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Rodrigo PÉREZ-SÁNCHEZ, María Ángeles GÓMEZ-SÁNCHEZ<sup>1</sup>**

## **EFFICIENCY OF GARDEN WASTE COMPOST TEAS ON POTATO GROWTH AND ITS SUPPRESSIVENESS AGAINST *RHIZOCTONIA***

### **SUMMARY**

Compost teas are organic solutions obtained by the fermentation of compost in a liquid phase for a few days, with or without aeration. The use of these teas in agriculture is emerging for supplementing or substituting fertilizers and for their ability to suppress soil-borne pathogens. In this study physical and chemical characterization of garden waste compost tea, its application effects on potato growth and its suppressive effect against *Rhizoctonia solani* were analyzed. N and K content were relevant (3200 and 3848 ppm, respectively). Humic acid level was 190 mg L<sup>-1</sup>. Field trials were carried out with three fried industrial cultivars (Agria, Hermes and Lady Amarilla) in soils affected by *Rhizoctonia* located in Rasueros municipality (Avila, Spain) during 2017. Different dosages of compost tea (d1:1l and d2:3l per plot-7.5m<sup>2</sup>) were applied in experimental potato crops, in order to evaluate growth and production parameters (plant height, SPA units, shoots number, yield, tuber size and fried quality) and the *Rhizoctonia solani* control (attack severity). The application of the dosage 1 (1333,3 l ha<sup>-1</sup>), with respect to control, increased the yield (9,47%), improved the culinary quality (40 %) and reduced the *Rhizoctonia* incidence (12,4-23,7%). These results show that the use of garden waste compost tea can be of great interest to organic and sustainable agriculture.

**Keywords:** *Solanum tuberosum*, Organic fertilizer, Biological control, *Rhizoctonia solani*.

### **INTRODUCTION**

Nowadays, potato (*Solanum tuberosum* L.) is the fourth important crop in the world (FAO, 2016) and millions of people depend directly from it for their food, being a staple in the diet, and driving the rural economy of entire regions in the world, especially in Latin America. This crop suffers from the attack of several diseases that affect its production, being one of the main *Rhizoctonia solani* Kühn, a fungus causing losses of up to 30% of the production, with the consequent reduction in economic yield and loss of food for the population. This

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fact combined with the current limitation of active materials for phytosanitary control and the new tendencies towards an increasingly ecological agriculture, shows that there is a serious problem at a global level. It will be necessary to find new forms of phytosanitary control of the disease, maintaining the yields and the quality of the crop and at the same time respecting the environment.

Compost teas are organic solutions obtained by the fermentation of compost in a liquid phase for a few days, with or without aeration. Generally, extracts are prepared by mixing mature compost with tap water in the ratios of 1:5 to 1:10 (v/v) (Al-Dahmani *et al.*, 2003). The use of these teas in agriculture is also emerging because of their ability to suppress a wide range of both soil and airborne pathogens (Martin, 2014). Morales-Corts *et al.* (2018) demonstrated the high potential of garden waste aerated compost and vermicompost teas on tomato growth and the suppressive effect on *R. solani* and *F. oxysporum* f. sp. *lycopersici* by “*in vitro*” and “pot” essays. Gomez-Sánchez *et al.* (2017) indicated the suppressive effect on *R. solani* produced by the same compost tea applied in potato crop grown in pot.

These studies support the use of garden waste compost teas as potential alternatives to the application of synthetic fungicides, and as plant promoters in crop production, for attaining environmental sustainability for farming and food safety. Reeve *et al.* (2010) also indicate the potential of compost teas for supplementing or substituting other types of fertilizers also seems promising, but further testing under both greenhouse conditions and in the open field is still required.

In this sense, the aims of this study were to carry out the physical and chemical characterization of garden waste compost and to analyse its application effects on potato growth and suppressive effect against *Rhizoctonia solani* in field conditions.

## MATERIAL AND METHODS

### Preparation of compost teas

Compost was based on green and pruning residues which came from gardens in the province of Salamanca (Spain). Most of the material collected consisted of the leaves and stems of different Cupressaceae species and grass clippings. The composting process was carried out using aerated-piles measuring 15 m by 2 m (sides) and 2 m in height. The piles were turned twice per week over 8 weeks and once a week during the rest of the bio-oxidative process. Pile moisture was controlled weekly and the composting process lasted 180 days. Then, compost was mixed with tap water in a ratio of 1:5 (v/v) in polyethylene non-degradable 1000 L containers at room temperature for brewing period lasting 5 days. Water had been previously aerated for 8 h to reduce the amount of chlorines present in it. The mixtures were aerated using an aquarium pump (4 h every day). Next, the liquid was filtered through a double layered cheesecloth to obtain the aerated compost tea which was stored in dark polyethylene containers at room temperature until use.



### **Analytical characterization of compost tea**

The pH and electrical conductivity (EC) were determined by using a CRISON pH-meter and a CRISON EC-meter ( $\text{dS m}^{-1}$ ), respectively. Total N was determined by a LECO-device analyser.  $\text{P}_2\text{O}_5$ ,  $\text{K}_2\text{O}$ , S, Ca and Mg were analysed by a HANNA HI 993310 photometer. Humic acids were determined using the alkali/acid fractionation method following the procedure indicated by Pant et al. (2012). Nine samples of the compost tea were analysed and the means of the parameters were calculated.

### **Potato production assays**

Field assays were carried out in soils affected by *Rhizoctonia* located in Rasueros municipality (Avila, Spain) during 2017. The growing region is 800 m above sea level and has an annual average temperature of  $12.2^\circ\text{C}$  and an annual precipitation of 375 mm (continental mediterranean climate). The value of the soil pH is 7.7 and materia organic content 1%. Three fried industrial cultivars were employed for the assays: Agria, Hermes and Lady Amarilla. Their sensibilities to *Rhizoctonia* are different. Hermes cultivar presents some resistance to *Rhizoctonia* attack, Agria is a sensible cultivar and Lady Amarilla is catalogued like medium sensibility cultivar.

The assay was established on April 1, using a random block design with three plots per treatment and cultivar. Twenty eight plants per plots ( $7.5 \text{ m}^2$ ) were sown in a disposition of 35 x 75 cm. Potatoes were collected on August 20. Different dosages of compost tea were applied as treatments in experimental potato crops (Control: no application, T1:1l per plot, T2: 3l per plot) in order to evaluate growth and production as well as the effect on the *Rhizoctonia solani* control (attack severity).

The first tea treatment was just applied into sowed line. The followings applications were carried out by pulverization each two weeks. Only a common pre-sow fertilization was practised and no phytosanitary products either post-sow mineral fertilizations were applied.

Analysed parameters were: Plant height (cm), chlorophyll content (SPAD-502) and shoots number after three weeks from planting. Yield (kg/plot), tuber size (mm) and fried quality were evaluated at the end of the essay. The quality for fried was assessed by simulating the industrial frying process using oil at  $178^\circ\text{C}$  for 3 minutes and determining the percentage of darkened potato slices.

Plants were watered by aspersion system when needed. Differences between treatments were determined by ANOVA and where significant differences were found, a Tukey range test ( $p < 0.05$ ) was also carried out.

## **RESULTS AND DISCUSSION**

Results of analytical characterization of compost tea are shown in Table 1. It is pointed that compost tea presents essential nutrients for growing plants. This fact was also described by Pant et al. (2012) who obtained similar EC values in different compost teas. pH was similar than others compost teas obtained from

variable materials (Martínez, 1996; Bollo, 1999; Masciandaro *et al.*, 2000). It is important to note that the N and K levels make this tea potentially interesting as fertilizer for growing crops. Segarra *et al.* (2009) supported this finding, indicating that compost tea prepared from garden wastes was rich in inorganic salts.

Table 1. Physic-chemical composition of compost tea.

pH	CE (dS/m)	N (ppm)	P <sub>2</sub> O <sub>5</sub> (ppm)	K <sub>2</sub> O (ppm)	S (ppm)	Ca (ppm)	Mg (ppm)	Humic acids (% ms)
7.16	1.2	2,240.4	61.4	2,851.2	20	280	20	10.3

However, other researchers such as Tognetti *et al.* (2005) obtained higher values of EC and nutrients due to the nature of the materials used for composting. In our study the content of humic acids is higher than the teas analyzed by Pant *et al.* (2012).

With respect to the potato production assays, Table 2 shows the results of the compost tea applications over the three cultivars.

Table 2. Growth effect on potato plants and attack severity of *Rhizoctonia solani* diseases using compost tea.

Treatments	Yield (Kg/ha)	Shoot High (cm)	Nº of shoots	Chloroph. content (SPA units)	Tuber Weight (Kg)	Tuber Size (mm)	Number of tubers per plant	Frying defects %	<i>Rhizoctonia</i> Attack severity
Agria Control	71,373a	9.25a	1.16a	53.41a	0.22a	73.82a	9.73a	46.67b	11.9
Agria T1	79,524b	18.3b	2.75b	51.5a	0.304b	96.38b	7.85a	7.26a	0
Agria T2	83,111b	20.5b	1.83b	53.8a	0.332b	92.38b	7.51a	8.67a	0
Hermes Control	85,387a	13.91a	2a	42.85a	0.207a	71a	12.37b	13.55b	4.76
Hermes T1	85,032a	24.33b	3.33b	44.63ab	0.281b	85.22b	9.08ab	0a	0
Hermes T2	92,098ab	20.41b	3.08b	50.36b	0.288b	85.6b	9.59ab	6.31a	0
Lady Amarilla Control	73,307a	13.75a	2.41a	50.95a	0.185a	76.83a	11.89a	15.55b	28.56
Lady Amarilla T1	72,529a	24.83b	4b	52.51a	0.232b	89.34b	9.38a	7.1a	1.19
Lady Amarilla T2	76,338ab	25.25b	4.66b	53.03a	0.222b	83.13b	10.32a	9.75a	2.38

\*Different letters in the same column per cultivar indicate significant differences (p<0.05)

As it is showed in Table 2, both T1 and T2 significantly increased control productions in Agria and Hermes cultivars. Moreover, the compost tea increased potato mean yield by 9.47% for the T2 and by 3.32% for the T1 dose. Even, in the Agria cultivar was got an increase value of 16.44% for T2. This fact reached up to 11 tons more production per hectare. It can also be observed a major number on stems in plants with compost tea application than in control.

Although it did not increase the number of tubers, the compost tea got up the tuber size and weight. Concretely, the tuber size was higher than control by 22.29% for T1 and by 17.96% for T2. The increase of tuber weight was significantly higher than control for the both compost tea dosages. Likewise, the improvement in potato weight reached on average of 68.33 and 76.67g for the T1 and T2, respectively. The effect as fertilizer of compost tea can be a reference for its use in ecological and conventional agriculture.

On this way, the analysis of the growth effect on tomato plants clearly indicates that compost tea when applied every two weeks produce a positive effect on tuber caliber, number of shoots, yield and fried quality compared to that on control plants. This improvement by using compost teas corroborates previous studies (Hargreaves et al., 2009; Marín et al., 2014). Pant et al. (2009; 2012) found a positive influence on the growth of *Brassica rapa* with the minerals of compost tea. This finding is in agreement with our results in which N, K levels together humic acids composition could be the principal explanations for the growth effect on potato plants.

In Figure 1, the results of the culinary tests are showed. It is observed a decrease in the level of average frying defects of 20.47% for T1 and 17.01% for the T2 with respects to the controls. This fact is especially important in Agria cultivar in which a reduction in the percentage of frying defects of 40% was achieved.

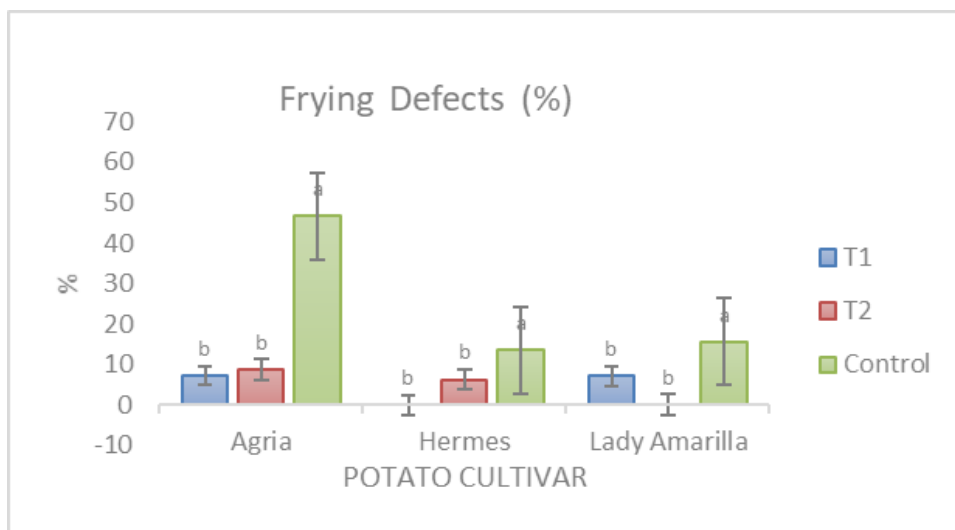


Figure 1. Percentage of frying defects in potatoes treated with compost tea.

The attack severity of *Rhizoctonia* is showed in Figure 2. Lady amarilla control suffered an important incidence which was reduced by application of compost tea both T1 and T2 dosages. Also, *Rhizoctonia* affection was controlled for Agria and Hermes cultivars where compost tea was applied (0% infected plants). Gómez-Sánchez *et al.* (2017) also confirm the effect of compost tea on controlling the pathogen in pot-trials. These authors consider that the effect is caused by the microbiological activity of garden waste compost tea.

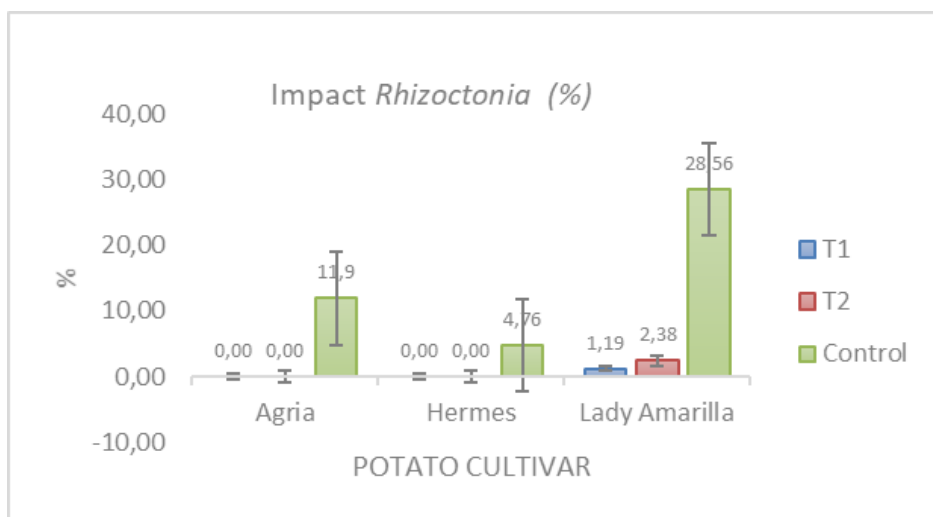


Figure 2. Impact of *Rhizoctonia solani* on potato cultivars treated with compost tea

Positive control of the pathogen was reported by Weltzien (1989) using composted organic materials, particularly green residues. Authors, such as Suárez-Estrella *et al.* (2012) and Tian and Zheng (2013), analysed in vitro the suppressive effect of different compost teas. The biocontrol of different crops has been studied (Tateda *et al.*, 2012 and Pane *et al.*, 2013), wherein the level of pathogenicity of *R. solani* was reduced using different compost teas.

Diáñez *et al.* (2007) reported that lignocellulosic wastes induce specific suppression of *R. solani* by *Trichoderma* spp., which are often present in garden waste mature compost. Additionally, Krause *et al.* (2001) related the suppression of *R. solani* to the presence of microbial antagonism in the compost. These results are in line with our study in which a clear suppressive effect on *R. solani* was obtained compared to the controls when using T1 and T2 dosages of compost tea.

