influenced the tuber yield of potato. The highest yield was obtained on the irrigation variant of 70% of FWC (50% of available water). That soil moisture could be used as the optimum when this crop is grown in a soil with medium texture. The water used on evapotranspiration, in that irrigation variant, of 470 mm may be considered as the water requirement of potato grown under climatic conditions of the Vojvodina region. High values of yield, IWUE and ETWUE coefficients, which were similar with those obtained under different climatic and soil conditions, confirmed that performed irrigation schedule was correct, i.e., fitted to biological characteristics of potato plants as well as to physical soil properties.

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IRRIGATION WATER SALINITY LIMITS FABA BEAN (*VICIA FABA* L.) PHOTOSYNTHESIS*

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SUMMARY: *Determination of plant salt stress responses is essential for stabilization of crop productivity under saline conditions. It is known that high soil salinity may adversely affect photosynthesis, which is a prerequisite for biomass production. For this reason, there is a considerable interest in measuring the photosynthetic rate of horticultural crops under saline conditions. A greenhouse pot experiment was set up to study the effects of saline irrigation water on faba bean (*Vicia faba* L.) photosynthetic rate and chlorophyll content index. Faba bean seedlings were transplanted into pots and treated with saline irrigation water. NaCl salinity was applied in a nutrient solution as follows: NaCl₀ – control (basic nutrient solution without added NaCl), NaCl₅₀ (control + 50 mM NaCl) and NaCl₁₀₀ (control + 100 mM NaCl). Five weeks after salinity treatment started faba bean leaf intercellular CO₂ concentration (Ci), stomatal conductance (g_s), transpiration rate (E), photosynthetic rate (A) and chlorophyll content index (CCI) were measured. Stomatal conductance, transpiration rate and photosynthetic rate of faba bean plants irrigated with saline water significantly decreased (P<0.01) in regard to control plants, but without significant difference amongst treatments. Intercellular CO₂ concentration was not significantly affected by application of saline irrigation water. Salinity treatments significantly altered (P=0.01) chlorophyll content index, although the influence was not linear to the salinity treatments. Raised soil salinity imposed water limitation for faba bean plants, which was suggested by lower stomatal conductance. Plant adaptive responses to salt stress include retention of water by stomatal closure, decreasing both transpiration and photosynthetic rate. Therefore application of saline irrigation water results in decreased rate of transpiration and limits faba bean photosynthesis as well.*

Key words: NaCl, stress, transpiration, photosynthetic rate, chlorophyll

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INTRODUCTION

Raising world food demands impose high productivity as the aim of the agricultural production, an objective inconceivable without extensive crop irrigation. However, extensive irrigation of crops favors secondary salinization of soil, deleteriously affecting soil properties and limiting the productivity of crops (Romic et al., 2008). Salinity affects 20% and up to 50% of the irrigated land worldwide (Pitman and Laüchli, 2002) and that percentage is expected to increase even more in the future (Tejera et al., 2006). Accumulation of soluble salts in the rhizosphere induces plant salt stress. Salt stress, amongst other physiological and morphological disorders which may emerge, disrupts plant pigment composition and decreases photosynthesis (Radwan et al., 2000), ultimately reflecting on crop productivity (Wang et al., 2003). It is essential to determine how plants respond to high soil salinity in order to improve crop productivity under saline conditions. Photosynthesis, as a prerequisite for biomass production, is considered a valuable parameter when studying plant adaptive responses to salinity (Radwan et al., 2000). In this context, measuring the photosynthetic rate of horticultural crops under saline conditions is useful tool in managing salinity stress.

Salinity affects photosynthesis via reduction in leaf area, chlorophyll content and stomatal conductance, but also through a decrease in photosystem II efficiency (Chinnusamy et al., 2006). Generally, restriction of photosynthesis by excessive salts in the rhizosphere may be divided to non-stomatal and stomatal limitations (Debez et al., 2006). Salt stress restrains product export in primary reactions of photosynthesis, which ultimately results in electron transport inhibition, increased chlorophyll fluorescence light emission, as well as enhanced reactive oxygen species (ROS) production (Koyro et al., 2010). Secondary effect of salt stress, mostly induced by abscisic acid (ABA) released from the plant roots, is stomatal closure and consequent inhibition of gas exchange (Koyro et al., 2010).

Faba bean (*Vicia faba* L.) is a legume crop produced worldwide because of its high yield and protein content (Daur et al., 2010). Based on the water stress day index, Katejri et al. (2000) specified faba bean as moderately sensitive to salinity, becoming less sensitive at later stages of development (Al-Tahir and Al-Abdulsalam 1997). Salinity effects on faba bean photosynthesis and chlorophyll content has been studied as well, yet the obtained results do not seem to show a consistent trend or a pattern. For example, El Sayed (2011) reported NaCl treatments did not generate a coherent trend in faba bean photosynthetic activity. Garg and Singla (2004) found that the response of photosynthesis of chickpea cultivars under salt stress was not as pronounced as the response of nitrogen fixation. Furthermore, Abdul Qados (2011) found differences in total chlorophyll content measured 10 days after NaCl salinity treatments started (decreased) and measured at the death of 40% of plants (increased in regard to control plants). For this reason, faba bean photosynthetic rate and total chlorophyll content under saline conditions was measured in order to elucidate plant responses to salt stress.

MATERIAL AND METHODS

The study was carried out from April, 2 – June, 15, in a greenhouse at the experimental station of the Faculty of Agriculture University of Zagreb, Croatia. Faba bean seedlings were grown from seeds in a polystyrene cups containing a peat soil (Potgrond P, Klastmann), and after three weeks uniform plants were transplanted into pots (one plant per pot) filled with agricultural soil. Pots were irrigated daily, using automatic drip irrigation system, with a basic nutrient solution (Poly-Feed Drip 20–20–20 with micronutrients). In order to provide aeration of soil and prevent waterlogging, good drainage was ensured. The fertigation rate and frequency was the same for all the treatments and adjusted to the plant phenology and to the climatic conditions in the greenhouse. Three weeks after transplanting, faba bean seedlings were treated with NaCl salinity applied in a nutrient solution as follows: NaCl₀ – control (basic nutrient solution without added NaCl), NaCl₅₀ (control + 50 mM NaCl) and NaCl₁₀₀ (control + 100 mM NaCl). Randomized block design with three replicates was applied in the experiment. Five weeks after salinity treatment started (at the pod filling stage), faba bean leaf intercellular CO₂ concentration (C_i, μmol mol⁻¹), stomatal conductance (g_s, mol H₂O m⁻² s⁻¹), transpiration rate (E, mol H₂O m⁻² s⁻¹) and photosynthetic rate (A, μmol CO₂ m⁻² s⁻¹) were measured in a triplicate, on a youngest fully developed leaf, with LCpro+ portable photosynthesis system (ADC BioScientific Ltd., Great Britain). Chlorophyll content index (CCI) was measured at the same time and on the same leaf with a CCM-200 plus Chlorophyll Content Meter (ADC BioScientific Ltd., Great Britain). Obtained data were subjected to the analysis of variance (ANOVA) using the SAS statistical software package (SAS Institute, 2007). The significance of differences between the means was determined with Tukey's HSD test at P≤0.05.

RESULTS AND DISCUSSION

Influence of raising irrigation water salinity on faba bean leaf intercellular CO₂ concentration, stomatal conductance, transpiration rate, photosynthetic rate and chlorophyll content index is presented in Table 1.

Table 1. Effect of irrigation water salinity (0, 50 and 100 mM NaCl) on faba bean leaf intercellular CO₂ concentration (C_i, μmol mol⁻¹), stomatal conductance (g_s, mol H₂O m⁻² s⁻¹), transpiration rate (E, mol H₂O m⁻² s⁻¹), photosynthetic rate (A, μmol CO₂ m⁻² s⁻¹) and chlorophyll content index (CCI).

NaCl treatment	Intercellular CO ₂ concentration (C _i)	Transpiration rate (E)	Stomatal conductance (g _s)	Photosynthetic rate (A)	Chlorophyll content index
	μmol mol ⁻¹	mol H ₂ O m ⁻² s ⁻¹	mol H ₂ O m ⁻² s ⁻¹	μmol CO ₂ m ⁻² s ⁻¹	(CCI)
NaCl ₀	144 _A	2.61 _A	0.16 _A	18 _A	64.7 _{AB}
NaCl ₅₀	126.6 _A	1.71 _B	0.09 _B	12.1 _B	83.7 _A
NaCl ₁₀₀	151.4 _A	1.46 _B	0.08 _B	10.2 _B	52.9 _B
<i>Statistical significance</i>	n.s.	P<0.01	P<0.01	P<0.01	P=0.01

Faba bean leaf C_i was not significantly affected by application of saline irrigation water (Table 1). C_i estimations, which are estimations of the CO_2 mole fraction at the evaporating sites, are assuming that photosynthesis and transpiration are fairly uniform over the leaf area (Meyer and Genty, 1998). However, this is proved not to be as adequate when g_s decreases due to environmental impacts, as is the case in this research, because of the non-uniform distribution of the stomatal aperture and photosynthesis (Meyer and Genty, 1998). It is considered that the concentration of CO_2 in chloroplasts decreases because of lower g_s , in spite of the apparent stability of CO_2 concentration in intercellular spaces (Gama et al., 2007). Thus, decreased g_s and A do suggest faba bean lower CO_2 uptake under saline conditions, even though this could not be assumed only by observing C_i values. In this context, decreased g_s and A , as lower CO_2 uptake rate leads to lower photosynthesis rate (Mansour et al., 2008), could prove to be a better indicators of plant CO_2 uptake rate under stressful environmental conditions, such as increased soil salinity.

Stomatal conductance, transpiration and photosynthetic rate of faba bean plants irrigated with saline water significantly decreased ($P < 0.01$) in regard to control plants, but without significant difference amongst salinity treatments (Table 1). Plant salt stress actually comprises (ionic and) osmotic stress, which reduces water availability to plants (Ondrasek et al., 2006). The osmotic effect of salinity stress, which is considered to continue for the duration of exposure of plants to salinity, may result in stomatal closure (Carillo et al., 2011). Lower g_s of salt stressed bean plants suggested that raised soil salinity imposed water limitation for plants, causing them osmotic stress. As a part of an adaptive response to salt stress, plants try to retain water by stomatal closure, causing a decrease of both, transpiration and photosynthetic rate, which is in agreement with our results (Table 1). Therefore, application of saline irrigation water, due to stomatal effects of plant salt stress, results in decreased rate of transpiration and limits faba bean photosynthesis as well. This is additionally confirmed by the same trend in g_s , E as well as A in regard to salinity treatments.

Even though osmotic adaptation was found to be low or even absent in faba beans (Stoddard et al., 2006), our results could suggest otherwise. Adaptation of plants to osmotic stress is actually a plant response to maintain water relations. It usually includes osmotically active substances (Stoddard et al., 2006), which were not a part of this research. Yet, decreased g_s along with the fact that all plants in the experiment completed their life cycle despite decreased soil water potential caused by salinity, point out the possibility that the osmotic adaptation is not yet well elucidated for faba beans. Furthermore, lower stomatal density, which we suggested earlier as a possibility, is actually associated with water conserving attributes and indicates better adaptation to water stress conditions (Khan et al., 2010). However, further research, focused on the faba bean possible adaptation to water stress, is needed.

Salinity treatments significantly affected CCI ($P = 0.01$), although the difference was found to be only between the salinity treatments themselves (Table 1). It is considered that plant salt stress may cause inhibition of chlorophyll biosynthesis, increase of its degradation by chlorophyllase, as well as the oxidative stress which could lead to degradation of chloroplast structure and decrease in chlorophyll content (Azooz et al., 2013). Absence of the significant difference between CCI of control plants and CCI of NaCl treated plants (Table 1) emphasized the importance of stomatal over non-stomatal effects of salt stress on faba bean photosynthesis. Otherwise, as non-stomatal effects of salinity on plant photosynthesis include

inhibition of chlorophyll biosynthesis or increase of its degradation which would have been reflected on CCI, significant difference between CCI of control plants and salinity treated plants is expected.

CONCLUSION

Decreased g_s and A suggest faba bean lower CO_2 uptake under saline conditions, even though this could not be assumed only by observing C_i values. In this context, decreased g_s and A could prove to be a better indicators of plant CO_2 uptake rate under saline conditions. Lower g_s of salt stressed bean plants suggested that raised soil salinity imposed water limitation for plants, causing them osmotic stress. Therefore, application of saline irrigation water, due to stomatal effects of plant salt stress, results in decreased rate of transpiration and limits faba bean photosynthesis as well. Absence of the significant difference between CCI of control plants and CCI of NaCl treated plants emphasized the importance of stomatal over non-stomatal effects of salt stress on faba bean photosynthesis. Screening for the crops that could maintain photosynthetic activity in a saline environment could provide a basis for identification of salt tolerance in horticultural crops.

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COMPACTION AND SOIL WATER CONTENT IN TILLAGE INDUCED SYSTEM

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SUMMARY: Soil compaction is a growing threat around the World for crop production. For that reason longtime stationary field experiment with six soil tillage systems was conducted in Central Croatia. This paper will try to provide an assessment of the impact of tillage system on soil compaction and soil moisture retention in the upper 40 cm soil layer. The aim is to determine the optimal tillage system due to the compaction and soil water content (SWC). During 2012 soil resistance was measured with penetrometer in six occasions. Disturbed soil samples were taken in six occasions from the depth of 0 - 20 cm and 20 - 40 cm in three replications. Statistical data evaluation showed significant differences in SWC between all tillage systems at all depths. SWC varied at depth 0 - 20 cm from 277 to 910 m³ ha⁻¹ and at depth 20 - 40 cm from 337 to 814 m³ ha⁻¹ depending on the period of the year. No-till (NT) system statistically shown highest SWC (699 m³ ha⁻¹) and had from 12.1% less to 5.8% more stored SWC in layer 0 - 40 cm than other tilled plots during 2012. Soil resistance at depth of 0 - 10 cm varied from 0.29 to 2.54 MPa, at depth of 10 - 20 cm from 0.37 to 3.36 MPa, at depth of 20 - 30 cm from 0.78 to 4.72, at depth of 30 - 40 cm from 1.25 to 7.19 MPa depending on SWC. Soil resistance showed significant differences in most of the soil layers between all tillage systems. It can be concluded that climate conditions and tillage systems through SWC differently affected soil resistance. Although NT system showed slightly higher soil resistance in layer 0 - 40 cm than variants with deep tillage (deep ploughing across the slope - DP and subsoiling with ploughing across the slope - SUB) results from 2012 suggest that, up to now, no-tillage could replace conventional tillage without adverse effects on SWC in the Central Croatia, but further detailed research is necessary for safer conclusion.

Key words: *soil compaction; soil water content; tillage system; water conservation*

INTRODUCTION

Soil compaction is recognized as a worldwide problem. Scientist all around the World reports about millions of hectares of degraded land (Carder and Grasby, 1986; Flovers and Lal, 1998; Akker and Canarache, 2001; Hamza and Anderson, 2005). In

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contrast to traffic induced compaction as the main interest in Western European countries, tillage-induced soil compaction has become a widely occurring Central-Eastern European problem (Birkás et al., 1996). Types of tillage-induced soil compaction can be grouped according to the depth of occurrence or by cause (Birkás et al., 2011). Wherever compacted layer is located, consequences are great for soil water management, root development and crop yields. There is insufficient moisture for crops in dry years, while in wet years soil above tillage pan becomes quickly saturated with threat for crops roots. Most crop species can exert maximal vertical pressure from 0.7 - 2.5 MPa (Gregory, 1994). In Croatia today, conventional tillage system dominates which mostly include moldboard ploughing, disking and seedbed preparation (Bogunovic et al., 2014). Consequences in form of compaction and erosion in Croatia, are already noticed by Basic et al. (2004); Filipovic et al. (2006), Tursic et al. (2008), Kisić (2008), Birkás et al. (2008, 2009). According to Kisić et al. (2004), 25 - 35% of total agricultural land is affected by human-induced degradation in Croatia. In this paper authors will try to present best tillage system on sandy loam soil for water conservation and compaction of soil horizons up to 40 cm.

MATERIAL AND METHODS

Long term tillage and crop management practices trial was established in 1994. Prior to the establishment of the experimental plots site have been conventionally tilled. Site is located 15 km southwest of Daruvar (45°33' N, 17°02' E, elevation 133 m) in Central Croatia. The soil is mapped as Stagnic Luvisol (FAO, 2006) with a slope of 9%.

Table 1. Particle size distribution on Stagnic Luvisols (from Kisić et al., 2002)

Soil depth (cm)	Soil horizon	Coarse sand (2-0.2 µm)	Texture (g kg ⁻¹)			Texture class
			Fine sand (0.2-0.02 µm)	Silt (0.02-0.002 µm)	Clay (<0.002 µm)	
0-24	Ap+Eg	18	586	242	154	Sandy loam
24-35	Eg+Btg	21	571	260	148	Sandy loam
35-95	Btg	5	545	254	196	Sandy loam

The experimental design consists of six plots. Soil on the experiment belongs by its texture to sandy loam (Table 1). Climate is semihumid to humid with annual precipitation of 878 mm and average annual temperature of 10.6 °C (Meteorological and hydrological institute of Croatia).

Tillage systems differed in tools that were used, depth and direction of tillage. Six tillage systems and implements, which were included in some system, are as follows: Check treatment (CT) – ploughing and other operations up and down the slope, black fallow; Conventional ploughing (25 - 30 cm) up and down the slope (CP) – other operations depending on the crop also up and down the slope; No-tillage system (NT)– no-till planter, sowing directly in mulch; Ploughing across of slope (PA) – conventional ploughing (25 - 30 cm) across of slope, other operations depending on

the crop also across of slope; Deep ploughing (50 cm) across of slope (DP) – operation repeats after termination of prolonged effect (every 3 - 4 years when crop rotation allows), other operations on conventional way depending on the crop; Conventional ploughing across of slope (30 cm) with subsoiling to the depth of 50 cm (SUB) – subsoiling repeats after termination of prolonged effect (every 3 years when crop rotation allows), other operations depending on the crop. Last deep tillage operation (subsoiling and deep plowing) was performed in 2010. The NT system is not tilled and weeds were controlled by total herbicides. Black fallow management in first tillage system consisted of weed control entirely with herbicides. Fertilizers and herbicides were applied according to soil fertility tests and weeds present at experimental site. NT system needed one to two more herbicide applications during growing season. Herbicides and fertilizers, and their rates, were the same for all tillage systems.

Soil samples for the determination of SWC was taken by hand sampling probe during six terms, from March to December at selected crop growth stages, once a month. SWC was determined gravimetrically to a depth of 40 cm in 20 cm increments, in three replicates. Water content results was converted to a volume basis using bulk densities previously determined by Kopecky's cylinders (100 cm³) from each depth increment, in three replicates. Soil water content data were grouped at layers 0 – 20 and 20 – 40 cm. SWC data from layer 0 – 40 cm represent average of two layers. Soil resistance was measured with penetrometer Eijkelkamp Penetrologger in six occasions. The conical point was 1 cm² in area and the point angle was 60°. Each term has 16 repetitions per variant and results were grouped at soil layers 0 - 10 cm, 10 - 20 cm, 20 - 30 cm, 30 - 40 cm and 0 - 40 cm, respectively. Observed data were subjected to analysis of variance (ANOVA) using SAS Institute 9.1.3 and mean values were separated by Fisher's LSD test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Detailed results of soil resistance are presented in table 2. values of investigated soil profile showed PA (4.07 MPa) in August, while the lowest penetration resistance showed CT (0.78 MPa) in March. Average soil resistance in a layer 0 - 40 cm showed significant differences in most of measurement. Highest Year averages indicate that lowest penetration resistance of whole profile recorded CT (1.78 MPa) followed by DP, while the highest penetration resistance recorded CP with 2.37 MPa. Soil resistance varied from normally values at depth of 0 - 10 cm (0.29 - 2.54 MPa) to high values at depth of 30 - 40 cm (1.25 - 7.19 MPa) depending on the period of the year. Temporal variability of data showed deviations from variant means with time. Soil record higher values from April to October after which it begins to decrease.

Table 2. Soil resistance (MPa) in soil layers per month and average values

Depth (cm)	Variant	Average	March	April	June	August	October	December
0-10	CT	1,13	0,29b	0,68c	1,13ab	2,29a	1,78a	0,60a
	CP	1,23	0,35b	1,28b	1,36a	1,68a	1,97a	0,75a
	NT	1,21	0,51a	2,01a	0,65c	2,15a	0,97b	0,97a
	PA	1,09	0,34b	0,86bc	1,11ab	2,54a	1,00b	0,70a
	DP	1,01	0,33b	1,00bc	1,14ab	2,11a	0,96b	0,52a
	SUB	0,97	0,35b	0,84c	1,02b	1,93a	1,00b	0,69a

10-20	CT	1.51	0,37c	0,86c	1,57a	2,96a	2,34a	0,99a
	CP	1.82	0,68b	1,81b	1,75a	2,49a	2,74a	1,47a
	NT	1.98	1,17a	3,17a	1,15a	3,19a	1,59b	1,62a
	PA	1.56	0,73b	1,25bc	1,64a	3,19a	1,33b	1,21a
	DP	1.50	0,46bc	1,30bc	1,43a	3,36a	1,38b	1,06a
	SUB	1.55	0,73b	1,59b	1,39a	3,06a	1,38b	1,14a
20-30	CT	1.96	0,78c	1,27c	2,20a	3,01a	2,87b	1,65a
	CP	2.62	1,38ab	2,45b	2,22a	3,30a	4,17a	2,21a
	NT	2.39	1,56a	3,12a	1,59b	3,90a	2,15bc	2,02a
	PA	2.58	1,71a	2,72ab	2,28a	4,72a	2,02bc	2,01a
	DP	1.88	1,02bc	1,50c	1,68b	3,49a	1,91c	1,69a
	SUB	2.26	1,61a	2,13b	1,90ab	4,19a	2,10bc	1,62a
30-40	CT	2.16	1,25c	2,21c	2,32bc	2,84d	3,14bc	2,27a
	CP	3.86	2,40a	3,11ab	2,75b	7,19a	4,87a	2,78a
	NT	3.25	1,82b	3,01ab	2,12c	6,05ab	2,75c	2,49a
	PA	3.87	2,78a	3,52a	3,37a	5,83ab	3,13bc	3,17a
	DP	2.62	2,25ab	2,58bc	2,64b	3,02cd	3,38bc	2,99a
	SUB	3.21	2,61a	3,13ab	2,38b	4,74bc	3,66b	2,69a
0-40	CT	1.78a	0,67b	1,26c	1,82ab	2,77a	2,67ab	1,49a
	CP	2.37a	1,19ab	2,08b	2,01a	3,66a	3,47a	1,81a
	NT	2.16a	1,29ab	2,80a	1,40b	3,82a	1,87b	1,80a
	PA	2.22a	1,39a	2,06b	2,12a	4,07a	1,88b	1,78a
	DP	1.79a	1,01ab	1,58bc	1,71ab	2,99a	1,91b	1,55a
	SUB	2.00a	1,31ab	1,89bc	1,75ab	3,48a	2,03b	1,54a

Differences in penetration resistance in soil layers indicate that they under directly influence of tillage depth and SWC. This is visible in DP and SUB variant at the depth 30 - 40 cm. Only these two variant have tillage deeper than 30 cm and result of this are lower resistance values of those variants.

According the previously research (Taylor and Gardner, 1963) value of 2 MPa is border for normal root growth, while at 2.5 MPa roots stops penetrate the soil (Taylor, 1971). Others (Håkansson and Lipiec, 2000; Hamza and Anderson, 2005) move that limit on the 2.5 - 3 MPa. In this study, due to the range of soil resistance values found over the experimental period, we chose 2.5 MPa as the most suitable reference value for comparison purposes. Critical values does not exceed in surface layer. In layer from 10 - 20 cm critical values marked in all variants in August and CP in October. In layer 20 - 30 cm year averages showed critical values in CP and PA, and in layer 30 - 40 cm in all variants. Individual measurements in dry months (June, August) show the results up to 6 MPa which indicates some limitations of these soils for crop production. Only variants with prolonged effect of deeper tillage (DP and SUB) have slightly better results in dry months than others conventionally tilled variants, but also with resistance above 2.5 MPa.

In most cases literature compares compaction under no-tillage and different variants of reduced tillage systems with conventional tillage systems (Quiroga et al, 1998; Tebrügge and Düring, 1999; Diaz-Zorita, 2000; Franzluebbers and Stuedemann, 2008; Alvarez and Steinbach, 2009). In this paper we study influence of no-tillage systems and five different conventional systems differed in tool, direction and tillage depth. It is visible that at 0 - 10 and 10 - 20 cm NT variant have higher resistance then tilled variant. Only CP have similar resistance as NT at these two layers. Same results reported others researchers (Hill, 1990; Lopez et al., 1996; Wander and Bollero, 1999). Resistance increase with depth at all variant, but below plowing depth showed

lower resistance in NT. Filipovic et al. (2006) on Albic Luvisol recorded lowest resistance in layer 0-40 cm on conventional system compared with no-tillage and conservation tillage system. They notice that penetration resistance increased at all tillage systems during the experiment, but the greatest increase was observed at conventional tillage system. Lopez et al. (1996) in tillage study presented soil resistance of layer 0 - 40 cm under no-tillage system from 2 - 4 MPa. Conventional tillage systems are more depended on the SWC. They data indicate that reduced and conventional tillage systems reduced penetration resistance in the depth of primary tillage.

Sajko et al. (2009) at same experimental site as this study recorded highest soil resistance at depth 0 - 10 cm on NT in both, dry and humid conditions. In dry conditions most favorable soil resistance was recorded on PA, and critically high soil resistance values were recorded on CP and DP (before repeated very deep ploughing and subsoiling). In humid climatic conditions most favorable soil resistance they recorded on DP and SUB, and the highest soil resistance on CT. Our results show that NT (1.21 MPa) in surface layer is similar in compaction as CP (1.23 MPa) and other variants recorded lower resistance. If we look averages of layer 0 – 40 cm in June - October highest resistance recorded CP (3.04 MPa), followed by PA (2.68 MPa). In this period most favorable resistance recorded on DP (2.31 MPa). In period of March, April and December in layer 0 – 40 cm CP (1.72 MPa) and PA (1.75 MPa) had highest resistance in conventional tillage systems, while lowest resistance recorded DP (1.39 MPa) if we exclude CT (1.10 MPa).

Statistical properties of the SWC, made within the variants on different dates, are presented in Figures 1- 3. Observing the entire depth (0 - 40 cm), the highest average SWC showed NT (699 m³ ha⁻¹), while the lowest SWC showed CP (623 m³ ha⁻¹). In conditions of northwest Slavonia Košutić et al. (2001) obtained same results in comparison no-tillage and other tillage systems. Špoljar et al. (2011) obtained more favorable results concerning the content of physiologically active and optimal soil moisture mainly on the reduced tillage treatments in similar soil and environmental conditions like this experiment. Generally, soils either humid or arid areas, under NT or some of reduced tillage systems have more stored water then conventionally tillage systems (Alvarez and Steinhilber, 2009) and this study is no exception.

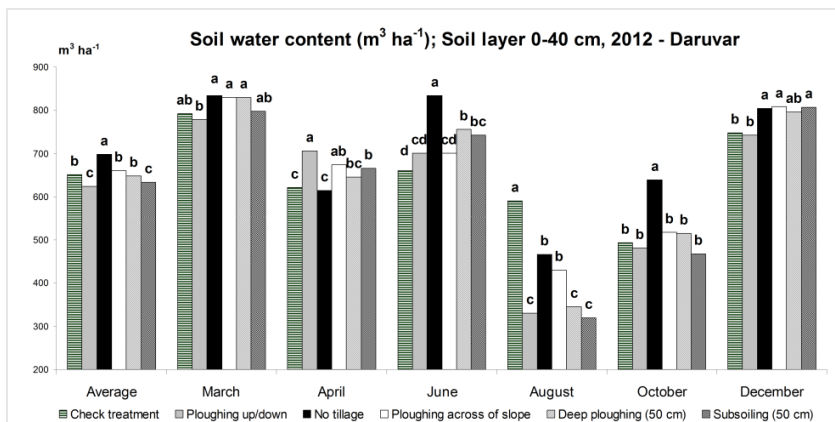


Figure 1. Average soil water content (m³ ha⁻¹) at depth 0 - 40 cm

In conventional tillage systems highest average SWC in layer 0 – 40 cm showed CP ($660 \text{ m}^3 \text{ ha}^{-1}$) while lowest results recorded PA ($623 \text{ m}^3 \text{ ha}^{-1}$). Statistically significant differences of whole profile (0 - 40 cm) were recorded between NT and conventional tillage and their different system of conventional tillage systems, except between CT, CP or PA. Observing each measurement there is a significant difference between all tillage systems in all months. Lowest SWC had SUB ($320 \text{ m}^3 \text{ ha}^{-1}$) in August and highest NT in June ($834 \text{ m}^3 \text{ ha}^{-1}$). In conventional tillage systems, except mentioned PA, best results except CT showed DP ($648 \text{ m}^3 \text{ ha}^{-1}$). Results indicate that SWC recharge during 2012 was greater under NT system than under different conventional tillage systems, probably due to reduced soil water evaporation because of crop residue on the soil. Other researchers (Blevins et al., 1971; Lindstrom et al., 1974; Martens, 2000; Nielsen et al., 2005) notice the same results. Soil water depletion during the growing season essentially followed the same pattern under all tillage systems, except the CT system in August. This can be justified by black fallow and rain that fell the day before sampling.

SWC showed greater values in surface layer in according to subsurface layer (Fig 2 – 3). In both layers NT recorded highest SWC (708 and $614 \text{ m}^3 \text{ ha}^{-1}$). Driest soil recorded CP (614 and $489 \text{ m}^3 \text{ ha}^{-1}$), also in both layers.

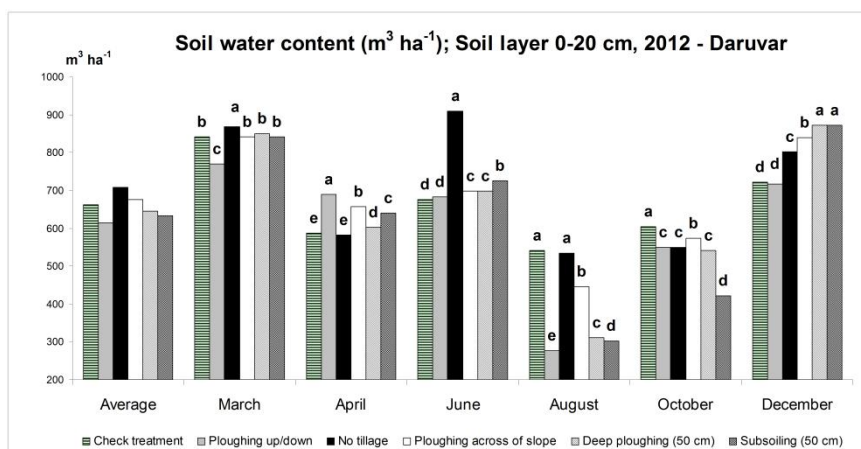


Figure 2. Soil water content ($\text{m}^3 \text{ ha}^{-1}$) at depth 0 – 20 cm

Statistically significant differences of water content in soil layers were recorded between NT and conventional tillage and their different variant of conventional tillage systems, at each measurement. In investigation in dryland conditions Jabro et al. (2008) did not found any significant difference in SWC between conventional tillage systems. Špoljar et al. (2011) found significant differences in moisture measurements at depth 0-30 cm between conventional tillage systems, but they recorded lowest SWC in more intensive conventional systems.

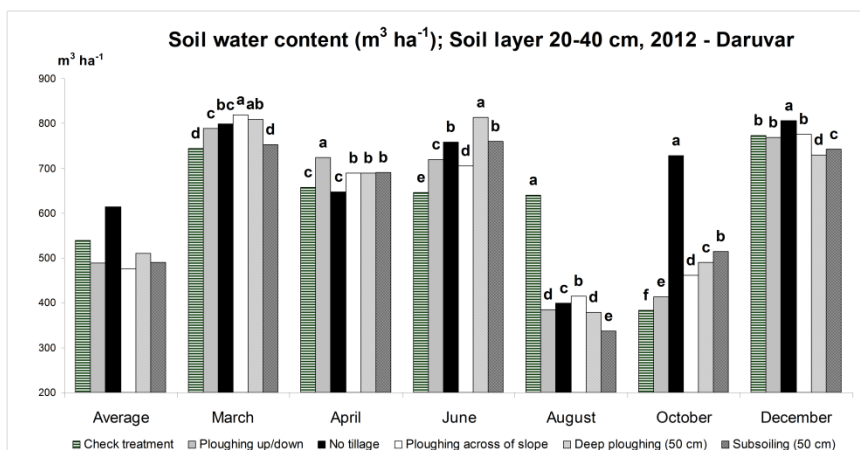


Figure 3. Soil water content ($\text{m}^3 \text{ha}^{-1}$) at depth 20 - 40 c

*Different letters means differ significantly at $p < 0.05$

CONCLUSION

Tillage has significant influence on soil resistance. The lowest average soil resistance at depth 0-40 cm showed CT, while the largest has shown PA. Soil resistance varied from normally values at depth of 0 - 10 cm (0.29 - 2.54 MPa) to high values at depth of 30 - 40 cm (1.25 - 7.19 MPa) depending on the period of the year. In conventionally tilled variants most favorable resistance showed DP and CT. Temporal variability of data showed deviations from variant means with time as direct influence of the SWC as an indirect effect of tillage. As soil became drier, soil resistance increased in all treatments, exceeding critical level of 2.5 MPa at all variants. Better results in dry months showed variants with deep tillage (DP and SUB). Some results from individual measurement marked values up to 6 MPa which indicates some limitations of these soils for crop production. Average SWC data point to the trend of increased moisture in NT and PA or DP compared to others variants. In conventional variants the adoption to soil moisture content is lower. It can be concluded that climate conditions and tillage systems through tillage depth directly, and SWC indirectly, differently affected soil resistance. Although NT system showed slightly higher soil resistance in layer 0-40 cm than variants with deep tillage (DP and SUB) results from 2012 suggest that, with point of view of SWC and soil resistance, no-tillage could replace conventional tillage in the Central Croatia. For safer conclusion and strong dependence of investigation factors on climate conditions further detailed research is necessary.