## CONCLUSIONS

The obtained results show that the application of compost tea in potato culture can be of great interest as a biofertilizer and for the *Rhizoctonia solani* control.

The waste of gardening can go from a residue to a very valuable resource for using in agronomy by composting and their after obtaining a tea. These trials should be repeated in a new campaign to corroborate the results.

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## EFFECT OF THE 1BL.1RS WHEAT-RYE TRANSLOCATION ON QUALITATIVE TRAITS IN BREAD WHEAT

### SUMMARY

Despite the positive effect on yield and resistance under stress conditions, cultivars carrying the 1BL.1RS wheat-rye chromosome translocation have a critical drawback, i.e. the deterioration of the end-product quality of the host cultivar. In order to study this deterioration, four bread wheat cultivars carrying and six without the aforementioned translocation, were evaluated in the field for two successive years. The experiments were established in the farm of the Western Macedonia University of Applied Sciences, which represents a rather cold and wet area. Each experiment consisted of four replications and every effort was made to grow the plants under optimum conditions. The following traits were studied: yield, 1000 kernel weight, hectoliter weight, protein %, moisture %, starch %, Zeleny test.

The results indicated that there was not any specific effect of the translocation on yield because two cultivars without the translocation were ranked first, although they did not differ from the following two cultivars carrying the translocation. On the other hand, there was no negative effect of the translocation in most of the qualitative traits in bread wheat. Furthermore, one of the cultivars carrying the translocation (cvr. Acheron) performed equally sufficient with the cultivars without the translocation in 1000 kernel weight. A similar performance was observed in the rest of the examined traits: cvr. Acheron was ranked first in protein content, wet gluten and Zeleny test. Cultivar Elissavet, also carrying the translocation, performed equally well with Acheron in protein content (%) and was ranked first in hectoliter weight. It could be concluded from all the aforementioned results that the translocation had no negative effect on bread wheat quality. However further study is needed to confirm the above results.

**Keywords:** yield, quality, 1000 kernel weight, protein, gluten, Zeleny test.

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## INTRODUCTION

One of the main problems in releasing new cultivars is that this new germplasm has to be grown on marginal environments due to the irrational waste of territorial resources noticed in previous years. Furthermore, the drought conditions prevailing in spring is the crucial obstacle of agricultural production in the southern regions of Europe (Yau and Saxena, 1997). Thus, one of the most decisive factors in all breeding programs is the identification and integration of genes into cultivated varieties that confer resistance or tolerance to drought (Blum, 1988). This led breeders and especially those working on wheat, to look for new gene pools to face the problem (Fehr, 1987). According to various reports bread wheat (*Triticum aestivum* L em Thell) cultivars carrying the 1BL.1RS wheat-rye chromosome translocation are characterized among other traits by high yield potential (Kim *et al.*, 2004; Xynias *et al.*, 2007) and resistance to drought (Hoffmann, 2008). The 1BL.RS translocation is originated from cv. Kavkaz/Cgn, and according to Weng *et al.* (2007) possesses resistance genes to different biotic and abiotic stress conditions. The unique traits of the translocation are attributed to genes located on the short arm of the first chromosome of rye (Schlegel and Meinel, 1994; Xynias *et al.*, 2007).

Despite the previously mentioned advantages of the 1BL.1RS translocation, there is a serious disadvantage: the short arm of the first chromosome of rye genome carries genes that decrease the quality of the end product (Graybosch *et al.* 1993; Fenn *et al.* 1994). There are certain parameters that determine quality in bread wheat: the 1000 kernel weight mark the maturity of the seeds, the hectoliter weight is an essential index of flour production seeds. Also, the protein content is desirable to be high enough and the same holds for the wet gluten content.

The aim of the present study was to investigate the effect of the 1BL.1RS wheat-rye chromosomal translocation on six qualitative traits and elucidate how they affect the performance of the host plants.

## MATERIAL AND METHODS

### Plant material

For the purpose of this study nine Hellenic bread wheat cultivars (eg. Acheron, Elissavet, Orfeas, Apolonia, Acheloos, Vergina, Doirani, Nestos and Strymonas) that were developed at the Cereal Institute of Thessaloniki (Anonymous, 1985) and the Russian cultivar Kavkaz/Cgn, one of the donors of the 1BL.1RS wheat-rye chromosome translocation (Xynias *et al.*, 2006; Weng *et al.*, 2007), were used. Three of the Hellenic cultivars were found to carry the 1BL.1RS wheat-rye chromosome translocation (cvs. Acheron, Elissavet and Orfeas) whereas the other six cultivars, were not carry this specific translocation (Xynias *et al.*, 2006; Peros *et al.*, 2015).

### Method

The experiments were established for two successive years 2015-16 and 2016-17 in the main Farm of the School of Agricultural Technology & Food



Technology and Nutrition, in Florina (40°46' N, 21°22' E, 707 m asl), in a sandy loam soil with pH 6.3, organic matter content 14.0 g kg<sup>-1</sup>, N-NO<sub>3</sub> 100 mg kg<sup>-1</sup>, P (Olsen) 50.3 mg kg<sup>-1</sup> and K 308 mg kg<sup>-1</sup> and water holding capacity 21.8% (0 to 30 cm depth). Seedbed preparation included mouldboard plough, disc harrow and cultivator. Nitrogen and P<sub>2</sub>O<sub>5</sub> at 80 and 40 kg ha<sup>-1</sup>, respectively, were incorporated into the soil as diammonium phosphate (20-10-0) before sowing. The crop was kept free of weeds by hand hoeing when necessary. The 1000 kernel weight was estimated by the mean weight of four random samples of 100 seeds and hectoliter weight using a hectolitre balance. The traits grain protein content, starch percentage, wet gluten and Zeleny value were measured with the Infratec 1241 Near Infrared Transmittance Grain analyzer (Foss, Denmark) using the standard grain network model for quality traits in wheat. A seed sample size of approximately 350 g was used. The NIT instrument analyses subsets of each sample (10 aliquots) before registering an average output reading.

The Randomized Complete Blocks (RCB) experimental design was applied (fixed model), with four replications (Steel and Torrie, 1960). The plots were consisted of five rows (plot area 3 m<sup>2</sup>) of which the three inner were threshed (harvest area 1.8 m<sup>2</sup>).

The means were compared according to the L.S.D. method. The data obtained were analyzed statistically with Mstat-C (Freed and Eisensmith, 1986).

## RESULTS AND DISCUSSION

No significant superiority of the cultivars with the translocation over the respective without was observed in yield (Table 1).

Table 1. Analysis of variance of bread wheat with and without the 1BL.1RS wheat-rye chromosomal translocation regarding yield and six qualitative traits.

Source	df	Yield	1000 kernel weight	Hectoliter	Protein %	Starch %	Wet gluten	Zeleny test
		MS	MS	MS	MS	MS	MS	MS
Environment	1							
Factor (A)	9	**	**	ns	ns	ns	ns	ns
E x A	9	**	*	ns	**	ns	**	**
Error	54							
CV		15.8	7.37	17.55	12.08	49.42	7.77	15.25

Source: Author's elaboration based on the obtained results.

\*,\*\* significant differences at  $p \leq 0.05$  and  $p \leq 0.01$  respectively

All the examined cultivars were classified into four groups according to their yield performance (Table 2). The cultivar (cv Orfeas) with the translocation, did not perform well and along with cultivar Yecora (a cultivar sensitive to low

temperatures) were ranked in the last position. The top group, marked as "A" consisted of three cultivars with and three more without the translocation.

Table 2. Ranking of the bread wheat cultivars according to yield and 1000 kernel weight.

Cultivar	Yield	Cultivar	1000 kernel weight
Acheloos	a	Apollonia	a
Doirani	a	Strymonas	a
Elissavet	a	Acheron	ab
Acheron	a	Nestos	ab
Apollonia	ab	Doirani	b
Kavkaz/Cgn	ab	Acheloos	b
Strymonas	bc	Kavkaz/Cgn	b
Nestos	c	Yecora	c
Orfeas	d	Orfeas	c
Yecora	d	Elissavet	c
LSD	135.2		4.77

Source: Author s' elaboration based on the obtained results

Cultivars followed by different letters are significantly different at  $p=0.05$  level

According to this observation one could conclude that there is no any visible effect of the translocation on yield performance. This is in disagreement with the results of Kim *et al.*, (2004) who reported a positive effect of the translocation on yield. The different performance of the cultivars carrying the translocation could be explained by the proposal of Lisova *et al* (2005) and Lazaridou *et al.* (2017) who studied the effect of the translocation on disease resistance and another culture response. They both concluded that the presence of the translocation does not guarantee any advantage and that the genetic background of the host cultivar is also important.

Regarding the qualitative traits, differences were recorded only in 1000 kernel weight (Table 1) whereas no significant differences were obtained in all other qualitative traits. In this case the cultivars were classified into three groups according to their 1000 kernel weight value: the top, marked as "A" consisted of three cultivars without and only one with the translocation (Table 2), cv Kavkaz/Cgn was classified last in the second group (marked "B") and the other two cultivars carrying the translocation were ranked in the last (marked "C"). The above results suggest the genetic background of the host cultivar is also important and that there is not any negative effect of the presence of the translocation on the quality of the host cultivar. Cultivar Acheron who was ranked in the top group and performed well in yield and 1000 kernel weight is a quite interesting cultivar, since it was found to respond well and in another culture (Lazaridou *et al.* 2017).

## CONCLUSIONS

The results of the present study suggest that there is no negative effect of the presence of the 1BL.1RS wheat-rye translocation on the qualitative traits studied. However, due to the fixed model used, this conclusion is valid only for the cultivars and environments studied.

For this reason, more research is needed in order to use the random model and become to conclusions that apply generally to all environments and cultivars despite the presence of the translocation.

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## **AUTOZYGOSITY ISLAND RESULTING FROM ARTIFICIAL SELECTION IN SLOVAK SPOTTED CATTLE**

### **SUMMARY**

The aim of this study was to identify the runs of homozygosity (ROH) segments within genome and to identify regions significantly affected by artificial selection in Slovak Spotted cattle. In total of 85 animals representing the nucleus of Slovak Spotted cattle were included in present study. The sampled population consisted of 37 AI sires and 48 dams of sires that were genotyped by using two platforms, Illumina BovineSNP50v2 BeadChip and ICBF International Dairy and Beef v3. The consensus map file, constructed to identify common SNPs within these platforms, consisted of overall 40,033 markers. Subsequently, all of loci with call rate lower than 90% and minor allele frequency lower than 0.01% were removed. The final database included genotyping information for 37,833 SNPs. The ROH segments were defined as genomic regions with 15 or more consecutive homozygous calls at density of one SNPs on every 100 kb and maximum gap between consecutive SNPs of 1 Mb. One heterozygous call was allowed for length >16 Mb. In addition, one missing call was allowed for length >4 Mb, 2 for >8 Mb and 4 for >16 Mb. The subsequent analyses of genome-wide selection signatures were based on the assumption that the most frequent homozygous regions in population (minimum ROHs with length 4 Mb) reflected the recent selection characteristic for Slovak Spotted breed. As expected due to the dual-purpose character of this breed the strongest signals of artificial selection was found direct or very close to genes associated with milk production and beef quality.

**Keywords:** Autozygosity, cattle, genotyping data, selection signatures.

### **INTRODUCTION**

Domestication followed by breed formation and selection schemes has allowed the formation of very diverse cattle breeds that are adapted to a wide variety of environments and specialized for either milk or meat production or raised as dual-purpose breeds in different regions of the globe. Natural and artificial selection strategies is likely to impose pressure on specific genome regions that control these traits (milk and meat production) as well as other

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important characteristics such as adaptation to different environments, reproduction, body conformation, behavior and resistance to diseases (de Simoni Gouveia *et al.*, 2014; Brito *et al.*, 2017). Thus, intensive selection pressure then resulted in specific changes in the patterns of variation across loci in particular genomic regions controlling traits of interest as well as in neutral loci linked to them (Zhao *et al.*, 2015). Such unique genetic patterns left behind in the genome of individuals under natural and/or artificial selection are defined as signatures of selection (Qanbari and Simianer, 2014).

The detection of selection signatures is a relevant topic since it has the potential to elucidate the identities of genes and mutations associated with phenotypic traits even if they are no longer segregating within any of the populations of interest and does not necessarily require phenotypes measures. Furthermore, this knowledge is important in order to better understand the evolution process and the mechanisms that underlie traits that have been exposed to intensive natural and artificial selection (Brito *et al.*, 2017).

In recent years, the availability of high-density genomic data and parallel progress in statistical techniques have allowed the identification of genomic regions that have been subjected to natural and artificial selection in cattle (Gutiérrez-Gil *et al.*, 2015). One of the methods that can be used to determine the impact of artificial selection on the genome architecture is the analysis of ROH segments distribution in the genome. Runs of homozygosity (ROH), defined as contiguous homozygous regions of the genome where the two haplotypes inherited from the parents are identical, reflect essentially the autozygosity of an individual (Ferenčaković *et al.*, 2013; Curik *et al.*, 2014). The length and frequency of ROH can be used for example to describe the history of the population in which an individual occurs, to describe the trend of increase in inbreeding within population or to reveal recent population bottleneck (Szmatola *et al.*, 2016). Moreover, it has been showed that the distribution of ROH islands in the genome isn't random and due to the various selection events the ROH are distributed and shared among related individuals (Zhang *et al.*, 2015). Because of this and based on the fact that the genomic regions sharing ROH potentially contain alleles associated with genetic improvement in cattle, they can be used to identify the genomic regions under the strongest selection pressure (Marras *et al.*, 2015; Peripolli *et al.*, 2018).

The aim of this study was to determine the impact of artificial selection on the Slovak Spotted cattle genome based on the analysis of genome-wide ROH distribution and to identify genomic regions resulting from the intensive selective breeding for traits of interest during the development of this breed.

## MATERIAL AND METHODS

In total of 85 animals representing the nucleus of Slovak Spotted cattle were included in present study. The sampled population consisted of 37 AI sires and 48 dams of sires that were genotyped by using two platforms, Illumina

BovineSNP50v2 BeadChip (sires) and ICBF International Dairy and Beef v3 (dams), in commercial lab.

The subsequent quality control of SNP genotyping data was performed by using PLINK 1.9 (Chang *et al.*, 2015). The quality control was carried out to remove all of SNP markers assigned to unmapped regions or with unknown chromosomal position according to the latest bovine genome assembly (Btau 4.0) and SNPs positioned to sex chromosomes. In next step, because of the two different genotyping platforms used for animals' genotyping, the consensus map have to be constructed.

The final consensus map file consisted of 40,033 markers. In the subsequent SNP pruning only samples with lower than 10 % of missing genotypes, autosomal SNPs with call rate higher than 90 % and minor allele frequency higher than 1 % that adhered to mendelian inheritance patterns were retained. The final database included genotyping information for 37,833 SNPs.

The ROH segments were defined as proposed Ferenčaković *et al.* (2013) as genomic regions with 15 or more consecutive homozygous calls with maximum gap between consecutive SNPs of 1 Mb and minimum density of one SNPs on every 100 kb. Because of the theoretical relationship between the distribution of identity by descent (IBD) fragments and the number of generation since common ancestor the minimum length of ROH segments was set to 1 Mb. Due to the fact that ROH segments with specific length can reflect different generation of ancestors (Ferenčaković *et al.*, 2013; Curik *et al.*, 2014) the distribution of ROH segments in the genome were analysed separately for five length categories (>1 Mb, >2 Mb, >4 Mb, >8 Mb, and >16 Mb). Heterozygous calls were not allowed across ROH categories, except length >16 Mb with one permissible call. Missing calls per windows were not allowed for lengths >1 Mb and >2 Mb, while one missing call were accepted for length >4 Mb, 2 for >8 Mb and 4 for >16 Mb. Subsequently, the total number of ROH detected, the average length of ROH (in Mb) and the sum of all ROH segments by animals were calculated for each ROH length category. The proportion of autosomes covered by ROH was then expressed as the pools of overlapping segments within animals per each breed by using R package detectRUNS (Biscarini *et al.*, 2018).