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ROLE OF CONSTRUCTED WETLAND SYSTEMS IN THE ENVIRONMENTAL PROTECTION OF VOJVODINA PROVINCE

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SUMMARY: *Constructed Wetland System (CWS) in the Glozan settlement is the first system of biologically active fields for wastewater treatment constructed in Vojvodina (Serbia), aimed at treating local municipal wastewaters. The common reed (*Phragmites australis* (Cav.) Trin. ex Steud.), naturally growing on the CWS location, is used as biofiltering vegetation. The second CWS in the Novo Milosevo settlement also uses reed as biofiltering vegetation. The investigation of the Glozan and Novo Milosevo CWS was carried out in the 2005-2012 period. The first set of results (2005-2008) confirm that the CWS Glozan can remove more than 90% of suspended solids while reducing organic matter, expressed in terms of biological oxygen demand (BOD_5) is more than 80%. The efficiency of removal of nitrogen compounds ranged from 47.3 – 78.3% (for nitrates, ammonia / ammonium, and nitrites), and the efficiency of total phosphorus removal was 29.1%. The CWS Novo Milosevo can remove more than 73% of suspended solids while reducing organic matter, (BOD_5) is more than 82%. The efficiency of removal of nitrogen compounds ranged from 33.6 – 79.0%, and the efficiency of total phosphorus removal was 6.6 – 23.8%. Results from 2012 year, after several years of operation of the system was confirmed a high degree of removal of pollutants. The CWS Glozan removes 64.6 – 99.3% of suspended matter, while the decrease in organic matter, (BOD_5), has been 54.4 – 89.0%. The efficiency of removal of nitrogen compounds ranged from 70.6 – 85.73%, while the efficiency of total phosphorus removal was 8.7 – 49.8%. The CWS Novo Milosevo removes 7.6 – 98.6% of suspended matter, while the decrease in organic matter, (BOD_5), has been 52.1 – 88.4%. The efficiency of removal of nitrogen compounds ranged from 74.4 – 90.1%, while the efficiency of total phosphorus removal was 31.1 – 51.6%.*

Based on the monitoring of the contents of ammonia/ammonium, nitrate and nitrite, it can be concluded that the nitrification is the main process, but denitrification and ammonification can also take place. The results of the chemical composition of the reed confirmed the bioaccumulation of nutrients in the plant organs. The results presented for the CWS Glozan and CWS Novo Milosevo

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demonstrate durability, reliability, and usability of such systems under the conditions of moderate continental climate, which could be an essential contribution to the sustainable development and environmental protection in Vojvodina Province.

Key words: wastewater; constructed wetland; microorganisms; *Phragmites australis*; purification

INTRODUCTION

Wetlands have been recognized as a natural resource throughout human history. Their importance is appreciated in their natural state by such people as the Marsh Arabs around the confluence of the rivers Tigris and Euphrates in southern Iraq, as well as in managed forms; e.g., rice paddies, particularly in South East Asia (Mitsch and Gosselink, 2000). Also, natural wetlands exist during the centuries on the Pannonian Basin (Szucsne-Murguly, 2006). The natural water purification processes occurring within these systems have become increasingly relevant to practical use of constructed or even semi-natural wetlands for water and wastewater treatment.

Wastewaters are often discharged into the recipients without any pretreatment, which represents a serious threat to the quality of water and its further usability. As a consequence, many water resources have been brought to the state of unsatisfactory water quality, which affects the health of people and all living world in the environment. The essence of the CWS method is in the utilization of phytofiltration and phytoaccumulation capacities of marsh plants (Brix, 1994; Nikolić et al., 2003; Nikolić et al., 2007; Urbanc-Berčić, 1997). Besides, due to the favorable conditions, the root zones of these plants are amply populated with microorganisms, which are of essential importance in the transformation of nitrogen and its compounds, through the processes of ammonification, nitrification and denitrification, as well as in the mineralization of organic matter (Greenway, 2007).

Constructed wetlands are used to remove a wide range of pollutants such as organic compounds, suspended solids, pathogens, metals, and excess nutrients (e.g., N and P) from various wastewaters including stormwater runoff and municipal wastewater (Chen, 2011; Cooper, 2009; Ghermandi et al., 2007; Kadlec, 2009; Snow et al., 2008; Vymazal, 2007). Because of high removal efficiency, low cost, water and nutrient reuse, and other ancillary benefits, CWS have become a popular option for wastewater treatment (Ghermandi et al., 2007; Kadlec, 2009; Llorens et al., 2009; Rousseau et al., 2008). Since the 1990s, wetland systems have been used for treating numerous domestic and industrial waste streams including those from tannery and textile industry, abattoirs, pulp and paper production, agriculture (animal farms and fish farm effluents), and various runoff waters (agriculture, airports, highway, and stormwater) (Carty et al., 2008; Diaz et al., 2012; Haberl et al., 2003; Lee et al., 2006; Vymazal, 2007).

The treatment efficiencies of constructed wetlands vary depending on the wetland design, the type of wetland system, climate, vegetation, and microbial communities. The design for CWS is based on free water surface flow (FWS), horizontal subsurface flow (HF), or vertical subsurface flow (VF) (Kadlec, 2009). Nowadays, the most popular are constructed wetlands with subsurface flow. Constructed wetlands with subsurface flow consist of gravel or rock beds sealed by an impermeable layer and planted with wetland vegetation.

Most articles have been based on either pilot plant-scale or laboratory-scale experimental systems. Very few articles have been carried out on the assessment of performance of full-scale constructed wetlands treating domestic wastewater. Constructed wetlands are often seen as complex “black box” systems, and the processes within an experimental wetland are difficult to model due to the complexity of the relationships between most water quality variables (Gernaey et al., 2004). However, it is necessary to monitor, control and predict the treatment processes in order to meet environmental and sustainability policies, and regulatory requirements such as secondary wastewater treatment standards.

The European Commission for Water proposed in 1991 the document: Implementation of Council Directive 91/271/EEC of May 1991 Concerning Urban Wastewater Treatment, which serves as the guidelines for treatment of urban wastewaters, where the method of WETLANDS is mentioned on equal footing as the approach to be used for small and medium settlements from 500 to 5,000 inhabitants. Despite of the fact that none of the national legislative systems imposes the use of any particular method, the practice in many developed countries (England, France, Austria, Germany, Italy, Slovenia, etc.) has shown certain advantages of the application of wetlands. In our country, the application of this method began in 2004, by putting into operation the constructed wetland system at Glozan. After that in 2008 CWS was put into operation at Novo Milosevo.

The objective of this study was to examine the efficiency of the wetlands system constructed to treat municipal wastewater of Glozan and Novo Milosevo settlements in Vojvodina (northern province of the Republic of Serbia). In the observed Glozan CWS and Novo Milosevo CWS the dominant species is reed - *Phragmites australis* (Cav.) Trin. ex Steud .. The CWS efficiency was assessed on the basis of the water balance, removal of suspended matter, reduction of the organic load, removal of nitrogen compounds and total phosphorus, bioaccumulation of nutrients in the reed plant organs, all measured in the investigation period of several years. Besides, the sanitary safety of the effluent was assessed via the count of the coliform bacteria and *Escherichia coli* (*E. coli*) under the eco-hydrological conditions of a moderate continental climate.

MATERIAL AND METHODS

The geographic coordinates of Glozan are 45°17' North latitude and 19°33' East longitude, while the altitude is 80-82m a.s.l. The regional surface waters are the Danube River and land drainage canals. The geographic coordinates of Novo Milosevo are 45°43' North latitude and 20°18' East longitude, while the altitude is 77-78m a.s.l. The regional surface waters are the Tisza River and land drainage canals.

The Glozan CWS consists of three cells of a total area of 1 ha. The Novo Milosevo CWS consists of a lagoon and the cells of a total area of 1.20 ha. Glozan CWS has a design capacity of 2,275 population equivalent, and Novo Milosevo CWS 8,450 population equivalent. The constructed CWSs are a horizontal subsurface flow. The investigation of the Glozan and Novo Milosevo CWS was carried out in the 2005–2012 period. The measurements encompassed chemical analyses of the wastewater, bioaccumulation of nutrients in the reed organs, and tests of sanitary safety (total counts of coliforms and *E. coli*). Measurements were made at distinctive points of the CWS.

Chemical analyses were carried out by the following methods: suspended matter was measured according to the standard SRPS H.Z1.160, BOD₅ according to the SRPS ISO 5815, ammonium (NH₄-N) according to the ISO 7150-1, nitrite (NO₂-N) and nitrate (NO₃-N) by the SEV 1973 and total phosphorus (P) by the ISO 6878/1. Numerical characteristics of the samples were expressed as mean values.

The total coliforms and *E. coli* were determined on the McConkey medium (Torlak) using the plate count method. Ten millilitres of the sample (water from wetlands) was transferred into 90 ml sterile water and decimal dilutions were prepared. One millilitre of each dilution was introduced into petri dishes and overflowed with nutrient medium. The characteristic colonies were counted after incubation.

Analyses of the chemical composition of reed encompassed the determination of nutrients, nitrogen and phosphorus, expressed on the dry mass basis. Plant material from all CWS cells was collected and analyzed at the end of the vegetation period according to the standard methods (APHA, 1995). Contents of nutrients (N and P) were determined in both the vegetative and generative reed parts. Total nitrogen in the dry mass was determined by standard micro-Kjeldahl method. Phosphorus was determined after the ignition and treatment with HCl, by spectrophotometric method using ammonium molybdate-vanadate reagent.

RESULTS AND DISCUSSION

Effects of the municipal wastewater treatment in the Glozan CWS (2005-2012)

The eco-hydrological effects on the Glozan CWS were analyzed via the water balance as the basic ecological factor in the wetland system. The results indicated that despite of an extreme nonuniformity of precipitation during the day, month, or year, and extreme temperature conditions, the wastewater treatment in the CWS has been efficient. The water balance results show that both the dilution caused by precipitation and water loss due to intensive evapotranspiration occurred in the CWS. The changes in the amount of the effluent were in the range from 5 to 12% due to dilution and the loss from 15 to 25%. However, it is evident from the water balance that wastewater has always been present in the substrate, ensuring thus the necessary conditions for microbiological processes and reed growth (Josimov-Dundjerski et al., 2012).

Suspended solids concentrations in influent are relatively unified with average value of 305.13 mg L⁻¹. Standard deviation of influent suspended solids for investigation period has shown small variability. Suspended solids concentrations in effluent are < 20 mg L⁻¹, with average value of 18.96 mg L⁻¹ (Table 1). Based on the average values, the efficiency of suspended matter removal amount of 96 – 93% (Josimov-Dundjerski et al., 2013). Suspended solids are retained predominantly by filtration and sedimentation and the removal efficiency is usually very high (Vymazal and Kröpfelová, 2008).

Measured concentrations of BOD₅ in influent are in the range 162.25 mg L⁻¹ to 838.40 mg L⁻¹ with the average value of 463.18 mg L⁻¹, and in effluent average value of 84.56 mg L⁻¹ (Table 1). The average reduction of the BOD₅ value amount of 84 – 79% (Josimov-Dundjerski et al., 2013). Pollutants are reduced effectively if the hydraulic retention time is relatively high (Carty et al., 2008). Retention time in Glozan CWS is relatively short and is 4.4 days and the influent pollution is high. It is

highly probable that hydraulic characteristics of CWS and influent pollution can affect and determine relatively high BOD₅ in effluent.

Raw municipal wastewater of the Glozan settlement has a high content of inorganic nitrogen, mostly in the form of ammonium, but nitrates and nitrites are also present. The efficiency of ammonium removal in the CWS was 47.5% (Table 1). While the content of ammonium in the cell I decreased, in the cells II and III it was transformed in the other forms and/or taken up by plants, which was significantly less pronounced. Nitrates were present in the majority of water samples, while nitrites were found only in a smaller number of them. The processes occurring most in the CWS are nitrification and ammonification (Belic et al., 2006). The former process takes place in the cells I and II, while ammonification is characteristic of the cell III. Hence, transformations of nitrates and nitrites, participating in these processes, take place just in concordance with these observations (Josimov-Dundjerski et al., 2012). On the basis of the average values, the mass load of total inorganic nitrogen was 8.0 kg day⁻¹, i.e. 2924.0 kg year⁻¹. It was found that a certain portion of nitrogen was taken up by reed and accumulated in its particular parts, mostly in the leaves, where nitrogen content may be up to 42 g kg⁻¹ DM (Table 2) (Josimov-Dundjerski et al., 2011).

The efficiency of phosphorus removal is 29.1%. Based on the average values, the mass load of total phosphorus is 0.8 kg day⁻¹ that is 292.0 kg year⁻¹. Chemical composition of the reed showed that a certain portion of phosphorus was taken up by plants. The reed bloom accumulated phosphorus most, where its content was up to 2.1 g kg⁻¹ DM (Table 2) (Josimov-Dundjerski et al., 2011). Most of the phosphorus that macrophytes take up from the substrate returns to the substrate in the form of dead plant material. By harvesting macrophytes at the end of vegetation season, phosphorus can be removed from its internal cycle in the CWS (Wang and Mitsch, 2000).

Table 3 shows the seasonal variation of treatment efficiency, which was highest in the autumn. The BOD₅ value of the influent was 403.3 mg/l, and of the effluent only 8 mg/l, the reduction being >95%. Content of suspended matter decreased from 230 mg/l to 19 mg/l, i.e. by 91.7%. The decrease in total phosphorus content was >95% (from 17 mg/l to 0.42 mg/l). The ammonium content in the influent was 93.23 mg/l and in the effluent 0.22 mg/l, the decrease being >95% (Josimov-Dundjerski et al., 2012).

In the examined CWS, coliform bacteria and *E. coli* have been found in all the samples. Coliform bacteria are heterotrophic microorganisms that use organic sources of carbon and nitrogen, which explains their highest number in the raw wastewater (influent). The number of microorganisms decreased by passing through one cell to another, which indicates the efficiency of the treatment. This is evident from table 4, under conditions of their maximum activity (autumn) (Josimov-Dundjerski et al., 2012).

Mature CWS Glozan does water treatment successfully. After eight years of operation of the system was confirmed a high degree of removal of pollutants. The measurement results from 2012 year confirm that the CWS Glozan removes 64.6 – 99.3% of suspended matter, while the decrease in organic matter, (BOD₅), has been 54.4 – 89.0%. The efficiency of removal of nitrogen compounds ranged from 70.6 – 85.73%, while the efficiency of total phosphorus removal was 8.7 – 49.8%.

Table 1. Effects of wastewater treatment in the Glozan CWS

Pollutants	Influent mg L ⁻¹	Effluent mg L ⁻¹	Treatment eff. %
Suspended matter	305.13	18.96	96 - 93
BOD ₅	463.18	84.65	84 - 79
Ammonium	86.25	45.28	47.5
Nitrite	0.092	0.020	78.3
Nitrate	0.23	0.12	47.3
Total phosphorus	14.46	10.25	29.1

Table 2. Content of nutrients (g/kg DM) in the CWS reed

	Cell	Plant organ			
		Bloom	Leaf	Stem	Rhizome + root
N	I	24.7-28.2	31.0-42.7	9.4-11.7	8.5-21.5
	II	25.8-30.1	29.3-36.5	7.0-10.2	8.7-11.4
	III	23.9-32.9	28.3-36.7	8.0-10.5	8.2-13.9
P	I	1.6-2.1	1.1-1.3	0.3-0.7	0.4-0.8
	II	1.5-2.0	0.8-1.4	0.2-0.7	0.2-0.4
	III	0.9-1.6	0.9-1.4	0.2-0.9	0.2-0.6

Table 3. Seasonal variations in treatment efficiency (%)

Pollutants	Spring	Summer	Autumn	Winter
Suspended matter	84	83	>95	66
BOD ₅	>95	93	92	94
Ammonium	74	18	>95	28
Total phosphorus	50	50	>95	46

Table 4. Average number of coliform bacteria and *E. coli* in the CWS water

	Summer (July)		Autumn (October)	
	Total colif. 10 ³ ml ⁻¹	<i>E. coli</i> 10 ¹ ml ⁻¹	Total colif. 10 ³ ml ⁻¹	<i>E. coli</i> 10 ¹ ml ⁻¹
Influent	420	287	340	104
Effluent	51	3.5	75	3

Effects of the municipal wastewater treatment in the Novo Milosevo CWS (2008-2012)

The first set of results of the constructed wetland at the Novo Milosevo settlement showed a relatively high efficiency of removal of organic pollutants (Table 5). The CWS Novo Milosevo can remove more than 73% of suspended solids while reducing organic matter, (BOD_5) is more than 82%. The efficiency of removal of nitrogen compounds ranged from 33.6 – 79.0%, and the efficiency of total phosphorus removal was 6.6 – 23.8% (Belic et al., 2010; Josimov-Dundjerski et al., 2010). Results from 2012 year of the system have confirmed high degree of removal of pollutants. The CWS Novo Milosevo removes 7.6 – 98.6% of suspended matter, while the decrease in organic matter, (BOD_5), has been 52.1 – 88.4%. The efficiency of removal of nitrogen compounds ranged from 74.4 – 90.1%, while the efficiency of total phosphorus removal was 31.1 – 51.6%. Also, microbiological investigations showed that the CWS was efficient in reducing the number of coliform bacteria and *E. coli*, ensuring thus the sanitary safety of the effluent (Table 6).

Table 5. Effects of wastewater treatment in the Novo Milosevo CWS

Pollutants	The first set of results		Results from the 2012.	
	Influent mg L ⁻¹	Effluent mg L ⁻¹	Treatment eff. %	Treatment eff. %
Suspended matter	80 - 180	10.0 - 49.0	> 73	7.60 - 98.6
BOD₅	324 - 394	49.5 – 59.5	> 82	52.1 - 88.4
Ammonium	56.2 – 59.8	32.6 – 49.8	11.9 - 45.5	80.0 - 90.1
Nitrite	0.112	0.023	79.0	89.0
Nitrate	0.26	0.17	33.6	74.4
Total phosphorus	13.5 – 16.0	10.4 - 14.9	6,6 - 23,8	31.1 - 51.6

Table 6. Average number of coliform bacteria and *E. coli* in the CWS water

	Total colif. 10 ³ ml ⁻¹	<i>E. coli</i> 10 ⁴ ml ⁻¹
Influent	260	37
Effluent	15	< 3

CONCLUSION

The results have shown that the removal of pollutants in both CWSs is very high. Serbian water quality standards for discharge treated wastewater are in coordination with Directive EU (91/271/EEC). According to limit concentration values and taking into consideration number of residents the quality of effluent are satisfied. Exception is only present in case of total phosphorus.

The CWS water balance confirmed the permanent presence of wastewater as the necessary ecological factor for the activity of microorganisms and vegetation, which yields the transformation and cycling of nutrients in the system. The efficiency of the system depends also on the activity of microorganisms and reed vegetations, optimum

conditions for both are in autumn. The relatively high N and P contents in the reed indicate that it is a very successful biological accumulator of nutrients. Microbiological investigations showed that the CWS was efficient in reducing the number of coliform bacteria and *E. coli*, ensuring thus the sanitary safety of the effluent.

Although the results of research on CWSs in Vojvodina are in favor of this method to the present constructed wetlands still have not taken a significant place in the purification of water in this region. On the other hand, in developed countries are increasingly favored as ecological way of treating waste water of different origin and apply constructed wetlands for all levels of purification (Vymazal, 2010; Kayranli et al., 2010).

Vojvodina has population of more than two million inhabitants who live in 467 settlements. Of this number, 404 are settlements with less than 5,000 inhabitants, having no systems for collecting and treating local wastewaters. The main reason for such a situation is the shortage of financial resources. Hence, in these settlements use is made of septic tanks, so that their leaks contaminate ground waters, whose level in this region is rather high. Contents of septic tanks are disposed on the fertile soil or to water bodies. Thus the soil and water resources are constantly contaminated. A promising solution to this problem would be to construct low-pressure sewerage systems for collecting used waters and treating them in CWSs. The results presented for the Glozan CWS and Novo Milosevo CWS demonstrate the usability of such systems under the conditions of moderate continental climate, which could be an essential contribution to the sustainable development and environmental protection.

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ASSESSMENT OF VULNERABILITY TO INLAND EXCESS WATER IN VOJVODINA REGION

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SUMMARY: One of the most important tasks of water management in Vojvodina is draining the excess water from arable lands in order to create favorable conditions for cultivation of plants. In this study, assessment of vulnerability to inland excess water is performed based on drainage properties of soil, geomorphologic characteristics of the area, average groundwater level in non-vegetation period, land use and land cover. It was performed by using GIS and AHP. The resulting map of vulnerability shows that approximately 20% of the territory in Vojvodina is very vulnerable to inland excess water.

Key words: vulnerability assessment, excess water, GIS, AHP

INTRODUCTION

Studying the phenomenon of inland flooding in Vojvodina region is of great importance because agricultural lands were frequently flooded in the past. In some cases these phenomena have reached catastrophic proportions, when tens and hundreds of thousands of hectares of arable land were flooded (Dragović et al., 2005). One of the most important tasks of water management in Vojvodina is draining the excess water from arable lands in order to create favorable water and air conditions in the soil for cultivation of plants (Belić et al., 2007; Škorić, 2002; Kolaković, 2006). Occurrences of inland excess waters are caused primarily by extreme amounts and uneven spatial and temporal distribution of rainfall. Arable land of Vojvodina are located in plain on which drainage problems occur because of poor surface runoff, whose residue partially evaporates but mostly infiltrates into the soil. Generally speaking, the need for drainage in Vojvodina occurs on flat and shallow soils; on those who lie on impermeable layer; at shallow depressions where water retains; on the land on which water comes from a higher terrain by subsurface or surface runoff and on soils where water infiltrates from rivers in the periods of high water levels (Škorić, 2007). The extent and duration of inland flooding depends (Milošev and

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Dedić, 2000, Zdravić et al., 2002) on: meteorological and hydrological conditions in previous years and in the year of flooding; elevation of the affected areas - the size and depth of the depression; the structure of the drainage system; soil properties; soil cultivation - particularly heavy equipment has a negative impact because it deteriorates water regime of soil and eventually form a soil layer that is largely impervious to water, so-called "plow pan"; etc. Similarly, the authors (Kuti et al., 2006; Van Leeuwen et al. 2012; Barta, 2013) point out that maps of vulnerability to excess water for the territory of Hungary were created mainly based on a limited number of factors such as relief, soil characteristics, groundwater, rainfall, and so on.

As the largest effect on reducing the risk of hazardous events can be achieved by reducing the vulnerability (Knutson et al., 1998, Wilhite and Svoboda, 2000; Wilhite, 2005; Thywissen, 2006), vulnerability assessment, such as the assessment of vulnerability to excess water, is a very important step in the risk assessment. Based on the assessment of vulnerability, appropriate measures can be planned and taken to prevent or mitigate adverse effects.

The Geographic information system (GIS) imposes as an indispensable tool for collecting, storing, and presenting spatial data and it is often combined with multi-criteria decision making tools for the analysis of spatial problems. The aim of this study is to assess the spatial vulnerability to inland excess water by using GIS and Analytic Hierarchy Process (AHP) multi-criteria decision making method in order to create thematic maps that could help decision makers to visualize the hazard and implement appropriate mitigation measures.

MATERIAL AND METHODS

Factors affecting the vulnerability of the area to the occurrences of excess water are determined based on the natural conditions in Vojvodina. These factors were selected based on their impact on the very phenomenon of inland excess water, on the basis of data availability and research of numerous authors (Belić et al., 1995; Milošev and Dedić, 2000; Zdravić et al., 2002; Škorić, 2007; Kolaković, 2006; Kuti et al., 2006; Van Leeuwen et al. 2012; Barta, 2013). It is estimated that following factors have the greatest impact on the vulnerability to inland excess water in Vojvodina: soil properties, geomorphologic characteristics of the area, average groundwater level in non-vegetation period and land use and land cover.

Soil characteristics are important in terms of drainage properties of the soil as they relate to the speed and the scale of removal of excess water due to the influx of precipitation, surface runoff and ground water flow. Soils in Vojvodina are classified into drainage classes based on their drainage properties (Miljkovic, 2005) and will be used as a factor in the excess water vulnerability assessment. In this study, soil drainage classes were derived from the digital soil map of Vojvodina (Benka i Salvai, 2006).

Geomorphologic characteristics of the area are very important in terms of generation and removal of excess water (VOS, 2001; Mijatović et al., 1995). On lower geomorphologic units, the occurrences of excess water and drainage needs are pronounced, while higher terrains provide water that gravitates towards lower geomorphologic units. For the purposes of this study, geomorphologic map of Vojvodina (Mijatović et al., 1995) is digitized.

The appearance of excess water is manifested by raising the groundwater table, so it is very important to know changes in groundwater levels (VOS, 2001; Putarić, 1994). In this study, a digitized map of average groundwater levels in non-vegetation period was used, because the amount of excess water generated in that period is also used for designing the drainage systems in Vojvodina (Putarić, 1994).

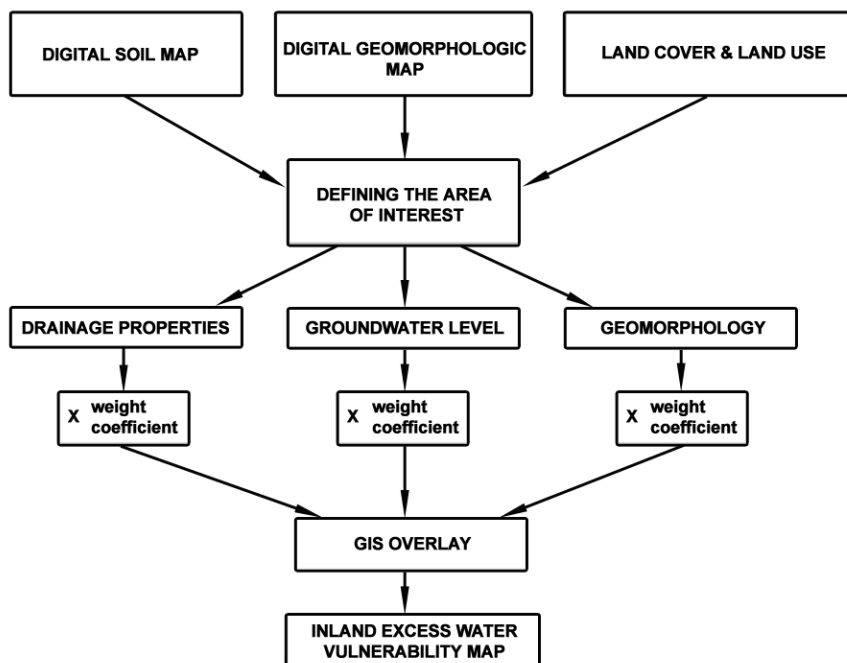


Figure 1. Flowchart of the inland excess water vulnerability assessment

Data on the land use and land cover makes possible to accurately determine the boundaries of the area of interest, or to isolate areas that are not of interest for the assessment of vulnerability to excess water, so vulnerability assessment is not performed on areas like forest areas and water bodies. Also, naturally well drained areas that do not require drainage and high terrains, where there is no need for the construction of drainage systems, belong to areas that are not of interest for assessment of vulnerability to inland excess water. In this study, data from CORINE Land Cover 2006 (EEA, 2007; Nestor and Protic, 2006) spatial database of land cover were used.

The procedure for assessment of vulnerability to inland excess water is performed according to the flowchart shown in Figure 1. It was performed in GIS environment by using techniques of classification, reclassification and GIS overlay and the weights of factors are determined by multi-criteria decision making method, called Analytic Hierarchy Process (AHP). The AHP method is proposed by Saaty (1980) and nowadays is one of the most popular decision support tools (Srdjevic and Srdjevic, 2013) which has received significant application in numerous fields (Basak, 2011).

RESULTS

To perform overlay operations in GIS environment on layers that could contain different types of data, the data layers should be standardized and classified according to certain criteria. By classification of layers according to certain criteria, the same type of data and in the same range is assigned to all layers. In this study, the data of each input layer was classified by assigning integer values in the range from 1 to 5, where 1 indicates the lowest vulnerability level and 5 indicates the highest.

Certain factors of vulnerability, due to the nature of the phenomena, may have a greater or lesser impact on the final result of a vulnerability assessment, and therefore they need to have appropriate weights assigned. In this study, AHP multi-criteria decision making method has been used in order to determine the weights of factors. The pairwise comparisons of factors are performed by using Satty's nine-point scale and the numerical values of judgments are placed in the matrix. By following the standard AHP procedure, in order to derive weights from this matrix, eigenvector prioritization method (EV) is used. The results showed that the greatest impact on vulnerability to inland excess water has drainage properties of soil (with weight of 0.595) whereas slightly smaller impact has groundwater levels (with weight of 0.276) and geomorphologic characteristics of the area (with weight of 0.129).

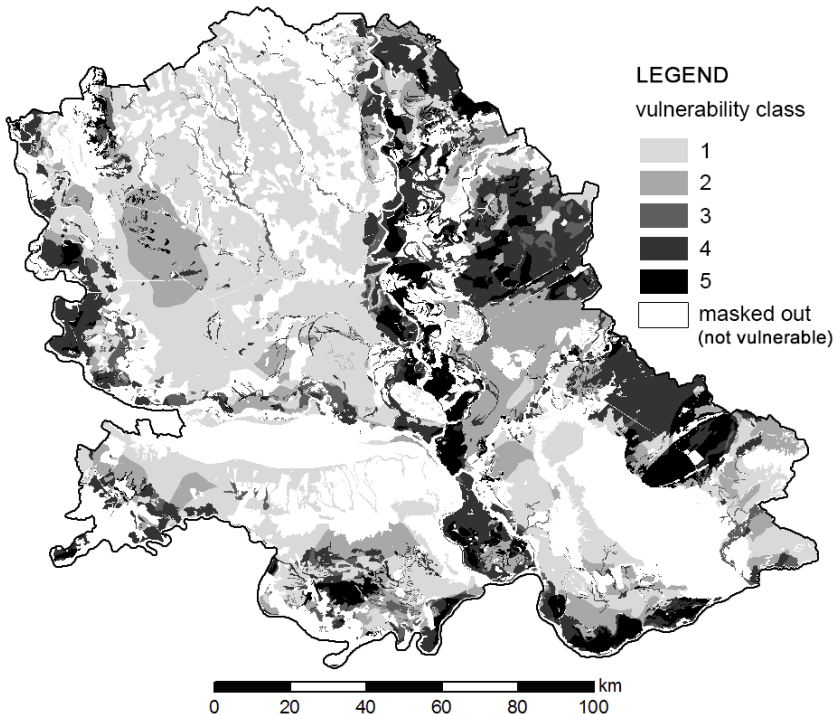


Figure 2. Map of vulnerability to inland excess water in Vojvodina

In order to define the area of interest for the assessment of vulnerability to excess water, certain areas are extracted and removed from all input layers, such as water

bodies, forest areas, naturally well drained areas and high terrains. The resulting map of vulnerability to inland excess water (Figure 2) is obtained by multiplying raster layers of vulnerability factors (drainage properties of soil, geomorphologic characteristics of the area and average groundwater level in non-vegetation period) with appropriate weights and then summing these layers in GIS. The resulting map was classified into five equidistant classes of vulnerability.

Due to a combination of adverse factors of vulnerability it is assessed that about 20% of the territory of Vojvodina is highly vulnerable to excess water (class of vulnerabilities 4 and 5). Most of those areas are located in Banat. Particularly vulnerable are Northeastern Banat, the entire coastal area of the Tisa River and the area bounded by the Northeast slopes of Deliblato sands from Vršac to the river Tamiš and coastal area of the Danube River. In Backa, vulnerable areas are located mostly by the left bank of the Danube River, and in Srem along the left bank of the Sava River. Around 3% of the territory has medium vulnerability (vulnerability class 3), while around 40% of the territory has low vulnerability to excess water (class of vulnerability 1 and 2). Around 37% of the territory of Vojvodina is not vulnerable to the occurrence of excess water because it is located on high terrain, or the soils in these areas are well drained and do not require drainage or it is forest area or water body.

DISCUSSION

Authors who have researched the occurrence of excess water and flooding from inland waters in our region point out that these events are caused by the relief and elevation of the affected areas, soil texture and soil water properties, soil cultivation, the structure of the drainage system and by meteorological and hydrological conditions (Belić et al., 1995; Škorić, 2007; Milošev and Dedić, 2000; Zdravić et al., 2002). Similarly in Hungary, maps of vulnerability to inland excess water were created mainly based on a limited number of factors (relief, soil characteristics, groundwater, precipitation, etc.) and by weighing those factors using coefficients that are derived experimentally (Kuti et al., 2006; Van Leeuwen et al. 2012; Barta, 2013).

As previously mentioned, vulnerability assessment in this study has been based on data availability and on their impact on the very phenomenon of inland excess water occurrence. As the data on soil cultivation is difficult to access; especially data on the formation of the so-called "plow pan", it could not be used in this research. Also, the meteorological conditions were not included in the assessment of vulnerability in this study. They are a natural hazard and they should be specifically analyzed in future studies of excess water risk assessment, because the risk assessment is usually based on assessment of hazard and assessment of vulnerability (Blaikie et al. 1994).

Van Leeuwen et al. (2012) stated that in studies of excess water in Hungary, weight of individual vulnerability factors were determined using regression functions or other linear statistical methods. In contrast to this approach, in this study the weights of vulnerability factors were determined using AHP multi-criteria decision making method. Factors of vulnerability were multiplied by appropriate weighting coefficients and then GIS layers were added together to create the resulting map of vulnerability to inland excess water.

Although the methodology of vulnerability assessment performed in this study is different, the results are consistent with the results of previous researches of inland excess water occurrences in Vojvodina region (Kolaković, 2006; Belić et al., 1995; Škorić, 2007; Milošev and Dedić, 2000; Zdravić et al., 2002). This framework, based on the combination of GIS and multi-criteria decision making tools, allows more flexible use of different spatial data sets than previous approaches and that could improve the quality and reliability of the results.

CONCLUSION

Frequent occurrence of excess water on arable lands can cause significant adverse effects on agricultural production. Strict requirements of agricultural production and special characteristics of agro-climatic conditions of the Vojvodina region make it very vulnerable to the occurrences of excess water. In this study following factors of vulnerability to inland excess water are identified: soil drainage class, geomorphologic characteristics, average groundwater level in non-vegetation period and land use and land cover. Result of application of the AHP multi-criteria decision making method for determining the weight coefficients of vulnerability factors showed that the greatest impact on vulnerability to inland excess water has drainage properties of soil whereas slightly smaller impact has groundwater levels and geomorphologic characteristics of the area. The spatial database of land cover and land use was used to define areas of interest for which assessment of vulnerability was performed. Results of vulnerability assessment showed that approximately 20% of the territory in Vojvodina region is very vulnerable to occurrence of inland excess water. Most of these areas are along coastal areas of rivers Danube, Sava and Tisa, as well as in Northeast and East of Banat. A clearer understanding of spatial extent of inland excess water could lead to taking appropriate measures of preparation and mitigation of the negative consequences before hazardous event occur and that could improve the management and maintenance of land reclamation drainage systems.

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POTENTIAL ANALYSIS OF AGRICULTURAL RESIDUES AS A SOURCE OF RENEWABLE ENERGY

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SUMMARY: *Over the past few decades Austria has seen a steady decline of agricultural farms. The reduced management of extensively used grassland areas (e.g. natural protected areas) subsequently enhances forestation. This process of succession, reinforced by climate change, has numerous negative effects on different sectors, including tourism. Ideally, a stable sod secures soil from erosion protecting against avalanches, landslides and other natural hazards. In the absence of agricultural farming, alternative management concepts are required in order to preserve an open countryside. Grassland biomass processed in a biogas plant to produce renewable energy is therefore seen as a sustainable alternative.*

The aim of this study was to determine and evaluate the technical potentials of grassland biomass and animal excrements as renewable energy sources for one model region in Austria. Further, a future scenario for the year 2030, based on a continuous reduction of animal stock, was considered. A novel calculation scheme was developed in order to calculate the technical potentials. The main results obtained from these calculations show an unused biomass from grassland of 557 t DM/year corresponding to an unused pasture area of 183 ha. Including animal excrements, the total methane potential is around 899.900 Nm³/year. This equals approximately 4.5 GWh of electricity and 4.4 GWh of heat, which can be used for power grid and district heating networks or as fuel in the case of biomethane. For the year 2030 the potential analysis predicted the area of unused pasture rising to 1,205 ha. The biomass potentials from grassland will increase to 3,663 t DM/year. Per year, about 1,864,300 Nm³ of methane can be expected. This corresponds to 6.4 GWh of electricity and 6.3 GWh of heat.

Key words: *renewable energy, biogas, agricultural residues, unused grassland, forestation, potential analysis*

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INTRODUCTION

In Austria, the quantity of agricultural farms has steadily decreased over the last few decades. This decline has particularly affected smaller farms and farms which are situated in less favorable regions (Bmlfuw, 2013). The main reason for giving up farming is the economic disadvantage towards industrialized farms, which produce greater quantities under cheaper conditions. The areas that are no longer managed face scrub encroachment and subsequent forestation, associated with a range of negative effects (Pötsch, 2008). Due to succession processes, numerous important functions of grassland, described by Pötsch (2008), gradually disappear. Soil is likely to be exposed to erosion, which reinforces avalanches, landslides or other natural hazards. Biodiversity also declines, as does the ability of grassland to store and filter water. Furthermore, forestation changes the landscape, negatively influencing tourism. During summertime, meadows attract leisure travelers and in winter, grassland is the central basis for various winter sport attractions.

Buchgraber (2004) showed an enormous potential of unused grassland biomass in Austria. For the year 2010 the author predicts that about 47 % of the pasture areas will be located in regions, which produce more grassland biomass (overproduction is about 2,500 to 3,500 t DM per year) than needed for animal feed. Aside from the socio-economic factors, climate change will also influence the harvest yields of grassland biomass in the future. Different climate scenarios show that regions with an annual precipitation of over 1,000 mm will profit from an extended vegetation period and warmer temperatures, whereas those regions with a precipitation rate less than 800 mm per year are more likely to suffer from increased water scarcity, reducing biomass yields (Fuhrer *et al.*, 2007; Haas *et al.*, 2008; Tamme, 2012). Moreover, increased fluctuations of biomass yields are expected through higher temperatures and rising evaporation in summer. Due to the aforementioned impacts of climate change, the quality of fodder from grassland biomass is likely to be reduced (Fuhrer *et al.*, 2006). Biomass from dry grassland is strongly lignified and is no longer proper fodder, however, it is still suitable for biogas production. A biomass pretreatment (for example with steam explosion) would increase both the decomposition rate of lignified biomass, as well as the biogas yields (Bauer *et al.*, 2009).

To maintain the positive aspects of grassland, an open countryside must be preserved. In the absence of agricultural farming, alternative management concepts are required. Grassland biomass as a feedstock for biogas production, a source of renewable energy, can be viewed as one sustainable alternative.

The aim of this work was to determine and evaluate the technical potentials of the unused grassland biomass and animal excrements for one exemplary model region in Austria. Beyond the evaluation of the technical potentials, the study provides the technical dimensioning of the proposed biogas plant. A future scenario for the year 2030 was also considered in order to highlight prospective developments based on a further reduction rate of animal stocks.

MATERIAL AND METHODS

The analyzed model region includes a grassland area of 5,680 ha. Around 910 ha are managed intensively, which means the pastures are cut at least three times per

year. The remaining area, 4,770 ha, is managed extensively with a maximum of two cuts per year. Furthermore, the region's farmers raise around 2,850 cattle, 970 other roughage eaters, 130 pigs and 1,300 chickens. Based on this data, the potentials of the unused grassland biomass and of the animal excrements were calculated. To calculate the technical potentials as realistically as possible, only 60 % of the extensively used grassland area is considered.

According to Kaltschmitt and Wiese (1993) different possibilities for calculating potentials exist. In this study only the technical potentials were considered. In comparison to the theoretical potential, the technical potential is more realistic, because it includes technical restrictions as described below. Biomass is going to be reduced by so-called losses on stock. Mechanical losses and losses due to biomass respiration (i.e. gross yields) are two examples of technical restrictions. Additionally, losses emerge due to the conservation of ensiling or hay-making (= net yields). In this study the gross yields were reduced by 5 % due to conservation losses. The theoretical potential of animal excrements is reduced by the duration of the free-range grazing period.

For the calculations, secondary data were used. These data included benchmarks to calculate the dry matter yields (depending on type of grassland), fodder demand and amount of manure (both depending on animal type, age and sex) as well as the gas potentials and nutrient loads of each organic material

The amount of unused biomass was obtained by calculating the net biomass yield of all grassland areas and the livestock fodder demand. The study assumes that net yields from grassland are low to middle due to the high percentage of extensively used areas. After covering fodder demand with biomass, the excess is intended to be used for biogas production. The entire amount of animal excrement was calculated assuming a free-range grazing period from one to five months and considering the manure system of the stall. Further, it was assumed that 80 % of cattle and pigs produce solid manure, the rest produce liquid manure. The calculation of the methane potential was carried out using the data from Table 1.

Table 1: Yields of Biogas.

	Content of DM [%]	Content of VS [%]	Yield of Biogas [Nm ³ t VS ⁻¹]	Content of CH ₄ [%]
Grassland biomass ³	40	84 – 90	546 – 603	54 – 56
Manure (solid and liquid) ⁴	5 – 25	75 – 85	300 – 450	55 – 65

Source: LfL(2004)

The calculation of the daily amounts of material to be introduced into the biogas plant was based on the assumption that the operating time for biogas production is 8,760 hours per year with a constant production rate. Although daily manure accumulation differs depending on the period of free-range grazing, this study assumes efficient storage management, allowing the daily amount introduced to the

³ These biogas yields refer to grass silage depending on the date of cut.

⁴ Depending on the type of animal, age and sex

biogas plant to remain constant. This also applies to the unused grassland biomass, which is recommended to be stored as silage. The required power of the CHP was calculated by multiplying the power of methane with the CHP's electrical and thermal efficiency (assumption of 38 and 42 %). The power of methane is obtained through the division of the whole energy output of methane (kWh) and the real operating hours, which are estimated with 7,468 h per year. In order to calculate the fermentation residues, we assumed that entire organic dry matter is degraded by 76 % and that the residues contain 4.97 % VS of FM.

The predicted changes in animal stock for the year 2030 are displayed in Table 2.

Table 2: Predicted changes in animal stock for the year 2030 opposite 2008 [%].

Type of animal	Predicted changes opposite 2008 [%]
	2030
Milk cows from 2 years	- 33,6
Other cows	- 22,0
Horses	+ 6,4
Sheep	+ 8,2
Goats	- 14,2

Source: LfL(2011)

The predicted changes in animal stock from 2008-2030 were calculated with linear regression (reference period for milk cows from 1991-2008, for all others from 2003-2008). The base year of the study, conducted by LfL, is 2008. In this study the base year is 2010. This deviation remained unconsidered.

RESULTS AND DISCUSSION

In the model region, the grassland areas produce about 11,461 t DM of biomass each year (conservation losses of 5 % already considered). According to the livestock population within the model region, the annual fodder demand is around 10,904 t DM every year. Despite this high fodder demand, there is still a considerable potential of unused grassland biomass that can be used as a feedstock for biogas production. This potential equates to 557 t of DM per year, which corresponds to an unused grassland area of 183 ha and a methane potential of 153,800 Nm³ or rather 1.5 GWh. The technical potential of animal excrements that can be fed into a biogas plant is, due to the large animal population, about 6,000 t of DM per year. Due to the fermentation process, 1,163,400 Nm³ of methane or 11.5 GWh can be produced. Together, these two organic materials could produce about 1,317,200 Nm³ of methane or 13 GWh from methane annually. Methane can be used as a fuel after a gas purification process or for producing electricity and heat. For the latter, a CHP with a power of 668 kW_{el} and 738 kW_{th} is necessary. The total amount of electricity is 4.5 GWh and of heat 4.4 GWh. The daily quantity of silage available to be fed into a biogas plant amounts to around 3.8 t FM, those from liquid manure 15.3 t FM and from solid manure approximately 59 t FM. In sum, 78 t FM could be fed into a biogas plant every day. The total amount of fermentation residue is 25,700 m³ per year, while the nitrogen amounts to 119,800 kg in total. This leads to a nitrogen availability of 32 kg per ha and year, when the entire fermentation residue is used as a fertilizer for

the same grassland areas assumed by the potential analysis. This would ensure a closed nutrient cycle, but also be logistically demanding. The logistical effort of the biogas substrates and the fermentation residues can be rated as high and has to be adapted to each region individually.

According to a steady decrease of animal stock, biogas potentials will change in future. The unused grassland area will increase in the model region. By 2030, this area will increase by 1,022 ha to 1,205 ha (plus of 558 %). The potential of unused biomass available to be fed into the biogas plant also will rise to 3,663 in 2030, whereas the potential for manure (solid and liquid) will decrease. In total the potential for the biogas plant will rise from 6,560 t DM in 2010 to 7,967 t DM by 2030 (plus of 21 %). This corresponds to a total methane production of 1,864,300 Nm³ in 2030, an increase of 547,100 Nm³ from 2010. In 2030, the final energy will be around 6.4 GWh of electricity and 6.3 GWh of heat (plus of 42 %). Due to an increase of unused biomass, the daily amount of this substrate available to be fed into the fermenter will be 25 t FM. In contrast, the daily amounts of liquid and solid manure available to be fed into the biogas plant will reduce to 10.7 and 42.2 t FM. As the potential analysis of the model region shows, the amount of fermentation residue will also increase from 25,706 m³ per year in 2010 to 32,300 m³ per year in 2030. The total nitrogen amount will be 148,200 kg per year, resulting in a nitrogen availability of 39 kg per ha and year. Thus, the entire amount of fermentation residues can be distributed on grassland fields.

The calculated technical potential of unused biomass and manure for 2010 as well as 2030 can be absolutely rated as realistic. Nevertheless, deviations from calculations can occur, when, for example, conservation losses increase, the free-range grazing period changes or animal stock develops differently than assumed in this study. Deviations can also emerge if farmers do not provide manure and grass for the biogas plant. In the future, the changing landscape will affect the model region due to an increase in unused grassland area amounting to around 1,022 ha between 2010 and 2030. This relatively large area will obviously bring changes for the overall appearance of the landscape as long as no adaptation measures are carried out. Effects on the environment due to fertilization can be rated as low, as the nutrition contents, obtained by the calculations for 2010 as well for 2030, complies with Austria's regulatory framework.

CONCLUSION

In order to ensure an open countryside, conserve the characteristic landscape, adapt to climate change and reduce natural risks associated with forestation, the use of grassland biomass for energy production appears to be a good alternative to dairy farming. As the potential analysis has shown, biomass shows a high level of potential as a feedstock for biogas production in the model region. Further research is needed to ascertain the feasibility of such a biogas concept, in which not only the potentials of the organic materials are calculated but also ecological, social and economic aspects. A holistic approach, which includes the logistic as well as the usage possibilities of fermentation residues and biogas, is therefore essential for implementing a biogas plant. The involvement of all relevant stakeholders, especially the farmers, is of key importance for the whole implementation process.

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MANAGEMENT OF INVASIVE ALIEN PLANT SPECIES IN RIPARIAN FOREST HABITATS

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*SUMMARY: Riparian forest habitats are affected by biological plant invasions. The results of this case study show that species rich forest habitats are less invaded than forests with higher human impact. Further forest habitats with a higher native species density in the herb layer, or with a developed shrub layer are less affected. In total, 41 plots (n=142) showed a proximity to frequented forest roads proximity to frequented forest roads (max 2m distance). Forest roads and other forestry-related infrastructure are "pathways" for the establishment of invasive plant species in an area. Specifically, the abundance of *Impatiens parviflora* increased significantly from 5.43% of the total plant cover in 2011 to 17% in 2013 ($P = 0.0002$). These results are valuable for the management of invasive species in protected areas and the monitoring of restoration projects.*

Key words: *biological invasion, *Impatiens parviflora*, riparian habitat, plant diversity*

INTRODUCTION

Riparian forest habitats are highly vulnerable to biological invasions. One characteristic of riparian plant communities is the structural diversification along vertical and horizontal gradients. Besides modification of river systems through dams and regulation measures, the occurrence of invasive alien species influences the ecological development of riparian forest habitats (Hager et al., 2005). The massive occurrence of invasive alien plant species (IAS) can be correlated with the immediate suppression of native plants (Pyšek and Prach, 1995; Richardson et al. 2007). Human activity supports the distribution of invasive species that cause dramatic environmental changes of global importance (Walter et al., 2005, Kowarik and Boye, 2003, Vitousek et al., 1996, Thuiller et al., 2008). The aim of the research is to: (1) investigate dominance in plant cover of IAS; (2) evaluate the abundance of the invasive alien species in the community of riparian forest habitats; (3) assess the intensity of human disturbances within forest habitats.

The study area is part of the Natura 2000 site "Tullnerfelder Donau-Auen" in Lower Austria. The Natura 2000 site "Tullnerfelder Donau-Auen" represents one of

the largest connected riparian ecosystems of Austria. The management targets are to support the development of a mosaic of typical riparian ecosystems, consisting of wooded and non-wooded ecosystems. The installation of power plants in the 1970th caused serious changes in hydrology to the area. Most abandoned meanders were disconnected from the main stream. Without this important cross-link to the main stream, the ecosystem is highly endangered. Furthermore, the reduction of river flooding has led to massive disturbances within the gallery forests (NÖ, 2013). With the project Life+ Traisen, an improvement of the hydro-ecological condition of the downstream section and the outlet of the river Traisen is expected within the following years. The planning of the project follows instructions of the European Water Framework Directive.

MATERIAL AND METHOD

The vegetation development of the riparian forest habitats along the outlet of the river Traisen was monitored for four years (2010 to 2013) on 142 plots (each 200m²). To assess the quality of plant diversity and vegetation coverage, a grid of coordinates was placed over the research area. The scientific nomenclature of all plant species followed the classification Book “Exkursionsflora für Österreich, Lichtenstein und Südtirol” (Adler et al., 1994). The following parameters were recorded within the vegetation mapping in the years 2011, 2012 and 2013: habitat type, plant cover of occurring species, number of species and site conditions. The cover (%) of all vascular plant species were recorded and estimated following the adapted Braun-Blanquet scale (Braun-Blanquet, 1964). The analysis of the vegetation sampling data was analyzed using Microsoft Excel 2010. Statistical computing and graphics “R” (32-bit) was used (R Core Team, 2012). The normality was tested on the model residuals using the Shapiro-Wilk test (Shapiro and Wilk, 1965; Shapiro and Francia, 1972). According to the Shapiro-Wilk test, the data of vegetation sampling did not follow a normal distribution ($P < 0.05$). Therefore the Pearson correlation was used to analyze the relationship between species number, plant cover, habitat types and cover of IAS (Gustafson 1998; Deutschewitz et al., 2003; Raes et al., 2009). In order to calculate the influence of the development of the occurrence of invasive alien plants on the native diversity, the Shannon diversity index H' was chosen (Magurran, 1988). Descriptive data of all 142 plots from 2011, 2012 and 2013 was further assessed by the H and J indices and analysed using the non-parametric Mann-Whitney-Wilcoxon Test (Mann-Whitney, Wilcoxon paired sample, or Spearman rank correlation) to detect significant changes.

RESULTS

The vegetation sampling of 142 recording surfaces (plots) apprehended the occurrence of 8 invasive alien plant species *Acer negundo*, *Ailanthus altissima*, *Bunias orientalis*, *Robinia pseudoacacia*, *Rudbeckia laciniata*, *Impatiens glandulifera*, *Impatiens parviflora*, *Solidago gigantea* that were recorded within four different vegetation layers. The comparison of the distribution from 2010 and 2013 showed an ongoing establishment of IAS. The stands of *Impatiens glandulifera* were related to riparian forest habitats under direct influence of open water bodies. Between the years

2010 and 2013, the major spread of *I. glandulifera* took place on the artificial embankments along the old river bed of the river Traisen. Water body proximity and anthropogenic influence promoted the expansion of *I. glandulifera*.

The 41 plots (n=142) showed a proximity to frequented forest roads. Vegetation development of plots with close proximity to direct roads showed significantly different IAS abundance to plots not in proximity to roads between 2011 and 2013 (Wilcoxon test; $P < 0.001$). The species number of plots along roads decreased significantly during the three years ($P < 0.001$). The invasive species *Acer negundo* and *Ailanthus altissima* were not found along roads. The invasive species *Solidago gigantea* (mean \pm SD = 7.24 ± 14.43) had the highest abundance along roads in 2011. The spread of *Solidago gigantea* in the study is related to disturbed habitats along roads and poplar tree forests. Proximity to forest roads is a significant factor for the spatial distribution of *S. gigantea*.

The increase of *Impatiens parviflora* between 2010 and 2013 was the highest of all invasive species in the study area. The distribution was extensively high within all forest habitats. The anthropogenic influence of road proximity did not significantly influence the distribution of *I. parviflora* (Wilcoxon test; $P > 0.05$). The spatial spread was extensive and rapid. The most significant differences among plots with and without road proximity existed for *I. parviflora* were seen between 2011 and 2013 (Wilcoxon test; $P < 0.001$). In 2011, invaded plots showed an IAS occurrence of 12.00% in comparison of plots proximal to roads. In 2013, invaded plots with road proximity showed an average abundance of 14.27%. Within the plots with road proximity *I. parviflora* shows an average total plant cover of 2.56% in 2011 and 6.04% in 2013 in the herb layer. Within plots with no proximity to roads, *I. parviflora* occurred with an mean plant cover of 3.18% in 2011 and 10.96% in 2013.

Analysis of the abundance of IAS in the plant cover of each plot showed differences among herb layer, shrub layer, tree layer I and tree layer II. The herb layer (0 to 1m) was most affected by the invasion of IAS. In total, 109 plots were invaded by IAS in the herb layer (total n=142). The IAS was least affected in the tree layer I and II (2m to 20m). The tree species *Acer negundo*, *Ailanthus altissima* and *Robinia pseudoacacia* were detected in the tree layer I and II within 23 plots (total n=142). The proximity to roads was significant for the positive occurrence of IAS in the tree layer I ($P < 0.005$). The comparison of the development of IAS for 2010 and 2013 showed no significant increase of IAS in the tree layer I and II.

IAS occurred in six different forest habitat types: Ash tree forest, grey alder tree forest, cutover land, poplar tree forest, red pine tree forest, and the willow tree alluvial forest. The result of the nonparametric Mann-Whitney-Wilcoxon test showed that the development of invasive species in the herb layer differs among habitat types ($P < 0.001$). The Pearson product-moment correlation showed a very low relationship among habitat type IAS cover as well as species number and IAS cover ($r < 0.05$). In total, 40 plots corresponded to the habitat type ash tree forest in 2012, which was invaded strongest. In 2013, one plot was deforested (2013, n=39). The mean plant cover increased from 73.15% in 2011 to 93.63% in 2013. On average 12.5 species were recorded in the herb layer in the years 2011 and 2012 and 13.5 species in 2013. The invasive species *Solidago gigantea*, *Impatiens glandulifera*, *Impatiens parviflora*, *Ailanthus altissima*, *Robinia pseudoacacia* and *Acer negundo* were detected. The abundance of IAS within the type Ash tree forest habitat increased. In 2011, invasive species represented in total 18.11% of the plant cover, in 2012 in total 34.0% and

39.41% in 2013. The changes of average abundance of *Impatiens parviflora* were strongest in 2012. The average abundance of *I. parviflora* increased from 7.48% in 2011 to 20.19% in 2012. The populations of *Solidago gigantea* increased constantly from 8.02 % in 2011, to 10.53 in 2012, to 15.31% in 2013. Comparing to the grey alder tree forest (n=18) The mean plant cover in 2011 was 65.35% and increased to 95.26% in 2013. On average, 12 species were recorded in the herb layer. The average number of species did not change in grey alder tree forests.



Picture 1. Monotypic stand of *Impatiens parviflora* in the study area in 2013 (habitat type: ash tree forest)

The analysis of vegetation development of plots with influence of canopy showed significant differences of IAS abundance and number of species comparing plots without influence of canopy in the years 2011, 2012 and 2013 ($P < 0.001$). The mean cover of *I. parviflora* increased from 3.11% in 2011 to 13.82% in 2013 on plots with a canopy, and from 0% to 0.22% on plots without a canopy. The invasive species *Bunias orientalis*, *Solidago gigantea* and *Impatiens glandulifera* occur with a higher mean on plots with higher human impact. *Impatiens parviflora* were more abundant in nature-near habitats ($P < 0.001$).

DISCUSSION

Table 1 : The ecology, invasiveness, habitat occurrence and ecological effects of IAS in the forest habitat of the study area.

	<i>Ailanthus altissima</i>	<i>Acer negundo</i>	<i>Impatiens glandulifera</i>	<i>Impatiens parviflora</i>	<i>Robinia pseudoacacia</i>	<i>Solidago gigantea</i>
Family	Simaroubiaceae	Aceraceae	Balsaminaceae	Balsaminaceae	Fabaceae	Asteraceae
Life form	phanerophyte	phanerophyte	hemicryptophyte	hemicryptophyte	phanerophyte	geophyte
Area of origin	China	N America	India	Central Asia	N America	N America
tolerant to canopy		+		+	+	+
EU climate distribution	Temp/Med	Temperate	Temperate	Temperate	Temperate	Temperate

AUT Conservation assessment *	Invasive	invasive	invasive	invasive	invasive	invasive
invasiveness (riparian area) **	no impact	potentially in.	temporary in.	invasive	potentially in.	invasive
Introduction reasons	ornamental	ornamental	indirect	forestry (indirect)	ornamental, forestry	ornamental
Ecological effects						
changes in succession pattern		+			+	+
changes in species composition	+	+	+	+	+	+
changes of nutrient cycles					+	
hybridisation*						
pathway - forest road				+	+	+
pathway - river stream		+	+			
ash tree forest		+		+	+	
grey alder tree forest		+				
cutover land		+		(+)	+	+
poplar tree forest		+		+		(+)
red pine tree forest						
willow tree alluvial forest		+	(+)	+		
embankment		(+)	+			
forest edge	+	+	+	+	+	+

* = (Water et al., 2005, Essl and Rabitsch, 2002), invasiveness riparian area ** = based on research data evaluation; habitat occurrence: + = main occurrence, (+) accessory occurrence; other categories: + = appropriate

Riparian forest habitats are the fringe between aquatic and terrestrial ecosystems of rivers or streams. Riparian habitats are important habitats for important ecological functions and European plant diversity (Gregory et al., 1991; Naiman et al., 1993; Naiman and Decamps, 1997; Hood and Naiman, 2000; Richardson et al., 2007). Riparian forest habitats play an essential role in the complex of wild life ecosystems (Elmore and Beschta, 2006). Many protected areas are related to riparian ecosystems (Manzano, 2000). The management of invasive alien plant species in riparian forest habitat is gaining attention for conservation of European biodiversity.

In most riparian plant communities the plant cover, abundance and species richness of the tree and shrub layer are more developed than the herb layer. This is an enormous advantage for the distribution of invasive alien plant species. Various studies discuss the connection between life strategies and habitat properties linking to the success of IAS (Binggeli, 1996; Obidzinski and Symonides, 2000). The in this study the evaluation of the life strategies of IAS within riparian habitats concentrates on invasive species with the highest impact on native species composition in the study area. The focus lies on the combination of riparian ecosystem characteristics and

factors of invasive plant ecology with a significant importance for the biological invasion. The results were correlated with recommended and tested management and control mechanisms of previously published case studies. The short life cycle, high seed predation and rapid growth of seedlings support the invasive success of *I. parviflora*, which is one of the most invasive alien plants in European temperate forests (Vervoort and Jacquemart, 2012, Chmura and Sierka, 2007, Pyšek et al., 1998). *Impatiens parviflora* DC. (Balsaminaceae) is one of the most invasive alien plants in European temperate forests (Trepl, 1984; Pyšek et al. 1998; Chmura and Sierka 2007; Vervoort and Jacquemart, 2012). The spread of *I. parviflora* is supported by anthropogenic disturbance such as forest management work (Falinski, 1998, Vervoort and Jacquemart, 2012). The introduction of *I. parviflora* was caused by indirect human activity. Fragmentation by roads, trampling and logging are supporting factors for the distribution of IAS (Obidzinski and Symonides, 2000; Kowarik, 2008). The data of the present study supports this hypothesis. *I. parviflora* successfully competed with native species of the herb layer. The decrease of species richness within the last three study years within the study area was related to the invasion of *I. parviflora*.

The results of this and other European case studies show that species-rich forest habitats are less invaded by IAS than forests with higher human impact (Case 1990; Chmura and Sierka, 2007). Forest habitats with a higher native species density in the herb layer, or with a developed shrub layer, are not as easily invaded (Pyšek and Pyšek, 1991; Chmura and Sierka, 2007). The results of this case study confirm that natural and semi-natural forest habitats with a higher abundance of native species in the herb layer had a slower rate of invasion. Special monitoring efforts within the forest management on these pathways are necessary to diminish the invasive spread (Chmura and Sierka, 2007). Active eradication of individuals of IAS is necessary to protect natural forest habitats. Conventional conservation strategies for natural forests are not sufficient though recent data show a higher resistance of natural forest habitats (Chmura and Sierka, 2007). A successful control is only possible in the early stages of invasion.

Preventing the introduction of alien plant species (IAS) is the cheapest and most effective option for the successful management of IAS (Species Survival Commission, 2000). Preventing invasions is also the most challenging step, where ecological functions and land use changes need to be considered in the long term (D'Antonio, 2002). Successful prevention includes actions against the introduction of IAS with a scientifically documented negative impact as well as the observation of IAS with scientific uncertain long-term impact on native species. It is useful to differ between the intentional and unintentional introduction of IAS. With regards to unintentionally introduced IAS, it is necessary to recognize that the impact of IAS is often strongly related to environmental conditions. The impact of established, non-native species varies between climate regions and ecosystems; therefore, the long-term impact is often unpredictable. The intentional introduction of invasive alien species with well-detected negative effects can be legislatively permitted to raise preventive efficiency (EEA) (Species Survival Commission, 2000), but the impact of many IAS is unpredictable and difficult to generalize. The early identification on a local level depends on supporting the establishment of IAS and can save costs associated with IAS management (Species Survival Commission, 2000). An updated database of

invasive alien species is a recommendable tool to identify the species before they cause unnoticed damage (Lowe et al., 2000). To support the knowledge of IAS specifically, conservation managers on policy makers need to be informed about the supervision of risk management. International programs are helpful to identify risks of IAS early enough (Shine, 2007). The eradication of individual alien plants is highly necessary in early plant population development stages (Species Survival Commission, 2000). The eradication of a few individual invasive plants is considered to be the best response the introduction of IAS (Genovesi 2005). Specifically, vulnerable ecosystems, like riparian areas, need consistent monitoring (SpeciesSurvivalCommission 2000). Since rivers are important pathways for the introduction of IAS (Johansson, Nilsson et al. 1996; Hood and Naiman 2000; Richardson et al., 2007), frequent monitoring of riparian vegetation is useful, economical and very efficient method for early detection of biological invasions. On an international level, it is useful to identify the important pathways of IAS concerning the variation between countries according to trade and tourism routes. This supports the early detection of the unintentional introduction of IAS (Species Survival Commission, 2000). Furthermore, stakeholders with any role contributing to the movement within these pathways need to be sufficiently warned and trained (Hiebert, 1997; Species Survival Commission, 2000; Pyšek and Richardson, 2010). Successful eradication management is achieved with the sustainable recovery of the native plant diversity (Genovesi, 2005). The re-introduction of native species helps the native diversity in the long-term.

CONCLUSION

The total eradication of invasive alien species (IAS) in European riparian areas is not to be expected, because of the high number of ecological and economical barriers. The current environmental changes are supporting biological invasion. Biological invasions are negatively impacting biodiversity on a global level (Hellmann et al., 2008). The future development of the global distribution of plant species is related to land use changes and climate changes (Pressey et al., 2007; Thuiller et al., 2008). Invasive alien species have become one of the most serious challenges for global and local nature conservation efforts (Sukopp, 2001; Walter et al., 2005; McNeely, 2006). The impact of IAS has become a fundamental issue for nature conservation strategies (EEA, 2012). Every year the amount of annual budget spent on the control of IAS is increasing (D'Antonio, 2002). The issue of invasive alien species was discussed in several international conventions and nature conservancy programs (Clout & Williams, 2009). In the case of riparian forest habitats, open and dynamic hydrological structures create individual and riparian vegetation compositions. A successful conservation management process for invasive species and ecological restoration of riparian vegetation asks for participative solutions including human land-use claims and natural ecosystem services. Preventing the introduction of alien plant species is the cheapest and most effective option for the successful management. The eradication of individuals is highly necessary in early plant population development stages. An active long-term monitoring needs to concern the local adaption of life strategies of invasive and native plant communities and environmental variations.