The analysis of genome-wide selection signatures was based on the assumption that the identified autozygosity islands across the genome of Slovak Spotted cattle are a results of selective breeding for traits of interest defined in their breeding objectives. The autozygosity islands, characterized by SNPs with extreme frequency in ROH segments >4 Mb across specific genomic regions, were determined by the calculation of runs incidence per each SNP using R package detectRUNS (Biscarini *et al.*, 2018). The genome-wide occurrence of SNPs in ROH was then expressed as the frequency (%) of overlapping ROH shared among individuals. Genomic regions under selection were defined by the top 0.01 percentile of signals. For identifying genes located directly in the detected regions the Genome data viewer of the bovine genome UMD3.1.1 was used (<https://www.ncbi.nlm.nih.gov/genome/gdv/>).

## RESULTS AND DISCUSSION

The final database of SNP genotyping data consisted of 37,833 markers covering overall length 2,496,829 kb of the genome. The average obtained distance between adjacent SNPs ( $66.05 \pm 70.27$  kb) was in accordance with previous studies that utilized 50K SNP panel for cattle genotyping (Flury *et al.*, 2010; Beghain *et al.*, 2012). The minimum distance between adjacent SNP markers was 0.001 kb and the maximum was 4428.95 kb. All of animals under consideration showed call rate greater than 90 %. The average call rate at level 99.16 % was comparable with results published for dairy as well as beef cattle (Cooper *et al.*, 2013; Mullen *et al.*, 2013).

As expected based on the previous studies the ROH segments were detected in the genome of all analysed animals. The average sum of ROHs calculated per animal was  $70.65 \pm 14.95$  and the average length of ROHs was  $153.24 \pm 51.52$  Mb. Overall, the ROH segments represents 6.14 % of the Slovak Spotted genome covered by SNP markers. However, the distribution of ROH segments across the autosomal genome wasn't uniform. The major fraction of autosome residing in ROH was found on BTA6 (13.5 %), whereas the lowest proportion of ROHs showed BTA24. The obtained proportion of overall autozygosity per animals was comparable with other cattle breeds (Kim *et al.*, 2015; Mastrangelo *et al.*, 2016; Szmatoła *et al.*, 2016; Kukučková *et al.*, 2017).

The analysis of ROHs distribution showed that in the genome of Slovak Spotted cattle across all of length ROH categories in total of 8,632 segments were located. As showed many studies the different length categories of ROH as well as the frequency of ROH occurrence in the genome give insight into the history of target population (Szmatoła *et al.*, 2016) and can be used to estimate the age of inbreeding (Curik *et al.*, 2014). It is generally accepted that very long autozygous segments (>16 Mb) are originated from recent common ancestors, whereas most of the short segments are likely derived from more remote ancestors (Curik *et al.*, 2017). In cattle, Ferenčaković *et al.* (2013) showed that the ROH >1 Mb date back ~50 generations, >2 Mb ~25 generations, >4 Mb ~12.5 generations, >8 Mb ~ 6 generations, and >16 Mb ~3 generations. In Slovak Spotted cattle the total length of ROHs was composed mostly from short segments >1 Mb that accounted for 69.57 %. The ROH segments of 2 – 4 Mb long, representing the 25 – 12.5 generations from common ancestor, accounted for 28.30 %. The lowest proportion within the total length of ROH was found for the longest segments (>16 Mb) that accounted for 0.43 % of all segments identified in the genome. From the point of view of genetic diversity it can be conclude that overall 1.47 % (genome coverage of ROH >16 Mb) of the Slovak Spotted genome can be affected by the increase in inbreeding in recent generation.

The analysis of selection signatures was based on the assumption that the genomic regions with extreme ROH frequencies are most likely consequences of selective breeding for traits of interest during the formation of target breed. Only ROH segments greater than 4 Mb were taken into account mainly due to the fact



that this length category date back ~12.5 generations of common ancestors, which correspond to the breeding history of Slovak Spotted cattle in Slovakia. Genomic regions under selection were defined by the top 0.01 percentile of signals (Figure 1).

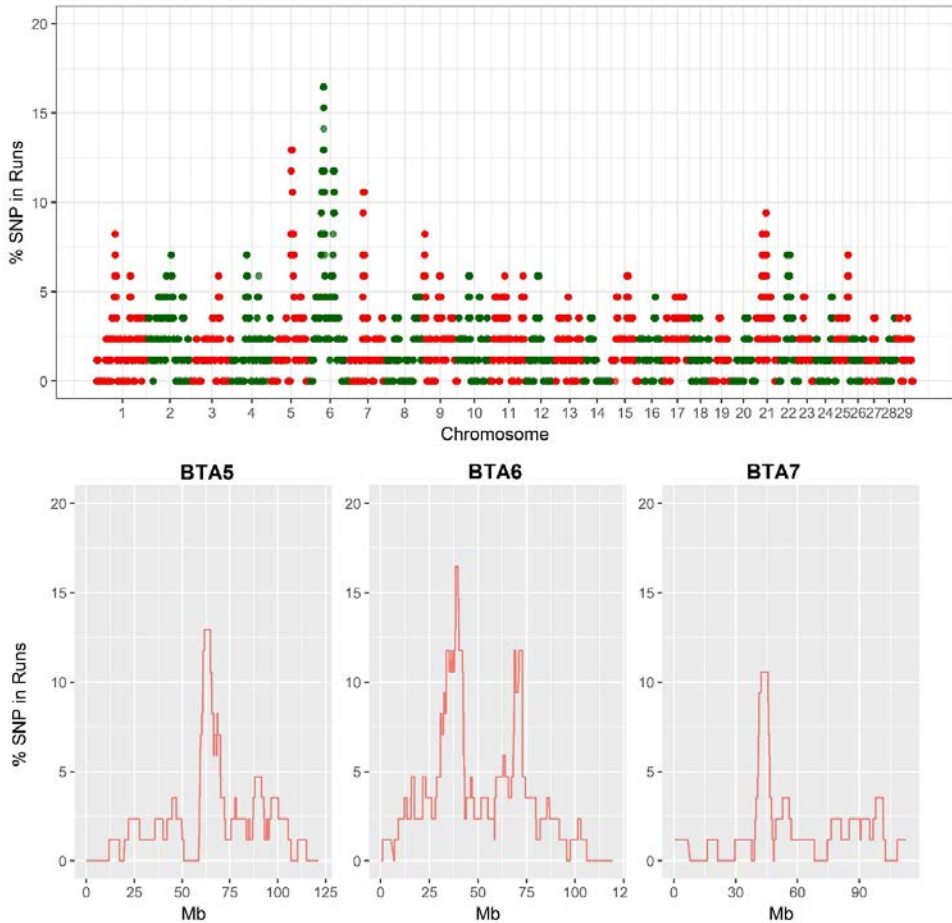


Figure 1. Genome-wide occurrence of SNPs in ROH (minimum ROH length set to 4 Mb) across autosomal genome of Slovak Spotted cattle.

According to this criteria overall four genomic regions located within three autosomes were found: BTA5 (59,665,562-68,880,383 bp), BTA6 (32,489,075-42,866,573 bp and 68,546,212-72,969,608 bp), and BTA7 (41,321,459-46,354,401 bp). Inside identified regions a number of genes were detected, including those involved in genetic control of milk production (ABCG2, SPP1, FAM13A), feed efficiency and growth (FAM184B, PKD2), muscle formation and body composition (PACRGL), reproduction (GDF9), and coat colour pattern (KIT, KDR).

The observed intensive selection pressure particularly in the genomic areas associated with economically important production traits was confirmed also in previous studies. The extensive study of The Bovine HapMap consortium (2009) showed that within the genomes of 19 geographically and biologically distinct cattle breeds the most genomic regions displaying selection signatures were related especially to milk yield, meat quality and feed efficiency.

Another studies identified in several dairy and beef breeds the extreme signals of selection mainly in the regions involved in genetic control of coat colour (MCR1, KIT) and body size (McClure *et al.*, 2010; Lee *et al.*, 2014; O'Brien *et al.*, 2014; Porto-Neto *et al.*, 2014; Kim *et al.*, 2017). Similarly, Rothammer *et al.* (2013) showed that the selection signatures are mostly limited to sequences of genes associated with QTL regions controlling economically important traits of cattle (TG, ABCG2, DGAT1, GH1, GHR, casein family genes).

### CONCLUSIONS

Our study showed that the autozygosity islands covered in average 6.14 % of the Slovak Spotted genome expressed by the SNP markers under consideration. The distribution of ROH segments across the autosomal genome wasn't uniform and the major fraction of autosome residing in ROH was found on BTA6. The intensive pressure of artificial selection on genomic regions on BTA6 was confirmed also by the subsequent analysis of selection signatures.

As expected due to the dual-purpose character of Slovak Spotted cattle the majority part of selection signatures was detected directly or very close to genes associated with milk production, feed efficiency and growth, muscle formation, body composition, reproduction, and coat colour pattern. The detailed analysis of SNP markers located in those regions can be valuable for genetic improvement of Slovak Spotted cattle in the future.

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## **SUSTAINABILITY TRANSITIONS IN BOSNIAN AGRO-FOOD SYSTEM**

### **SUMMARY**

Agriculture plays an important socio-economic role in rural areas of Bosnia and Herzegovina (BiH); the rural population accounts for 61% and almost half of the rural households is still engaged in agriculture. Faced with several environmental, economic and social problems, Bosnian agriculture needs a deep transformation to achieve both food security and food system sustainability. This paper explores the dynamics of past and ongoing transition towards sustainability in Bosnian agro-food system through the lens of the Multi-Level Perspective (MLP) on socio-technical transitions. MLP heuristic posits that transitions come about through interacting processes within and between niches (locus of radical innovations), regimes (locus of established and dominant socio-technical system) and an exogenous landscape. There are different agro-food niches in BiH (e.g. organic farming) but they are still marginal both in terms of land use and market share. Bosnia is characterised by a dual agro-food regime i.e. traditional farming and intensive agriculture. Landscape factors (e.g. civil war, Common Agricultural Policy, climate change) have shaped transformation in both sub-regimes. It is argued that changing climate and harmonisation with the regulatory acquis of the European Union will put pressure on the agro-food regime, whose de-alignment will create opportunities for nascent agro-food niches in the country. This also implies that transition pathways will likely be diverse; from technological/input substitution, to transformation and reconfiguration. Diversity of pathways means that different, tailored policy interventions are needed to foster transition-in-the-making towards sustainable agro-food system in BiH. MLP is useful to map sustainability transitions but further refinement is needed to adapt it to agro-food systems.

**Keywords:** sustainability transitions, Multi-Level Perspective, agro-food system, agriculture, Bosnia and Herzegovina.

### **INTRODUCTION**

Bosnia and Herzegovina has to cope with serious socio-structural problems, such as high rates of unemployment (especially youth unemployment).

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The rural population, which accounts to 61%, is faced with missing job opportunities in sectors other than the primary one (UNDP, 2013), because the presence and growth of industry is insufficient in rural areas (Martinovska Stojcheska *et al.*, 2016). The economic importance of agriculture is shown in its contribution to the gross domestic product (6.4% in 2016) and in the high number of people being employed in agriculture (19.2% in 2016) (World Bank, 2018). Nevertheless, Bosnia and Herzegovina (BiH) is not capable of producing self-sufficiently. Import of foreign agro-food goods was nearly 3 times higher than exports in 2017 (MoFTER, 2018a).

Agriculture in BiH is typically small-scale, fragmented and organised in family farms that mainly produce for local markets or for self-consumption. In 2012, only half of the potential arable land was used for agricultural production. The vast majority of agricultural land (80%) is in the mountainous parts (typically grasslands for livestock farming), whereas only 20% of agricultural land (located in the northern flatter region) is suited for intensive agriculture (Zurovec *et al.*, 2015; Sakic and Crnkic, 2015).

Agro-environmental problems identified in the First National Report on the Implementation of the United Nations Convention to Combat Desertification/Land Degradation in BiH include low quality soils due to poor soil management (e.g. inadequate use of pesticides or fertilisers, livestock production practices, increasing soil acidity), soil degradation and destruction caused by exploitation of raw materials and war activities, erosion and deforestation (MAFWM, 2007). Apart from the numerous environmental problems and the general underrepresentation of environmental issues in the legislation, the lack of environmental awareness by producers and the lack of available official data on the status of the environment are evidently reinforcing the difficulties. The fragmentation of competencies in the administration regarding agriculture and environment constitutes another hindrance for successful measures dealing with the amelioration of the environmental condition. Despite all these complications, there have been small achievements in environmental protection, mainly due to the EU accession process and increased public awareness for nature conservation (MoFTER, 2013).

Bosnian agriculture needs a genuine transition to achieve both food security/self-sufficiency and food system sustainability. The objective of this paper is to explore transitions towards sustainability in the agro-food sector of BiH, with a particular focus on conversion to organic agriculture.

The Multi-Level Perspective on socio-technical transitions (MLP) is the framework used in analysing change dynamics and processes in the Bosnian agro-food sector. This paper discusses the emergence of organic agriculture within the agro-food regime in the Bosnian political and socio-economic context (cf. landscape). In line with the MLP, interactions and processes are analysed within and between three levels: niche (micro-level), socio-technical regime (meso-level) and a socio-technical landscape (macro-level) (Geels, 2002; Geels, 2011). Emerging problems (environmental, economic, social) or new

developments in the socio-technical landscape put pressure on the regime, forcing its adaptation or creating windows of opportunity for new niches to break through. Once a niche-innovation gains momentum and creates a new equilibrium, changes in the regime are possible (Geels, 2002). In sustainability transitions in the agro-food sector, institutional, regulatory and political support is of particular importance. Often sustainability is an intended, long-term goal, but the inherent broad meaning of sustainability can have different directions dependent on the network of actors involved (Smith et al., 2005; Markard et al., 2012). Depending on the constellation of actors from the political arena or from agricultural organisations, alternative practices in the agro-food sector may have a true chance to evolve or will be highly suppressed (Darnhofer, 2015).

## MATERIAL AND METHODS

The agro-food sector can be conceptualised as a ‘socio-technical system’ (Geels, 2002; Geels, 2004), whose societal function is primarily food supply. Multiple factors within or outside a sector can initiate a transformation of an established system, whereas the function per se remains. To structure relevant elements of an evolving transition and to see the implications and challenges for the development of organic agriculture (OA) in BiH, the analytical framework of MLP is employed. In order to develop the analysis, secondary data was searched on the databases Scopus and Google Scholar between 23 May and 16 June 2018. Publications were reviewed first by title relevance, followed by abstract scrutiny and full-text reading. Further literature was included from the reference lists in the found publications. Recent data and statistics on BiH were found on several websites by targeted search via Google. The findings of the relevant literature were conceptualised in the framework suggested by El Bilali and Probst (2017) to map sustainability transitions in Bosnian agro-food system. The identification of processes and dynamics, influential factors and incorporation into the conceptual framework was carried out stepwise. First, influential factors are identified to understand the ongoing change processes in the agro-food regime as well as the emergence and development of niches in BiH. These external factors, that are historically relevant, have shaped the current agro-food regime. Second, the configuration of the agro-food regime is described, including specific agricultural characteristics, institutions and policy framework.

Hereinafter, some insights are highlighted about the constraints for agricultural governance, but also about potential that Bosnian agriculture holds. The last part consists of a description of OA in BiH, the processes within the niche and how OA is linked internally and externally to the regime and the landscape. At this point, it was useful to apply analytical frameworks of Transition Management (TM) (Rotmans and Loorbach, 2009) and Strategic Niche Management (SNM) (Kemp et al., 1998) to understand if organic niche is supported by policy measures, how niche actors are organised and if niche-regime networks are established.