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EFFECT OF NITROGEN ON PHYCOBILIPROTEIN CONTENT IN NITROGEN-FIXING CYANOBACTERIA ISOLATED FROM SERBIAN FOREST ECOSYSTEMS*

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SUMMARY: *Cyanobacteria can be found in different terrestrial habitats with widely fluctuating environmental factors. They possess phycobiliproteins, a family of highly soluble pigments, which represent biotechnologically valuable compounds. Cyanobacteria can regulate their pigment content and composition in response to different environmental factors. In this study, the effect of nitrogen on the phycobiliprotein content in six terrestrial filamentous N₂-fixing cyanobacterial strains belonging to the Anabaena and Nostoc genera has been investigated. All the tested strains have been isolated from the forest ecosystems of two Serbian mountains, Stara Planina and Morović. The mineral, nutrient medium BG-11 has been used for the growth of the cyanobacterial strains. The concentration of three phycobiliproteins (phycocyanin, allophycocyanine and phycoerythrin) has been quantified spectrophotometrically during different growth phases. At the beginning of the exponential phase of growth (the 14th day), the concentrations of the phycobiliproteins were higher in the most of the tested cyanobacterial strains (Nostoc SP₂, Anabaena SP₂, Nostoc M₁ and Anabaena M₂) grown in the presence of nitrogen (the concentration of 1.5 g/L) compared to the same strains grown in the absence of nitrogen, suggesting that the presence of this element in the medium could increase the pigment production. The detected concentrations of all three phycobiliproteins were higher in the nitrogen free medium in the case of two strains, Anabaena M₁ and Nostoc M₂. Pigment contents were also affected by the growth phase of strains. On the 21th day of cultivation, in the three tested strains (Anabaena SP₂, Nostoc M₁ and Anabaena M₂), phycobiliproteins concentration were higher in the presence of nitrogen whereas in the other three cyanobacterial strains (Nostoc SP₂, Anabaena M₁ and Nostoc M₂), the concentrations were higher in the nitrogen free medium. During the stationary phase of growth (the 28th - 50th day of cultivation), the concentrations of APC, PC and PE of each strain separately were not correlated with respect to the medium probably as a result of nitrogen consumption. In the present study, PE*

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content was the highest on the fourteenth day of cultivation (BG+N) in the cyanobacterial strain *Nostoc* SP₂ with the recorded value of 0.0428 mg/ml. The highest concentration of APC (0.0164 mg/ml) was observed in the cyanobacterial strain *Nostoc* M₁ on the thirty-fifth day of cultivation in the absence of nitrogen in the medium. The highest PC content (0.0762 mg/ml) was found in the cyanobacterial strains *Anabaena* SP₂ (the fiftieth day) cultivated in the medium with nitrogen. These results indicate that the phycobiliprotein content is a strain-specific property, which is strongly dependent on the growth phase of the tested cyanobacteria. Also, the results obtained in this study suggest that the nitrogen availability affects the composition and content of phycobiliproteins in all tested strains. Based on the presented results, tested terrestrial cyanobacterial strains, especially strains of the *Nostoc* genus, can be a concern as a potential natural source of phycobiliproteins being biotechnologically valuable compounds.

Key words: forest ecosystems, cyanobacteria, nitrogen, phycobiliproteins

INTRODUCTION

Cyanobacteria represent photosynthetic prokaryotes which are very abundant in terrestrial as well as aquatic habitats contributing significantly to global carbon fixation (Schwarz et al., 2006). The cyanobacteria have developed unique physiological and morphological features as a respond to different environmental changes, which allow them collectively to colonize nearly all ecosystems (Tandeau de Marsac and Houmard, 1993; Tandeau de Marsac et al., 1996). Their simple nutritional requirements (such as light, water, carbon dioxide, and inorganic salts) enable these organisms to occupy highly diverse ecological niches (Madamwar et al., 2012). As photosynthetic organisms, cyanobacteria are able to tune light-harvesting and metabolic capacities for optimization of growth, due to the fact that the number and composition of their pigments are sensitive to environmental changes, in particular to light and nutrient availability (Tandeau de Marsac and Houmard, 1993; Sauer et al., 2001; Grossman et al., 2001; Houmard et al., 2004). Phycobiliproteins represent the major light harvesting supramolecular complex structure (Grossman et al., 1993) situated on the outer surface of thylakoid membrane in the form of phycobilisomes (PBSs) (Madamwar et al., 2012). The phycobiliproteins form tightly bound heterodimers that belong to one of four groups: allophycocyanin (APC, λ_{max} ~650 nm), phycocyanin (PC, λ_{max} ~620 nm), phycoerythrin (PE, λ_{max} ~545-565 nm) and phycoerythrocyanin (PEC, λ_{max} ~575 nm) (Tamary et al., 2012). Blue pigments, phycocyanin and allophycocyanin are present in all cyanobacteria, while, phycoerythrin (red pigment) is widely spread but it is not found in all cyanobacteria as well as phycoerythrocyanin which is usually found in filamentous species (Hoffmann et al., 1990). Phycobiliproteins are widely used in different branches of industry (cosmetic, pharmaceutical, food, etc.) and they are gaining more importance as natural colorants over synthetic ones, as they are non-carcinogenic and they are not toxic (Spolaore et al., 2006).

The objective of the study was to analyze the influence of nitrogen on phycobiliprotein production in six nitrogen-fixing filamentous cyanobacteria isolated from two mountains in Serbia. Also, the aim of this study was establishing the differences in the content of phycobiliprotein between tested cyanobacteria as

potential producers of phycobiliprotein pigments during different phases of their growth.

MATERIAL AND METHOD

In this study, the effect of nitrogen on the phycobiliprotein content in six terrestrial filamentous N₂-fixing cyanobacterial strains belonging to the *Anabaena* and *Nostoc* genera was investigated. All the tested strains were isolated from the forest ecosystems of two Serbian mountains, Stara Planina and Fruška gora (area Morović). The mineral, nutrient medium BG-11 with nitrogen (the concentration of 1.5 g/L) and without nitrogen (Rippka et al., 1979), were used for the growth of the cyanobacterial strains. The cultures were incubated photoautotrophically at 22-24°C under illumination of cool white fluorescent light (50 μmol m⁻²s⁻¹). Used mode of dark and light period was 12 hours light and 12 hours of darkness. The concentration of three phycobiliproteins (phycocyanin, allophycocyanin and phycoerythrin) was quantified spectrophotometrically on the 14th day, 21th day, 28th day, 35th day, 42th day and 50th day of cultivation.

Phycobiliproteins contents were determined using spectrophotometric method described by Bennett and Bogorod (1973). Cyanobacterial strains were grown in Erlen-Meyer vessels where the inoculum of 2 ml was streaked in the 200 ml of medium. After 14 days of incubation, 10 ml of samples (cultures) were filtrated using vacuum pump and stored on the 4° C over night. The qualitative extraction of biliproteins was achieved by suspension in 5ml of 1M Tris-HCl buffer (pH 8.1). Further destruction of the cell wall and releasing of intracellular content was performed by sonication of samples (10 minutes with cycles of 30 seconds). The cell debris was removed by centrifugation at 5000 rpm for 15 minutes at a room temperature. After centrifugation, all supernatants of tested cyanobacteria where separated. The concentration of phycocyanin (PC), allophycocyanin (APC) and phycoerythrin (PE) was calculated spectrophotometrically at wavelengths of 562 nm for PE, 615 nm for PC and 652 nm for APC. Absorbance measurement was performed on the spectrophotometer "NICOLET Evolution 100" (Thermo Electron Corporation). Tris-HCl buffer was used as a blank. The following equations were used for estimation of pigment concentration which was expressed in mg/ml:

$$\text{PE [mg/ml]} = (A_{562} - 2.41 \cdot \text{PC} - 0.849 \cdot \text{APC}) / 9.62$$

$$\text{PC [mg/ml]} = (A_{615} - 0.474 \cdot A_{652}) / 5.34$$

$$\text{APC [mg/ml]} = (A_{652} - 0.208 \cdot A_{615}) / 5.09$$

RESULTS

At the beginning of the exponential phase of growth (the 14th day), in the most of the tested cyanobacterial strains (*Nostoc* SP₂, *Anabaena* SP₂, *Nostoc* M₁ and *Anabaena* M₂) the concentrations of the phycobiliproteins were higher in the medium with nitrogen compared to the medium without nitrogen. Only in the case of two strains, *Anabaena* M₁ and *Nostoc* M₂, the detected concentrations of all three phycobiliproteins were higher in the nitrogen free medium. The highest amount of phycobiliproteins was recorded in the two strains belonging to the genus *Nostoc*. In *Nostoc* strain M₂ grown in the nitrogen free medium the contents of APC (0.0063 mg/ml) and PC (0.0076 mg/ml) were the highest. However, the concentration of PE

(0.0428 mg/ml) was the highest in *Nostoc* strain SP₂ grown in the medium with nitrogen. Also, the obtained value for PE represents the highest recorded value in the present study (Figure 1). During the exponential phase of growth, the 21th day of cultivation, in the three tested strains (*Anabaena* SP₂, *Nostoc* M₁ and *Anabaena* M₂), the concentrations of APC, PC and PE were higher in the presence of nitrogen. However, in the three other cyanobacterial strains (*Nostoc* SP₂, *Anabaena* M₁ and *Nostoc* M₂), the concentrations of phycobiliproteins were higher in the medium without nitrogen. Also in this phase of growth, the production of APC and PC was increased in all tested strains compared to the 14th day (Figure 2). *Nostoc* strain SP₂ growing in the absence of nitrogen contained the highest PE amount (0.0053 mg/ml) among all tested strains, while the highest concentration of PC (0.0409 mg/ml) and APC (0.0139 mg/ml) was observed in *Anabaena* strain M₂ grown in the medium with nitrogen (Figure 2). On the 28th day of cultivation, in the most of the tested strains (*Nostoc* SP₂, *Anabaena* M₁, *Nostoc* M₁, *Nostoc* M₂) the contents of PC and APC were higher in the nitrogen-free medium except in the two strains (*Anabaena* SP₂ and *Anabaena* M₂). On the other hand, in most of the tested strains (five of six) the concentration of PE varied with respect to concentrations of APC and PC and medium. In this phase of growth, the production of two phycobiliproteins (APC and PC) was increased in all tested strains. The highest amount of phycobiliproteins was observed in two strains (*Nostoc* M₂, *Anabaena* SP₂) grown without NaNO₃ as a nitrogen source (Figure 3). On the 35th day of cultivation, the production of phycobiliproteins was lower in relation to the 28th day. The influence of nitrogen on the production of APC, PC, PE was observed only in the case of two strains. In strain *Anabaena* M₁ phycobiliproteins content was higher in medium with nitrogen. In the case of strains *Nostoc* M₁, phycobiliproteins content was higher in the medium without nitrogen. In the majority of tested cyanobacterial strains, the concentrations of phycobiliproteins were different with respect to the medium. Two strains belonging to the *Nostoc* genus grown in the absence of nitrogen had the highest content of phycobiliproteins. Concentrations of APC (0.0164 mg/ml) as well as PE (0.0045 mg/ml) were the highest in strain *Nostoc* M₁. The highest amount of PC (0.0160 mg/ml) was observed in strain *Nostoc* M₂ (Figure 4). During the later periods of growth, the production of phycobiliproteins was decreased compared to the exponential phase of growth. On the 42th day of cultivation, the production of PC was significantly higher compared to the APC and PE content in all tested strains (Figure 5). The highest recorded values for all three phycobiliproteins were recorded in the case of strain *Nostoc* M₂. Also, on the fiftieth day, in five of six strains grown in the nitrogen free medium the content of PC was higher compared to the APC, PE concentration. Only in the case of cyanobacterial strains *Anabaena* SP₂ cultivated in the medium with nitrogen, the concentration of PE was higher. Also, the obtained value for PC represents the highest recorded value in the present study (0.0762 mg/ml) (Figure 6).

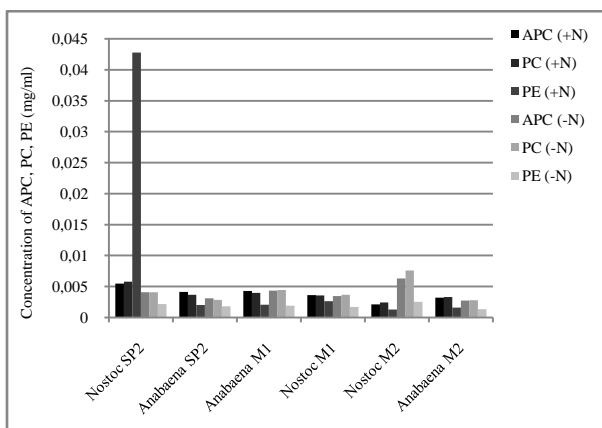


Fig. 1 Content of phycobiliproteins in tested cyanobacteria strains during 14th day of cultivation

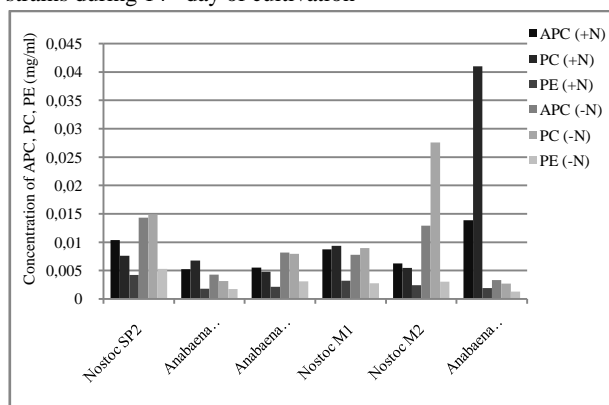


Fig. 2 Content of phycobiliproteins in tested cyanobacteria strains during 21th day of cultivation

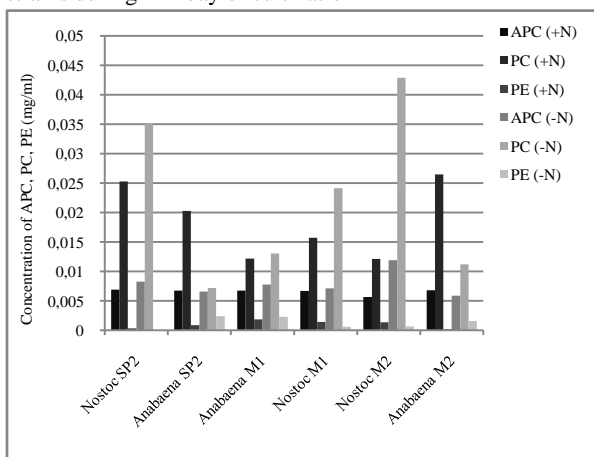


Fig. 3 Content of phycobiliproteins in tested cyanobacteria strains during 28th day of cultivation

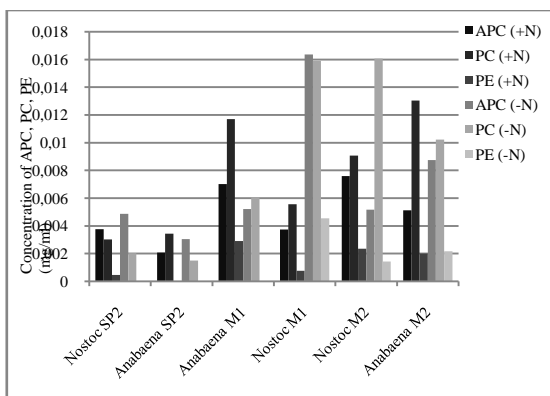


Fig. 4 Content of phycobiliproteins in tested cyanobacteria strains during 35th day of cultivation

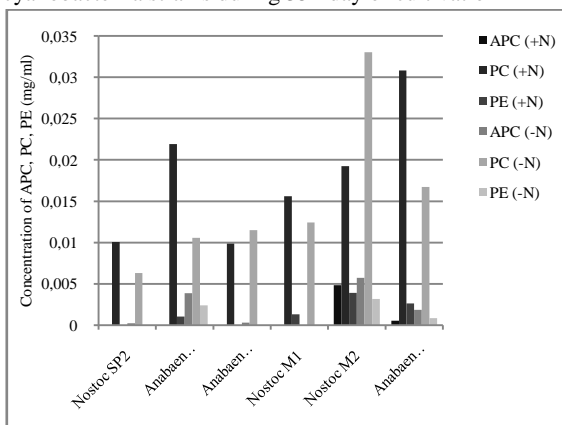


Fig. 5 Content of phycobiliproteins in tested cyanobacteria strains during 42th day of cultivation

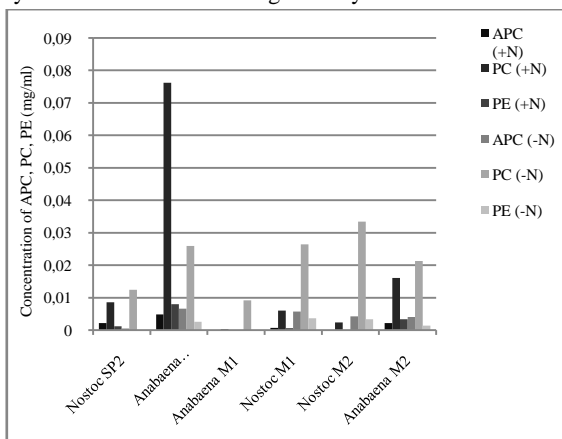


Fig. 6 Content of phycobiliproteins in tested cyanobacteria strains during 50th day of cultivation

DISCUSSION

In the present study, changes in phycobiliprotein content at different points in the growth cycle were detected in all tested filamentous nitrogen-fixing strains. These results indicate that the phycobiliprotein content is a strain –specific property, which is strongly dependent on the growth phase of tested cyanobacterial strains. Simeunović et al. (2012) showed that qualitative and quantitative content of total and individual phycobilin pigments were different in 10 filamentous cyanobacteria indicating the existence of specific features in the pigment composition of every tested strain. On the basis of the obtained results, most of the *Nostoc* strains showed higher content of phycobiliproteins compared to *Anabaena* strains during different phases of growth. Simeunović et al. (2013) pointed out that although these two genera are phylogenetically very close (Schirmeister et al., 2011), phycobiliprotein distribution in all tested strains of *Nostoc* and *Anabaena* genera showed the presence of phycobilins in different proportion.

Differences in the pigment concentration and composition of phycobiliproteins were also noticed in the medium with and without nitrogen suggesting that nitrogen availability affects the composition and content of phycobiliproteins in all tested strains. In the majority of tested strains, at the beginning of cultivation period, the phycobiliprotein production was increased in the medium with nitrogen. These results indicate that the presence of nitrogen in the medium could increase the pigment production. Based on the presented results, the highest recorded values obtained for phycoerythrin (PE) as well phycocyanin (PC) were obtained in the medium with nitrogen in cyanobacterial strains *Nostoc* SP₂ and *Anabaena* SP₂. These results are in agreement with the results obtained by Simeunović et al. (2012) who showed that the content of phycoerythrin was the highest in the two strains (*Nostoc* strain 2C₁ and *Anabaena* strain LC₁B) grown in the medium with NaNO₃ as a nitrogen source. Soltani et al. (2007) also showed that cyanobacteria *Fischerella* produced more phycobiliprotein under nitrate grown cells than nitrogen free media and ammonium grown cells. However, during the later phase of growth (the 28th-50th day of cultivation), the concentration of each of the phycobiliproteins were different with respect to the medium, probably as a result of nitrogen consumption. As heterocystous, N₂-fixing cyanobacteria, *Anabaena* and *Nostoc* strains could escape nitrogen deficiency during growth in the nitrogen-free medium, using molecular dinitrogen as a source (Simeunović et al., 2013). During stationary phase of growth, N₂-fixing condition could cause increase in concentrations of blue pigment (PC and APC) in most of the tested strains. Simeunović et al. (2013) demonstrated that PC and APC contents changed markedly depending on the nitrogen source, while PE content did not differ significantly. In the same study, N₂-fixing conditions caused increases in PC and APC concentrations in most tested strains (Simeunović et al., 2013). Also, in the present study the concentration of allophycocyanin (APC) (0.0164 mg/ml) was the highest in the cyanobacterial strain *Nostoc* M₁ (35th day) cultivated in the medium without nitrogen. The obtained results are in accordance with the results of other studies where heterocystous, N₂-fixing cyanobacterial strains showed higher phycobiliprotein content in the absence of nitrogen. Hifney et al. (2012) found that phycobiliprotein fractions (phycocyanin, allophycocyanin and phycoerythrin) were elevated in nitrogen deficiency as well as phosphorus and sulphur deficiency, whereby the presence of HCl (0.3 and 0.6M) also increased the production of

phycobiliproteins. In the study Hemlata and Fatma (2009), *Anabaena* NCCU-9 produced the highest amount of phycobiliprotein under nitrogen free environment. Similar results were obtained by Loreto et al. (2003), who showed that the strain *Anabaena* 7120 cultivated in the nitrogen-free medium exceeded the amount of phycobiliprotein compared to the nitrate grown cultures. Tandeau de Marsac et al. (1996) also noticed that the nitrogen source specifically alters the phycobiliprotein content in the cyanobacterial strain *Calothrix* PCC 7601. As the major component of the phycobilisome during starvation, the level of phycocyanin (PC) reflects the balance between the synthesis and degradation rates of this antenna pigment system (Schwarz et al., 2006).

Besides nutrient availability, light intensity, light wavelength, temperature (Prassana et al., 2004) as well as the presence of toxic compounds, pH, and their combination have great influence on phycobiliprotein production (Madamwar et al., 2012). In response to different environmental signals, cyanobacteria can regulate their tetrapyrrole content and composition (Prasana et al., 2004). Tamary et al. (2012) showed that disassembly of the PBS antenna under high-light stress may result in their degradation, possibly similarly to their degradation during nitrogen/phosphate starvation (Grossman et al., 1993). Degradation of phycobiliproteins can supply amino acids for the synthesis of essential proteins under nutrient-limited conditions as they constitute a substantial fraction of the total soluble proteins (Ughyt and Ajlani, 2004). It is also important to mention that all examined cyanobacterial strains which were isolated from different forest ecosystems were grown in constant and usual conditions of cultivation. Due to the fact that in their natural habitats they are often exposed to changes of the environmental factors, contribution of various parameters on phycobiliprotein production is of great importance for further analysis, as they could have great impact on their production.

CONCLUSION

The results of the present study showed that some autochthonous terrestrial, filamentous N₂-fixing cyanobacterial strains isolated from the forest ecosystems of two Serbian mountains, Stara Planina and Fruška gora, had good potential for phycobiliprotein production. The obtained results provided evidence that every examined strain showed different phycobiliprotein content which depends on the nitrogen source and growth phase. The highest amount of all three phycobiliprotein was detected in cyanobacterial strains *Nostoc* M₁, *Anabaena* SP₂ and *Nostoc* SP₂. Thus, the tested terrestrial cyanobacterial strains, especially the strains of *Nostoc* genus, can be a concern as a potential natural source of phycobiliproteins being biotechnologically valuable components. Also, it is possible that the phycobiliprotein content would be increased if other factors were used, which requires an additional research.

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FOREST ECOSYSTEMS IN THE SERBIAN PART OF THE PANNONIAN BASIN – THEIR ROLE IN ENVIRONMENTAL PROTECTION (A REVIEW)

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SUMMARY: *The Vojvodina Province (Northern part of Serbia) is mostly a plain region with the agriculture as a dominant activity. The forests cover about 140.000 hectares and contribute with 6.5% to the total area. Nevertheless, importance of forests in soil protection, carbon sequestration, and the development of vast number of activities that are significant to the population and rural development in the Serbian part of the Pannonian Basin (Vojvodina province) is enormous. The main challenge is to harmonize the interests of agriculture, forestry and environment protection at the same area and achieve targeted social and economic development.*

Key words: *forestry, agroforestry, agriculture, environment*

INTRODUCTION

The Vojvodina Province (Northern part of Serbia) is mostly a plain region with the agriculture as a dominant activity. The forests cover about 140.000 hectares and contribute with 6.5% to the total area (Spatial plan, 2011). The forests are situated along the lowland rivers (Danube, Sava, Tisa, Tamiš) and cover Fruska Gora and Vrsac mountain. According to the watershed regulation lowland forests along the rivers are divided into floodplain forests and protective areas. In both, the most common forest species are poplars (*Populus x euramericana*) and willows (*Salix alba*), growing as plantations. In natural mixed forests the dominant species are pedunculate oak (*Quercus robur*), usually mixed with leaved ash (*Fraxinus angustifolia*) and hornbeam (*Carpinus betulus*). On the Fruska Gora mountain the dominant species are linden (*Tilia sp.*), Sessile oak (*Quercus petraeae*) and beech (*Fagus sylvatica*). In other parts of Vojvodina the most abundant forest species is Black locust (*Robinia pseudoacacia*). The Spatial Plans of the Republic of Serbia and Vojvodina Province aim to increase the forest cover up to 14.2 %, which means that another 150,000 ha of new forests should be established, mainly on the agricultural

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land. This new forests, consisting of different types of multifunctional plantations and agroforestry systems, should protect farmlands from wind, improve bee keeping, serve for the phytoremediation of contaminated industrial and agricultural land, preserve and promote the diversity and provide biomass for energy production. Increase of the forest area and agroforestry systems should be in favor of wood production for industrial development, tourism, sport and recreation, diversification of income of the rural population as well as contribute to the energy stability of the farms.

The aim of the paper is to describe how enlargement of forestry and agro-forestry areas in Vojvodina can contribute climate change mitigation, environmental sustainability and improvement of rural landscapes.

SOILS IN VOJVODINA

Chernozem, as the dominant soil type, occupies 938,881 ha (45% of total area). This soil type is highly productive agricultural soil and it is subject to, and endangered by, different destructive processes, primarily wind erosion, i.e. climatic extremes.

Hydromorphic soils cover 572,709 ha. In the past, deforestation occurred mostly on humogley 348,846 ha. This soil type is now used by agriculture. The land use change was not the best solution for this soil type because the suitability for agricultural production depends climatic conditions i.e precipitation.

Another potential soil type for afforestation is solonetz. The selection of tree species for the afforestation of halomorphic soils in Vojvodina is a special problem, considering the very low productivity as the consequence of a series of unfavourable soil properties (Galic, 2008; Ivanisevic et al., 2008). Low productivity determines the potential, level and character of this soil type utilisation in forestry.

WIND EROSION AND SOIL DEGRADATION

Major challenges in the 21st century at the global scale include the severe degradation of agricultural soils (Sartori et al., 2007). Sustainable ecological solutions are required to restore degraded lands and to prevent further land degradation, but soil erosion is an increasing environmental problem. One third of the planet Earth is affected by wind erosion, transport and deposition of material (Chen and Fryrear 1996). Soil loss caused by wind erosion presents a problem on cultivated lands and pastures in Europe (Goossens et al., 2001), Africa (Biielders et al. 2000), Asia (Zhibao et al. 2000), Australia (Gillieson et al. 1996), and North America (Buschiazzo et al. 1999). The main agent of soil erosion in Vojvodina is wind. The intensity of wind erosion in Vojvodina is shown in Figure 1.

The soil with the highest wind erosion rates in Vojvodina are the soils with the highest yields, the chernozem (Sapundzic 1978; Letic et al. 1984; Vlatkovic 2001; Galic 2004; Orlovic 2004). This region is the most unafforested region in Vojvodina too.

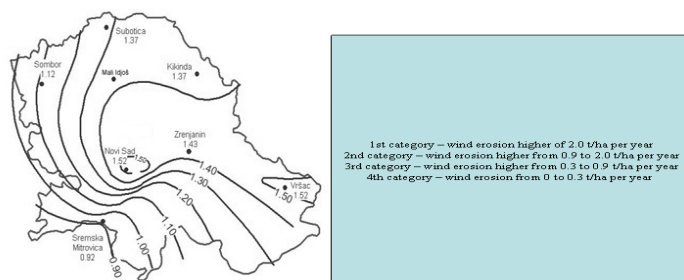


Figure 1 Intensity of wind erosion in Vojvodina

ENVIRONMENTAL HAZARDS DUE TO INTENSIVE AGRICULTURE

Soil, surface water and groundwater may become contaminated with hazardous compounds as a consequence result of either natural or human activities from different traces with both inorganic and organic compounds (heavy metals, radionuclide, nitrate, phosphate, inorganic acids and organic chemicals) from sources including waste materials, explosives, pesticides, fertilizers, pharmaceuticals, acidic deposition and radioactive fallout (Arthur et al. 2005). Among the various numbers of contaminants present in the environment, nitrates play significant role in ground and surface water contamination. Clean and safe drinking water is justifiably considered to be basic human right, and water quality will undoubtedly be one of the most important ecopolitical issues in the coming decades (Landis et al., 1992). According to Newbould (1989), if nitrates present in drinking water are converted to nitrites they can cause methemoglobinemia in infants, while excess nitrate in surface waters, together with phosphorus, can cause eutrophication. This contamination is mostly caused by agricultural practices, where fertilization with the increased application of mineral fertilizers plays a significant role in increase of nitrate concentrations both in plant tissues and in the environment (Kastori and Petrović 2003). Also, excess of nitrates in the soil promotes their denitrification and emission of nitrous oxides, such as N_2O , thus causing even up to 300 times more harmful effects than CO_2 (Schepers et al. 2005). Other contaminants in agricultural soils are heavy metals and pesticides. Amongst heavy metals, cadmium (Cd) is considered as hazardous heavy metal with high toxicity to plants and animals. Primary sources of Cd in agriculture are phosphate fertilizers, while rock mineralization presents other source of Cd in soil (Kastori et al. 2001). The entry of herbicides into surface and subsurface waters through interaction of hydrology and agricultural practices may be the largest factor degrading water quality (Rice et al. 1997). Most important persistent herbicides are atrazine and bentazone due to the high toxicity of these compounds and their residues (U.S. Environmental Protection Agency 2007).

THE MAIN FOREST SPECIES AND FOREST TYPES

The forests are situated along the lowland rivers (Danube, Sava, Tisa, Tamis) and cover Fruska Gora and Vrsac mountains. According to the watershed regulation lowland forests along the rivers are divided into floodplain forests and protective areas. In both, the most common forest species are poplars (*Populus x euramericana*) and willows (*Salix alba*), growing as plantations. In natural mixed forests the dominant species are pedunculate oak (*Quercus robur*), usually mixed with leaved ash (*Fraxinus angustifolia*) and hornbeam (*Carpinus betulus*). On the Fruska Gora mountain the dominant species are linden (*Tilia sp.*), Sessile oak (*Quercus petraeae*) and beech (*Fagus sylvatica*). In other parts of Vojvodina the mostly distributed forest species is Black locust (*Robinia pseudoacacia*).

Most important association in lowland forests are *Salicion albe* and *Alno – Quercion*. In alliance *Salicion albe* occurs species *Salix alba*, *Populus nigra*, *Populus alba*, *Ulmus effusa*, *Ulmus minor*, *Fraxinus angustifolia*, *Frangula alnus*, *Viburnum opulus*, *Cornus sanguinea*, *Crataegus sp.*, *Rubus caesius*. In alliance *Alno - Quercion* the most important species are: *Quercus robur*, *Fraxinus angustifolia*, *Ulmus effusa*, *Ulmus minor*, *Alnus glutinosa*, *Carpinus betulus*, *Acer campestre*, *Acer tataricum*, *Populus alba*, *Viburnum opulus*, *Genista eleata*, *Crataegus oxycantha*, *Crataegus monogyna*.

Association *Aceri tatarici-Quercion* the most continental alliance distributed in the Pannonian plain. This habitat type encompasses remnants of isolated woods of *Quercus sp.* These communities develop on typical *chernozems*. The most important associations are: *Festuco pseudovinae – Quercetum roboris*; *Convallario – Quercetum roboris*; *Violo – Quercetum roboris*; *Pruno mahaleb – Quercetum roboris* with species: *Quercus robur*, *Quercus petraea*, *Quercus cerris*, kao i *Pyrus piraster*, *Acer tataricum*, *Crataegus sp.*, *Rosa sp.*, *Sorbus torminalis*, *Euonymus sp.*, *Prunus spinosa*.

STRATEGIC DOCUMENTS RELATED TO AFFORESTATION

The main strategic documents related to forestry and agroforestry in Serbia are: Law of forests, Law of agricultural land, Spatial Plan.

Forest Law

This Law is adopted in 2010. The most important issue is that Fund for forests was established. It is possible to use financial sources from Fund for afforestation of private land.

Law of agriculture land

Law of agriculture land is adopted in 2009. Some Articles in this Law are important for forestry. The Article which approves an afforestation all classes of agriculture land is extremely important for increasing forest area in Vojvodina due to the fact that the forest land is lacking. It practically means that it is possible to increase forest area on agricultural land in the area where it is necessary.

Spatial Plan

The Spatial Plan is adopted in 2011. In that Plan an increase of forest area from 6.5 to 14.2% was planned. It means that new 140.000 hectares of the forests has to be established in Vojvodina for the 10 years.

THE POSSIBILITIES FOR INCREASING FOREST AREA OR AGRO-FOREST AREA

Potentially, the most common soil type for afforestation could be a fluvisol and humogley. Large area of this soil types are now used by agriculture and water management. In the alluvial plain of the Danube it is possible to determine the area of fluvisol which is linked to monostorski, Apatinski and Koviljski rit. The alluvial plain of the Tisza situation is slightly more complicated since the river is much more controlled and thus reduced the flow through Serbia.

Another potential soil type for afforestation is solonetz. The selection of tree species for the afforestation of halomorphic soils in Vojvodina is a special problem, considering the very low productivity as the consequence of a series of unfavourable soil properties (Galić 2008; Ivanišević et al. 2008; Galic et al. 2009). Low productivity determines the potential, level and character of this soil type utilisation in forestry. The selection of tree species for the afforestation of halomorphic soils in Vojvodina is a special problem, considering the very low productivity, as the consequence of a series of unfavourable properties of this site type.

Agroforestry plantations are divided to more than 50 different types (Wojtkovski, 1998) but most significant for Vojvodina province from productional point could be: (I) alley crops which presents agroforestry practice intended to place trees within agricultural cropland systems and combines timber production with inter rowed crops such as wheat or maize; (II) Silvopasture with combination of pasture with trees organized as small groups or wider spaced grid; (III) biomass plantations of fast growing woody crops with short rotation periods (SRC) and (IV) beekeeping plantations with abundant flowering tree species. Besides production purpose, some agroforestry plantations have significant role in environmental improvement and protection.

Windbreak shelterbelts present single or multiple rows of trees, or a strip of retained natural vegetation (Brouwer and Dutton 1992). Besides the wind protection and mitigation of eolic erosion, shelterbelts also serve as wildlife habitat and food source. The height of shelterbelts determines what area of land is to be protected where the area of land protected by the shelterbelt is approximately 20 times the height of the tallest trees in the shelterbelt (Breckwoldt, 1983).