## RESULTS AND DISCUSSION

The current Bosnian agro-food regime evolved from past processes and changes summarised in several landscape elements. Changes in political ideologies, demography and migration, macro-economic trends and the civil war (1992-1995) changed the regime and introduced characteristic patterns. Transition in the agro-food sector is strongly linked to political transition – rather convulsed and incomplete – that BiH has undergone. The Yugoslavian socialist government had great difficulties in managing agricultural issues and was mainly concerned with industrialisation of the country. After a period of economic reforms, where market mechanisms were introduced, efficiency of the agricultural sector increased (Hofler and Payne, 1995), thanks to higher output of private agriculture in the 1950s despite the discrimination of private farmers (Dyker, 2011). Subsequent redistributions of agricultural land, land-maximum holding and prohibition on the sale of land rendered the agricultural population with low possibilities of agricultural rationalisation. All in all, the main obstacles for the improvement of the agricultural sector lied in the obsession of the socialist government and party for a top-down regulation without considering the fatal effects of non-implementation by the agricultural population (Dyker, 2011).

After the fall of Yugoslavia, followed by the recent civil war in BiH, agriculture sector was devastated. Post-war recovery was characterised by international donor funds for social support. Around ten years after the war more budgetary support was dedicated, mainly for traditional production (Bajramović *et al.*, 2014). The Dayton agreement marked the end of the war and still marks the complex structure of the government in BiH. Today agricultural policy management is divided into several administrative levels. There is a Division for Agriculture, Food and Rural Development within the Ministry of Foreign Trade and Economic Affairs (MoFTER) at the state level. At the entity level (Federation of BiH and Republic of Srpska) several ministries are responsible for agricultural policy measures, along with a Division for Agriculture in Brčko District (BD). In the entity Federation of BiH (FBiH), the cantonal level has as well certain competencies for agriculture, veterinary, forestry and water management.

Evidently, Bosnian administration brings a complicate framework for governance and policy management and the agricultural population must cope with instable funding, constantly changing requirements for payments and complex regulations (different regulations depending on entity) (Bajramović *et al.*, 2014). Additional constraints originate from different political ideologies where cooperation among the administrative units is challenged by ethnic contention. As a consequence, agricultural measures are implemented at a very slow rate or not effectively, because of unclear responsibilities and split competencies in the administration (Martin and Fahey, 2009). BiH's current fragmented structure of agricultural land is the consequence of Yugoslavian collectivisation and policy measures for egalitarian land ownership. It is estimated that over 80% of the farms are smaller than 2 ha, most of the times in



separated, multiple plots (Dimitrije and Tomic, 2010). Additionally, migration before, during and after the war left more arable land unused. Only a few farms gain their total income from their agricultural source. The majority is engaged in traditional agriculture with low or inefficient use of inputs and machinery, but also knowledge and technology utilisation, are not favourable (Bajramović et al., 2014).

Historical developments shape the agro-food regime and its elements (policy, culture, practices and technology, markets and consumer preferences). Current exogenous landscape factors, such as trade and competition, market prices, environmental problems and climate change, put pressure on the regime and will influence the relevant regime elements. Agricultural production in BiH is generally weakly linked to the needs of the food industry and the consumers (Kuipers et al. 2013; Vanzetti and Nikolić, 2013), explaining the increasing imports of highly processed food (Vanzetti and Nikolić, 2013). Food processors need larger quantities of raw materials, but the fragmented structure of agriculture and farmers missing to organise themselves in cooperatives to deliver collectively, block this essential coordinated supply (Martin and Fahey, 2009). In 2006, BiH signed the CEFTA (Central European Free Trade Agreement), along with other countries to prepare for accession to the European Union (EU). The anticipation for membership in the EU and a regional free trade cooperation would serve as impulses for integrating political and institutional values of the EU into domestic policy making. Apart from socio-economic and political factors, pressure on the agro-food regime evolves also from environmental problems and climate change. During the last two decades, agricultural production was economically damaged by a few extreme weather events, such as periods of extreme droughts or floods (Žurovec et al., 2017). The Germanwatch Climate Risk Index (CRI) ranks BiH 69 based on weather and socio-economic related impacts (Eckstein et al., 2017). The high share of rural population and their dependence on agriculture, leaves this population considerably vulnerable to climate change because of their livelihood options (UNDP, 2013; Žurovec et al., 2017).

BiH will need a transition from the current agricultural regime, to gain in productivity, to meet future market needs and to produce sustainably. First, modernisation of conventional agriculture is necessary to deliver competitively to the domestic and the European market (Lampietti et al., 2009). Second, as stated by several scholars, the characteristics of traditional farming (e.g. low use of pesticides and fertilisers, small-scale) in BiH are convenient qualifications for a conversion to economically and environmentally sustainable agriculture, e.g. organic agriculture (OA) (Dimitrije and Tomic, 2010; Driouech et al., 2013). A transformation from a dominant to a sustainable system is a long-term, multidimensional process, bearing fundamental or large-scale disruptive changes. Loorbach et al. (2017) refers to sustainability transitions (STs) as “large-scale societal changes, deemed necessary to solve grand societal challenges”. Transition from conventional and/or traditional to organic farming is already

underway in BiH. Moreover, the organic market in BiH is growing at an annual rate of 10-20% and an increase by 17% in organically cultivated land surface was registered in 2010. Despite this growth, less than 1% of agricultural land was devoted to organic farming (Dimitrije and Tomic, 2010).

In order to support sustainable food systems, it is important to identify the already ongoing dynamics and linkages between and within (i) organic agriculture (niche), (ii) the political and institutional framework, institutional linkings, market and consumers (regime), and (iii) the external market and consumer preferences and EU originating processes (landscape). The trend and progress of organic production is mainly influenced by developments outside of BiH. EU accession and gradual market opening for Bosnian products are fruitful incentives for OA. It is expected that organic products will be profitable due to exportability and compatibility to European countries, if measures for certification and controlling are implemented (Natos *et al.*, 2014). Most of the exported organic food products are raw material goods without further processing (except drying and freezing) to add value to the production (Dimitrije and Tomic, 2010; MoFTER, 2018b), hence organic producers experience the same missing links to food processors as conventional farmers. The agricultural market structure grounds major hindrances for further development of organic farming. Inside the country, the supply-demand chain is poorly established. Organic operators, who can bear the costly certification, export their products mainly to European countries (Renko *et al.*, 2010). Some producers have promotional agreements with and sell their organic products at local supermarkets and shops. Food is also retailed directly on-site or at local green markets. Medicinal or healthy plants, produced or wildy collected, are also sold at drugstores and pharmacies and make a significant share of organic production (Dimitrije and Tomic, 2010). Studies show that there is an awareness for organically produced products and that people's choice to buy them is because of their ecologically and socially sustainable production (Vukasović, 2013; Nikolić *et al.*, 2014). On the other hand, Bosnian consumers cannot pay higher prices for certified organic products and are satisfied with the taste and quality of locally produced food. They put them in the same category of food, because of the rare use of pesticides (Dimitrije and Tomic, 2010; Giraud *et al.*, 2013; Nikolić *et al.*, 2014). Nevertheless, the domestic organic market is small (about 0.4% of the total food market) but growing (Dimitrije and Tomic, 2010).

Acknowledging the pre-developmental phase of organic agriculture in BiH, there are many reasons for the low land and market share. The main constraint faced by farmers to enter into or to stay in organic production is the lack of support from municipalities and other public institutions. Institutionally evoked problems, such as lack of financial resources and training on organic farming, irregular payments, weak organisation at the national level and high certification costs, cause difficulties specifically for organic farmers (Driouech *et al.*, 2013). Legislative frameworks for organic agriculture are developed at the entity level. The Republic of Srpska (RS) adopted The Law on Organic

Production already in 2013 (Official Gazette of RS, No. 12/13), whereas the Law on organic production has not been introduced until September 2016 in FBiH (Official Gazette of FBiH, No. 72/16). The adopted laws comply with the EU regulations on organic farming and food processing.

Farmers are generally linked to diverse associations and institutions: municipalities, entities' ministries of agriculture, farmers' cooperatives, research institutes, certification bodies or extension services. Around 2000 organic producers and 17 institutional members (other smaller associations dealing with organic production) connect their knowledge, vision and promotion of organic farming via the Union of organic producers ORGANSKO FBiH. Likewise, an association of organic producers was established by the ministry of agricultural in RS in 2015.

To strengthen the linkages between micro-level niche and meso-level regime, Elzen et al. (2012) suggested to "anchor" emerging links i.e. intensifying exchange between niche and regime actors, and then making more robust links between the two levels. Because of undeveloped institutional capacity in BiH (Bajramović et al., 2014), disbelief that policy measures can bring change (Martinovska Stojcheska et al., 2016) and weak extension services (Driouech et al., 2013; Martinovska Stojcheska et al., 2016), dedicated niche actors supporting and strengthening the networks are crucial for the emergence and establishment of niche-innovations. Farmers who had already experience with rural development support (that also encompasses measures dealing with OA) were more likely to apply again (Martinovska Stojcheska et al., 2016) and this willingness is a good prerequisite for future measures as they can play an active role in supporting unexperienced farmers to engage in rural development programs. Therefore, subsidy programs or policy measures have a higher legitimisation and are more likely to be implemented. Extension services are asked to coordinate such transfer of knowledge but also to provide adequate advisory assistance to increase the knowledge about organic production (Driouech et al., 2013; Pestek et al., 2017). Beneficial effects of organic farming could be delivered by information campaigns to increase the public awareness on the whole concept of organic agriculture. A concerted way of collaboration between different institutions and actors (e.g. State, entities, municipalities/cantons, cooperatives, extension services, academia) is needed to push the institutional progress of organic agriculture. But most importantly is to identify enthusiastic niche actors and bring them together to catalyse synergistically organic agriculture development in order to change the agro-food regime in BiH.

## CONCLUSIONS

The current agro-food regime in BiH is shaped by historical processes connected to former macro-economic trends and political ideologies in the Yugoslavian socialism, ethnic conflicts and the recent civil war, and former political agreements. Past developments define present agricultural practices,

routines in production and consumption, shared and contested beliefs and institutional norms and regulations. Implications for governance derive from practices during the socialist periods, the slow policy implementation due to the Dayton agreement and politics along ethnic lines.

However, the agro-food system is changing, due to internal and external pressures. Accession to the EU forces the political harmonisation with the *acquis communautaire* and the opening of the market due to the CEFTA agreement changes the rules for trade and competition. Climate change and environmental problems are also shaping future rural development policy. Cooperation within the administration at all levels and with the private sector are needed for a gradual alignment and harmonisation of efficient policy measures in the agro-food sector. BiH needs to draw upon the given natural potentials (e.g. unused agricultural land, untouched forests), the low labour costs and its geographical position (proximity to EU market) to develop a comparative advantage, especially in OA. A transition towards a sustainable agro-food system will require several transformational steps: reducing policy inconsistencies, changing priorities towards sustainable practices, improving provision of knowledge regarding sustainable agriculture, and strengthening institutional capacity. To foster organic agriculture development, better organisation at the state level is needed to protect organic producers' interests. Reducing certification costs and securing premium prices for organic products could improve market access.

Further research is necessary to understand farmers' awareness of sustainable agro-food production and how they perceive conversion towards organic farming. It is also necessary to analyse worldviews and narratives of actors involved in the niche, those providing institutional and political support to OA as well as the agro-food regime actors in BiH in order to better understand the factors hindering sustainability transitions in the country. MLP is useful in understanding the ongoing change processes but an integration with other transition frameworks (e.g. TM, SNM) might be needed.

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## MOLECULAR AND BIOLOGICAL PROPERTIES OF SOYBEAN MOSAIC VIRUS AND ITS INFLUENCE ON THE YIELD AND QUALITY OF SOYBEAN UNDER CLIMATE CHANGE CONDITIONS

### SUMMARY

Soil and climatic conditions of Ukraine provide the obtaining of ecologically sound yields of grain crops and legumes, among which the soybean occupies the forefront. In 2017, the average soybean yield was significantly (by 3.4 - 13 centner/ha) lower than in 2016.

The aim of the work was to investigate the molecular and biological properties of the Soybean mosaic virus (SMV) isolate SKP-16, its effect on soybean yield and grain quality under agro-climatic change conditions. In 2015-2017, monitoring inspections of soybean crops showed that viral infections, occupied a significant place, mainly SMV. Also, seed transmission of SMV infected several soybean varieties was proved. In 2017, a very dry year for Ukraine, and especially for Poltava region, we found a significant infecting of many varieties with SMV.

Hydrothermal coefficient of G.T. Selyaninov (HTC) was less than 1, only 0.53. This indicator takes into account simultaneously the actions of the main climate elements – temperature and precipitation. The low HTC provided a significant number of aphids – vectors of SMV.

We have studied in detail the SMV isolate SKP-16 from soybean variety Kophy (GenBank Accession No MG940990). The yield of soybeans in the SMV-infected plants was reduced by 2.6 times, compared with healthy ones in 2017. But in 2016, the difference was considerably smaller, indicating a synergistic harmful effect of both factors - the virus and climatic conditions. The analysis of the nucleotide and amino acid sequences of the SKP-16 capsid protein gene revealed the highest percentage of identity (97.9% and 97.2% respectively) with the isolates UA1Gr, Ar33, Lo3, VA2. Four amino acid substitutions were found in position 1 (Ser → Cys), position 2 (Lys → Ser), position 3 (Gly → Leu), and position 5 (Val → Leu).

**Keywords:** soybean, Soybean mosaic virus, sequencing, yield, quality.

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## INTRODUCTION

Soil and climatic conditions of Ukraine provide the obtaining of ecologically sound yields of grain crops and legumes, among which the soybean occupies the forefront. Since 1990 the area of the collected culture has been constantly growing (from 87.8 thousand hectares in 1990 to 2 million hectares in 2017). However, soybean suffers from a lot of species of fungal, bacterial and viral diseases. To date, over 30 fungal, 10 bacterial and about 67 viral diseases are known which cause significant damage and can occur at different stages of soybean plant growth and development: from seed germination to the end of the vegetation. Particularly acute is the problem of viral diseases, which can affect soybean throughout the growing season, taking into account its ability to branch and appearance of young leaves that are very attractive to many aphid species. It was noted that the average soybean yield in 2017 was significantly (by 3.4 centner/ha, in many farms by 10-13 centner/ha) lower than in the previous year (23.4 centner/ha). This is due to a significant drought in 2017, since the soybean is needs moisture. It was investigated that Soybean mosaic virus (SMV) is affect transgenic soybean (Mishchenko *et al.*, 2018a). But biochemical studies of soybean grain and harvest under the influence of viral infection under conditions of climate change and molecular biological properties of the SMV (isolate SKP-16) was not conducted, which was the aim of our study.

## MATERIAL AND METHODS

Inspections of soybean plants were conducted by visual diagnostics method (Peresyphkin *et al.*, 2000). Biometrics, crop and its structure were carried out by generally accepted methods (Dospekhov, 1985).

Content of protein, fat, isoflavones in soybean seeds was investigated by (Molodchenkova *et al.*, 2016). Meteorological data (the amount of precipitation, the amount of active temperatures, and the HTC for vegetation) were provided from the agrometopost of the Ustimovka Experimental Station of Plant Production, Plant Production Institute nd. a. V. Ya. Yuryev of NAAS, Poltava region.

Hydrothermal coefficient of Selyaninov (HTC) calculated using the formula:  $r / (0,1 \cdot \sum t > 10)$ , where  $r$  - total rainfall during the growing season (May-August), mm;  $\sum t > 10$  - the average daily air temperature more than 10 ° C for the same period. Identification of the viruses was performed by DAS-ELISA using commercial antibodies against Soybean mosaic virus (Loewe, Germany). The results were recorded on Termo Labsystems Opsi MR reader (USA) with Dynex Revelation Quicklink software at wavelengths of 405 nm. Samples were considered positive when their absorbance values at 405 nm were at least three times higher those of negative controls (Crowther, 1995). Statistical data processing was carried out according Dospekhov (1985).