According to USDA (1997), riparian buffers are aquatic ecosystem and the portions of the adjacent terrestrial ecosystem that directly affect or are affected by the aquatic environment. This includes streams, rivers, lakes, and bays and their adjacent side channels, floodplain, and wetlands. In specific cases, the riparian buffer may also include a portion of the hill slope that directly serves as streamside habitats for wildlife. Lowrance et al. (1985) defines riparian buffers as "A complex assemblage of plants and other organisms in an environment adjacent to water. Without definitive boundaries, it may include stream banks, floodplain, and wetlands, as well as sub-irrigated sites forming a transitional zone between upland and aquatic habitat. Mainly linear in shape and extent, they are characterized by laterally flowing water that rises

and falls at least once within a growing season.” Riparian buffers have been shown to be effective in controlling nonpoint source pollution and according to Licht and Schnoor (1993), buffer strip of poplars can decrease nitrate concentration in groundwater from 150 to 3 mgNO₃/l H₂O.

POSSIBILITIES FOR INCREASING FOREST AREA ACCORDING TO THE CLIMATE CHANGE SCENARIOS

According to the IPCC SRES A2 CO₂ emission scenario and regional climate change projections, temperature in some parts of Serbia will rise for about 3.8 degrees Celsius before the end of 21st century (Figure 2). Existing forests, as well as those which are planted today, will be exposed to different climate conditions than they are now.

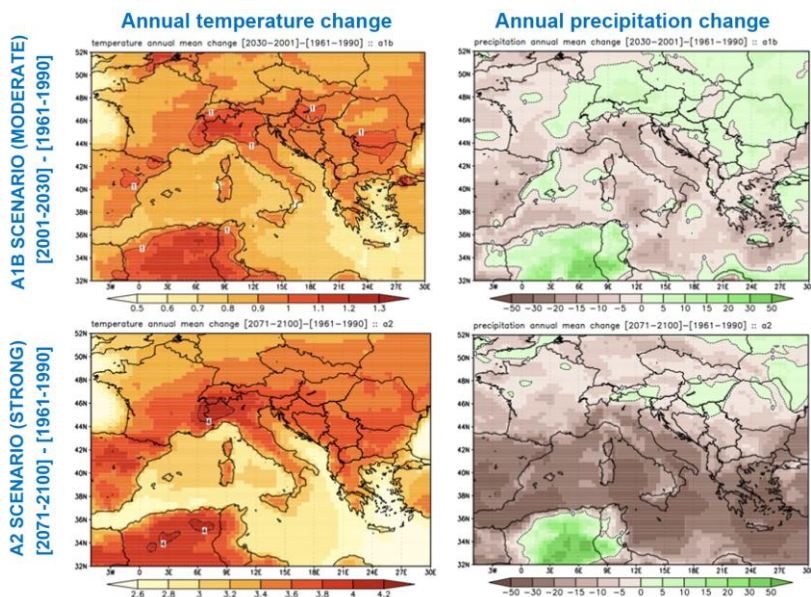


Figure 2 Increase of mean annual temperatures and change of precipitation for Europe and Serbia for the periods 2001-2030 and 2071-2100 in comparison to the period 1961-1990 according to A1B and A2 scenarios (<http://www.seevccc.rs>)

For consideration of the potential suitability of habitats in Vojvodina for afforestation the projections obtained in the FORESTMOD project can be used. FORESTMOD is supported by the European Joint Research Centre (EC Joint Research Centre). Methods used in that project were established by Casaletto et al. (2010), Guisan et al. (2005) and Austin (2007). Bioclimatic data consisted of 47 different maps resolution of 1 km were used (two soil maps, six morphological parameters obtained from SRTM digital elevation model and 39 bioclimatic variables obtained from the WORLDCLIM meteorological database). We shortly reviewed the potential of pedunculate oak for future afforestation.

In the Figure 3 presented were suitable habitats for pedunculate oak in the 20th century. Figure 4, 5 and 6 represent potential habitats for oak under A2 emission

scenario in 2020, 2050 and 2080. Maps are available from the European Forest Data Centre (EFDAC).

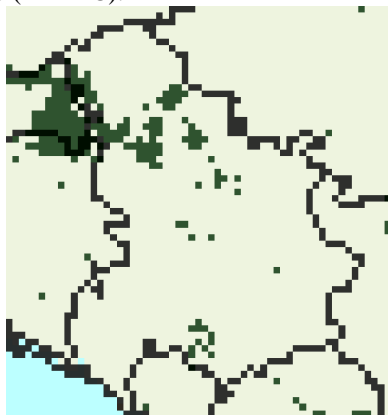


Figure 2. Suitability of habitats in Serbia for pedunculate oak in 20th century

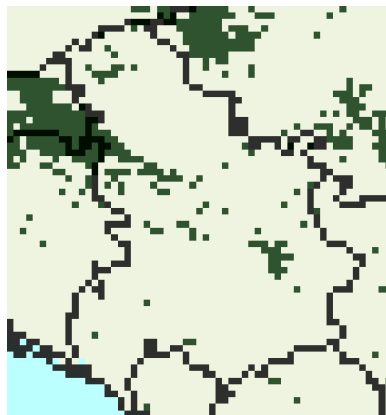


Figure 3. Suitability of habitats in Serbia for pedunculate oak in 2020 according to A2 scenario and CCMA model



Figure 4. Suitability of habitats in Serbia for pedunculate oak in 2050 according to A2 scenario and CCMA model



Figure 5. Suitability of habitats in Serbia for pedunculate oak in 2080 according to A2 scenario and CCMA model

Decrease of potentially suitable oak habitats is obvious. These results suggest that most of the current habitats in Vojvodina probably won't be suitable for pedunculate oak by the end of 20th century. This fact imposes the necessity for selection of tree species which are less prone to drought (e.g. Black locust).

APPROPRIATE TREE SPECIES FOR AFFORESTATION AN AGROFORESTRY SYSTEMS

Although most of area of Vojvodina province is the best for establishment and growing of poplar plantations, it should not be neglected the possibility of planting other tree species. According to Marković and Tatalović (1995), the area of Vojvodina

province is suitable for a wide range of woody species and the new forests should be established of various forest trees and shrubs. Among the others, special attention should be paid on valuable broadleaved tree species. Ducci et al. (2010) suggest that noble hardwoods are best planted where soil conditions are not suitable for poplars, generally far from rivers.

Scattered broadleaved tree species such as ashes (*Fraxinus excelsior* L. and *Fraxinus angustifolia* Vahl.), black alder (*Alnus glutinosa* (L.) Gaertn.), limes (*Tilia cordata* Mill. and *Tilia platyphyllos* Scop.), maples (*Acer platanoides* L. and *Acer pseudoplatanus* L.), wild service tree (*Sorbus domestica* L. and *Sorbus torminalis* L. Crantz), walnuts (*Juglans regia* L. and *Juglans nigra* L.), wild apple (*Malus sylvestris*(L.) Mill), wild pear (*Pyrus pyraster*(L.) Burgsd.) and wild cherry (*Prunus avium* L.) could be important components of new forests in Vojvodina province. As they often produce high value timber, these species are now increasingly planted in Europe, both in afforestation of abandoned farm land and enrichment of forests. Hemery et al. (2010) stated that increasing of area under these species would provide multiple benefits, as the world timber trade trends indicate a continuing decrease in hardwood roundwood exports from tropical forests and increasing consumption of timber in industrializing countries. Also, new forests and shelterbelts, established of noble hardwoods, will provide numerous benefits to ecosystem and society, such as increasing of biodiversity, improvement of microclimate conditions, reduction of upwind velocity, shelter for wildlife, etc.

Of all the noble hardwood species, wild cherry has been the most treated species in Serbia. Wild cherry breeding program in Serbia have started in 1999, when the first seed orchard, composed of half-sib families of 35 „plus trees“, were established (Orlović et al., 2006). Further, in 2005, inventory of wild cherry plus trees were conducted according to natural distribution of the species and information about seed sources on the entire territory of Serbia. On that occasion, totally 58 genotypes, from five localities were described. Geographical coordinates of plus trees were determined and stored in the database together with information about stem straightness, branching, diameter, height and vitality of trees (Pilipović et al. 2011). Similar activities were continued in the following years, as well. During 2011, seeds of 61 genotypes from 12 localities in Serbia were collected and sown in seedbeds for the purposes of establishing of progeny test. After two years of testing in nursery conditions, the genotypes which satisfied breeding goals (high CO₂ accumulation, fast growth, straight stem, low susceptibility to leaf diseases, etc.) were moved on the field. Of these seedlings, gene collection was established in 2014 and amount 730 genotypes, from 44 families. The main purpose of this object is further testing of genotypes, as well as production of seed for commercial forestry.

However, except of selection of genotypes with desirable genetic characteristics, important issues related to growing of noble hardwoods are silvicultural treatments aimed in getting timber with a high value, used for the veneer and furniture industries. There is a big price difference between low quality veneer, which amounts to a price of less than 10 Euro per m³ and high quality veneer, which amount to a price of between 200 to over 1,000 Euro per m³ (Oosterbaan et al., 2008).

CONCLUSIONS

The Vojvodina Province is typical agricultural area with small forest area. In order to increase production in agriculture and to provide conditions for development other activities, such as beekeeping, hunting, tourism, recreation, the afforestation is necessary on the area of 140.000 hectares. Afforestation and establishment of agroforestry systems have to be harmonized with climate change scenarios and possibilities of tree species to grow in changed conditions.

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IMPACT OF CLIMATE CHANGE AND DIFFERENT MANAGEMENT REGIMES TO VARIOUS ECOSYSTEM SERVICES IN EUROPEAN BEECH FORESTS IN SERBIA

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SUMMARY: According to the IPCC SRES A2 CO₂ emission scenario and climate change projections on regional level, temperature in some parts of Serbia will rise for about four degrees Celsius before the end of 21st century. Current forests, as well as those that are planted today, will be exposed to drastically different climate conditions. The aim of this research was to evaluate climate change impacts on various ecosystem services in European beech forests in Serbia. In this paper we integrated stakeholder preferences, experts' opinion and forest growth modeling in a way to get total utility for used climate and management scenarios. Particular ecosystem services considered in this study were biodiversity support, carbon sequestration and timber production. Nineteen different management scenarios and three climate periods (1971-2000, 2011-2040, 2051-2080) were used. Provided results allowed detailed analyses of complex interactions among management operations, climate conditions and implications to three key ecosystem services. Results showed that forest management together with stakeholder preferences will play much bigger role in providing of better support for ecosystem service in comparison to climate conditions and that more frequent felling operations provide better result than less frequent operations for the same amount of harvested wood.

Key words: climate change, beech forests, biodiversity, carbon sequestration, timber production

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INTRODUCTION

Climate model simulations coordinated by the Intergovernmental Panel on Climate Change suggest that the region of southeast Europe will suffer major climate changes in the 21st century (IPCC, 2007). They predict that average annual temperature for the most pessimistic A2 scenario will rise by as much as 3.8 degree Celsius for Serbia. It is expected that the summers will be longer and warmer, with more extreme weather events and less precipitation (Božanić and Gasperić, 2010).

Many existing forests will thus face growing conditions which they have not experienced for centuries. Contrary to agriculture, decisions in forestry (for instance: choice of tree species) remain irreversible for decades or even centuries. The Food and Agriculture Organization (FAO, 2011) recommends several options for reducing vulnerability and strengthening the adaptive capacity of forests in sensitive ecosystems. One of the important options is adjusting of forest management practices, which also implies adjusting of thinning operations.

Unfortunately, decision makers in forestry in general lack knowledge concerning available adaptive management options that could support their decision-making. This research is a step forward to develop adaptive management plans and to analyse their effects on the forest stands, which will on the one hand ensure greater sustainability of forests and on the other hand support multifunctional forest management practice and diverse ecosystem services.

This study directed focus on dynamics of originally natural beech forest ecosystems (Matović, 2012), with deep consideration of management intensities and trade-offs among various stakeholder preferences and ecosystem services provision in the overall framework of coupled natural and human systems.

One of the key concepts and research priorities for landscape sustainability according to Musacchio(2013) is the sustainability of managed landscapes. This study is effort in that direction with focus on the European beech managed forests as the one of the most important ecosystems in Europe. With a forecasted rise in average global surface temperature and changes in precipitation and disturbance regimes at an unprecedented rate (Nakicenovic et al., 2000), climate change will bring forth new challenges for forest ecosystems, as well as for current management concepts, specific objectives and various ecosystem services. Climate change impacts are likely to be aggravated by other anthropogenic pressures such as land use change and pollution (Camill, 2010).

Current management of forests have to be reassessed and improved to become reliable and effective in the light of altering climate (Lindner, 2000) as well as to pay more attention to landscape sustainability (Musacchio, 2009) and to include necessities of human society for diverse ecosystem services (Nelson et al., 2009). The intensive continuation of anthropogenic greenhouse gas emissions and the lack of an international agreement on binding climate change mitigation policy increase the importance of development and implementation of appropriate forest adaptation management measures for coping with inevitable global change of climate (Seidl and Lexer, 2013). A well-timed implementation is thus essential for the reduction of the substantial climate vulnerability of forest ecosystems.

The aim of this study was to test 19 different forest management scenarios in a beech stand with a process-based forest growth model to find out which management

strategy will give the best performance under different stakeholder preferences and various climate conditions.

The novelty of this study is the robust evaluation of impacts of different variables (particular soil and forest stand structure diversity, various forest management measures, variable climatic conditions and different stakeholder preferences) to trade-offs among ecosystem services in sustainable forest management planning.

MATERIAL AND METHOD

Studied forest sites

European beech forests in Serbia are mostly uneven-aged characterized by virgin origin (Matović, 2012). They cover 660,400 ha or 29.3 % of forest land which makes them the most abundant tree species in Serbia, while about 68% of these forests are publicly owned (Banković et al., 2009). In comparison to private once, public beech forests have higher growing stock and annual increment due to the higher proportion of high forests. They are mainly managed by public enterprise “Serbian forests” (srp. Srbijašume), and by National parks “Djerdap, “Fruškagora”, “Tara” and “Kopaonik” according to single tree or group selection felling (Koprivica et al., 2013).

Forest survey was conducted from 2005 to 2007 at nine publicly owned beech forest sites in Serbia (Table 1). Representative sampling was performed with special attention to covering all beech forest types. The investigated beech stands belong to high forests (Matović, 2012).

Table 1 Detailed description of nine examined stands

Localities	Javor	ZapadnaB oranja	Cemernik -Ostrozub	Homoljsk eplanine	Kucajske planine	Zeljcin	Kukavica	Jastrebac	IstocnaBo ranja
Dominant soil type	Humus- siliceous soil	Pseudogley	Illimerised acid brown soil	Illimerised soil on limestone	Humus- siliceous soil	Acid brown soil	Acid brown soil	Acid brown soil	Acid brown soil
Longituda	20.07	19.16	22.21	21.61	21.96	20.78	21.90	21.37	19.26
Latituda	43.45	44.37	42.84	44.40	44.13	43.50	42.81	43.42	44.34
Elevation (m)	1270- 1380	480-630	880-1060	400-540	920-990	1000- 1170	780-1030	530-670	690-830
Exposure	Northwes t	West - southwest	West - southwest	West - northwest	Northwes t	Northwes t	North	East - northeast	North
Slope	5-20°	5-40°	5-40°	5-30°	5-20°	15-35°	15-45°	5-40°	5-35°
Stand size (ha)	26.3	20.2	31.6	22.7	28.3	22.6	28.3	32.3	29.5
Number of sample plots within stand	26	20	32	23	28	23	28	33	29
Size of sample	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05

plots (ha)									
Total sample size (ha)	1.3	1.0	1.6	1.15	1.4	1.15	1.4	1.65	1.45
Number of trees/ha	402	246	301	274	333	296	221	315	214
Initial growing stock (m ³ /ha)	396	346	312	543	398	523	333	308	526
Mean diameter (cm)	30.95	33.66	30.12	39.42	35.44	36.57	31.43	29.95	41.59
Dominant height (m)	27.31	31.47	30.43	32.14	24.75	33.65	29.11	28.41	35.15

Management regimes and climate data

Nineteen different sets of silvicultural management measures (Table 2) were designed in a way to be close to current management practice and to examine modifications in the sense of increase of intensity. All simulations were performed under three climate periods (1971-2000, 2011-2040 and 2051-2080) (Table 3 and 4). ECHAM5 global circulation model (Hagemann et al., 2006) together with A2 SRES emission scenario (Nakicenovic et al., 2000) was used for prediction of global climate and later downscaled with regional climate model EBU-POM (Djurdjevic and Rajkovic, 2008). Resolution of climate data was 25 km x 25 km.

Table 2 Description of management measures

	Felling type	Felling intensity (% of growing stock)	Felling interval (years)
1	Without felling	0	0
2	Felling of higher trees	10	10
3	Felling of higher trees	20	10
4	Felling of higher trees	30	10
5	Felling of lower trees	10	10
6	Felling of lower trees	20	10
7	Felling of lower trees	30	10
8	Selective felling	10	10
9	Selective felling	20	10
10	Selective felling	30	10
11	Felling of higher trees	5	5
12	Felling of higher trees	10	5

13	Felling of higher trees	15	5
14	Felling of lower trees	5	5
15	Felling of lower trees	10	5
16	Felling of lower trees	15	5
17	Selective felling	5	5
18	Selective felling	10	5
19	Selective felling	15	5

Table 3 Mean temperatures for simulated 30-years' periods

Climate periods	Javor	Zapad na Boranj a	Čemer nik-Ostroz ub	Homol jske planin e	Kucajs ke planin e	Zelj in	Kukav ica	Jastre bac	Istocna Boranj a
1971-2000 20c3m	5,9	9,5	8,9	9,3	7,2	9,0	9,7	9,3	9,5
2011-2040 A2	6,2	9,8	9,2	9,5	7,5	9,3	10	9,6	9,8
2051-2080 A2	8,3	11,9	11,2	11,6	9,7	11,3	12	11,7	11,9

Table 4 Mean annual sum of precipitation for simulated 30-years' periods

Climate periods	Javor	Zapad na Boranj a	Cemer nik-Ostroz ub	Homo ljske planin e	Kucaj ske planin e	Zelj in	Kukav ica	Jastre bac	Istocn a Boranj a
1971-2000 20c3m	724	786	606	674	729	709	616	666	786
2011-2040 A2	802	859	697	786	839	808	712	756	859
2051-2080 A2	709	745	612	667	704	695	616	666	745

The model 4C (FORESEE – Forest Ecosystems in a Changing Environment)

The process-based forest growth model 4C (Lasch et al., 2005) was used to simulate the effects of four forest management regimes on beech forests in changing environmental conditions. It was developed in order to predict the long-term effects of climate change on forests. For the simulations, 4C requires descriptions of tree species composition, soil characteristics, and climate parameters on a daily basis. Model application and validation was performed in numerous studies (Suckow et al., 2001; Lasch et al., 2002, Reyer et al., 2010). Also in cases where there were more than one forest layer (Gutsch et al., 2011), or when regeneration and three size diversity were included (Kint et al., 2009).

Integration of ecosystem services, model outputs, experts' opinions and stakeholder preferences in multi-functional sustainable forest management

Multi-criteria decision analysis (MCDA) or multiple criteria decision support (MCDS) methods, as an umbrella approach in natural resource management (J. Kangas and A. Kangas, 2005; Mendoza and Martins, 2006) were used for assessing multifunctional sustainable forest management. Three major forest ecosystem services (provisioning of biodiversity, carbon sequestration and timber production) were taken into consideration.

Model outputs for this research were chosen to correspond as much as possible to the MCPFE indicators (MCPFE, 2002). Several model outputs (biomass, harvested wood, dead wood, annual increment, total soil carbon and Gini coefficient as a measure of tree size diversity (Lexerød and Eid, 2006)) were ranked by five experts according to three targeted ecosystem services using pair-wise comparison technique (Saaty, 1988; Goepel, 2013).

Then, total performance index (TPI) (Gutsch et al., 2011) was calculated for every partial objective of forest management by the using weighting factors. The TPI makes aggregation of the normalised output variables of the model in following way:

- 1) Calculation of the $X_{max,j,i}$ (1),

$$X_{max,j,i} \quad (j = 1, \dots, 6, i = 1, \dots, 19) \quad (1)$$

where j is number of model output variables, and i is number of management scenarios;

- 2) Calculation of maximum values over the four management scenarios (2):

$$X_{max,j} = \text{maximum} (X_{max,j,i}) \quad (2)$$

for $i = 1, \dots, 19$.

- 3) Calculation of a set of normalised values $X_{j,i}^n$ for output variable j ($j = 1, \dots, 6$) and management i ($i = 1, \dots, 19$) (3)

$$X_{j,i}^n = \frac{X_{j,i}}{X_{max,j}} \quad (3)$$

- 4) Calculation of the TPI for every management scenario i ($i = 1, \dots, 19$) as weighted average (4):

$$TPI_i = \frac{\sum_{j=1}^6 w_j X_{j,i}^n}{\sum_{j=1}^6 w_j} \quad (4)$$

- 5) TPI_i values calculation were performed for each targeted ecosystem service (provisioning of biodiversity, carbon sequestration and timber production), all nine sites and three climate periods (1971-2000, 2011-2040, 2051-2080).

TPI values calculation were performed for each targeted ecosystem service (provisioning of biodiversity, carbon sequestration and timber production), all nine sites and three climate periods (1971-2000, 2011-2040, 2051-2080).

Weighting factors for three stakeholder preference groups were created using pair-wise comparison technique (Saaty, 1988; Goepel, 2013). Representatives from three stakeholder groups: lawmaker (Ministry), forest management companies (public enterprises) and nature conservation sector (national parks and nature conservational NGOs) were interviewed through dialogue and ranking questionnaire.

TPI was recalculated to fit into interval [0-100] % and then values for each management scenario, each site, climate period and each targeted ecosystem service were multiplied by stakeholder weighting factors and summed up into total (overall) utility (Fürstenau et al., 2006; Schwenk et al., 2012) (Figure 1).

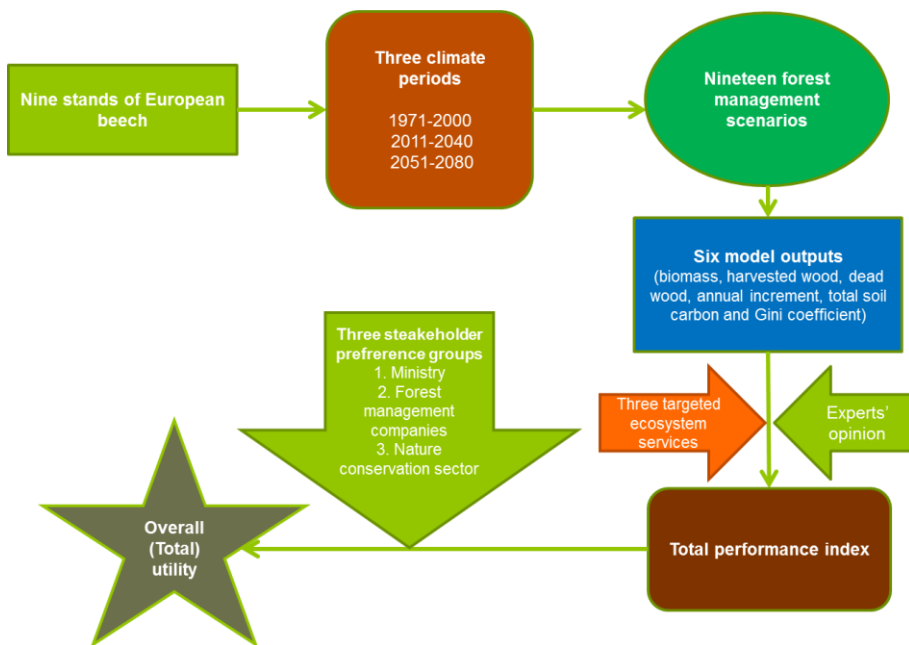


Figure 1 Overall study flow

RESULTS

Experts' and stakeholders' ranking

Five forest experts crossed and ranked different model outputs with three targeted ecosystem services (Table 5).

Table 5 Weighting factors for model outputs according to experts' opinion

Ecosystem services (or partial objectives in forest management)	Biomass	Harvest	Dead wood	Growth (Stem increment)	Total carbon in soil	Gini (structural diversity index)
Carbon sequestration on site	0.25	0.13	0.08	0.30	0.17	0.07
Provisioning of biodiversity	0.15	0.07	0.19	0.12	0.14	0.33
Timber production	0.16	0.29	0.06	0.30	0.07	0.12

Stakeholder preference groups in Serbia provide their vision how forest should be managed to provide specific shares of ecosystem services consistent with their values (Table 6).

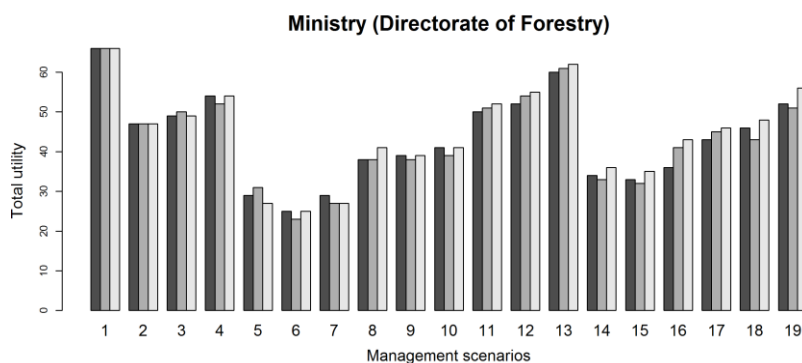
Table 6 Weighting factors for four scenarios and three objectives

Ecosystem services (or partial objectives in forest management)	Stakeholder preference groups		
	Lawmaker	Nature conservation sector	Forest management companies
Carbon sequestration on site	0.33	0.15	0.19
Provisioning of biodiversity	0.33	0.7	0.13
Timber production	0.33	0.15	0.68

Provision of various ecosystem services under different management and climate regimes

Obtained results showed that different management scenarios provide different total utilities for various stakeholder group preferences. The highest average values of total utility for nine stands for preference scenarios of Ministry and Nature Conservation Sector was achieved in no-felling management regime (Figure 2). That is not the case for the preferences of forest management public enterprises for which management scenarios with the most intensive felling operations provided the best results (4, 10, 13 and 19).

It was noticed that management regimes which included more frequent felling operations (regimes from 2 to 10) provided better results than others with less frequent felling operations (regimes from 11 to 19) for the same volume harvested.



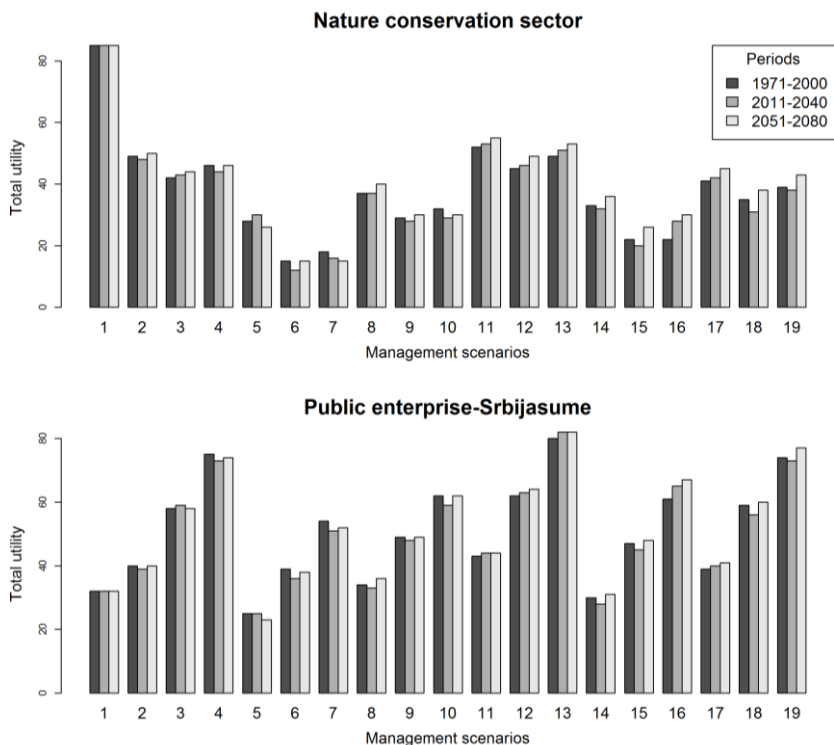


Figure 2 Mean values of total utility for nine stands, 19 forest management scenarios, three climate periods and three stakeholder preference scenarios

DISCUSSION

Several studies have reviewed different forest models from the perspective of their usability for assessing sustainable forest management (for review see Mäkelä et al. 2012 and Monserud, 2003). Sustainable forest management requires from current models to expand their predictions to a variety of ecosystem services and to evaluate more deeply trade-offs among them (Mäkelä et al., 2012). Idea of constant evaluation in nature conservation (Milad et al., 2011) (which can be applied in any planning, even for sustainable forest management and assessment of various ecosystem services in the dynamic landscapes) stating that this concept need to be adapted either within a constant evaluation process or through the inclusion of further changes *a priori*, even if they are to some extent unpredictable.

Forest resource planning is a very complex problem mainly owing to the numerous criteria involved in the underlying decision-making process and heterogeneity among different forest management units. Every decision made has influence to different criteria: economical (e.g., timber production, hunting, etc.), environmental (e.g., soil erosion, biodiversity conservation, carbon sequestration, etc.) and social (e.g., recreation, employment, population distribution, etc.) (Diaz-Balteiro and Romero, 2008).

The optimization problem in the base of most real forest management planning problems needs to be expressed within the multiple criteria decision-making (MCDM) paradigm (Diaz-Balteiro and Romero, 2008). Question “How do models provide information to assess sustainable forest management?”, is very important and closely connected to conceptual framework for better understanding of the models role in assessing sustainable forest management at the stand level using sustainability criteria and indicators (C & I) (Mäkelä et al., 2012). C & I were proposed by the Ministerial Conference on the Protection of Forests in Europe (MCPFE, 2002) to reinforce evaluation of the sustainability of forest management. Despite the fact that C & I were primarily defined at the national scale, their implications range from forest stands to the forest management unit. Sustainable forest management undoubtedly have to deal with trade-offs among ecosystem services, so the tools used for sustainable forest management were appropriate for quantification of spatial heterogeneity and landscape dynamics in this paper. Quantification of ecosystem services and analysis of trade-offs among them can help in making natural resource decisions more effective and defensible (Nelson et al., 2009).

Common sense is that intensive exploitation of forest resources will bring decrease of biodiversity, but where are the boundaries in management intensity on spatial and temporal scale that will make adequate compromises in order to sustain several ecosystem services? This is the one of the most difficult questions in management of forest ecosystems. If you take amount of dead wood and forest structure (Gini index) as the leading indicators of biodiversity, as it is in this study, then the decrease of biodiversity as a consequence of management intensity increase is not surprising. What is interesting is that the most intensive management regime (4) has slightly better total utility than the less intensive one (3) for first two stakeholder preference scenarios. That may happen due to the fact that intensive felling of higher trees gave more space for growth of lower trees.

Some studies suggest that there is no simple inverse relationship between harvested timber and the amount of carbon stored and that management should mimic natural forest disturbance for achieving of high wood yield and general storage of carbon (Thornley and Cannell, 2000). What is noticeable is that there were no larger differences in performance among three climate periods for different ecosystem services. Generally, growth was slightly decreased in the period 2051-2080 in comparison to 2011-2040, which was the most productive one, but not so drastically to introduce significant change in ecosystem services provision. Some other methodological approaches suggested more dramatic outcomes concerning beech future in the region. Models based on ecological niches predict severe decline of beech forests in southeast and central Europe before the end of 21st century (Czucz et al., 2011; Rasztovits, 2011; Stojanović et al., 2012; Stojanović et al., 2013; Hanewinkel et al., 2013).

Management was proved to be the most influential variable in forest management panning. Stronger variance caused by forest management in comparison to climate in altered environmental conditions was noticed also in mixed oak-pine forests in Germany in similar study (Gutsch et al., 2011). This result put more responsibility to decision makers entailing more thoughtfulness and vision.

Other studies emphasize the key importance of land-use change followed by climate change, nitrogen deposition, biotic exchange, and elevated carbon dioxide concentration, as the threatening factors to biodiversity (Sala et al., 2000). Extreme

case of 30% felling per 10 years and 15% every five years (management scenario 4, 7, 10, 13, 16 and 19) goes beyond 10 years' increment for all nine sites, which in long run will cause deforestation and could be characterized as "land-use changing" management scenario. Considerable high variation among sites should be also considered carefully.

There were suggestions that no single solution fits all future challenges, especially in the context of changing climate, while the best strategy is to combine different approaches in different occasions (Millar et al., 2007). If we add stakeholder preferences, situation becomes more complex. Some studies advised protection of ecosystem services through better decision making which implies their valuation into effective policy and finance mechanisms (Daily et al., 2009).

Current average annual increments for nine beech stands in Serbia ranged from 5 to 10 m³/ha, while the growing stock is approximately from 300 to 550 m³/ha. In all cases, felling of 10% of growing stock per 10 years was below the increment, but somewhere about the current management practice in Serbia.

Earlier, clear gap regarding need for more specific, functional examples of adaptation principles that are consistent with unavoidable uncertainty referring to the future were recognized (Heller and Zavaleta, 2009). Our study through complex modeling approach fill that gap with clear result that suggest low intensity felling for satisfying various stakeholders and provision of multiple ecosystem services.

Regarding future of various ecosystem services in European forests, it is likely that they will be impacted by climate change, but the knowledge about the extent is still scarce. (Lindner et al., 2010). Better understanding of forests adaptive capacity and regional vulnerability to climate change demand more research endeavor around the world.