Viral particle morphology was studied by transmission electron microscopy. Negative staining of virions was performed with the 2% solution of phosphotungstic acid and studied by electron microscope JEM 1400, JEOL,



Japan (Center for collective use, Danylo Zabolotny Institute of Microbiology and Virology of National Academy of Science of Ukraine). Total RNA was extracted from fresh leaves using Genomic DNA purification kit (Thermo Scientific, USA) following the manufacturer's instructions.

Two step RT-PCR was performed. The reverse transcription was performed using RevertAid Reverse Transcriptase – genetically modified MMuLV RT (Thermo Scientific, USA) according to the manufacturers' instructions using specific oligonucleotide primers to part of SMV CP gene (469 bp) (Mishchenko et al., 2018 a,b) CP gene sequences of the Ukrainian SMV isolate were compared with SMV sequences in the NCBI database with the BLAST program. Nucleotide and amino acid sequences were aligned using Clustal W in MEGA 7 (Kumar et al., 2016).

Phylogenetic trees for the part of SMV coat protein gene were constructed by the maximum-likelihood method (ML) (Huelsenbeck JP, Rannala, 1997) using the best-fitting evolutionary models. To check the reliability of the constructed trees used bootstrap test with 1000 bootstrap replications. The values at the nodes indicate the percentage of replicate trees in which associated taxa clustered together (number of bootstrap trails: 1000 replicates). The scale bar shows the number of substitutions per base. Aligned CP amino acid sequences were visualized and compared using BioEdit sequence alignment editor.

The percentage of the nucleotide sequences identity was presented as color blocks using the software package SDT v.1 (Sequence Demarcation Tool Version 1.1). Statistical analysis of experimental data was carried out according to the parametric criteria of the normal distribution option, the standard deviation of the mean values - according to the generally accepted method.

## RESULTS AND DISCUSSION

The analysis of annual gross of soybean gross showed that in the last four years it was almost stable due to the increase of harvested areas and the highest yield in 2016, which was 2.34 t / ha. The following yield was noted: in 2000 – 1.06 t / ha, in 2010 – 1.62; 2012 – 1.72; 2013 – 2.05; 2014 - 2.16; 2015 - 1.84 t / ha, 2016 - 2.34, and 2017 - 2.00 t / ha. Beginning in 2010, soybean yield was less than 2.0 t / ha in 2010, 2012, and 2015 (Fig.1).

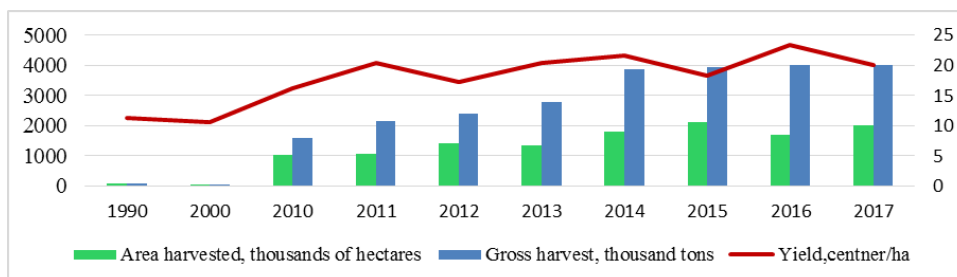


Figure 1. Soybean area harvested, yield and gross harvest in Ukraine for the period from 1990 to 2017

Our inspections of the soybean fields showed significant affecting of soybean plants with viral diseases in 2012 in the Kyiv region and in 2015, 2017 under the conditions of Poltava region. It was detected that the most common and wide spread was SMV. Symptoms caused with SMV on soybean plants showed on Fig.2.



Figure 2. Symptoms caused by SMV on soybean plants cv. Kophy (a) and Kano (b), Poltava region, 2016

In symptom soybeans, filamentous particles with sizes from  $500$  to  $850 \times 13-17$  nm were found. It is typical for the viruses of *Potyvirus*, *Potyviridae* family which are have size  $680-900 \times 11-13$  nm (Virus taxonomy, 2012). All major deficiencies of soybeans are correlated with weather conditions. Analyzing our about viral infections of soybean, it is safe to state that the its productivity depends on climate change that affects both the host and the virus vectors - aphids.-Previously, we have shown that the SMV infection leads to a significant decline in the yield of soybean cv. Kano and the transgenic varieties Grimo and Monro (Mishchenko *et al.*, 2018a, b). But biochemical studies of soybean grains under viral infection in Ukraine were not conducted. The studies on the influence of SMV infection on the quality of soybean seeds showed ambiguous results. In general, it is known that grain (seed) quality deteriorates under viral infection. On the variability of the parameters had effect the time when the infection of soybean plants occurred (phases of growth and development of plants), variety, weather conditions, etc. For example, we present 1). Kano soybeans in a very dry 2017, when the HTC in the Poltava region was significantly less than 1; 2). less arid in 2016, when the HTC was about 1; 3). transgenic Grimo soybeans in 2017. There was an insignificant decrease in the content of oil (fat) in all variants with the damage of plants with viral infections. In comparison, protein content decreased by 2.34% in case of viral infection only in 2017 in the variety Kano. But in the variety of transgenic soybean Grimo, protein content increase by 5.33%. This can be explained by the accumulation of lectins and isoflavones (Table 1).

Table1. Influence of SMV infection on the biochemical parameters of soybean seeds

Variant/ sampling year	Protein, % per abs. dry substance	Fat, % per abs. dry substance	Lipoxygenase activity, CU/min/mg protein	Lectins activity, ( $\mu\text{g/g}$ protein /ml) $^{-1} \times 10^{-3}$	Isoflavo -nes, $\mu\text{g/g}$
Kano, healthy/2016	38,15	23, 21	1,067	2, 651	73
Kano, SMV- infected/2016	39,22	21,90	0,588	2,354	102
Kano, healthy /2017	40,49	23,75	0,975	1,281	79
Kano, SMV- infected/2017	38,15	22,73	0,888	2,746	98
Grimo, helthly/ 2017	30,49	27,01	0,923	0,304	79
Grimo, SMV- infected/2017	35,82	24,22	0,812	1,325	73

An important function of flavonoids is the protection of plants from external unfavorable abiotic and biotic factors, in particular viruses (Panche et al., 2016). It is well seen that in the Kano variety infected with SMV, the content of isoflavones increases from 24 to 39%. In 2017, there was also marked increase in lectins content under SMV infection in both varieties (Kano – in 2.1 and Grimo – in 4.4 times).

In the case of a viral infection, decreased activity of lipoxygenase was also noted. Thus, the viral infection contributes to the accumulation of isoflavones in the soybean variety Kano and lectins in Grimo. It was determined that the maximum July temperature, the minimum temperature in August and the total amount of precipitation in July-August are significant factors affecting the productivity of wheat, soybeans and corn (Hatfield et al., 2017), as well as the number of viruses vectors. Therefore, to assess the effect on soybean yield, our attention was focused on the indicators: the amount of precipitation, the amount of active temperatures, and HTC during vegetation (May-August). An analysis of these meteorological data showed that 2017 was characterized by a significantly lower HTC and slightly higher temperatures than in the previous 2016 (Fig. 3).

It is clearly visible from Fig.3 that the HTC was significantly lower than the 1 in 2007 (0.58), 2009 (0.59), 2010 (0.61), 2012 (0.65), 2013 (0.46 - very dry zone), and 2017 (0.53 - very dry zone). It is considered that if the HTC is more than 1, then moisture is good, and for the forest-steppe zone this indicator is 1.2-1.4. Coefficient of variation (V) for the HTC is 32.9%, which indicates its significant variability in the period from 1995 to 2017. But by the sum of active temperatures during vegetation period, the variability was insignificant and the coefficient of variation was only 6.1%. In the amount of precipitation for vegetation in the same period also marked significant variability (V = 29.0%). The results of our research showed that the yield of soybeans of the Grimo

variety in 2016 (HTC about 1) was twice higher than yield of 2017 (a very dry year) in our experiment in the Poltava region. (Mishchenko *et al.*, 2018 a). In Ukraine, soybean yields in 2016 were higher by 3.4 tons / ha compared to 2017.

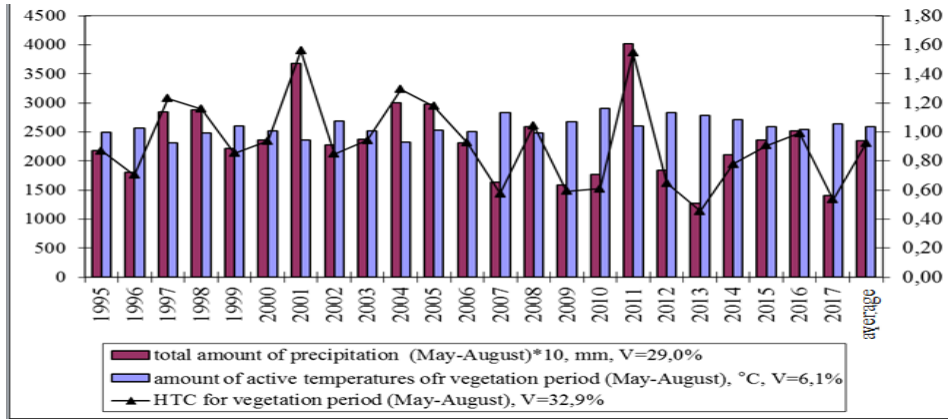
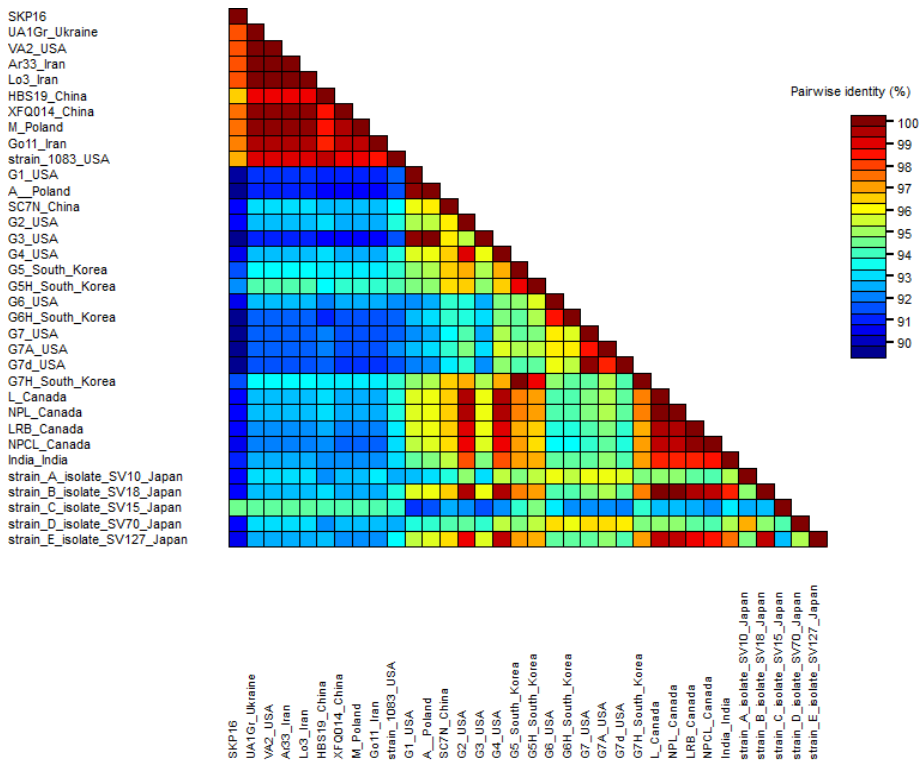
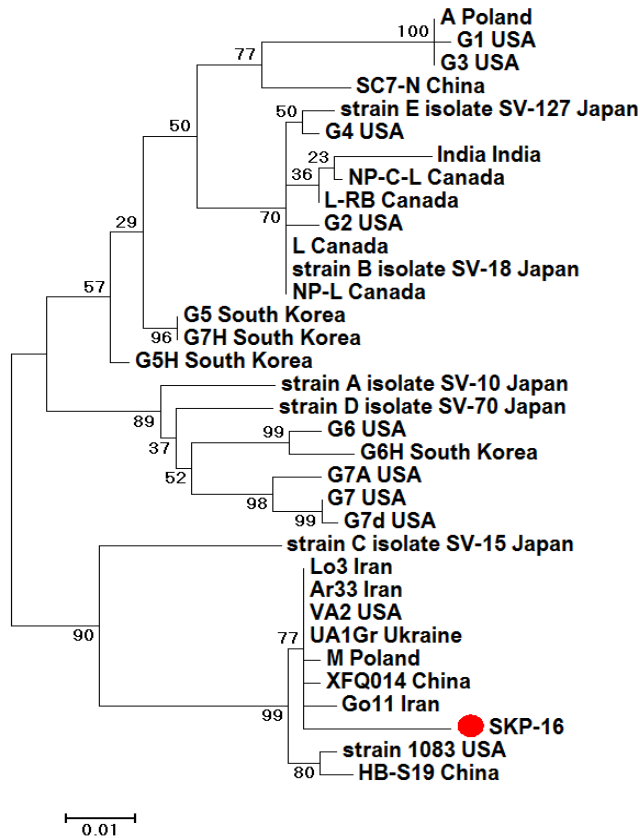


Figure 3. Meteorological data during vegetation periods May-August 1995-2017, Poltava region



(a)



(b)

Figure 4. Nucleotide identity of SMV isolate SKP –16 and isolates from other countries: (a) -graphical representation of pairwise nucleotide identity of SMV isolates (percentage of identity is represented on the scale); (b) - maximum likelihood (ML) phylogenetic tree resulting nucleotide sequences of 430 bp part of the CP gene of isolates. Tree is constructed using Jukes-Cantor evolutionary model.

Next stage of the work was to study molecular properties of the SMV isolated from soybean cv. Kophy, named as SKP – 16 (GenBank Accession No MG940990). Nucleotide (nt) and amino acid (aa) sequence 430 nt of the coat protein (CP) gene region of the SMV isolate SKP – 16, localizing at the genomic position 8640-9069 were compared with sequences of 33 SMV isolates/strains from GenBank. SKP–16 has the highest percent of the identity with Ukrainian isolate UA1Gr, Iranian Ar33, Lo3 and American VA2: aa – 97.2%, nt – 97.9%. SKP–16 has a high nt identity with other isolates studied in China - XFQ014 and HB-S19, Poland – M, Iran - Go11, and in USA - the strain 1083 (96,7-97,6%) (Fig.4a).

The phylogenetic tree presented in the Fig. 4b is fully consistent with the data in Fig.4a - isolate SKS-18 is located in one cluster with isolates with the

highest nucleotide identity: Ar33 and Lo3, VA2, UA1Gr, XFQ014, HB-S19, M, Go11 strain 1083, as well as strain C, isolate SV-15 that may be due to their similar variability. Isolate SKP –16 have 4 aa substitutions: at position 1 (Ser→Cys), position 2 (Lys→Ser), position 3 (Gly→Leu), and position 5 (Val→Leu) in the 171 aa investigated part of CP gene. It has been established that the aa substitutions of SKP –16 are unique in comparison with all SMV isolates taken for the analysis.

## CONCLUSIONS

Analysis of the meteorological data in the agroecosis was carried out and it was established that the yield of soybean crops is closely related to the ratio of precipitation and air temperature (HTC). From these parameters also depended the frequency of SMV detection, because they have a direct impact on insects – vectors of the viruses and degree of SMV influence on the on the seeds quality. The molecular genetic properties of the SMV isolate SKP-16 were studied.

It is determined that it has the highest percent of nt and aa identity with Iranian, American, Chinese, Polish isolates and isolate from Ukraine. The changes in the molecular genetic level in CP gene of SKP-16 can be involved in the it's harmfulness.

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**THE ESSENTIAL OIL EXTRACTED FROM *Thymus kotschyanus* BOISS. & HOHEN AS A NATURAL SUBSTANCE FOR MANAGEMENT OF THE LESSER GRAIN BORER, *Rhyzopertha dominica* F.**

**SUMMARY**

Plant essential oils as promising bio-rational agents have been considered for management of damaging insect pests because they are naturally biodegradable and safe to the non-target organisms. In the present study, the fumigant toxicity of essential oil from *Thymus kotschyanus* aerial parts was assessed against a major Coleopteran insect pest *Rhyzopertha dominica* and this bio-effect was optimised and modelled using Response Surface Methodology (RSM).

Optimization of the fumigant toxicity displayed a concentration of 51.720 µl L<sup>-1</sup> was adequate to kill 72.752% of insect population after 60.0 h exposure time. The best model for predicting of insecticidal effect was a third-grade model. Results of the present study recommended a high potential of *T. kotschyanus* essential oil for management of a major stored-product insect pest *R. dominica* and prediction of this bio-effect using response surface methodology.

**Keywords:** Essential oil, fumigant toxicity, response surface, *Thymus kotschyanus*.

**INTRODUCTION**

Although effective synthetic chemicals for management of different detrimental pests are available, there adverse side-effects such as environmental pollution, toxicity to the non-target organisms and resistance of treated species encouraged researchers to search safe and efficient alternatives (Damalas and Eleftherohorinos, 2011; Lorini *et al.*, 2007; Pimentel *et al.*, 2008).

Plant-derived essential oils are complex blends of volatile components including terpenic, aromatic and aliphatic groups (Bakkali *et al.*, 2008). These materials produce by almost all living organisms of plants such as leaf, stem, root, and flowers against phytophagous pests and/or phytopathogenic fungi (Batish *et al.*, 2008). The volatile components have diverse biological effects and bio-efficiency of essential oils affected by them (Bakkali *et al.*, 2008). Essential oil biosynthesis is dependent on many different factors such as genetic make-up, climatic conditions, geographical position, cultivation measures, soil quality, water stress, harvesting time, and isolation method (Bakkali *et al.*, 2008; Tripathi

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*et al.*, 2009). Essential oils have generally considered as safe and biodegradable materials (Isman, 2005). They have broad biological effects such as insecticidal, acaricidal, antifungal, antibacterial, and antioxidant activities (Isman *et al.*, 2011; Isman and Grieneisen, 2014; Asbahani *et al.*, 2015; Ebadollahi *et al.*, 2017). Further, it was found that the essential oils have multiple modes of actions and for this reason development of pest resistance to them is very low (Tangtrakulwanich and Reddy, 2014).

*Thymus kotschyanus* Boiss & Hohen with the name of “Avishane-Kohi” is one of the most popular medicinal plants in Iran (Mozaffarian, 1998). Aerial parts of this species are used in traditional medicine and as a herbal tea (Bahmani *et al.*, 2014). Further, some biological effects of the essential oil of *T. kotschyanus* comprising antibacterial, antifungal, antigenotoxic, and antioxidant activities were documented (Afshari *et al.*, 2016; Rasooli and Mirmostafa, 2003; Sevindik *et al.*, 2016).

According to our knowledge, there are not any documents on the application of a mathematical model to predict the insecticidal effect. Accordingly, the primary goals of present study were 1) evaluation of fumigant toxicity of *T. kotschyanus* essential oil against the lesser grain borer (*Rhyzopertha dominica* F.) as a destructive stored-product insect pest (Edde, 2012) 2) development of mathematical model to predict the insecticidal activity and 3) finding of the optimized conditions for this bio-effect.

## MATERIAL AND METHODS

*Extraction of the essential oil:* Aerial parts of and *Thymus kotschyanus* were collected, respectfully, from Hassan Baroog and Sardabeh regions (Ardabil province, Iran). After drying at room temperature, the parts were chopped with an electric grinder. Extraction of essential oil was done using a Clevenger apparatus with 100 g of each plant sample, and 1500 ml distilled water within 3 hours.

*Rearing of the insect:* Adults of *Rhyzopertha dominica* were collected from infected wheat grains at the Moghan college of Agriculture and Natural Resources, University of Mohaghegh Ardabili, Iran. One-litre cylindrical flasks filled with wheat kernels hosted the rearing of insect pest. In order to vent the kernels, a fine mesh cloth stopped each flasks mouth. Fifty pairs of adult insects were transferred in each flask and removed 48 hours later. Infested grains were kept at  $27 \pm 2$  °C and  $65 \pm 5$  % relative humidity and 1-3 days old adult insects were selected for fumigation bioassay.

*Toxicity of the essential oil against R. dominica:* To investigate the fumigant toxicity of *T. kotschyanus*, 232 millilitres containers were used as a fumigant chamber. Twenty 1-3 days old adults of insects were located in plastic canisters ( $3.5 \times 5$  cm), which were cut on one side and covered with a fine mesh cloth, and were hanged from the centre of the fumigant chamber. Essential oil concentrations from 8.60 to 50.72  $\mu\text{l L}^{-1}$  was calculated and prepared during preliminary experiences. Each concentration was poured on  $2 \times 3$  cm filter

papers rectangles, which were placed in the fumigant chambers. The lids of the containers were air-tightly closed, and mortality of the insect was counted at 12 hours intervals after treatment. All experiments were conducted for control groups without essential oil concentrations, and each experiment was repeated four times.

*Modelling of the insecticidal effect of the essential oil and statistical analysis:* The data were analysed by Design Expert versions 7.0.0 (2007, Stat-Ease company, USA). The coded independent variables for fumigant toxicity of *T. kotschyanus* essential oil against *R. dominica* are essential oil concentrations ( $X_1$ ) and exposure time ( $X_2$ ) in 5 levels and four replications. Mathematical model between the independent variables [Concentration ( $\mu\text{l L}^{-1}$ ) and time (h)] and dependent variable [Mortality (%)] evaluated employing multiple linear regression analysis in the following form (Khuri and Cornell 1987):

$$Y = \beta_0 + \sum_{i=1}^n \beta_i X_i + \sum_{i=1}^n \beta_{ii} X_i^2 + \sum_{i=1}^n \sum_{j=i+1}^{n-1} \beta_{ij} X_i X_j + e$$

where  $\beta_0$ ,  $\beta_i$ ,  $\beta_{ii}$ ,  $\beta_{ij}$  are constant coefficients of regression,  $X_i$  and  $X_j$  are the independent variables,  $Y$  is the dependent variable,  $n$  is some independent variables, and  $e$  is the random error term. The relationships between the responses were checked by correlation coefficients of determination ( $R^2$ ), adjusted  $R^2$ , and predicted  $R^2$ . A good model will have a large predicted  $R^2$  and a low PRESS. ANOVA found the significant terms in the model. The significance was analysed with a confidence level of 95% ( $P < 0.05$ ).

## RESULTS AND DISCUSSION

The results of bioassays confirmed that the essential oil of *T. kotschyanus* has significant toxicity in the adults of *R. dominica*. Figure 1 shows the effect of different concentrations of essential oil and various exposure times on the mortality of insect pest: with the time and concentration increase, the mean mortality percentage increases.

The results of analysis of the variance for fumigant toxicity data, in Table 1, show that the effects of exposure time (A) and essential oil concentration (B) and their interactions (AB) on pest mortality are statistically significant. Also, the effects of factors  $AB^2$ ,  $B^2$ , and  $B^3$  on the mean mortality were significant ( $P < 0.05$ ). The regression model is significant at 95% confidence level. The most significant effect based on the sum of squares was established in the essential oil concentration factor (76%), which indicates that this factor is more important than others (Table 1).

To model for the high estimating data potential, it is necessary to push the predicted and adjusted  $R^2$  values for the highest count. The Coefficient of determination ( $R^2$ ) is expressed as the ratio of described variations by the model to total variations. As  $R^2$  value is closer to 1.0 (at least 0.8), the fitted model will have more ability to describe the response variations according to independent variables.

Table 1. Results of the analysis of variance of data on the toxicity of *T. kotschyanus* essential oil against the adults of *R. dominica*

Source	Sum of Squares	df	Mean Square	F value	p-value
Model	18506.62	6	3084.44	219.91	< 0.0001
A-Time	202.74	1	202.74	14.45	0.0003
B-Concentration	1782.44	1	1782.44	127.08	< 0.0001
AB	76.58	1	76.58	5.46	0.0216
B <sup>2</sup>	69.59	1	69.59	4.96	0.0283
AB <sup>2</sup>	115.18	1	115.18	8.21	0.0051
B <sup>3</sup>	90.34	1	90.34	6.44	0.0128
Residual	1304.38	93	14.03		
Lack of Fit	141.88	18	7.88	0.51	0.9462
Pure Error	1162.50	75	15.50		

A and B, respectively, are the exposure time (h) and essential oil concentrations ( $\mu\text{l L}^{-1}$ ).

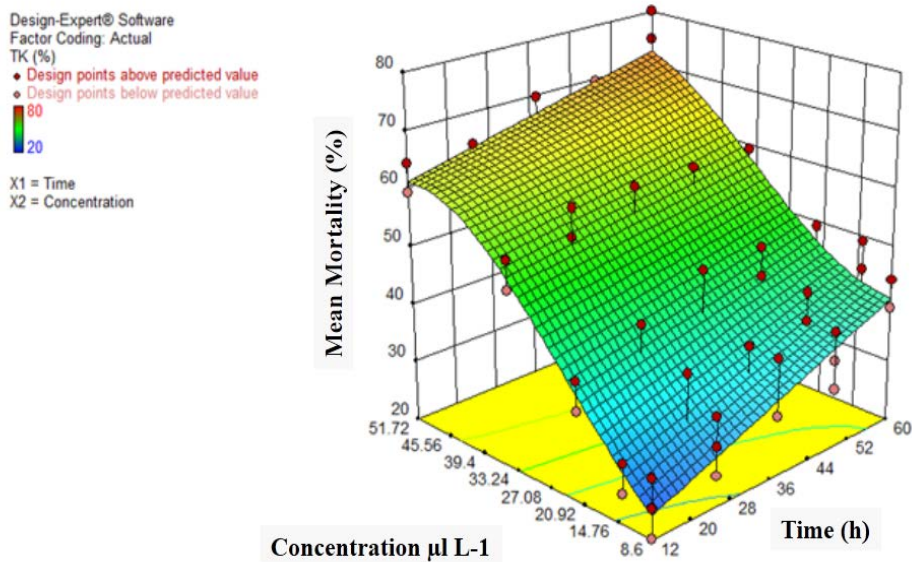


Figure 1. Three-dimensional diagrams of the fumigant toxicity of *T. kotschyanus* essential oil against *R. dominica*.

According to the model presented in Table 2 and the coefficient of variations, it can be concluded that this is the best model for estimating of the insecticidal effect of *T. kotschyanus* essential oil. The coefficients of the independent variables (concentration and time) are positive: an increase in each of the variables is an incremental effect on the response variable. The negative sign of the variables in the model indicates the decreasing effect of the variable on the amount of insect mortality.

Table 2. Estimated regression models of fumigant toxicity of *T. kotschyanus* essential oil against *R. dominica*.

Equation	R <sup>2</sup> value	Adj R <sup>2</sup>	Pred R <sup>2</sup>	C.V. (%)
$52.08 + 3.49A + 22.84B - 1.72AB - 2.06B^2 + 3.73AB^2 - 5.60B^3$	0.93	0.93	0.93	8.02

\*A and B, respectively, are exposure time (h) and essential oil concentrations ( $\mu\text{l L}^{-1}$ ).

The optimum conditions for achieving 50% and maximum mortality of insect pest are shown in Table 3. Essential oil concentrations and the time were selected from 60.68 to 51.72  $\mu\text{l/l}$  and from 12 to 60 hours. The concentration of 51.72  $\mu\text{l/l}$  and 60 hours-time was estimated as the optimum conditions for 75.72% mortality with the desirability of 88%. Based on the results, the amount of mortality increased with increasing concentrations and time. For 50% mortality of the pest population, a concentration of 24.62  $\mu\text{l/l}$  and a time of 98.57 hours are adequate (Table 3).

Table 3. Optimization of the fumigant toxicity of *T. kotschyanus* essential oil against *R. dominica*.

Response variable	Time (h)	Concentration ( $\mu\text{l L}^{-1}$ )	Desirability
Mortality (%) = 50	57.98	24.62	100
Mortality (%) = 72.75	60.00	51.72	0.88

Although susceptibility of the key stored-products insect pest *R. dominica* to the plant essential oils was documented in some recent researches (Ebadollahi *et al.*, 2010; Hanif *et al.*, 2016), fumigant toxicity of the essential oil of *T. kotschyanus* was initially recognized in the present study against this pest. However, the antifeedant, repellency and oviposition detergency of *T. kotschyanus* essential oil were documented against *Tribolium castaneum* Herbst and *Callosobruchus maculatus* Fabricus (Akrami *et al.*, 2011a and b). Further, the modelling and optimisation of this bio-effect were assessed in the present study for the first time.

Chemical profile of the *T. kotschyanus* essential oil has investigated in some of the recent studies. For example, carvacrol (22.75%), thymol (16.52%), myrcene (12.65%), thymoquinone (11.39%), and borneol (4.52%) were introduced as main components in the essential oil of *T. kotschyanus* (Rasooli and Mirmostafa, 2003). In another study, thymol (46.72%), benzene (6.88%), carvacrol (3.73%), caryophyllene (3.39%), and  $\gamma$ -terpinene (3.58%) were the main components in the *T. kotschyanus* essential oil (Mohammadi *et al.*, 2014). 1,8-cineole (4.6%), p-cymene (16.6%),  $\gamma$ -terpinene (6.6%), and thymol (52.2%) were recognised as the major components in the essential oil of *T. kotschyanus* in the other work (Sevindik *et al.*, 2016).

The differences can be due to various geographic location, weather conditions, plant organs, extraction methods, soil types of the harvested areas and

some other factors (Afshari *et al.*, 2016; Pluhár *et al.*, 2016; Rowshan *et al.*, 2013). Further, recent studies have shown that the biological effects of plant essential oils are directly related to their principal components and even their synergistic effects with other components (Isman *et al.*, 2011; Regnault-Roger *et al.*, 2011). Some terpenic compounds such as borneol, camphene, Linalool, linalyl acetate, and  $\beta$ -ocimene indicated potential insecticidal activities (Ogendo *et al.*, 2008; Rozman *et al.*, 2007). So, it can be said that the insecticidal effect of *T. kotschyanus* essential oil may be related to the mentioned components.

## CONCLUSIONS

*R. dominica* is among the most detrimental agent in the many countries, which has economic damage to many agricultural stored-products. Control of this destructive agent is currently carried out using chemical pesticides, but utilisation of synthetic chemicals caused several adverse side-effects such as environmental pollution, resistance to pests, outbreaks of secondary pests and effects on non-target organisms. Therefore, the use of low-risk and natural compounds in the management of such detrimental agent is necessary.

In the present study, the fumigant toxicity of *T. kotschyanus* essential oil was proved against *R. dominica*. Further, results of the present study were also revealed that the response surface methodology (RSM) was efficaciously used for the modelling and optimisation of the insecticidal effect of *T. kotschyanus* essential oil.