CONCLUSION

Making the relation among the concept of multifunctionality with the ecosystem services (Mastrangelo et al., 2013), as well as with multiple management measures, climate conditions and stakeholder preferences is a promising approach for producing scientific evidence for forest management planners. We concluded that management has the larger impact on variations in ecosystem services provision than climate conditions. Also, we noticed that management regimes which included more frequent felling operations provided better results in multifunctional forest management under different stakeholder preference scenarios than regimes with less frequent felling operations for the same harvested volume.

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DEFICIT OF GREEN SPACES AS AN ENVIRONMENTAL ISSUE IN URBAN AREAS

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SUMMARY: Due to intensive technological developments and deficit of green spaces, a number of environmental issues becoming more and more visible in urban areas. The aim of this paper is to emphasize a need for determining the strategy for system of greenery, especially in the parts of the city where environmental parameters are significantly poor. At nine different locations, there were measured noise levels. Parallel to this process, it was analyzed an existing greenery. Some conclusions about the current environmental problems and possibilities of their transformation were set out.

Key words: *quality of the environment, greenery, noise level, strategy for the system of greenery*

INTRODUCTION

An environment represents a complex of biotic and abiotic impacts on the living organisms in the area, where they are living. The organisms are unbreakable connected with the environment, throughout a complex of relationships (Vratuša, 2005). Human influence on the changes in the environment is remarkable. During the development of human civilization, man has contributed to significant changes in the biosphere and transformation of natural ecosystems into the anthropogenic. Later, it came to the resource spending, changes in natural areas, pollution of the air, surface and ground water, soil degradation and changes in biodiversity.

New approach to the urban planning is based on sustainable urban development, which includes environmental, economic, social and cultural development of the city. Urban environment, as a part of human environment, is a space and ambient, where people, by shaping it for their own purposes, live and work (Cvejić, 2002). Urban noise and deficit of green areas represent one of the biggest problems in the urban environment.

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As Atkinson (2007) points out, "the noise is a sound which is out of place". Any unpleasant sound, but strong enough to be singled out from the rest ones, is determined under term of noise. Noise is a subjective feeling and it isn't seen by all people in the same way. What is noise to one person, the same, to other person is a melody. The noise is unpleasant ingredient of modern life, not only in urban and industrial areas, but also in rural areas.

Long time in the past, people were exposed only to the noise which originated from natural phenomena: thunder with a burst of lightning, volcanic explosion, roar of wind, sea waves and roaring animals. Today, there are almost no towns or workplaces, that aren't more or less exposed to the noise. It is considered that the noise is most prevalent and serious danger of modern urbanized society (Stansfeld et al., 2001). Oscillation occurs when the air in the form of longitudinal waves, reaches the ear. The frequency of these waves is in the range of 20 - 20 000Hz. The volume - zero to decibel scale, corresponding to the threshold of audibility, while the limits of pain is at an altitude of 130dB. The human ear can receive audio from 16 - 20 000Hz. The best receiving of sound waves is under frequencies of 500 - 4000Hz. Unit of measurement for sound is decibel, and it is noted as dB (Đukanović, 1996).

In every urban environment, greenery provides hygienic living conditions. Its significance is multifold. The greenery restores oxygen in the atmosphere and has a biological function in the process of exchanging oxygen and carbon dioxide (Vratuša, 2005). Urban greenery regulates air temperature, pressure and air flow. What is more, it increases humidity and perform ionization, absorbs dust and soot, mitigates the impact of street noise, protects against a gust of wind, sand and snowdrifts (Rajic et al., 2010). Leaf moisture of deciduous species slows the spread of fire. In the parks and squares greenery has social, cultural and educational importance. It has positive effect on the physiological functions of people. Also, it has a positive role in maintaining degraded soils and protects them from the erosion, reduces the reflection, and bactericidal effect in a polluted environment. In the city greenery has an invaluable aesthetic significance (Vratuša, 2005).

Vegetation, both from the ecological and the aesthetic aspect, has positive influence on human, its health and physiological functions. Spending time in a rich green area is connected with decrease of hearth pulse, which can reach 4 to 8 beats per minute. It also acts on the skin temperature, decreasing it by 1 to 1,3°C (Đukanović, 1996).

As Cvejić (2002) points out, the effective protection of road noise can be achieved by certain forms of plant species, with their substantial width and density (Table 1). The crowns of deciduous trees absorb about 25% of sound energy, 75% of it is dispersed and rejected. The noise on the street, without plants, is five times stronger in the man's height (180cm). Urban greenery, especially trees, reduces the influence of noise up to 25%. As 60 - 80% of noise sources in urban areas, are coming from transportation equipment, the protection of the greenery must be significant. When barrier of 2 to 3 rows of green plants, trees and shrubs exists, it is possible to decrease the noise level by 15 to 18dB. The first protected line (10 - 15m of vegetation) is the biggest sound absorber (Đukanović, 1996; Nzama et al., 1995).

In order to create a network of greenery, there is condition to reach a sufficient mass of vegetation, expressed in m² in relation to population. The basic normative is based on the fact that one hectare of forest, consuming during the day amount of carbon dioxide that breathing out around 200 people. That means one man needs 50m²

of greenery. In the urban environment, this standard is much lower, depends on the size and structure of the city, also natural conditions. The European normative ranging from 15m²/citizen to 25m²/citizen of greenery. In some more developed countries normative reaches up to 50m²/citizen (Vujković, 2003, Tišma et al., 2009).

Table 1. Sound volume in relation to the width of vegetation (source: Cvejić, 2002)

SOUND VOLUME (dB)			WIDTH AND HIGHT OF GREEN BELT, TYPE OF GREEN AREA AND SPECIES
before green belt	after green belt	difference	
76.7	69.0	7.7	Green belt with the width of 10m and hight 5m. Tree-lines of <i>Tilia</i> sp. and hedge line with <i>Ligustrum</i> sp.
80.0	74.0	6.0	Green belt with the width of 11m and hight 8m. Tree-line of <i>Tilia</i> sp.
83.0	75.6	7.4	Green belt with the width of 10m and hight 8m. Tree-lines of <i>Tilia</i> sp. and hedge line <i>Ligustrum</i> sp.
77.7	66.1	11.6	Green belt with the width of 36m and hight 12m. Group of deciduous plants and shrubs.

According to official data from the Public enterprise JKP "Zelenilo" - the greenery of Novi Sad, 430 ha of the city is under the greenery. That means 13m²/citizen of greenery. Far from the satisfied level.

MATERIAL AND METHOD

The paper discusses about the environmental quality in the city of Novi Sad. Ecological parameter that has been examined was the noise level at defined locations in the city (Figure 1). Parallel to this process, it was analyzed an existing greenery. Some conclusions about the current environmental problems and possibilities of their transformation were set out.

The survey was conducted during 2013 year in the period from April to July. At nine different locations (1 - settlement "Novo Naselje"; 2 - settlement "Detelinara"; 3 - a gas station next at Liman 3; 4 - a gas station at "SPENS"; 5 - Boulevard of Empirior Dušan; 6 - Futoški park; 7 - Dunavski park; 8 - Limanski park and 9 -Kamenički park), there were measured noise levels at various intervals during the day.

Two of the first five locations were allocated in a perifer zone of the city and the rest three ones in the zone next to the city center. For the next four locations were chosen representative parks of the city of Novi Sad. The research has the aim to compare the quality of environment between assigned sites by observing the level of noise and greenery composition. Qualitative literature review brought results in terms of environmental improvement of urban landscape. For the conducted research, it was given a photo analyze of the investigated localities.



Figure 1. Location of researched localities in the city of Novi Sad (source: Plan plus, modified by Ivana Blagojević, 2014)

RESULTS

In order to determine the quality of environment and to make a proper selection of preventive measures for preserving it (also to improve human health), it was carried out monitoring of the noise level according to the existing legal and professional basis (Ekobilten, 2013). The research has shown that the settlements Detelinara and Novo Naselje (Figure 2a, Figure 2b) are the least threaten with the noise, while a gas stations next to the Mercator center and "SPENS", also the area of Boulevard of Empirior Dušan are substantially more threaten (Figure 2c, Figure 2d, Figure 2e). Location "SPENS" proved itself to be the loudest in all tested intervals (Table 2). Localities are burdened by the noise due to transport vehicles in traffic. The reason for slightly lower noise levels at localities 1 and 2 is a distance from the city center, that means less traffic load to which the localities are exposed, as the main cause of noise.



a. settlement "Novo Naselje"



b. settlement "Detelinara"



c. gas station at Liman 3



d. gas station at "SPENS"



e. Boulevard of Empirior Dušan

Figure 2. Photos of the researched localities (source: Ivana Blagojević, 2013)

Table 2. The values of noise in the environment at the first five researched localities (source: Ekobilten, 2013)

N ^o	Measured locality in the city of Novi Sad	ID br. measurement	Date of measurement	L _{day} [*] dB (A)	L _{evening} [*] dB (A)	L _{night} [*] dB (A)	L _{den} dB (A)
1.	Novo Naselje	861	10.04.2013.	57.9	55.9	50.0	59.3
2.	Detelinara	865	18.04.2013.	58.7	56.4	50.3	59.8
3.	Liman 3	863	12.04.2013.	66.7	65.9	59.9	68.8
4.	"SPENS"	869	24.04.2013.	69.4	69.9	63.8	72.4
5.	Boulevard of the Empirior Dušan	873	28.04.2013.	65.6	64.9	61.8	69.3
*reference interval for the day is from 6:00 to 18:00 p.m., for the evening is from 18:00 p.m. to 22:00, and for the night is from 22:00-06:00, in terms of national norms, Službeni glasnik 75/2010							

Selection of the plant species is mainly focused on highlighting their aesthetic values, while the functional values are ignored. Generally, the vegetation is mainly composed of tree-line deciduous plantings, with transparent tree canopies. A graduation and multi rowed planting in the composition is not applied, so the residential areas are directly exposed to the noise that is coming from the road. The

most common plant species that occur in the research area are: poplar, acacia, cypress, birch, cherry laurel, cherry plum, berberis and the most common form *Ligustrum* sp. hedges.

In the Study (Tišma et al., 2009), urban green spaces are treated as part of the urban public area. Directly, they are used for active or passive recreation, while at the same time their influence is indirectly, because with their presence affects improving the quality of the urban environment. Presumably, the green areas of this nature should be with a favorable microclimate and environmental parameters within the allowed limits. For the purpose of this research, there were examined four city parks (Figure 3). Danube Park (Figure 3a), is located in the city center, while Liman park (Figure 3b) and Futoški park (Figure 3c), are located in the wider area of the city center. Kamenički park (Figure 3d) is the only park outside the area of the city core and is located on the opposite side of the city (the area of Srem region).



Figure 3. A prominent parks in the urban area of Novi Sad (source: Ivana Blagojević, 2011-2013)

During the month of May, it was measured a noise level (Ekobilten, 2013) in the parks (Table 3). An increased noise value (allowed 65dB) was identified at all researched sites, parks. The level of noise in Futoška street, near the Futoški park, was recorded 69,5 dB; while in Liman 3, next to Liman park was 69,9 dB (Figure 1). Research has shown that in the area of the city center and the city's roads, that 48% of the population is affected by urban noise (70,4dB), while in the area of leisure and recreation, the percentage reaching even 37% (65,9 dB).

These are really devastating data, since the word is about park areas where it should not be set a question related to satisfy the ecological parameters. Not surprisingly is the fact that the noise level deviates from the tolerant zones in residential areas right next to the traffic road, but it is certainly a surprising that the

situation is reflected in the park areas as well. It is worrying that Kamenički park, which is a recreational park, located at some distance from the city central core, also has higher noise level. For sure, this is affected by crowded traffics, coming from the Bridge "Sloboda", that passes through the edge of the park and connects the city of Novi Sad with Fruška gora.

Graph 1. Level of noise in diferent parts of the city (source: Ekobilten, 2013)

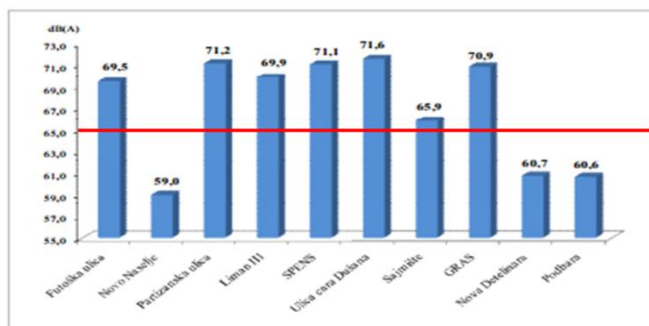


Table 3. The values of noise in the environment in the zones of parks (izvor: Ekobilten, 2013)

Nº	Measured locality in the city of Novi Sad	Date of measurement	[*] L _{day} dB (A)	%A	%NA
1.	Zone of city center and traffics corridors	10.04.2013.	70.4	48	26
2.	Sport and recreational areas	18.04.2013.	65.9	37	18
*mean monthly values - %A: percentage of people threaten by noise, %A - percentage of people threaten by noise on high level					

DISCUSSION

The research has shown that measured levels of noise close to, or in sourroindings of green areas, such as parks, are lower than the value of same in the other areas. That is reasonable, because vegetation is the greatest assistant in the fight against environmental problems. What is not courages, the noise level was above the limited (65dB). Deficit of green areas proved to be an environmental issue in urban areas and that has to be changed in future.

During the last decades, European cities have adopted "compact cities" strategies for their urban development (Nefs, 2006). In Serbia, this is out of the case because a degradation of the environment is at high level. One of the important steps of

development the country, certainly need to be focused on sustainable urban development and the improvement of the urban environment, by reducing environmental problems in the cities and started to monitor them regularly.

The idea is to connect all green areas by specific green corridors into one unique green system (Figure 4). This will develop environmental conditions and improve quality of life in the city of Novi Sad.



Figure 4. Proposed green corridors as a connections of the system of greenery

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URBAN GARDENING AND ITS ROLE IN THE CITIES*

MARKÉTA MIKOVCOVÁ¹

SUMMARY: Vegetable or fruit self-provisioning is according to many authors beneficial not only for the gardener himself but it has also positive impacts on the landscape and nature in general. Gardening has potential to comply with the sustainability principles. The aim of this paper is to determine through data triangulation method to what extent sustainability of gardening is publicised in the Czech Republic and what rules gardeners observe in their activities.

Key words: sustainability, urban gardening, expert perspective, lay persons acting

INTRODUCTION

The self food provisioning is often seen as the main motivation for the gardeners, but it is not always the truth. Sumner, Mair and Nelson (2010) suggest to view urban gardening as an alternative that gives the culture back into agriculture. As such urban gardening holds not only connotation of production (food self-provision) but also the connotation of ethics, rules and relations to environment which are also important element of culture (Giddens, 1989)

The economical benefit of urban gardening is obvious. Gardeners do not need to buy their food because they can grow their own. Corrigan (2011) documents in his case study among community gardeners in Baltimore that some of them appreciate when they do not need to make a trip to a distant supermarket.

Urban gardening has various impacts on environment. The authors specify that next to the enhancing urban greenery and lowering the carbon foot print, people rediscover ecosystems there; they start to become more interested in environment. (Howe, 1999; Okvat a Zautra, 2011; Macias, 2008)

Some authors deal with the impact of gardens on the individual gardeners. They are usually focused on the health issues from the point of view of diet and psychology, and they emphasize the impact on the physical health. Okvat and Zautra (2011)

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outline a wide range of studies showing positive psychological impacts for children as well as for the adults involved in gardening and being in the green vegetation; they discuss the contribution of gardening to improving the mental concentration, cognitive functions and emotional stability. Authors also show that gardeners eat more fresh vegetable often in organic quality and they have healthier diet without increasing financial costs. There are also several documented impacts of gardening on the community of gardeners. Primarily it is meeting new people and creating contacts which contradicts anonymity of urban way of life coined by Wirth (1938), people help each other in problems concerning gardening but often concerning also personal life. These contacts connect people across generations, gender, religion and ethnicity. The fact that the movement is bottom-up, empowers the community confidence and recreates the citizenship. (Corrigan, 2011; Howe, 1999; Okvat and Zautra, 2011)

Wills, Chinemana and Rudolph (2009) in their study from community garden in Johannesburg point out that in the countries with high level of poverty, where food security is low, the satisfaction of individual needs dominates over the community development. In contrast in Western countries the individual and community needs are of the same importance, in some cases the community ones dominants. (Sumner, Mair and Nelson, 2010)

The studies on urban gardening highlight three categories shaping this activity: economic, environmental and social. These categories suggest urban gardening has a potential to be sustainable (Bent, Barthel and Colding, 2013; Colasanti, Hamm, Litjens, 2012; Howe, 1999; Sprecht *et al*, 2014). It raises the questions of how is the image of sustainability of urban gardening constructed by experts and if it does differ from the gardeners views and practices (do they follow this constructed picture in their activities?). The distinctions in images of sustainability of urban gardening could be understood through the concept of “images of the world”. The first set of images is created mainly by experts operating in the public space, the second set is constructed by the gardeners as lay person. (Lošťák, 2002) The aim of this paper is to determine to what extent environmentally friendly urban gardening, socially embedded urban gardening and economically beneficial urban gardening is supported and publicised in the Czech Republic and to recognise whether the gardeners observe and continue to develop this image or they create their own.

MATERIAL AND METHODS

To answer the questions outlined above the content analysis of “*Zahrádkář*” (the monthly magazine for gardeners published by Czech Union of Allotment and Leisure Gardeners) was done. For the analysis all twelve issues of years 2009, 2013 and first four issues of year 2014 were used with emphasis on food growing, soil care and its fertilization, and use of herbicides all in terms how these issues are addressed in the magazine. These years were selected with regard to the years when the data collections for public opinion pools used in this text were done (gardening season 2010) as well as the interviews (at the end of gardening season 2013) and the selection was based on the assumption that the gardeners (in the case they do read the magazine) are acting according to the knowledge they gained past season. These data were compared with secondary analysed data from Public Opinion Research “Our Society 2010 - September” provided by Centre for Public Opinion Research of the Institute of Sociology of the Academy of Sciences of the Czech Republic about

gardening activities. To achieve the requests of triangulation in the research method (Giddens, 1989) the results of content analysis and secondary data analysis from Public opinion research were crosschecked through data gathered in ten qualitative semi-structured interviews with community gardeners operating in six existing Prague community gardens.

RESULTS

The experts' view

If one has an interest in gardening and s/he wants to become a gardener s/he does not have to rely on the method of trial and error. There are various television programs about gardening, information is also available on the internet. Those who are interested can get advices from sellers in the gardening stores or from experienced colleagues and friends. There is also whole range of literature of experts. Almost every bookstore has department called “Hobbies” or in larger stores there are even departments called “Garden” which can contain two bookshelves and offer almost two hundreds of books about gardening, fruit trees, herbs, medicinal, utility or decorative plants for planting inside or outside but also about pests treating or garden lakes (based on authors' inventory research in these shops in Prague). Today's trend is return back to natural cultivation therefore books about natural, organic or “eco” gardens of permaculture are available in relatively high number. In case that gardener does not need to buy the book in the shop, there is a possibility to borrow one from the Public Library in Prague. It has 6252 books about gardens and 3538 books about cultivation available.

Since 1969 The Czech Union of Allotment and Leisure Gardeners publishes monthly magazine called *Zahrádkář* (The Gardener) with number of copies 104 thousands and audience consisting of more than 250 thousands readers. Given the institutional embedding of the magazine, its tradition and with respect to the outlook of the front page the expectation of advices with emphasis on productivity of the garden with strong attention to environmental issues might be in place. But this expectation is not confirmed. Contrary to the expectation generated through the outlook of front page, the content of the magazine highlights modern trends related to environmental concerns such as organic cultivating which is similar to the content of majority of popular books about gardening.

The editorial board of the magazine consists of experts with achieving master degree in higher education system, only one member has the bachelor degree. In the editorial board there are five experts with PhD. degree. Three members are associated professors (2 at Mendel University in Brno and one at Czech University of Life Sciences Prague).

The important information the magazine provides is the calendar for the gardener informing about seasonal gardening activities, types of vegetables for particular seasons, fruits, decorative plants. This information is often presented in the form of advertising article and it covers essential part of the content of the magazine. In one issue there is an article about problems resulting from own cultivation of seeds. In all reported years there are several articles that highlight the return to traditional historical types of fruit trees especially apple trees because of their higher resistance caused by broader genetic stuff. The magazine observes follows the modern trends in

gardening and brings the information about flower lawns and natural lawns. There is also a serial concerning natural gardens that brings reports about existing natural gardens. The number of articles split into categories is in the Table 1.

Table 1 Number of Articles addressing particular issues

Category	Number of Articles
Fertilization and Soil Care	16
Composting	13
Pests and Fungi	14
Animals Beneficial in the Garden	6
Eco, Organic and Natural Gardens	18

Source: Own calculation.

Important part of the content is covered by the articles concerning soil quality, fertilization and composting. It emphasises traditional procedures, fertilization using minerals and chemical fertilizers is only on the margin of the discussion. The importance and utility of humus and composting is emphasised as well. There is a comprehensive series of articles addressing composting. There are several articles concerning social character combined with composting (article about composting in the town Opava and every year event /beauty contest/ Miss Compost). Other themes addressed are the rules for selection of substrate for various plants, mulching as the activity protecting soil against erosion, green fertilization and other possibilities of organic fertilization such as using the ash or extracts from plants or livestock manure.

The importance of animals in the garden is mentioned several times. The activities against pests and fungi are also obvious part of the articles. In cases where it is possible experts from the magazine recommend biological protection, chemical protection is recommended only when necessary and there is also a warning about increasing the toxicity of spraying when they are mixed.

The comprehensive series of articles called Organic Garden emphasises the need of long-term sustainability in gardening and cultivation of food in general. The serial deals with the soil fertility and quality, important soil organisms, compost using and mulching and how to operate a garden without chemicals – i.e. so called eco, organic and natural gardens.

The magazine provides also legal advices concerning gardening. However it is only a marginal issue. As a part of each issue there is an enclosure for women outlining mostly traditional and healthy recipes. Invitations to seminars and exhibitions concerning gardening are at the end of each issue.

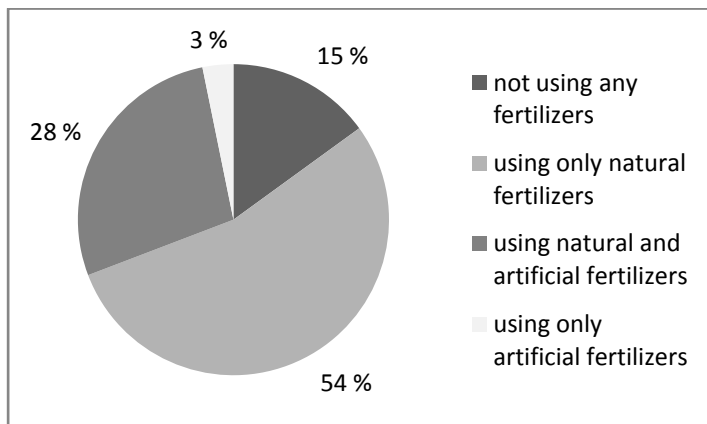
In contrast to overall environmentally friendly impression of the magazine there are some advertisements for chemicals, for example the Roundup gel and so on.

The Gardeners' activities

The research of the Center for Public Opinion Research shows that in Czech Republic 43.1 % of inhabitants operate their own garden, field or orchard; 65.2 % of them lives in the town with less than 15,000 residents, in the city of Prague (with more than a million residents) only 7,3 % of population operate their own garden,

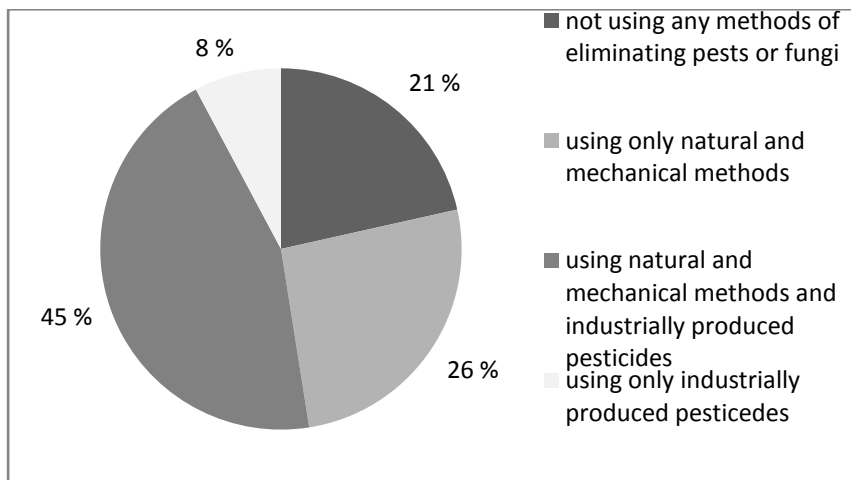
field or orchard. Those who operate any sort of soil/land evaluate their living standard as “not good, not bad” or as “rather good” without differences in the size of the town they live in. But they differ in age groups. There are almost no gardeners in the age group 60+ in the large cities, the age group from 45 to 59 years uses gardens in towns or villages of all sizes without differences, the age group from 30 to 44 years uses gardens rather in small villages, the number of own gardening in younger age groups is low.

Graph 1 The use of fertilizers



Source: Own processing of the data from CVVM Our society - September 2010.

Graph 2 The method of eliminating pests or fungi



Source: Own processing of the data from CVVM Our society - September 2010.

More than a half of gardeners (54 %) use only natural fertilizers without differences in municipality size, 15 % does not use any fertilizers at all, and 28 % of

gardeners use natural as well as artificial fertilizers. Only a minimum of respondents use only a chemical fertilizers (see Graph 1). Similarly almost a half use natural or mechanical methods of gardening but also a chemicals treating pests and fungi (see Graph 2). The analysis of the data shows that gardeners who do not use fertilizers also do not apply any activities against the pests of fungi. Those who use only natural fertilizers as well as those who use together natural and chemical fertilizers also use natural, mechanical and chemical methods of eliminating pests and fungi.

The main reason for using own garden is the same for urban and rural residents. As the research demonstrated it is the hobby. The second reason in the number is to produce fresh food. It is important primarily to habitants of suburb areas and villages. The third reason is to have healthy food. It is important especially for rural dwellers. Almost 55% of gardeners who use only natural fertilizers and about one third of gardeners who use natural and industrial fertilizers are those who identified these tree main reasons for their gardening.

Almost two thirds (60 %) of the people operating own garden, field or orchard exchanges or gives at least a part (minimum 10 %) of their production mainly to close family members, friends, wider family members or to neighbours.

The community garden experience

The reason for cultivation in community gardens for the gardeners is that they consider this activity as a hobby. Their production does not cover their consumption, it represents only food variation with fresh and own food. They emphasise the importance of cultivation of land for raising children.

“Well, there are herbs, tomatoes, cucumbers, zucchini, some melons, I sow there eggplant, a lot of strawberries, spinach, also beans and pea pods ... “ (Martina, 13/09/13) “We bought redcurrants and gooseberry bushes, blueberries, so children from the building pecked the berries. ... We always try for the children the best, so they have something out of it and so they can help out with it.” (Emilie, 13/11/08)

At the same time they think highly of the building of social relations, these are developed through communal plant growing but also through social events that usually concern food. *“We had a great time, everyone brought something, cake or what we had there? The spread, we had these herbs on it ... we eat everything, it was great.” (Katka, 13/11/20)* They also share their products during these events and activities. *“From time to time one comes with some strawberries, so she or he offers everyone, great. Or a radish ... Someone even gives it all away, she or he comes, harvests something washes it and gives to people around and that is how it is.” (Martin, 13/12/10)*

Next to their production they share also their experiences about cultivation in the gardens.

“It is very nice there are peoples with no experiences with gardening, right? ... And we are grateful for people that moved to Prague from some region and they have the know-how about how it works. And these people they are the “family gold” of the community garden, they can tell you – hey, that won’t grow, you can’t do it this way ... People are very open to this kind of sharing.” (Milan, 13/11/13)

All community gardens offer the possibility of composting for their members as well as for the inhabitants of the neighbourhood. The members appreciate it. *“I recognized that I have quite a lot of organic waste in my garbage.” (Hana, 13/11/13)* They become understood how important part of garden the compost is and appreciate

quality of such soil fertilizer. They do not use any additive fertilizers. They collect rain water in some cases.

“And back there are two composts, it is sure thing we have it, right ... And next to the building there is a barrel collecting the rain water, quite big, so it is enough when it is hot outside, we can water.” (Emilie, 13/11/8) “The compost is there from the very beginning, it is divided into three parts so we turn it over from time to time. So there is no need for fertilizers and we grow food. Everything grows fantastically.”(Soňa, 13/11/19)

DISCUSION

From the very beginning of the discussion about sustainable development all three pillars – economic, social and environmental, are always mentioned. It is because they are not isolated, they mutually complement each other and influence each other (see Meadows Randers and Meadows, (1992) 1995; Our common future, 1995; The Agenda 21, 1992). Contemporary publications for the gardeners are addressing mainly the issues of cultivating practices giving mostly environmentally friendly advices that could be included under the environmental pillar of sustainability. This is the case of the monthly magazine *Zahrádkář* (The Gardener) which had been analysed. In this magazine there are sometime mentioned also projects with social content as examples of community gardens, composting projects and competition events in the cities, invitations to seminars or exhibitions concerning gardening. But there has not been any reference to economic issues. It implies that in contrast to political and academic discourse which contains all three pillars, the image created for gardeners by experts is narrowed especially to environmental practices. Social issues are rare and economic are completely missing.

The assumption that the environmental pillar is important for the gardeners surveyed in Public Opinion Research might be in place, based on the fact they use mainly the natural fertilizers. But they mostly use with natural pesticides also chemical ones. It could be explained by the fact that they prefer their yields over the nature protection. It questions the importance of the environment for the gardeners but the fact that they are aware of is the significance of environment protection is without doubts. Hou (2009) similarly documents these findings and emphasises day-to-day experiences of gardening, which raises the awareness of seasonal, hydrological, and nutrient cycles. The preference for yields suggests the inclination towards an economic pillar, growing fresh and healthy food was the second and third most common reason for gardening. It might rather echo environment and social concerns (health as social issue as well as consumer demands of certain types of foods. Howe (1999) shows the importance of food supply and food safety issues. The impact of gardening on the social pillar can be validated by the fact that most of the gardeners share the food they grow with their relatives, friends or neighbours. Even stronger social impact gives as an example of Hou (2009) who shows there are actually gardeners who donate part of their yield to local food bank. To confirm this fact, Lawson (2005) claims that gardening have large social concerns, such as economic relief, education reform, and civic accord. In this case, image of gardening includes especially social and economic pillars of sustainability.

Both pillars are important for community gardens as well, although the environmental pillar is more significant. It illustrates the accent on composting, rain water collecting and non-use of pesticides. Community gardeners they also organise educative events and workshops for public. These arguments mentions Hou (2009) as well.

CONCLUSION

Urban gardening, although might be considered as small in its size indicated high importance in the concept of sustainable development in the cities. However, the paper found out different approaches of actors (lay persons and experts) toward this initiative. They differ in viewing the role of urban gardening in terms of its images and how it is articulated. They also differ in terms of emphasis concerning the role of urban gardening in the pillars of sustainable development (economic, environmental or social). The experts highlight environmental issues of the gardening. Gardeners who are considered as lay persons do not echo the image of urban gardening as solely environmental activity. They construct their own views through which they present urban garden. The lay persons emphasis economic and partially social pillars, while environmental pillar is not articulated too much. The last is not, however, the case of community gardens where also environmental issues are valued highly by gardeners – lay persons.

Nevertheless gardeners to some extent perceive the importance of the environmental pillar of sustainability in general. They manifest it through their practices when they use natural fertilizers and do compost their organic waste, collect rain water and in the case of community gardens organise environmentally oriented educative events and workshops (social pillar) and together with natural fertilizers they use artificial ones as well. These practices are in line with the issues the experts raise when addressing most of the problems of natural and ecological gardens, soil quality, natural fertilization and composting. The experts also question pesticides (which is similar with practices of lay persons-gardeners) and although they recommend whole range of natural pesticides the use of chemical ones is in some cases recommended too.

Generally speaking that although views and images developed by experts in gardening and lay persons in gardening indicate different importance in terms of their emphasis of three pillars of sustainability (social, economic and environmental), in the case of environmental pillar their images are overlapping in their content. In means in understanding environmental impacts of urban gardening both experts and lay persons coincide in their views which is promising finding for the future development of urban gardening.