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## **AGROECOLOGY AND HIGHER SECONDARY EDUCATION: EDUCATIONAL PRACTICES FOCUSED TO CURRICULUM GREENING**

### **SUMMARY**

The problem of the environmental pollution and the indiscriminate abuse of natural resources as a result of the human practices, have affected many parts of the world through the global warming. In the last few decades it has been observed that climate variation affects to diverse ecosystems; the increase or decrease of the temperature in geographical zones that use to have a constant temperature, the water shortage, droughts and floods in places where these phenomena did not occur, are some examples caused by the irresponsible human actions. Although it is acknowledged that the large majority of the countries has a capitalist economic system has been an important cause of the environmental deterioration, is possible to make a change starting with social groups to replace these habits of consumerism and to raise awareness about care and environmental conservation. Against this background, as educational institution as an agent for change it is proposed the development of a curricular proposal that links the agroecology disciplinary knowledge in educational practices. A curriculum greening is achieved through the curricular contents of the subjects offered at high school, where the entire school community participates on sustainable practices that encourage their development and a students comprehensive education. In this paper, we present the case of the development of this project in the No. 100 Official High School, located in Texcoco, Mexico, likewise, demonstrates the progress, issues and challenges that have arisen

**Keywords:** Agroecology, environmental education, curricular environmentalization, curriculum greening, sustainability.

### **INTRODUCTION**

A society is a group of people who share a common habitat and depend on each other for their survival and well-being. The culture of a society tends to be similar in many aspects from one generation to another (Harris, 2001, Miranda, 2013). Neighboring societies may have both very similar cultures and very

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different cultures. The enculturation, according to Harris (2001), focuses on generational control transmissible from parents to children or the elderly at younger ages. Given this, the environmental culture, for the purposes of generational transmission, conceives a set of attitudes, behaviors and environmental knowledge that a person has (Kilbert, 2000). People can formulate appropriate values, norms, and codes of conduct for the development of their daily activities (Harris, 2001).

Therefore, culture is an inherited characteristic of human beings. For Harris (2001), González (2018) and Miranda (2013), every culture is an environmental culture because it can be defined as the way in which social groups relate to their environment within a symbolic and practical way. In fact, the environmental culture is manifested in each society lifestyles (González, 2018). Since its appearance, humans have transformed its environment and have transformed himself. But what repercussions exist in the environment when there is no sustainable relationship between the practices of a culture and the transformation of nature by human beings? Undoubtedly, humanity has walked contrary to the laws of nature and its ecological processes, because the management given to organic and inorganic matter as a polluting waste deteriorates the environment and causes diseases. In this sense, the human being, in his eagerness to achieve the mastery of nature and the subjugation of those of his own species, appeals to instrumental reason to justify the means that have served to fulfill his purposes. Science achieves its task of dominating nature but causes the oppression of man, who, in his intention to be the master of nature, ends up getting rid of it (Adorno and Horkheimer, 1988).

This is reflected in the high rates of waste generation associated with deficiencies in collection and treatment services that are a source of negative impacts; the inadequate disposition of the material linked to an incorrect separation is causing proliferation of plague species, bad odors, formation of toxic gases, fumes and dust that contribute to the contamination of ecosystems (Velázquez et al, 2017). Therefore, it is considered, as an alternative, to resort to the state of knowledge of agro-ecology, interdisciplinary linking agronomy, and ecology is responsible for the development of agricultural techniques oriented to an environmental approach (Granados and Lopez, 1996). From this interdisciplinary, the concepts of the ecosystem (environment), agro-system (modified ecosystem) and eco-technology (sustainable use technique) are immersed. Agro-ecology is a field of knowledge generated in the last decades of the twentieth century, we cannot currently refer to Environmental Education (EE) if this interdisciplinary approach is not contemplated, which leads to the analysis and understanding of ecological and biological bases (Victorino et al. , 1992). The knowledge and application of these terms are of great importance for the development of educational practices oriented towards “Curricular Environmentalization” (CE) in the educational plans and programs, reflected in the teaching practices.

At the international level, the first advances in CA were made in countries such as the United States, Canada, Australia and the United Kingdom (Sterling and Scott, 2008). In Colombia, efforts in environmental education in higher education began in the seventies with the introduction of studies of ecology and the environment, as well as the conservation of natural resources (Pabón, 2006, Gómez and Botero, 2012). According to Gómez and Botero (2012), in the eighties, professional training programs were established in the environmental field, incorporating this subject in other professions and disciplines. In a qualitative study carried out in three institutions in Colombia (National University of Colombia-Headquarters Medellín, Universidad San Buenaventura-Sede Medellín and the National Service of Learning) in 2012 on the degree of incorporation of the environmental issue in the educational curriculum, reflected advances achieved in the search for the environmentalization of their mission processes and that these processes should seek the adoption of pro-environmental principles that guide the institutional practice, promoting greater awareness and participation of the entire community (Gómez and Botero, 2012). According to Gómez and Botero (2012), in the eighties, professional training programs were established in the environmental field, incorporating this subject in other professions and disciplines. In a qualitative study carried out in three institutions in Colombia (National University of Colombia-Headquarters Medellín, Universidad San Buenaventura-Sede Medellín and the National Service of Learning) in 2012 on the degree of incorporation of the environmental issue in the educational curriculum, reflected advances achieved in the search for the “environmentalization” of their mission processes and that these processes should seek the adoption of pro-environmental principles that guide the institutional practice, promoting greater awareness and participation of the entire community (Gómez and Botero, 2012).

This paper presents the evolution of the planning and execution of an intervention project whose main objective is to promote Environmental Education (EE) in educational institutions through the curriculum. Thanks to the follow-up of this project, we can appreciate the real impact that the development of these actions has generated in a group of high school students, who participated in the academic activities of this project (Velázquez and Blanc, 2018).

## **MATERIAL AND METHODS**

From a qualitative approach, two methods of this nature were considered. The first is the documentary research that leads to the theoretical review which allows to identify the conceptualization of culture and how it can move towards an environmental culture and in turn, sustainable. In addition, it was important to consider linkages between this concept with the CE matter and its agroecological relationship. The second is the use of participatory action research, since the researcher is involved as coordinator of the intervention project, conceiving it as a set of systematically planned actions, based on needs fully identified and

oriented to goals that respond to what happens, which must contain theoretical elements that support it (Rodríguez *et al.*, 1990). This involvement allows you to guide the search, refer to the research and give a close follow-up that gives you more information about the development of the project.

To make the analysis as part of this study, the systematization method was used. The fundamental principles of this methodology are the following: the analysis is based on a set of indicators, is directly related to the evaluation of the experience, consider goals and objectives to determine levels of progress or success; the evaluation of the experience is carried out through the representation of opinions, judgments, and questions of what has been done and what was lived (Chávez, 2006). In this work, a critical opinion is extracted about the experiences of the development of environmental culture in the institutions of upper secondary education and when evaluating it, we obtain evidence of the advances of a CE, under an agroecological approach, in the educational practices.

The research instrument to obtain information and with this guide, the analysis based on the application of a questionnaire, designed based on the instruments made in the diagnosis for the implementation of the EPO 100 project towards sustainability in September 2016. This questionnaire is made available to previous and current students who have participated in the project, through a database with information to contact them (name, email, Facebook account), In addition, from the beginning of the project, an account was created Facebook called "EPO 100 towards sustainability", in which information related to the project was published and followers interacted with their comments and dissemination of this page. With this, it allows having a fairly broad view of the impact of learning activities, under the EE approach, on the environmental culture of the students. The project began in September 2016 and is still ongoing. So this questionnaire highlights the vision of the CE that students have at this point in the project, comparing them with the perspective they had before participating in this project of educational intervention. With this instrument, one can consider a perception of the average environmental culture of high school students after participating in sustainable practices in the school setting (Velázquez and Blanc, 2018).

The procedure used in the development of this questionnaire was through a digital application called *surveyMonkey.com*, the participants accessed the survey through this link <https://www.surveymonkey.com/r/Q8BZYBH>. In this program, the questions are designed and the link is sent to the participants who have a social network or email to answer it. The information obtained is graphed and ordered by the same application.

This questionnaire was applied on May 3, 2018, and the results of the first 84 students surveyed were trained, of the 840 students (number of students and alumni who participated in this project) to whom this instrument was sent for their participation via email and through the use of the social network Facebook page <https://www.facebook.com/epo100.com.mx/> where they are followers. With this, an analysis of their opinions and their relationship with the sustainable

practices that they have carried out in the academic activities under the CE approach was made.

## RESULTS AND DISCUSSION

Working directly with the students of EPO 100 makes it possible to develop these questionnaires in an explicit way regarding the evolution of an environmental education. Indeed, students have participated in various activities related to the preservation of the environment during the development of this project, among the most outstanding is the collection of PET containers for recycling and the development of biodigester bales: the latter consists of reusing food waste to create organic fertilizer for crops, is much simpler than a compost, can be done quickly and with basic tools, plus laboratory studies for organic fertilizer have proven their effectiveness in their physicochemical properties and the content of macro and micronutrients (Velázquez et al, 2017).



Figure 1. Activities developed in the EPO 100 project towards sustainability linked to the subjects of Innovation and technological development, Chemistry and Creativity.

38.5% of respondents believe that the care and conservation of the environment should be part of a habit of life, a percentage that exceeds those who responded that should be a need, a duty and obligation. Although in question (P) 6, 50% expressed an intermediate participation in the development of sustainable actions, giving a score of three on a scale, where one is the lowest score and five the highest. This reflects that considering these sustainable practices as a habit is not synonymous with being carried out in a constant and active way by the participants. Another data to consider, is the level of impact of this project in the EPO100, in this study 78% of respondents report that the conceptualization of sustainability has been heard constantly in school, compared to the results applied in the diagnosis prior to the implementation of this project in 2016 that was 55%.

In addition, 83 of the 84 respondents are in favor of this type of projects being institutionalized and part of school activities, where 40% of participants considered that their level of participation in this project is 4, on a scale where the one is the lowest score and five the highest. For this, in P10 more than half of the students expressed that for EPO100 to be consolidated towards sustainability,

there must be greater participation of students, teachers, and managers. Although 30% say that it is important that this project is part of the institutional regulation for compliance.

In P3, more than 70% of the students expressed as the main problem that affects their local environment is the excessive generation of garbage and the lack of efficient methods for their management, a percentage that increases compared to the study applied in 2016, where to this same question 56% expressed this same answer. The most surprising fact is that thrown by the P8 where 61% say that the school has been the medium that has driven their habits for a practice of sustainable actions, followed by a 31% that expresses the family as the means to the development of these actions. Data that is related to P3 where it is observed that 3 out of 4 participants have implemented the activities of this intervention project at home or in another space outside the school context. Before this, more than half of the respondents expressed that in the family, the school and the media, a sustainable culture should be promoted for the benefit of the conservation of the natural and social environment.

Given this, education is a factor of change for the development of culture for the benefit of society. Environmental education is the way that facilitates the transmission of cultural keys so that the individual and social groups adapt responsibly. The curriculum around the environment should be reformulated from an integral perspective (Mata, 2004). This will make it possible to redefine the culture environmentally because every culture in itself is environmental and if the environment is related to sustainable growth, it propitiates the maintenance of the capitalist system that currently influences society. A change that extends to all sectors of society, promoting a culture of sustainability, not only in the political sphere but also in social agents and citizens as a whole (Aznar, 2003, Meira and Caride, 2006).

In the case of EPO 100, for two years now the project "EPO 100 towards sustainability" has been implemented, which aims to guide this institution to sustainable practices through curricular environmentalization in teaching plans by teachers and that these are reflected in the learning activities and in the promotion of an environmental culture, a different institutional dynamic has been manifested since its application, where the participants conceive this environmental conceptualization as part of their daily work, although this does not yet mean that all their daily practices are sustainable for the care and conservation of natural resources (Velázquez and Blanc, 2018).

## CONCLUSIONS

The environmental issue influenced by culture is appreciated with greater objectivity, which, in turn, is in constant epistemological dynamism due to external factors, such as globalization, among one of the main causes. It is through the process of enculturation where mechanisms can be given that favor the incorporation of elements of an environmental culture for the development of sustainable practices. It is important that the family promotes the construction of

a sustainable culture among its members. It is expected that this intervention project in educational institutions will allow participants to carry out this enculturation in a medium or short term.

On the other hand, environmental problems must be addressed from different areas of knowledge, such as agroecology, in a transversal manner, making use of innovation to search for alternatives and orientation towards sustainable practices.

When addressing curricular environmentalization, the comprehensive education of upper secondary education students is considered under an approach that links learning with the development of an environmental culture that affects the context in which students interact. Given this, education is a factor of change for the development of culture for the benefit of society, it is the EE that facilitates the transmission of cultural keys for the individual and social groups to adopt responsibly.

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## INCREASING OF STORAGE PERIOD ALTERS EMBRYO DEVELOPMENT AND HATCHING CHARACTERISTICS OF PEKIN DUCK EGGS

### SUMMARY

This research was performed to determine the effects of storage period on embryo development and hatching characteristics in Pekin ducks. A total of 360 Pekin duck eggs was divided into four groups as 5-7 d, 8-10 d, 11-13 d and 14-16 d storage period and each group were stored at 15-18 °C. Eggs were incubated at 37,5°C and a relative humidity of 55 to 60% during the first 24 days of incubation. These eggs were transferred into hatching machine for the last four days. A hatcher temperature of 37.0°C and a relative humidity of 72% were provided during hatching period.

The effects of storage period on embryo development, embryonic mortality, hatchability of fertile eggs, hatchability of total eggs and chick hatching weight were significant ( $P<0.01$ ). Results showed that a longer storage period caused a decline in yolk absorption and therefore decline of embryo growth parameters including body weight and length during incubation period. Hatchability declined with increasing of storage duration, and a storage period less than 7 d appeared to be the best for maximum hatchability. Egg weight loss increased with increased storage length ( $P<0.01$ ), and the chick weight tended to decline in relation with storage period longer than 5-7 days.

**Keywords:** Pekin duck, egg storage, embryo development, hatching characteristics.

### INTRODUCTION

Recently, meat-type duck production, which has a huge economical value in Asian countries, has gained increasingly importance in other countries as an alternative poultry species for animal protein requirement. Due to the rapid growth in the world population, there is a growing trend in meat-type duck production (Ipek and Sözcü, 2017).

At that point, white Pekin duck is one of the most popular meat-type duck strains and is widely produced in most countries, for example China, Korea, England, and France (Heo et al., 2015; Wen et al., 2015).

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In commercial practices, hatching eggs are stored for short or long time until the setting time to obtain sufficient number for maximum capacity of incubator. It is known that prolonged of storage period has detrimental effects for embryo growth, embryonic mortalities, hatchability and in fact chick quality at hatching (Tona et al., 2003, 2004; Reijrink et al., 2009).

This research was performed to determine the effects of four storage periods (5-7 d, 8-10 d, 11-13 d and 14-16 d) on embryo development and hatching characteristics in Pekin ducks.

### MATERIAL AND METHODS

The research was performed at the Research and Experimental Farm of the Department of Animal Science in Uludağ University in Turkey. A total of 360 hatching eggs were collected from a breeder flock at 36 to 38 wk of age. The breeder flock was kept according to the standard industry practices in the experimental farm of the faculty. All eggs were numbered and weighted with  $\pm 0.1$ g precision before storage period. The eggs were stored for 5-7 d (S-I), 8-10 d (S-II), 11-13 d (S-III) and 14-16 d (S-IV, n=90 eggs/storage period). During storage period, eggs were kept at a temperature of 17.0°C and a relative humidity of 75%.

After the storage period, all eggs were weighted again and incubated in a fully automated, ventilated, programmable incubator at 37.5°C and a RH of 55 to 60% during the first 24 d of incubation. The eggs from each storage period group were randomly placed into incubator trays consisting of 30 eggs (n = 3 trays/storage period group). On d 25 of incubation, eggs were weighed to determine the weight loss during incubation, and all eggs were transferred to a hatcher. The hatcher was operated at 37.0°C and 72% relative humidity.

A total of 10 eggs per each group were randomly sampled for measurement of embryo development on d 25 of incubation. The eggs were carefully cracked, and embryos were killed by cervical dislocation. The embryos were separated from the yolk sac. Excessive embryonic fluid was dried off, and the embryos were weighed for embryo weight and yolk sac weight to calculate the yolk free body weight, relative embryo and yolk sac weights (Willemsen *et al.*, 2010; Ipek *et al.*, 2014). The embryo length was measured from the tip of the beak to the tip of the middle toe by placing the embryo face down on a flat surface and straightening the right leg (Hill, 2001; Nangsuay *et al.*, 2011). The shank length was measured from right knee joint to the tip of the middle toe (Willemsen *et al.*, 2008).