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MICROBIOLOGICAL SAFETY OF AUTOCHTHONOUS LAYERED CHEESE MADE FROM RAW MILK

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SUMMARY: *The technology of this cheese is specific and different from other autochthonous cheeses from Montenegro. The unique technology is the result of a specific method of preparation of fresh milk for cheese production and the specific ways of coagulation and curd processing. The raw milk used for the production of this cheese originates from a small dairy farm in the conversion process. Milk used in the production of this cheese was not pasteurised. Based on the results of analysis of chemical parameters of cheese, it can be observed that the layered cheese made without cooking of cheese body, which was done in these experiments, belongs in semi-fat soft cheeses (>67% of water in non-fat cheese matter, and 45-60% of fat in dry matter), by the Regulations on the quality of dairy products and starter cultures. This layered cheese has another specificity – it can be stored at temperatures below -20 °C for several years. When the cheese is defrosted, the difference can not be told in the sensory, nutritional, qualitative, and quantitative properties compared to fresh. Shelf life of layered cheese made without cooking of cheese body in terms of keeping in the fridge is 40-60 days, at 4 °C. Microbiological testing done in the JPS Dairy Institute, Belgrade, showed that there was no increase in the number of strains of Listeria, Salmonella, Stafilococci, which are analyzed in fresh cheeses according to the Regulations on the general and specific requirements of food hygiene at any stage of production, and that the cheese is therefore safe to use. It is observed that the microbiological quality of the layered cheese made from raw organic milk is satisfactory, while the absence of the process of milk pasteurization helped retaining the quality of milk as the basic raw material for cheesemaking.*

Keywords: food safety; raw milk; autochthonous cheese; cheese microbiology

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INTRODUCTION

Milk has a high nutritive value, not only for the new-born mammal and for the human consumer, but also for microbes. Raw milk kept at room temperature will be liable to microbial spoilage. After some days, the milk will spontaneously become sour. This is generally due to the activity of lactic acid bacteria. A flora of these bacteria may develop, which can be transferred deliberately to fresh milk in order to maintain or even strengthen it. This principle is the basis for controlled acidification of milk towards products, sustainable and safe, with most often an attractive flavour (Wouters et al., 2001). Raw (unpasteurized) milk can be a source of food-borne pathogens. Raw milk consumption results in sporadic disease outbreaks. Pasteurization is designed to destroy all bacterial pathogens common to raw milk, excluding spore-forming bacteria and possibly *Mycobacterium paratuberculosis*, but some people continue to drink raw milk, believing it to be safe (Steele et al., 1997). Raw or incompletely pasteurized milk can cause infection from several pathogens, most commonly from *Salmonella* and *Campylobacter*, but also from *Escherichia coli* and *Listeria monocytogenes*, among others (Cody et al., 1999).

It is interesting that the three classical methods of food preservation, i.e. fermentation, dehydration and salting, are all exploited in cheese manufacture and in fact are interdependent. The fourth common method of food preservation, i.e. use of high and/or low temperatures, was less widespread than the others because the exploitation of low temperatures was confined to relatively few areas until the development of mechanical refrigeration about 1870 and, although heating was probably used to extend the shelf-life of foods throughout civilization, its controlled use dates from the work of Nicolas Appert in 1794 and Louis Pasteur in 1840. Among the principal effects of salt are control of microbial growth and activity, control of the various enzyme activities in cheese, syneresis of the curd resulting in whey expulsion and thus in a reduction of cheese moisture that also influence previous two effects, physical changes in cheese proteins which influence cheese texture, protein solubility and probably protein conformation (Guinee and Fox, 1993). Hardness, adhesiveness, and initial rate of cheese flow increases, and cohesiveness decreases upon salt injection. However, the final extent of cheese flow or melting is unaffected (Pastorino et al., 2003).

In some of the researches made in the past, it was found that poor quality of silage (pH >4.0), inadequate frequency of cleaning the exercise area, poor cow cleanliness, insufficient lighting of milking barns and parlors, and incorrect disinfection of towels between milking were significantly associated with milk contamination by *L. monocytogenes*. More attention to preparing silage and good milking and barn hygiene are important for diminishing the risks of exogenous contamination of raw milk by *L. monocytogenes* (Sanaa et al., 1993). Some outbreaks demonstrate the ability of drug-resistant *Salmonella* to spread from the animal to the human reservoir and, in a suitable host, produce a fatal infection (Tacket et al., 1985). The overall incidence of *Listeria* spp. in raw milk samples surveyed was found to be 25.0% (*Listeria monocytogenes* 15.3%), with the incidence in samples from processing centres 54.0% (*L. monocytogenes* 33.3%); this was higher than that in samples from dairy farms (*Listeria* spp. 8.8% *L. monocytogenes* 5.3%). *Listeria* spp. were isolated

from 1 of 95 pasteurized milk samples (*L. monocytogenes*) and 1 of 33 soft cheese samples (*L. seeligeri*) (Harvey and Gilmour, 1992).

On the other hand, high heat treatment of milk causes denaturation of whey proteins and complex interactions among denatured whey proteins, casein micelles, minerals and fat globules (Singh et al., 2001). Heat-induced changes in milk are: whey protein denaturation and aggregation, interactions of whey proteins with casein micelles, reaction between lactose and protein (Maillard reactions), changes in casein micelle structure, transfer of soluble calcium and phosphate to colloidal phase, changes in fat globule membrane and a decrease in pH.

Layered cheese was rarely the subject of scientific papers, and hence there is a very limited number of papers on its technology and quality. The earliest work on the technology of layered cheese dates back to 1947 (Zdanovska, 1947). The scientific work is known as "soft" (Dozet et al., 1987), but for the people of Montenegro is known as *Kolašin cheese*, named after the city where it is produced the most, or as layered cheese due to the shape (Rakočević, 1952). The technology of this cheese is specific and different from other autochthonous cheeses from Montenegro. The unique technology is the result of a specific method of preparation of fresh milk for cheese production and the specific ways of coagulation and curd processing (Mirecki et al., 2012). After evening milking, raw cow milk is heated to 40-45 °C, after which it is left to cool down at room temperature for 12 hours, process which increases the milk acidity. After the morning milking, the acidified milk left over night and the fresh milk are mixed in 1:1 ratio. Milk is heated to 38-42 °C. Once the mixture reaches this temperature, rennet is added. Coagulation of milk lasts 30-45 minutes, during which time the milk begins to coagulate, forming concentric circles. Start of syneresis is the first sign for cutting the curd. Curd is cut into 4 equal pieces and allowed to mature for 15-30 minutes at a constant temperature of 40 °C. Ripening of curd promotes syneresis, whey begins to separate on the surface, curd fall to the bottom and it is a sign that it is necessary to gradually separate the whey. While curd ripens in whey its structure becomes smooth, soft and elastic. Such curd is subjected to a combination of pressure and intense frequent bending. Dry salting occurs on the end of this process, and cheese is stored in refrigerator at 4 °C

This layered cheese has another specificity – it can be stored at temperatures below -20 °C for several years. When the cheese is defrosted, the difference can not be told in the sensory, nutritional, qualitative, and quantitative properties compared to fresh. Longest shelf life of layered cheese made without cooking of cheese body in terms of keeping in the fridge is 40-60 days, at 4 °C. Based on the results of analysis of chemical parameters of cheese, it can be observed that the layered cheese made without cooking of cheese body, which was done in these experiments, belongs in semi-fat soft cheeses (>67% of water in non-fat cheese matter, and 45-60% of fat in dry matter), by the Regulations on the quality of dairy products and starter cultures (Official Gazette of RS no. 33/2010). Findings of Mirecki and Ivanović (2013), regarding microbiological safety of this cheese made in Montenegro, showed that microbiological examination revealed an insufficient level of hygiene in the process of preparing and processing this cheese. Due to those results, a research was made to determine the safety of making this type of cheese using sterile laboratory equipment in Dairy Laboratory.

MATERIAL AND METHOD

On the basis of autochthonous layered cheese technology, three variations of cheese are made in the Dairy Laboratory in the Department of Animal Science, Faculty of Agriculture, University of Novi Sad, where samples of cheese were kept in refrigerator on 4 °C, and their shelf life was determined. Five cheeses are made from raw organic cow milk for every of three desired salt contents (0,5%, 1,5% and 3% of salt in dry matter, salt is added after the process of fermentation stopped), for a total of 15 cheeses. Microbiological testing was done in the JPS Dairy Institute, Belgrade. Samples were sent for testing right after the cooling of refrigerated, finished cheese. Strains of *Listeria*, *Salmonella*, *Stafilococci* were analyzed in fresh cheeses according to the Regulations on the general and specific requirements of food hygiene at any stage of production (Official Gazette of RS no. 72/2010). Methods for microbiological testing are the following: horizontal method for detection and enumeration of *Listeria monocytogenes* - Part 1: Detection method SRPS EN ISO 11290-1:2010 - used incubation temperature is 30 °C and 37 °C; horizontal method for the detection of *Salmonella* spp SRPS EN ISO 6575:2008; horizontal method for the enumeration of coagulase-positive *Staphylococci* (*Staphylococcus aureus* and other species) - Part 1: Technique using agar by Berd Parker - used incubation temperature is 37 °C.

RESULTS

Results of microbiological testing done are presented in the following tables:

Table 1. Results of microbiological testing of layered cheese with low salt level (0,5% of salt in dry matter)

Sample size		Testing	Samples		Threshold value: cfu/g(ml)		Results cfu/g(ml)				
		METHOD OF TESTING	n	c	m	M	n1	n2	n3	n4	n5
25	g(ml)	<i>Listeria monocytogenes</i> SRPS EN ISO 11290-1:2010	5	0	must not be found in 25g		not found	not found	not found	not found	not found
25	g(ml)	<i>Salmonella</i> spp. SRPS EN ISO 6575:2008	5	0	must not be found in 25g		not found	not found	not found	not found	not found
1	g(ml)	Coagulase-positive <i>Staphylococci</i> SRPS EN ISO 6888-1:2009	5	2	10.000	1000.000	<10	<10	<10	<10	<10

Table 2. Results of microbiological testing of layered cheese with medium salt level (1,5% of salt in dry matter)

Sample size		Testing	Samples		Threshold value: cfu/g(ml)		Results cfu/g(ml)				
		METHOD OF TESTING	n	c	m	M	n1	n2	n3	n4	n5
25	g(ml)	<i>Listeria monocytogenes</i> SRPS EN ISO 11290-1:2010	5	0	must not be found in 25g		not found	not found	not found	not found	not found
25	g(ml)	<i>Salmonella</i> spp. SRPS EN ISO 6575:2008	5	0	must not be found in 25g		not found	not found	not found	not found	not found
1	g(ml)	Coagulase-positive	5	2	10.0	1000.000	<10	<10	<10	<10	<10

		Staphylococci			00						
		SRPS EN ISO 6888-1:2009									

Table 3. Results of microbiological testing of layered cheese with high salt level (3,0% of salt in dry matter)

Sample size		Testing	Samples		Threshold value: cfu/g(ml)		Results cfu/g(ml)				
		METHOD OF TESTING	n	c	m	M	n1	n2	n3	n4	n5
25	g(ml)	<i>Listeria monocytogenes</i>	5	0	must not be found in 25g		not found	not found	not found	not found	not found
		SRPS EN ISO 11290-1:2010									
25	g(ml)	<i>Salmonella</i> spp.	5	0	must not be found in 25g		not found	not found	not found	not found	not found
		SRPS EN ISO 6575:2008									
1	g(ml)	Coagulase-positive Staphylococci	5	2	10.000	1000.000	<10	<10	<10	<10	<10
		SRPS EN ISO 6888-1:2009									

Legend:

n - the number of units that make up the sample; **c** - number of sample units that produce values between m and M; **m, M** - Minimum and maximum allowed value; **n1, 2,3,4,5** - designation of the units that make up the sample

Table 4. Shelf life comparison of layered cheeses with different salt levels (in days)

	Sample I	Sample II	Sample III	Sample IV	Sample V
Layered cheese, low salt level (0,5%)	33	31	28	30	32
Layered cheese, medium salt level (1,5%)	34	35	32	36	33
Layered cheese, high salt level (3,0%)	37	36	39	37	34



Figure 1. Elastic curd



Figure 2. Layered cheese made without cooking of cheese curd

DISCUSSION

Cheese that was made in this experiment shows good sensory characteristics, cheese body bends well, the flavor is mild and pleasant, scent is milky. Different levels of salt used in the process of making cheese are noticeable. It can be seen that with the rising of salt level from 0,5% to 3,0% of salt in dry matter shelf life can be extended for up to almost a week. Average shelf life for layered cheese with low salt level (0,5%) kept in refrigerator at 4 °C is 30,8 days, for layered cheese with medium salt level (1,5%) is 34 days, and for layered cheese with high salt level (3,0%), the average shelf life is 36,6 days.

Test results of cheese samples are satisfactory according to the section 1.1. and 1.10 of food security criteria and satisfactory according to the section 2.2.3 criteria of hygiene in the production process, defined by the Regulations on the general and specific requirements of food hygiene at any stage of production. This leads to conclusion that using raw milk in highly hygienic conditions of cheesemaking, it is possible to make a product that doesn't pose health risks to human health. All three types of cheese made in this experiment are safe for human consumption.

CONCLUSION

Layered cheese made without the process of cooking of cheese body, produced in this experiment, was made using raw cow milk. Goal was to make safe cheese for human consumption, using traditional technology found in Montenegro. In this experiment, five cheeses are made for every of three desired salt contents (0,5%, 1,5% and 3% of salt in dry matter), for a total of 15 cheeses. Cheese that was made in this experiment shows good sensory characteristics, and bends well. Microbiological testing done in the JPS Dairy Institute, Belgrade, showed that there was no increase in the number of strains of *Listeria*, *Salmonella* and *Stafilococci*, which are analyzed in fresh cheeses according to the Regulations on the general and specific requirements of food hygiene at any stage of production, and that the cheese is therefore safe to use. All three types of cheese made in this experiment are safe for human consumption, and it can be seen that with the rising of salt level from 0,5% to 3,0% of salt in dry matter shelf life can be extended for almost a week. It is observed that the

microbiological quality of the layered cheese made from raw organic milk is satisfactory, while the absence of the process of milk pasteurization helped retaining the quality of milk as the basic raw material for cheesemaking.

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EFFECT OF USING A BROILER DIET WITH DIFFERENT LEVELS OF KTI FREE SOYBEAN TO RETENTION OF NITROGEN

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SUMMARY: Soybean is the most used nutrient in the diet of broiler chickens to meet the needs of protein and essential amino acids. Full use of high quality vegetable protein in soybeans is only possible with the previous heat treatment, which significantly increases production costs. Therefore, in order to reduce the cost of many tests conducted on the possibility of the use of raw soybeans in the diet of poultry, from which it came to the realization that the main carriers of inhibitory activity of raw soybeans protease inhibitors. The study of 42 days duration was conducted to investigate the effect of different levels of the KTI and soybean extrusion of broiler chickens. One day male broiler chicks Ross 308 were measured and divided into 16 boxes, with each box to be 60 chickens. Initial average body weight was uniform and was 42.5 ± 7 g. The experiment included four groups with four replicates. Housing system is a floor with litter and food and water were available ad libitum, with a light regime of 24 hours. Temperature and air flow in the facility are controlled and regulated in accordance with technological standards for hybrid Ross-308th. Dead chicks were removed from the facility daily and mortality was less than 3%. Chickens were fed diets based on corn and soybeans. Starter mixture contained 23% crude protein and 12.65 MJ metabolic energy and was used from 1 to 10 days. Starter mixture consumed all birds involved in this experiment, and it contained 51.77% corn, 24.46% full-fat soybean meal, soybean meal 15:34%, 4% yeast, chalk, mono-calcium phosphate, salt and premix. From the experimental diet was started by the eleventh day after soybean strains with low KTI can be successfully used in the diet of young chicks without heat treatment. Experimental diets were grower and finisher and differed in two groups depending on whether it includes raw or heat-treated soybean standard varietie or KTI free varieties ("Lana"). The lowest level of nitrogen retention by marker method was recorded in the group of SS (39%) and SL of (48%), followed by LG and SG groups nitrogen retention was 55% and 57%. With the total collection method as well as the marker method, the obtained values indicate better nitrogen retention of mixtures in which the soybena was heat treated. It is evident that

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the heat unprocessed soybean variety "Lana" without KTI levels in diets for broilers had better nitrogen retention (in both methods) than the standard variety, but worst than in heat treated soybeans

Keywords: KTI-free ; soybean ; broiler ; nitrogen retention

INTRODUCTION

Soybeans and soybean meal are a staple protein source in livestock diets all over the world. Soybean meal is often referred to as the gold standard to which all other protein sources are compared, but in spite of its virtues, soybean meal is not perfect (Cromwell, 2000). Soybeans and therefore soybean meal contains numerous antinutritional compounds including protease inhibitors, lectins, oligosaccharides, and beta-mannans. New research is being directed to solving some of the problems with the undesirable factors in soybeans and the co-product soybean meal, focusing specifically on antinutritional factors that negatively affect nutrient utilization. To ensure that soybean use continues to be economically feasible in animal feed rations, plant breeders are designing seed compositions that will enhance animal production by increasing nutrient utilization. Raw soybeans are known to contain two separate protease inhibitors: 1) proteins with a molecular weight of about 20,000 Da and a specificity directed primarily against trypsin, known as the Kunitz trypsin inhibitor (Kunitz, 1945), and 2) those that have a molecular weight between 6,000 to 12,000 Da and are capable of The protease inhibitors in soybean, Kunitz trypsin inhibitor (KTI) and the Bowman–Birk inhibitor (BBI) constitute at least 6% of the protein present in soybean seed (Ryan, 1973) and with lectin, represent the main anti nutritional factors of soybeans. Approximately, 80% of the trypsin inhibition is caused by KTI (Brandon, 1993), which strongly inhibits trypsin, and therefore reduces protein digestibility and food intake. Beside that, KTI is responsible for hyper secretion of pancreatic enzymes, leading to demands of sulphur-containing amino acids and along side previous, resulting in growth depression in non-ruminant animals. Due to this, row soybean can not be used for animal feeding and needs to be heat processed to eliminate thermolabile anti nutritional factors. Heat processing inactivates anti-nutritional factors and modifies the structure. In an attempt to cut costs and avoid the heat treatment which in inadequate implementation may also have an adverse effect on the production, selection produced varieties with low levels of inhibitors and compatible for broilers. Hymowitz (1986) found soybean seed with low levels of Kunitz trypsin inhibitors. In the world there are Kunitz free , BBT-free (Bovman-Birck trypsin inhibitor) or lectin-free varieties of soybean under different commercial names. Production results obtained from these varieties of soybean and results of the research are contradictory. It's significant depended of the specific characteristics by the given properties of soybean varieties. Thus, Palacios et al., (2004) found better production results when the of genetically improved soybean varieties subjected to A part of the soybean breeding program at the Maize Research Institute »Zemun Polje« is aimed at developing the cultivar with reduced trypsin inhibitors content. As a result, this Kunitz-free (KTI free) variety Lana, were released. TI content in new cultivars was about 50% reduced as compared with the conventional cultivars (standard grain type). Numerous studies investigated the effect of soybean variety and processing on growth

performance of pigs (Cook et al., 1988; Palacios et al., 2004). They demonstrated that the inclusion of raw Kunitz-free soybean in diet was beneficial in terms of better growth performance compared with conventional cultivars, but still inferior to the growth performance obtained by soybean meal, where the KTI and other anti nutritional factors are inactivated by heating.

In trial with broilers Beukovic et al (2010) examined the effect of soybean variety “Lana” to the size of target organs, and noted hypertrophy of the pancreas in group with raw soybean which containing conventional level of KTI, and a mild form of hypertrophy in the group with KTI-free soybean in diets. Beside KTI in raw soybean there is, and some other thermo-labile anti-nutritive factors that negatively affect to the final weight and target organ size of broilers.

MATERIALS AND METHODS

Animals in experiment and diets

One day male broiler chicks Ross 308 were measured and divided into 16 boxes, with each box to be 60 chickens. Initial average body weight was uniform and was 42.5 ± 7 g. The experiment included four groups with four replicates. Housing system is a floor with litter and food and water were available ad libitum, with a light regime of 24 hours. Temperature and air flow in the facility are controlled and regulated in accordance with technological standards for hybrid Ross-308th. Dead chicks were removed from the facility daily and mortality was less than 3%.

Chickens were fed diets based on corn and soybeans. Starter mixture contained 23% crude protein and 12.65 MJ metabolic energy and was used from 1 to 10 days. Starter mixture consumed all birds involved in this experiment, and it contained 51.77% corn, 24.46% full-fat soybean meal, soybean meal 15:34%, 4% yeast, chalk, mono-calcium phosphate, salt and premix. From the experimental diet was started by the tenth day after soybean strains with low KTI can be successfully used in the diet of young chicks without heat treatment. Experimental diets were grower and finisher and differed in two groups depending on whether it includes raw or heat-treated soybean standard varieties (North America) or varieties with KTI free (Lana). One day before a finisher diet were start, chickens in the experiment were transferred to metabolic cages, which are specially designed for this type of trial research.

Table 1. Structure composition of diet used in grover mixture, %

Ingredients	KTI-free		Conventional level of KTI	
	Raw	Extruded	Raw	Extruded
	(SL)	(LG)	(SS)	(SG)
Maze (%)	49,87	49,95	53,65	53,35
Soybean meal (%)	12,03	11,95	8,22	8,52
Raw Soybean KTI-free (%)	30	-	-	-
Soybean KTI-free extruded (%)	-	30	-	-

Raw Soybean conventional level of KTI (%)	-	-	30	-
Soybean conventional level of KTI extruded (%)	-	-	-	30
Yeast (%)	4	4	4	4
Chalk (%)	1,4	1,4	1,4	1,4
MCP (%)	1,35	1,35	1,37	1,37
Salt (%)	0,35	0,36	0,35	0,36
Premix (%)	1	1	1	1
Ukupno	100	100	100	100

In the experiment, the four groups were fed identical food and between groups was not significant difference. From the eleventh day, began a treatment diet which, containing levels of 30% raw or heat-treated soybean varieties with different levels of KTI (Table 1).

Finisher diet has been shown in Table 2. In the mixture was added a Celite ® 545 marker in concentration of 1%. The Celite ® 545 is an indigestible substance which maintains a constant level during the passage through the digestive tract of animals

Table 2. Structure composition of diet used in finisher mixture, %

Ingredients	KTI-free		Conventional level of KTI	
	Raw	Extruded	Raw	Extruded
	(SL)	(LG)	(SS)	(SG)
Maze (%)	51,71	51,82	55,56	54,36
Soybean meal (%)	9,16	9,08	5,32	6,54
Raw Soybean KTI-free (%)	30	-	-	-
Soybean KTI-free extruded (%)	-	30	-	-
Raw Soybean conventional level of KTI (%)	-	-	30	-
Soybean conventional level of KTI extruded (%)	-	-	-	30
Yeast (%)	4	4	4	4
Chalk (%)	1,4	1,4	1,4	1,4
MCP (%)	1,38	1,35	1,35	1,35
Salt (%)	0,35	0,35	0,37	0,35
Premix (%)	1	1	1	1
Celite ® 545 - marker	1	1	1	1
Tottal	100	100	100	100

Analyse

In the preparatory period, which began a 28-day old chicks were used for equipment and housing conditions. Eating foods containing marker (Celite ® 545) was launched 33 days, during three days was carried out "cleansing" of the digestive tract from the remnants of food that did not contain marker. After the 36th day followed by a collection period, which lasted three days. Once a day was performed collection of feces samples. During collection period All given and rest feed to the birds were measured, all feces also. For calculation of digestibility by Total collection method were used Formula 1.

$$\text{Digestibility (X) \%} = 1 - \frac{\text{excretion (X) kg}}{\text{consumation (X) kg}} * 100$$

Formula 1. Calculation digestibility by total collection method

$$\text{Digestibility (X) \%} = 1 - \frac{M \text{ in diet (X)} * X \text{ excretion (\%)}}{M \text{ in excretion (X)} * X \text{ u diet (\%)}} * 100$$

Formula 2. Calculation digestibility by marker method

The analysis of the nitrogen, was performed in the fresh sample of feces. Marker, acid insoluble ash (AIA), the method is defined by (Vogtman et al., 1975), the samples were annealed and then incinerated during night time (5 hours), after annealing, the ash added hydrochloric acid (2N HCl), which was heated to boiling. The samples were cooked at boiling temperature for 10 minutes in a capped crucible. The hot hydrolyzate is then let in through a filter paper (diameter of 25-30 µm) and washed with hot distilled water. Rinse lasted until all traces were washed of hydrochloric acid, a check is performed litmus indicator. Then, the filter paper and allowed to drain and dry. After the filter paper was completely dried in the first combustion is performed, and in that annealing at 450 ° C overnight (5 hours), the resulting residue is weighed to obtain the amount of acid insoluble ash. As previously described in addition to fecal samples, and the content of food samples were processed.

Statistical analyses

For investigated traits adjusted mean (LSM - Least Square Means) were calculated, and TuckyLSD post-hoc test for significance determination between the groups were performed. within the statistical software STATISTICA 12.

RESULTS AND DISCUSSION

Results given in table 3 shows that the nitrogen retention obtained by using the marker method has a minimum value in the SS group, 39% of the SL group, and the 48%. In the above groups, was not heat treated, and there is significant difference ($p < 0,01$) in the values of the nitrogen retention which has been lower, if compared to the group with the heat treated soybean. The highest value of nitrogen retention observed in the SG group 57% and a slightly lower value of 55% at LG Group , (Table 3).

Retention of nitrogen obtained using total collection had the same arrangement of significance ($p < 0.05$) between heat treated and raw soybean groups. As in the previous method, it's depended of soybean heat treatment presence. Low retention

was observed in the SS group, the value of 46%, and the SL group value was 51%. Between these two groups (there was no heat treatment of the soybean) there is no significant difference ($p > 0.05$) in retention of nitrogen. (Table 3). When it comes to the LG and SG groups between which there is also no significant difference ($p > 0.05$), nitrogen retention was 62% in the LG group and 65% in the SG group. The differences between nitrogen retention, which is obtained if the marker method or the method of total collection of feces is clearly visible, Figure 1. Banaszkiewicz et al , (2009) and Hernandez et al . (2004) have obtained the retention of 62 % , Schneitz et al . (1998) , 48 % , and Mahmood et al . (2006) 69 % . Some studies suggest that a higher concentration of oil in high fat soybean meal results in improved digestibility of amino acids than in comparison with non peeled soybean meal (Cervantes - Pahn and Stein , 2008) . Mentioned above may be due to a slower gastric and intestinal emptying , which allows for extended exposure to proteolytic enzymes (Gentilcore et al . , 2006) . In their study on broiler chickens that had consumed a mixture of soy , which contained 44 % (T1) , 46 % (T2) and 48 % (T3) of crude protein , Gerber et al. (2004) found that chickens in last week had a lower digestibility as the protein content of soybeans increased. Nitrogen retention results ranged from 62.1 % to T1 (Soya in a mixture with 44 % SP) to T3 52.9 % (Soy in mixture with 48 % SP) (Gerber , 2004) . A statistically significant difference when it comes to retention of nitrogen marker method can be associated with the presence of anti-nutritional factors, primarily KTI which prevented proper digestion and thus absorption of protein. But it is evident that the absence of KTI in the SL group where nitrogen retention was better than in the SS group.

Table 2. Retention of nitrogen in diet for broilers with different levels of KTI free soybean, %

	SL	SS	LG	SG
Retention of nitrogen- marker method (%)	48	48	57	60
	50	46	59	56
	52	46	51	56
	43	48	55	57
\bar{x}	48^a	39^a	55^b	57^b
Std.dev	±3,9	±1,2	±3,5	±1,9
Retention of nitrogen – method total collection (%)	54	51	64	69
	49	39	66	63
	48	43	58	62
	51	52	60	65
\bar{x}	51^a	46^a	62^b	65^b
Std.dev	±2,5	±6,3	±3,9	±2,9

a-b, - letter in super script by columns indicates to significant differences, ($p < 0,01$).

a-a, b-b - letter in super script by columns indicates to no significant differences, ($p > 0,05$)

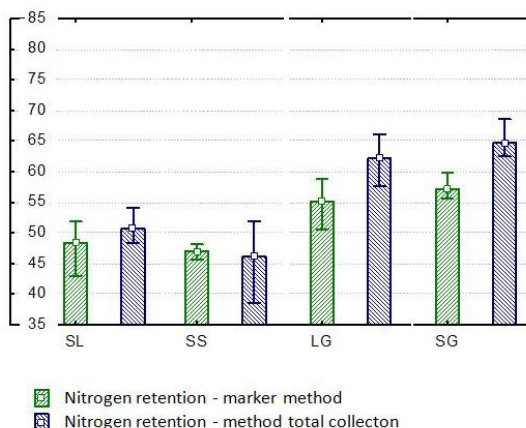


Figure 1. Retention of nitrogen in diet for broilers with different levels of KTI free soybean

CONCLUSION

The absence of heat treatment had a significant effect to nitrogen retention, regardless of whether it is the group that consumed a mixture consist a soybean with conventional level of KTI or KTI free. Observing only a groups without heat treatment (SS and SL), it can be seen better retention of nitrogen in the SL group, but there is no significant differences between these groups. Since it is a soybean variety without KTI significant impact to poor retention of nitrogen in the SL group certainly has contributed to the presence of other heat-labile anti-nutritional factors.

Therefore, it can be concluded that use whole (raw) soybean KTI-free, variety “Lana” is not suitable from the standpoint of nitrogen retention, for the chicken nutrition from 11th day at a concentration of 30% in diet.

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EFFECT OF SELENIUM ON THE INFLAMMATORY RESPONSE OF THE COWS UDDER

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SUMMARY: *Benefits of selenium supplementation to periparturient health have been largely documented in mastitis. The dietary selenium requirement is important for livestock health, and has been associated with a reduction in somatic cell count and the incidence of mastitis. A somatic cell count of 500.000/ml or more indicates an inflammation of the udder. The research was conducted on thirty dairy cows Holstein breed, who received daily 0,3 mg/kg selenium per os. We took samples two times: at first and at sixth lactating months. Mean selenium blood serum concentrations were found to be lower within first lactating month, and then increased in the sixth lactating month. Average somatic cell count at first lactating month was 450.000/ml of milk and at sixth lactating month was 355.000/ml. The aim of this study was to find out the effect selenium blood concentration on the inflammatory response of the cows udder. On the basis of these results it can be conclude that selenium has importance in positive inflammatory response of the cow udder.*

Key words: *selenium, inflammation, somatic cell count, milk, cow.*

INTRODUCTION

Trace minerals play an important role in dairy cow immune function (Spears, 2000), fertility (Rabiee et al., 2010) and growth (Enjalbert et al., 2006). Selenium is recognized as an essential trace element for domestic animals, and it functions in the antioxidant system as an essential component of the glutathione peroxidase, which is responsible for reduction of H_2O_2 and free O_2 to H_2O (NRC, 2001).

As a micronutrient who is involved in the cellular antioxidant system (Engle, 2001; Spears and Weiss, 2006) selenium also plays a vital role in protecting both the intra- and extra-cellular lipid membranes against oxidative damage. The majority of

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this essential element in body tissues and fluids is present as either selenocysteine, which functions as an active center for selenoproteins, or selenomethionine, which is incorporated into general proteins and acts as a biological pool for selenium (Juniper et al., 2006).

Bovine mammary endothelial cells grown in Se-deficient cell culture media were found to exhibit enhanced neutrophil adherence when stimulated with tumor necrosis factor (TNF)- α , IL-1, or hydrogen peroxide (Maddox et al., 1999).

Research in both human and veterinary medicine shows a clear relationship among nutrition, inflammation and disease susceptibility (Calder, 2008; Wood et al., 2009). In humans selenium deficiency can in extreme cases cause severe cardiomyopathy (Kashan disease) and joint abnormalities (Kaschin-Back disease) (Phipps et al., 2008).

Selenium deficiency impairs the antioxidant and immune system, and consequently disease resistance (Grasso et al., 1990; Hogan et al., 1990; Spears, 2000). Thus, its deficiency is associated with impaired growth, fertility and health in farm livestock (Weiss et al., 1990; Phipps et al., 2008).

Selenium deficiency in dairy cows reduced the blood ability (Hogan et al., 1990.) and milk neutrophils (Grasso et al., 1990.) to kill bacteria. Hence, whole blood selenium concentrations were positively correlated with neutrophil adhesion (Cebra et al., 2003).

National Research Council (2001) recommended that the level of selenium in dairy diets is 0,3 mg/kg dry matter and should be closely monitored to ensure that over supplementation does not occur. Selenium supplementation is regulated due to potential intoxication, however, values above recommended levels may provide additional benefits to neutrophil function in postparturient cows (Cebra et al., 2003).

Early studies about benefits of selenium supplementation to periparturient health demonstrated decreased clinical mastitis symptoms, decreased rate of new intramammary infections, enhanced rate of milk somatic cell response with fewer bacterial isolates and more rapid clearance of intramammary infections in selenium supplemented cows compared to those with a deficient diet (Smith et al., 1984; Erskine et al., 1989). Also, high serum selenium concentration was associated with reduced rates of inflammation of mammary gland and lower bulk-tank somatic cell counts in Ohio dairy herds (Weiss et al., 1990).

Blood concentration of selenium or glutathione peroxidase activities in dairy herds have been related to mammary gland health in several studies (Erskine et al., 1987; Weiss et al., 1990; Jukola et al., 1996).

The dietary selenium requirement is important for livestock health, and has been associated with a reduction in somatic cell count and the incidence of inflammation of mammary gland (Weiss et al., 1990; Weiss, 2002).

Somatic cell count measures the amount of leukocytes, such as macrophages, lymphocytes and polymorphonuclear neutrophilic leukocytes. A somatic cell count of 500.000/ml or more indicates an inflammation of the udder (Boboš et al., 1997).

According to the NMC (2011) clinical mastitis is characterized by visible abnormalities in the milk or the udder. The most obvious abnormalities in the milk are flakes, clots, and a watery appearance while subclinical mastitis is inflammation of the mammary gland that does not create visible changes in the milk or the udder.

The aim of this study was to find out the effect selenium blood concentration on the inflammatory respond of the cows udder because of literature lack data about not visible changes in udder tissue.

MATERIAL AND METHODS

The study was performed on thirty Holstein cows approximate same body weight, ages 3 to 5 years and in first to third lactation, and they giving approximately the same amount of milk. All cows were stabling with dry straw for bedding and with *ad libitum* access to potable water, and feed by total mixed ration. The total mixed ration contained maize silage, grass silage, cracked wheat, soyabean meal, rapeseed meal, sugar beet and hay.

The milk samples were taken in two productive periods, in the first and sixth lactating month for somatic cell count. The same sampling were used each time.

Blood samples were taken after the morning milking from the caudal vein by applying the principles of asepsis and antisepsis. Tubes with appropriate needle were used for taking blood. The blood in tubes was left at room temperature for 24 hours to separate the serum. The level of selenium in blood serum was determined by mineralizing 1g of sample in 4 ml of 16 M HNO₃ and 2 ml of 9.8 M H₂O₂ within a closed-vessel heating block system. The solution was further diluted with water and selenium was subsequently determined using inductively coupled plasma mass spectrometry

(Perkin Elmer Elan 6100 ICPMS, Massachusetts, USA).

The findings were evaluated using test of correlation between selenium blood concentration and milk somatic cell count at first and sixth lactating month. The evaluation was performed using Microsoft Excel 2007 software.

RESULTS AND DISCUSSION

Selenium blood serum concentration was measured on 60 samples. The results on selenium blood serum concentration at first lactating month are in table 1. and at the sixth lactating month are in table 2.

Table 1. Selenium blood serum concentration at first lactating month

No. Cows	1	2	3	4	5	6	7	8	9	10
Se conc. ($\mu\text{mol/l}$)	0.276	0.350	0.254	0.303	0.394	0.736	0.202	0.514	0.312	0.83
No. Cows	11	12	13	14	15	16	17	18	19	20
Se conc. ($\mu\text{mol/l}$)	0.801	0.612	0.897	0.776	0.679	0.688	0.769	0.815	0.900	0.242
No. Cows	21	22	23	24	25	26	27	28	29	30
Se conc. ($\mu\text{mol/l}$)	0.302	0.514	0.586	0.264	0.347	0.364	0.759	0.495	0.612	0.628

Table 2. Selenium blood serum concentration at sixth lactating month

No. Cows	1	2	3	4	5	6	7	8	9	10
Se conc. (μmol/l)	0.296	0.353	0.354	0.400	0.494	0.834	0.222	0.714	0.382	0.783
No. Cows	11	12	13	14	15	16	17	18	19	20
Se conc. (μmol/l)	0.842	0.741	0.998	0.896	0.772	0.812	0.869	0.855	0.903	0.242
No. Cows	21	22	23	24	25	26	27	28	29	30
Se conc. (μmol/l)	0.307	0.541	0.658	0.284	0.379	0.446	0.799	0.549	0.643	0.662

The mean estimate of selenium blood serum concentration at first lactating month was 0.536μmol/l and standard deviation was 0.224789. At sixth lactating month of lactation mean estimate of selenium blood serum concentration was 0.601μmol/l, and standard deviation was 0.236800. Mean selenium blood serum concentrations were found to be lower within first lactating month, and then increased in the sixth lactating month.

Values of milk somatic cell count in table 3. and table 4., which were measured on the 60 samples of milk. Table 3. is value of milk at first lactating month while table 4. is value at sixth lactating month.

Table 3. Milk somatic cell count at first lactating month

No. Cows	1	2	3	4	5	6	7	8	9	10
Somatic cell count/ml	570.000	510.000	530.000	490.000	480.000	390.000	540.000	410.000	520.000	320.000
No. Cows	11	12	13	14	15	16	17	18	19	20
Somatic cell count/ml	330.000	410.000	350.000	400.000	380.000	390.000	410.000	420.000	360.000	540.000
No. Cows	21	22	23	24	25	26	27	28	29	30
Somatic cell count/ml	450.000	480.000	430.000	580.000	560.000	530.000	430.000	480.000	420.000	420.000

Table 4. Milk somatic cell count at sixth lactating month

No. Cows	1	2	3	4	5	6	7	8	9	10
Somatic cell count/ml	490.000	420.000	470.000	400.000	390.000	290.000	510.000	330.000	450.000	220.000
No. Cows	11	12	13	14	15	16	17	18	19	20
Somatic cell count/ml	230.000	310.000	170.000	210.000	280.000	330.000	270.000	240.000	160.000	490.000
No. Cows	21	22	23	24	25	26	27	28	29	30
Somatic cell count/ml	460.000	410.000	390.000	490.000	450.000	430.000	280.000	380.000	370.000	330.000

Average somatic cell count at first lactating month was 450.000/ml of milk and at sixth lactating month was 355.000/ml

Table 5. Test correlation between selenium blood concentration and milk somatic cell count at first lactating month

Somatic cell count/ml	Selenium conc. ($\mu\text{mol/l}$)	n=30
320.000-380.000	0-0.24	5
390.000-450.000	0.25-0.49	12
460.000-530.000	0.5-0.74	8
540.000-580.000	0.75-1	5
		-0.89935*
*Negative correlation		

A negative correlation was noticed within blood serum selenium concentration and milk somatic cell count. These results are shown in table 5. The increasing levels of selenium in blood serum decline the number of milk somatic cell count.

Since there is a negative correlation between blood serum selenium concentration and milk somatic cell count it was noticed that increasing levels of selenium in blood serum cause a decline in the number of milk somatic cell count. These results are shown in table 6.

A reduction in somatic cell count and the low incidence of tissue inflammation are present with blood serum selenium concentration with estimate value $0.601\mu\text{mol/l}$.

This is shown in the results of the other authors like Weiss et al. (1990), Weiss (2002), Smith et al. (1984), Erskine et al. (1989), Cebra et al. (2003), Jukola et al. (1996); who claim that selenium have an important influence on reducing somatic cell count and the low incidence of tissue inflammation.

Table 6. Test correlation between selenium blood concentration and milk somatic cell count at sixth lactating month

Somatic cell count/ml	Selenium conc. (µmol/l)	n=30
150.000-240.000	0-0.24	6
250.000-340.000	0.25-0.49	8
350.000-440.000	0.5-0.74	8
450.000-540.000	0.75-1	8
		-0.956*
*Negative correlation		

A negative correlation between blood serum selenium concentration and milk somatic cell count in this rearsrch are similar to results of Cebra et al. (2003) where whole blood selenium concentrations were positively correlated with neutrophil adhesion.

CONCLUSION

There is relationship between blood serum concentration of selenium and incidence of tissue inflammation of dairy cows mammary gland. Since there is a lack of available results in literature about not visible changes in udder tissue it could be concluded that blood selenium concentration has effect on the inflammatory respond of the cows udder.

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PARAGENETIC FACTORS AND THEIR EFFECT ON HOLSTEIN-FRIESIAN COWS MILK YIELD IN FIRST LACTATION

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SUMMARY: *All over the world, dairy cattle production is one of the most important branches of livestock production, both in terms of value, and absolutely the greatest production of proteins of animal origin for human consumption. For these reasons, the aim of this study was to investigate the effect of paragenetic factors (farm, year and season of calving) and age at first successful fertilization on the lactation length, milk yield, amount of total milk fat and milk fat percentage. Investigation of the above mentioned factors is done in Holstein-Friesian heifers that are housed on two farms (farm I of 1182 and farm II of 598), which had first calving in the period from 2002 to 2006. Each year was divided into three seasons. Based on these results, it can be concluded that the year and season of calving had highly significant ($p < 0.01$) effects on all observed milk production properties. Regarding the effect of the farm, it was concluded that the farm has a significant ($p < 0.01$) impact on the total amount of milk yield and total amount of milk fat, while on the length of lactation farm did not had a significant ($p > 0.05$) impact. Regression analysis of dependence between age at first fertile insemination of heifers and mentioned dairy traits recorded negative regression coefficients (length of lactation $b = -0.04$; total amount of milk $b = -1.17$; amount of fat $b = -0.05$). Observed differences related to decreasing the length of the lactation were not significant ($p > 0.05$), as opposed to the total milk amount ($p < 0.05$) and amount of milk fat ($p < 0.01$).*

Key words: *lactation, milk yield, fat content, paragenetic factors*

INTRODUCTION

For the most dairy cows in the world were considered Holstein cows. In the dairy industry, a productive trait directly affects the profitability of the farm. These traits depend largely on the genetic potential of the dam and sire. In addition, in breeding of dairy cows, the most important aims are to obtain a calf in a year and high milk yield from cows.

Original scientific paper / Originalni naučni rad

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One of the basic assumptions of a successful selection work in dairy cattle is to create the greatest possible genetic improvement in animals, in order to increase the volume, the level and effectiveness of their environmental production. Achieving a certain genetic progress in cattle populations do not depends only on the intensity of selection and generation interval, but also from paragenetic factors (Vidović, 2009). Therefore, when assessing the value of cattle we must provide elimination of systemic influences that prevent accurate assessment of genetic variability such as impacts of farm, year, season, as well as many other factors.

Leading environmental factors affecting lactation performance in cattle are farm management, nutrition, lactation turn or the age, year and season in which lactation started. Also, lactation performance in dairy cattle depends upon genetic and environmental factors. Genetic background, climate, diseases, nutrition, year and calving season have been reported to affect milk production and lactation (Msanga et al., 2000; Hansen et al., 2006).

Under the farm influence is included the effects arising as result effects of specific housing conditions, nutrition, care, i.e. the entire management of the farm. All these effects are associated with each other and, as such, they are expressed separately for each farm. As a result of these factors, or different production conditions on the farm there are phenotypic differences between farms. Production conditions on farms are often changing due to changes in nutrition, the appearance of a disease, i.e. changes of the farm technology. How these changes are generally occurring every year we can say that the influence of year has a great impact in assessment of the breeding value of cows. For these reasons, the programs that are used to evaluate the breeding value of cows eliminate the influence of year. Inside every year we have also seasons which significantly affect the total production of milk and dairy properties, due to seasonal fluctuations in climatic factors such as temperature and relative humidity.

Body development and age of heifers at the time of insemination have significant influence in the breeding selection, because only harmoniously built heifers, with solid bone structure and a good constitution is expected to have a good life production. Most authors agree that early introduction of heifers in reproduction allows a longer productive life and increasing the efficiency and profitability of dairy cattle.

Paragenetic factors affecting variability in daily milk yield are widely documented in dairy cattle (Schutz et al., 1990; Singh et al., 2002; Petrović et al., 2009; Cilek, 2009). Paragenetic factors such as year of calving, season of calving and age at calving affect productivity (Essl, 1998; Hansen et al., 2006).

Based on aforementioned, the present research work was designed to investigate paragenetic factors of first lactation length, milk yield, amount of total milk fat and milk fat percentage of Holstein cows raised under Serbian conditions.

MATERIALS AND METHODS

In this paper are used data from the 1780 Holstein-Friesian heifers from two dairy farms in Vojvodina, in the calving period from January 2002, to December of year 2006. After examining the system records, for each cow was determined the age at first successful fertilization, duration of first lactation, milk and milk fat yield and milk fat percentage. To examine the effect of season on the observed characteristics,

the year was divided into three seasons (I season - November, December, January, February; II season - March, April, September, October; III season - May, June, July, August).

For the determination of the significance and impact of some paragenetic influences, general linear model (GLM) was used:

$$Y_{ijkm} = \mu + F_i + G_j + S_k + e_{ijkm}$$

Where is:

Y_{ijkm} = value of characteristics depending on the influence of the i -th farm, j -th year, and k -th season

μ = trait overall mean value

F_i = fixed influence of the i -th farm ($i = 1, 2$)

G_j = fixed influence of the j -th year calving ($j = 1, \dots, 5$)

S_k = fixed influence of the k -th season calving ($k = 1, \dots, 3$)

e_{ijkm} = random error that occurs as the uncontrolled influence of environmental factors

For investigated traits adjusted mean (LSM - Least Square Means) were calculated, and LSD post-hoc test for significance determination between the farm, year and seasons. To test the dependence between age at insemination and the observed properties, multiple linear regression was used with in the statistical software XLSTAT 2013.

RESULTS AND DISCUSSION

In tables 1, 2 and 3 are presented the results of analysis of the effect of paragenetic factors on the production traits of milk production, i.e. least-squares means (LSM), standard errors of the means (SE_{LSM}) and significance of the analyzed effects.

From the results shown in Table 1, it can be seen that the calving year of heifers had significant impact ($P < 0.01$) on the lactation length, the amount of milk and milk fat, and the percentage of milk fat. Also, within the observed years, there were statistically significant differences ($P < 0.01$) between the observed properties. The longest recorded lactations of 402 days were recorded in year 2003, and the shortest was recorded in year 2004 with a statistically significant differences ($P < 0.01$). When it comes to the amount of milk and milk fat, heifers that have calved in year 2002 had the highest amount of milk (7476 kg) and the lowest percentage of milk fat (3.32%), while heifers calved in year 2006 had the least amount of milk (6384 kg), but with the highest percentage of milk fat (3.90%).

The similar results have been obtained by many other authors. Inci et al. (2007) and Zambrano et al. (2006) reported that the effect of calving year on lactation duration was significant. Many researchers (Erdem et al., 2007; Inci et al., 2007; Zambrano et al., 2006) found that effect of calving year on all milk yield traits had significant influence.

When it comes to the influence of calving season, from the data presented in table 2, it can be noted that the calving season had highly significant influence ($P < 0.01$) on the observed milk properties of Holstein-Friesian cows. The best production results were observed in cows in the first calving season (I) with significant differences

($P < 0.01$) which amount in the row 390 days, 7230 kg of milk, 252 kg fat with the recorded percentage of milk fat negligibly lower than in the other two seasons of 3.49%.

Table 1. Influence of year calving on the observed traits

Calvin g Year	Numb er of cows	Lactation length, days		Milk yield (kg)		Fat content			
		LSM	SE _{LS} m	LSM	SE _{LS} m	(kg)		(%)	
						LSM	SE _{LS} m	LSM	SE _{LS} m
2002	395	376.69 ^A	5.61	7475.96 ^A	66.6 5	247.71 ^A	2.19	3.32 ^A	0.01
2003	205	401.98 ^a B	7.78	7217.91 ^a B	85.9 4	249.94 ^B	2.82	3.47 ^{aB}	0.02
2004	335	362.06 ^b C	6.09	7208.32 ^a C	75.3 5	255.02 ^{aC}	2.47	3.55 ^{ab} C	0.02
2005	418	373.08 ^b D	5.44	7273.18 ^a D	63.8 8	261.76 ^{abc} D	2.10	3.60 ^{abc} D	0.01
2006	427	394.67 ^a cd	5.39	6384.49 ^{ab} cd	61.2 4	235.38 ^{abc} d	2.01	3.90 ^{abc} d	0.01
p value		0.0003		0.0000		0.0000		0.0000	
F value		6.493		44.706		22.214		99.45	

The same capital and small letters - a highly statistically significant difference ($P < 0.01$)

The same small and different capital letters - there is no statistically significant difference ($P > 0.05$)

Also in their research Kocak et al. (2007), Inci at al. (2007) and Erdem et al. (2007) come to the conclusion that the influence of calving season was significant and indicated that milk yield was higher in autumn and winter, unlike Bilgiç and Alic (2005) and Pelister et al. (2000) which reported that effect of calving season was non-significant.

Table 2. Influence of season calving on the observed traits

Calvin g season	Numbe r of cows	Lactation length, days		Milk yield (kg)		Fat content			
		LSM	SE _{LS} m	LSM	SE _{LS} m	(kg)		(%)	
						LSM	SE _{LS} m	LSM	SE _{LS} m
I	630	390.14 ^A	4.57	7229.53 ^A	56.06	251.95 ^A	1,79	3.49 ^A	0.01
II	620	388.30 ^B	4.69	7105.98 ^B	56.72	251.29 ^B	1,81	3.54 ^a	0.01
III	530	363.66 ^a b	4.47	6874.92 ^a b	57.27	244.73 ^a b	1,83	3.55 ^a	0.01
p value		0.0000		0.0005		0.0083		0.0003	
F value		10.678		10.047		4.802		8.036	

The same capital and small letters - a highly statistically significant difference ($P < 0.01$)

The same small and different capital letters - there is no statistically significant difference ($P > 0.05$)

Opposite to the year and season of calving, which are significantly influenced ($P<0.01$) by the length of lactation, the farm had no a significant impact ($P>0.05$) when it comes to this feature. Highly significant differences in production traits such as milk yield, milk fat percentage and milk fat, farm has had an influence ($P<0.01$). Larger quantities of milk and milk fat was recorded on a farm I, while the larger realized amount of milk fat percentage was observed in the milk of cows from the farm II, which can be seen from the results shown in Table 3.

Table 3. Influence of farms on the observed traits

Farms	Number of cows	Lactation length, days		Milk yield (kg) /		Fat content			
		LSM	SE _{LSM}	LSM	SE _{LSM}	(kg)		(%)	
						LSM	SE _{LSM}	LSM	SE _{LSM}
I	1182	380.68	3.26	7348.19 ^A	38.15	258.22 ^A	1,21	3.53 ^A	0.01
II	598	379.58	4.58	6526.11 ^a	53.72	231.82 ^a	1,70	3.57 ^a	0.01
p value		0.8440		0.0000		0.0000		0.0163	
F value		0,038		155.63		158.54		5.783	

The same capital and small letters - a highly statistically significant difference ($P<0.01$)

The same small and different capital letters - there is no statistically significant difference ($P>0.05$)

The significant effect of farm on the productive performance of dairy cows could be attributed to the changes in nutrition and managerial systems and environmental conditions which occurred from year to another as well as to differences between years in the quantity and quality of available forage.

With regression analysis (Table 4) was found that the linear regression coefficient (b) had slightly negative values for all the observed features. Age at first insemination of cows had no significant ($p>0.05$) influence on the length of the lactation period, and the percentage of the milk fat, compared to the amount of milk and milk fat ($P<0.05$).

Table 4. Regression analyses and influence of age at heifers first fertile insemination on the observed traits.

Traits	a	b	Equation y	SE _b	r	p-value
Lactation length (days)	357	- 0.04	$y = -0.04x + 357$	0.04	0.03	0.24
Milk yield (kg)	6493	- 1.17	$y = -1.17x + 6493$	0.53	0.06	0.02
Amount of milk fat (kg)	222.3	- 0.05	$y = -0.05x + 222.3$	0.01	0.09	0.00
Percentage of milk fat	3.45	- 0.00	$y = -0.00x + 3.45$	0.00	0.04	0.12

a= intercept; b= coefficient of linear regression; SE_b= standard error of b; r = coefficient of correlation

Erdem et al. (2007) stated that age at calving had no significant effect on lactation duration. On the other hand, the effect of calving age on lactation duration was stated as significant (Ozelik et al., 2000).

CONCLUSION

Based on the obtained results it can be concluded that the variability of characteristics significantly ($P < 0.01$) was affected by farm, year, season and age at first successful fertilization. Regression analysis of dependence between age at first fertile insemination of heifers and mentioned dairy traits was observed negative regression coefficients ($b = -0.04$ length of lactation, milk yield $b = -1.17$; amount of milk fat $b = -0.05$). Observed differences related to shortening of the lactation length were not significant ($P > 0.05$), as opposed to the total amount of milk ($P < 0.05$) and milk fat ($P < 0.01$). Gained phenotypic variability of dairy traits in the observed impacts indicate the feasibility of including these effects in mixed models for the calculation of genetic parameters and breeding value estimation, because in this way the accuracy and reliability of the parameters is gained.

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CORRELATION OF THE EXTRUDED CORN MEAL ON FAT DEPOSITION AND CARCASS QUALITY OF BROILER CHICKENS

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SUMMARY: *The aim of this study was to investigate the effect of extruded corn meal in the diet of broiler chickens, as well as the correlation between the applied thermal extrusion process on the abdominal fat deposition and chickens carcass quality. At the beginning of the experiment, the two treatments were formed, the control (T1) and experimental (T2), with a total of 600 one-day old chicks of hybrid line Ross 308 by a treatment with four replications. During the experimental period which lasted for 42 days, chickens were fed and watered ad libitum. The control group was fed with a standard commercial feed mixture, while the experimental groups were fed with diet with the same amount of corn as well as the control group, provided that in the experimental group corn was extruded. At the end of the experiment, body mass of chickens prior to slaughter was measured and determinate carcasses yield as well as portions of less and more valuable parts of chicken carcasses. Statistical analysis of the data led to the conclusion that the application of heat treatment of extrusion did not have a statistically significant ($p>0.05$) effect on final body weight and carcass yield. With the usage of extruded corn in the chickens diet was observed positive correlations when it comes to weight of thigh (T2: 237.87g; $r = 0.11$), breast (T2: 652.39 g; $r = 0.23$) and liver (T2: 62.29g, $r = 0.19$), and a negative correlation when it comes to mass of legs (T2: 237.41g, $r = -0.06$) and wings (T2: 198.44 g; $r = -0.24$), as compared to the chicks at the treatment T1, with no statistically significant differences ($p>0.05$). When it comes to the deposition of abdominal fat, it can be concluded that the usage of extruded corn in the chickens diet led to statistically significant ($p<0.01$) reduction of abdominal fat in chickens at the T2 treatment (24.77 g; $r = -0.60$) compared with the contents of the abdominal fat of chickens in the control treatment T1 (30.81 g). It can be concluded that the usage of extruded corn in the diet of chickens can be one successful solution for getting chicks with lower content of fat in the carcass, as the production of chicks with increased amount of fat in the carcass increases cost of production.*

Keywords: correlation, extrusion, chickens, nutrition, abdominal fat

Original scientific paper / Originalni naučni rad

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INTRODUCTION

Processing of food represents extremely important activity in developed and undeveloped countries. Due to rapid global population growth, severe food shortages could be faced. To prevent this, food and feed production must be increased through application of new technologies in biotechnology, that is, bio industry. Numerous technological processes aimed at improving nutritional value of food and feed products intended for human and animal consumption and efficiently utilization of primary and processed agricultural and food by products have been developed worldwide. Today, various heat treatment processes are in usage for cereals and other grain processing, such as toasting, extrusion, hydrothermal treatment, micronization, microwave heat treatment, however, according to the practical experience and literature data, extrusion is most commonly used process in our country (Filipović et al., 2010; Lević and Sredanović, 2010; Puvača, 2011). Properly applied heat treatment process has been shown to reduce antinutritional factors to an acceptable level, enhance digestibility of some nutrients such as protein, oil, carbohydrate, and improve sensory properties and microbiological quality of final product. Along with the antinutritional content reduction, thermo labile nutritive components must be preserved, therefore, process need to compromise these two demands. Cereals and milling by products are primary source of energy in animal diets, and major ingredients in commercial feed. Corn is the most commonly used grain in the animal diet production in Serbia due to its high energy content, starch content, relatively high oil content and low fibre content (Kovčín, 1993). In addition to its excellent digestibility, corn is considered to have better flavour than other grains. Thermal treatment is typically used to enhance nutritional, hygienic, physic-chemical and other properties of grains, i.e. to improve nutritive value of some ingredients, upgrade sensory characteristics and inactivate thermo labile antinutrients (Živančev et al., 2010). Extrusion is a heat treatment which involves high temperature/short time principle of extrusion cooking, namely, the process in which material is exposed to high temperature, up to 200°C for short time, up to 2 minutes (Jansen, 1991; Riaz, 2007; Riaz, 2009). Extrusion processing of corn, which is the major raw material in the animal feed production, as well as extrusion of corn dry milling, contributes to improved feed utilization efficiency. During extrusion, carbohydrate fraction of corn meal undergoes changes resulting in starch content decrease due to starch degradation and dextrin production. These changes result in *in vitro* and *in vivo* enhancement of starch digestibility, since starch gelatinization enables improved availability of starch degrading enzymes, and consequently, inactivation of amylase inhibitor. During corn meal extrusion, content of total and reducing sugars is decreased as the result of Maillard reaction, considered as the least desirable reaction in food chemistry and involving sugar-protein interaction, primarily with the amino acid residues of lysine (Filipović et al., 2008; Filipović et al., 2009; Stanačev et al., 2012). At the same time, thermal treatment assures increased oil digestibility of extrudates, although it is followed by the increased susceptibility to the lipid oxidation due to the increase of surface contact with air.

Selection of the broiler chicken for faster growth rate resulted in some negative effects, such as ascites, skeletal abnormalities, and a higher percentage of deposited fat in the carcass (Emmerson, 1997; Ljubojević et al., 2011). The genetic correlation between the abdominal fat and other lipid deposits and the total amount of lipids in the

carcass is very high (Chambers, 1990). In contrast to the internal organs, which size is limited mutual relations and physiological activity, abdominal fat is relatively unrestricted tissue that can dramatically increase or decrease, without the effect on other physiological mechanisms (Deeb and Lamont, 2002). With the increase in poultry production parameters, growth and the need for poultry feed is also increased. Feed presents a major cost in the production of broilers. Definitely, feed ingredients make up the largest part of the cost of feed. However, the cost of feed processing are significant part of the cost of broilers and processing itself, in addition to feed ingredients, and provides the greatest opportunity to influence on broilers performance (Milošević et al., 2006; Wu and Ravindran, 2004; Gracia et al., 2009; Stanačev et al., 2012; Puvača and Stanačev, 2012). Almost every processing of feed can have a positive or negative impact on the performance of the animals and of course can affect the production profitability (Buchanan et al., 2010). Improving the nutritional value of corn is of particular interest, since corn is the dominant energy source in poultry nutrition (Milošević et al., 2006). Extrusion can increase the digestibility of starch, starch becomes accessible as digestive enzymes, resulting in a greater body mass of chickens (El-Halek and Janssens, 2010). Extrusion leads to the formation of complexes of fat and carbohydrates and enhances the stability, ie. preventing the oxidation process through inactivation of lipolytic enzymes (Strugar et al., 2006). In addition to the positive effects of extrusion, in the present research are identified and some negative consequences. The experiment of Moritz et al. (2005), extrusion process has reduced nutritional value of corn in broilers aged from zero to three weeks. In research of Amorntthewaphataet al. (2005), extruded crushed corn has a negative effect on the performance of broilers. Their results showed that the weight gain and feed conversion in poultry can be improved if the extruded corn is pelleted.

The aim of this study was to investigate the effect of extruded corn meal in the diet of broiler chickens, as well as the correlation between the applied thermal extrusion process on the abdominal fat deposition and chickens carcass quality.

MATERIALS AND METHODS

Extrusion of corn

Corn with moisture content of 12%, pre-ground in a hammer-mill, screen size Ø 5 mm, and then moistened for 6 hours to reach 18% moisture, was used for extrusion. The extruder used had throughput capacity 900 kg/h, electric motor power 100 kW and screw feeder driven by 1.1 kW electric motor. Extrusion temperature was 95°C.

Animal trials

Biological experiments were conducted at experimental farm "Pustara" in property of Faculty of Agriculture, Department of Animal Science in Novi Sad. At the beginning of the experiment, the two treatments were formed, the control (T1) and experimental (T2), with a total of 600 one-day old chicks of hybrid line Ross 308 by a treatment with four replications. During the experimental period which lasted for 42 days, chickens were fed and watered ad libitum. The control group was fed with a standard commercial feed mixture, while the experimental groups were fed with diet

with the same amount of corn as well as the control group, provided that in the experimental group corn was extruded (Table 1). At the end of the experiment, body mass of chickens prior to slaughter was measured and determinate carcasses yield, after what then chicken per treatment were sacrificed for portions determination of less and more valuable parts of chicken carcasses.

Statistical analyses

For investigated traits adjusted mean (LSM - Least Square Means) were calculated, and TuckyLSD post-hoc test for significance determination between the groups were performed. To test the dependence between deposited abdominal fat and used extruded corn in broiler nutrition, Pearson's coefficient of correlation was performed within the statistical software STATISTICA 12.

Table 1. Structure and chemical composition of used diet mixtures, %

Feedstuffs and nutrients	Starter		Grover		Finisher	
	0 – 10 days		11 – 28 days		29 – 42 days	
	T1	T2	T1	T2	T1	T2
Corn	47.36	0.00	51.93	0.00	58.14	0.00
Extruded corn	0.00	47.36	0.00	51.93	0.00	58.14
Wheat meal	9.00	9.00	6.00	6.00	5.90	5.90
Soybean meal 44%	28.48	28.48	16.12	16.12	10.77	10.77
Sojbean grits	16.75	16.75	22.35	22.35	21.72	21.72
MCP	1.15	1.15	1.01	1.01	0.92	0.92
Salt	0.33	0.33	0.33	0.33	0.33	0.33
Lime stone	1.56	1.56	1.26	1.26	1.22	1.22
Lizyne L	0.19	0.19	0.00	0.00	0.00	0.00
Methionine DL	0.10	0.10	0.00	0.00	0.00	0.00
Treonine L	0.09	0.09	0.00	0.00	0.00	0.00
Premix	1.00	1.00	1.00	1.00	1.00	1.00
Total	100.00	100.00	100.00	100.00	100.00	100.00
Croude proteins	22.00	22.00	21.00	21.00	19.00	19.00
Crude fat	5.44	5.44	6.43	6.43	6.50	6.50
Crude fibre	4.36	4.36	4.08	4.08	3.82	3.82
ME, MJ/kg	12.65	12.65	13.20	13.20	13.40	13.40

RESULTS AND DISCUSSION

Results given in table 2 shows that the chicken in control group (T1) achieved final body weight of 2612g, while the chicken in experimental group with the extruded corn in the diet mixture have achieved body weight of 2654g, at the end of experimental treatment without statistically significant differences ($p>0.05$). The same tendency was observed when in question was carcass processed classically, then for roasting and for grilling ($p>0.05$), what is normal because of dependence between the body weight of chicken and mass of chilled carcass according to the research of certain authors (Škrbić et al., 2008; Stanaćev et al., 2012). When chickens was separated by the gender in the research of Folasade and Obinna (2009) and Ljubojević

et al. (2011), statistically significant higher final body weight was observed in benefit of male broiler chickens.

Table 2. Chickens processed carcass yields, g

Parameters	Control (T1)		Extruded corn (T2)		p	r
	LSM	SE _{Lsm}	LSM	SE _{Lsm}		
Live body weight, g	2612.00	77.61	2654.00	77.61	0.71 ^{ns}	0.090
Classical processed carcass, g	2127.50	76.62	2146.90	76.62	0.86 ^{ns}	0.042
Ready for roasting, g	1983.50	68.88	2003.00	68.88	0.84 ^{ns}	0.047
Ready for grill, g	1798.50	64.60	1825.00	64.60	0.77 ^{ns}	0.068

p<0.05 - *; p<0.01 - **; p>0.05 – ^{ns}

Table 3 presents the results of share of more valuable parts of carcass such as thigh, drumstick, breast, wings back with pelvis and etc. From the given results it can be seen that statistically significant differences in weight of these economically and nutritive valuable parts of chicken carcasses fed with addition of thermally untreated and thermally treated corn was absent (p>0.05). In investigation of Bogosavljević-Bošković et al. (2006) share of thigh, drumstick and wings was higher at male chickens, compared to the female chickens. From the obtain results in the same table, can be seen the negative correlation coefficients for the mass of thigh (r=-0.06), wings (r=-0.24), head (r=-0.11) and neck (r=-0.05) without statistically significant differences.

Table 3. Weight of more valuable carcass parts, g

Carcass parts	Control (T1)		Extruded corn (T2)		p	r
	LSM	SE _{Lsm}	LSM	SE _{Lsm}		
Thigh, g	241.18	10.22	237.41	10.22	0.80 ^{ns}	-0.06
Drumstick, g	230.14	11.46	237.87	11.46	0.64 ^{ns}	0.11
Breast, g	620.88	21.79	652.39	21.79	0.32 ^{ns}	0.23
Wings, g	205.17	4.48	198.44	4.48	0.30 ^{ns}	-0.24
Back with pelvis, g	469.14	17.06	472.73	17.06	0.88 ^{ns}	0.04
Head, g	52.45	1.75	51.28	1.75	0.64 ^{ns}	-0.11
Neck, g	69.77	1.74	69.23	1.74	0.83 ^{ns}	-0.05
Legs, g	91.23	6.11	92.14	6.11	0.92 ^{ns}	0.03

p<0.05 - *; p<0.01 - **; p>0.05 – ^{ns}

When it comes to the mass of less valuable carcass parts, statistically high significant (p<0.01) differences was observed. Usage of extruded corn in broiler chicken nutrition led to significant reduction in deposition of abdominal fat (p<0.01) in carcass with correlation coefficient of r=-0.60. Investigation of Hopić et al., 1996; Zerehdan et al., 2004; Nikolova et al., 2009; Ljubojević et al., 2011 and Stanačev et al., 2012, led to a conclusion that the deposition of abdominal fat is significantly

affected by chicken gender, and that the female chickens had much more higher amounts of abdominal fat deposited in carcass when it is compared with male chickens. This difference is probably due to differences in metabolism and capacity of poultry for fat accumulation (Ljubojević et al., 2011).

Table 4. Weight of less valuable carcass parts, g

Carcass parts	Control (T1)		Extruded corn (T2)		p	r
	LSM	SE _{LSM}	LSM	SE _{LSM}		
Abdominal fat, g	30.81	1.37	24.77	1.37	0.00**	-0.60
Liver, g	59.69	2.29	62.29	2.29	0.43 ^{ns}	0.19
Heart, g	15.09	0.87	14.17	0.87	0.47 ^{ns}	-0.17
Gizzard, g	39.00	0.47	32.18	0.47	0.00**	-0.92
Edible offals, g	114.18	2.89	108.74	2.89	0.20 ^{ns}	-0.30

p<0.05 - *; p<0.01 - **, p>0.05 – ^{ns}

In opposite to these results, in earlier investigation of Milošević et al. (2007) was recorded that the chicken fed with thermally untreated corn meal deposited less abdominal fat in carcass, compared to the group of chickens fed with the same amount of extruded corn meal in the diet. Usage of extruded corn in nutrition of chicken led to a decrease in weight of heart (r=-0.17), gizzard (r=-0.92) and edible offals (r=0.30) with no statistically significant differences (p>0.05).

CONCLUSION

Based on obtained results it can be concluded that the usage of extruded corn in the diet of chickens can be one successful solution for getting chicks with lower content of fat in the carcass, as the production of chicks with increased amount of fat in the carcass increases cost of production.

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