After the completing of hatching process, all of the hatched chicks were pulled out according to standard hatchery procedures, and were weighed with  $\pm 0.1$  precision to determine the chick hatching weight. Then, the chicks were classified into two categories as saleable and cull chicks. The percentages of saleable and cull chick were determined as a percentage of fertile eggs. Unhatched eggs were opened to macroscopically determine fertility and embryonic mortality (early-term, mid-term and late-term embryonic mortalities).

Mortality and fertility were calculated as the percentage of total eggs at set to fertile eggs.

The data were subjected to analysis of variance (SAS, 1998) utilizing ANOVA procedures for balanced data. The parameters were analyzed using the general linear model (GLM) procedure. In the study, 3 replicate trays (30 eggs per tray) were used for each treatment group, and the trays were considered an experimental unit. Analyses for the percentage data were conducted after square root of the arc sine transformation of the data. Significant differences among treatment means were determined by Duncan's multiple range test. Data are presented as means  $\pm$  SE. Differences were considered significant at  $P < 0.05$ .

## RESULTS AND DISCUSSION

The effects of different storage periods on embryo development and yolk absorption on day 25 of incubation period are presented in Table 1. Embryos in S-I and S-II groups were found to be heavier compared to embryos in S-III and S-IV groups (36.1 g and 35.4 g vs. 33.2 g and 31.2 g, respectively). Similar difference was also observed for relative embryo weight. A higher yolk sac weight and relative yolk sac weight (respectively 18.7 g and 23.9%) were found in embryos of S-IV group. On the other hand, yolk free body weight, embryo body and shank length were found to be higher in S-I and S-II groups compared to other groups. Results showed that prolonged storage periods retarded embryo growth during incubation.

This could be related with some changes in the embryo growth and egg characteristics during storage period (Lapão *et al.*, 1999), for example an increment in albumen pH (Lapão *et al.*, 1999), a decline in albumen height (Burley and Vadehra, 1989), and strength of the vitelline membrane (Fromm, 1966). Also a retarded of embryo growth could be associated with a higher egg weight loss during storage in S-IV group, compared to the other groups. This finding is supported by Reijrink *et al.* (2010).

Table 1. The effect of different storage periods on embryo development and yolk absorption on day 25 of incubation period

Parameters	Storage periods			
	S-I	S-II	S-III	S-IV
Egg sampling weight (g)	77.2 $\pm$ 1.6	78.4 $\pm$ 1.4	77.7 $\pm$ 1.5	78.1 $\pm$ 1.5
Embryo weight (g)	36.1 $\pm$ 1.4 <sup>a</sup>	35.4 $\pm$ 1.7 <sup>a</sup>	33.2 $\pm$ 1.1 <sup>b</sup>	31.2 $\pm$ 1.1 <sup>c</sup>
Relative embryo weight (%)	46.8 $\pm$ 1.8 <sup>a</sup>	45.1 $\pm$ 1.9 <sup>a</sup>	42.7 $\pm$ 1.5 <sup>b</sup>	39.9 $\pm$ 1.4 <sup>c</sup>
Yolk sac weight (g)	14.6 $\pm$ 1.1 <sup>c</sup>	15.3 $\pm$ 1.2 <sup>c</sup>	17.5 $\pm$ 1.2 <sup>b</sup>	18.7 $\pm$ 1.1 <sup>a</sup>
Relative yolk sac weight (%)	18.9 $\pm$ 1.4 <sup>c</sup>	20.2 $\pm$ 1.3 <sup>c</sup>	22.5 $\pm$ 1.2 <sup>b</sup>	23.9 $\pm$ 1.3 <sup>a</sup>
Yolk free body weight	21.5 $\pm$ 2.3 <sup>a</sup>	20.1 $\pm$ 2.0 <sup>a</sup>	15.7 $\pm$ 1.4 <sup>b</sup>	12.5 $\pm$ 1.6 <sup>c</sup>
Body length (mm)	187.1 $\pm$ 4.1 <sup>a</sup>	184.6 $\pm$ 5.3 <sup>a</sup>	177.2 $\pm$ 4.5 <sup>b</sup>	166.9 $\pm$ 4.3 <sup>c</sup>
Shank length (mm)	45.0 $\pm$ 1.0 <sup>a</sup>	44.7 $\pm$ 1.1 <sup>a</sup>	43.6 $\pm$ 1.1 <sup>b</sup>	42.1 $\pm$ 1.2 <sup>b</sup>

<sup>a-c</sup> Means in the rows with different letters significantly ( $P < 0.05$ )

S-I: 5-7 d, S-II: 8-10 d, S-III: 11-13 d, S-IV: 14-16 d

n: 10 eggs/storage period

The effects of different storage periods on incubation results are presented in Table 2. Initial egg weight was 77.4 g in S-I, 78.1 g in S-II, 78.0 g in S-III, 77.9 g in S-IV group. Egg weight loss until transfer was found to be the highest with a value 18.4% in S-IV group compared to other groups. Hatchability of fertile and total eggs was higher in S-I (84.8% and 74.4%, respectively) and S-II (83.1% and 71.1%, respectively) groups.

The highest percentages for early and late term embryonic mortalities were observed in S-IV group, whereas mid-term embryonic mortalities were higher in S-III and S-IV groups. The percentage of cull chick was the lowest with a value of 1.4% in S-I group. On the other hand, chick hatching weight and relative chick weight were the highest in S-I (48.4 g and 62.5%) and S-II (47.7 g and 61.2%) groups.

Table 2. The effect of different storage periods on incubation results

Parameters	Storage periods			
	5-7 d	8-10 d	11-13 d	14-16
Number of eggs	90	90	90	90
Average egg weight (g)	77.4±1.2	78.1±1.3	78.0±1.0	77.9±1.2
Egg weight at transfer (g)	69.5±1.4 <sup>a</sup>	68.1±1.3 <sup>b</sup>	65.3±1.2 <sup>c</sup>	63.5±1.1 <sup>d</sup>
Egg weight loss (%)	10.2±1.5 <sup>d</sup>	12.8±1.6 <sup>c</sup>	16.2±2.1 <sup>b</sup>	18.4±2.2 <sup>a</sup>
Fertility (%)	87.8±2.8	85.6±2.4	86.7±2.1	85.6±2.6
Hatchability of fertile eggs (%)	84.8±2.5 <sup>a</sup>	83.1±2.6 <sup>a</sup>	78.2±2.7 <sup>b</sup>	74.0±2.7 <sup>c</sup>
Hatchability of total eggs (%)	74.4±3.6 <sup>a</sup>	71.1±3.4 <sup>a</sup>	67.8±3.1 <sup>b</sup>	63.3±2.9 <sup>c</sup>
Early term TEM* (%)	6.3±0.9 <sup>c</sup>	6.5±1.1 <sup>c</sup>	7.7±1.0 <sup>b</sup>	9.1±1.4 <sup>a</sup>
Mid TEM* (%)	1.3±0.9 <sup>b</sup>	1.3±0.8 <sup>b</sup>	2.6±0.8 <sup>a</sup>	2.5±0.7 <sup>a</sup>
Late TEM* (%)	7.6±1.3 <sup>c</sup>	9.1±1.8 <sup>c</sup>	11.5±2.2 <sup>b</sup>	14.3±2.7 <sup>a</sup>
Contaminate egg ratio (%)	0.0±0.0	1.6±1.2	1.6±1.3	1.8±1.3
Cull chick (%)	1.4±0.7 <sup>c</sup>	1.7±0.6 <sup>c</sup>	3.3±1.1 <sup>b</sup>	7.1±1.4 <sup>a</sup>
Chick hatching weight (g)	48.4±1.9 <sup>a</sup>	47.8±1.7 <sup>a</sup>	45.4±1.4 <sup>b</sup>	43.5±1.6 <sup>c</sup>
Chick/egg weight ratio (%)	62.5±2.1 <sup>a</sup>	61.2±1.8 <sup>a</sup>	58.2±1.5 <sup>b</sup>	55.8±2.0 <sup>c</sup>

<sup>a-d</sup> Means in the rows with different letters significantly (P<0.05)

\*TEM: term embryonic mortalities n: 3 trays per each egg weight group

Observed higher egg weight loss and embryonic mortalities and a decline in hatchability in prolonged storage period (S-III and S-IV) are consist with previous findings by Wilson (1991), Renema *et al.* (2006), Reijrink *et al.* (2010).

Similarly, the negative effects of prolonged storage in S-III and S-IV on chick quality parameters are also reported by Tona *et al.* (2003) for chick weight and chick quality parameters, by Wolanski *et al.* (2004), Lourens *et al.* (2005) for yolk-free body weight, and by Hill (2001), Wolanski *et al.* (2004), Molenaar *et al.* (2008) for body length.

## CONCLUSIONS

In conclusion, it seems to the storage length had negative effects for egg weight loss, embryo development, embryonic mortalities, hatchability and chick quality in Pekin duck eggs.

Therefore, during storage period, some environmental factor associated with storage (storage temperature and humidity) and storage length could be considered to minimise the negative effects of storage.

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## **IDENTIFICATION OF PHYTOPATHOGENIC BACTERIA IN MAIZE SEEDS IN UKRAINE**

### **SUMMARY**

The main bacterial diseases of maize: bacterial wilt of maize (*Pantoea stewartii* subsp. *stewartii*), goss's bacterial wilt (*Clavibacter michiganensis* subsp. *nebraskensis*), seed rot-seedling blight of maize (*Bacillus subtilis*), bacterial spot of maize (*Pseudomonas syringae* pv. *syringae*), bacterial leaf spot of maize (*Pantoea agglomerans*) and bacterial stalk rot of maize caused by several causative agents, were described. The causative agents of these diseases are often stored and transmitted with seeds, so seed analysis for the presence of phytopathogenic bacteria is an important step in the pathogen control system. For analysis, the corn seeds that were grown in the Poltava and Kiev regions of Ukraine were used. Microbiological analysis of seeds was carried out by classical methods. From visually healthy corn seeds, that form shoots in the field conditions, strongly affected by root rot, were isolated the strains of *Pseudomonas fluorescens*. Isolated *P. fluorescens* strains were the reason for the damage of soft rot in the field. This type of bacteria is an opportunistic pathogen and is capable of causing soft rot of a number of crops. It has been established that *P. fluorescens* cause root rot of maize seedlings. From visually healthy seeds, from which healthy corn seedlings form, *Pantoea agglomerans* and *Pantoea ananatis* were isolated. *P. agglomerans* are widespread plant epiphyte. *P. ananatis* causes disease symptoms in a wide range of economically important crops and forest tree species worldwide. It is regarded as a pathogen based on the increasing number of diseases reports on previously unrecorded hosts in different parts of the world and can cause damage to maize seedlings in the field.

**Keywords:** maize, seeds, bacterial diseases, *Pantoea agglomerans*, *Pseudomonas fluorescens*, *Pantoea ananatis*.

### **INTRODUCTION**

Maize – is one of the main crops of modern world agriculture. This crop is of various uses and has high crop yield. About 20 % of kernels of maize are used

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for food requirements in the world, 15 – 25 % are used for the technical purposes, in particular, the trunks and maize ears are used as alternative energy sources and nearly two-thirds is used for fodder. Corn ethanol is the most widely used biofuel in the USA and other countries.

Losses in the yield of maize from diseases are equal to 2-8 %, and in some years may reach 15-20 % (Gvozdyak *et al.*, 2011). Loss of maize crops to major diseases and pests make up in southern Russia on average 25-30% (Ivashchenko, 2010). The widespread use of pesticides leads to changes the pathogens composition of corn and an increase of bacterial diseases of plants (Patyka *et al.*, 2016).

The main bacterial diseases of maize are the bacterial wilt of maize (*Pantoea stewartii* subsp. *stewartii*), goss's bacterial wilt and blight (*Clavibacter michiganensis* subsp. *nebraskensis*), seed rot-seedling blight of maize (*Bacillus subtilis*), bacterial spot of maize (*Pseudomonas syringae* pv. *syringae*), bacterial leaf spot of maize (*Pantoea agglomerans*) and bacterial stalk rot of maize caused by several causative agents (Gvozdyak *et al.*, 2011).

**Bacterial wilt of maize;** *pathogen name: Pantoea stewartii* subsp. *stewartii* (Smith) Mergaert *et al.* Wilt of maize was newly diagnosed by F. Stewart in the USA in 1897. According to the official report European and Mediterranean Plant Protection Organization (EPPO) Stewart's wilt distributed on the American continent: Argentina, Bolivia, Canada, Mexico, Peru, Puerto Rico, United States of America. *P. stewartii* subsp. *stewartii* also detected in India, Korea, Benin, Togo. Stewart's wilt absents in Europe as of 11.04.2017 (EPPO Global Database, 2017). State service of Ukraine for food safety and consumer protection reported as of 01.01.2017 on the identifying bacterial wilt of corn in Ukraine (SSU, 2017).

The harmfulness of wilt is very big. Plants die in the case of infection of maize in the phase of underground seedlings. It is revealed that at average affection of plants, the yield decreases by 50-65%. The planting of maize die almost completely during epiphytotic (OEPP/EPPO, 2016; Pataky, 2003). *P. stewartii* subsp. *stewartii* bacteria spread by the vessels of the whole plant, they get even into the root system and grain. From the vascular system they can get into the parenchymal tissue of leaves, stalk, damaging it. By accumulating in vessels, they occlude them and violate the water regime. Rate of spread of bacteria in the plant is connected with the speed of transpiration. The wilt is happening not only as the result of vascular occlusion, but also under the influence of toxin of polysaccharide nature, which are produced by *P. stewartii* subsp. *stewartii*. The bacteria that cause Stewart's wilt (*P. stewartii* subsp. *stewartii*) is vectored by corn flea beetles (*Chaetocnema pulicaria*) that overwinter in the soil and feed on young corn plants in the early season. The small, shiny black beetles are very active. Small percentage of these flea beetles survive the winter with the *P. stewartii* subsp. *stewartii* bacteria in their guts.

*Management of Stewart's wilt.* One of the most effective methods of protection is the pre-sowing treatment of corn seeds with insecticides and



compliance with the schedules of insect protection measures throughout the growing season. It is shown that an effective method of *P. stewartii* subsp. *stewartii* control can be previous treatment of maize seeds with imuclopride.

**Goss's bacterial wilt and blight;** *pathogen name: Clavibacter michiganensis* subsp. *nebraskensis* (Vidaver *et* Mandel) Davis *et al.*. Disease was newly diagnosed on sugar maize in the USA (Nebraska State) in 1969, and later in other states throughout the Midwest, including Illinois, Wisconsin, and Iowa. It is the vascular disease by its symptoms, it practically doesn't differ from bacterial wilt of maize caused by *P. stewartii* subsp. *stewartii* (Mallowa *et al.*, 2016).

*Clavibacter michiganensis* subsp. *nebraskensis* can survive on and in seed, and can be transmitted from seeds to seedlings. Seed-to-seedling transmission is low (about 2 percent), but infested seed may introduce the disease into new areas. Grass weeds such as green foxtail and shattercane can also serve as perennial hosts for the bacteria, and be a reservoir from which bacteria spread to corn plants. Low levels of the disease may go undetected until environmental conditions favor widespread disease (Wise *et al.*, 2010).

**Seed rot-seedling blight of maize;** *pathogen name: Bacillus subtilis* (Ehrenberg) Cohn. In 1936 the seedling blight in Ukraine was discovered by Ph. E. Nemlienko, who allocated the pathogen (Gvozdyak *et al.*, 2011). The disease happens in the steppe and forest-steppe regions of Ukraine, in Russia, Kabardino-Balkaria, Bashkiria, Uzbekistan and Georgia (Ivashchenko, 2010). Bacterial disease does great damage to seed maize: the storability of seeds and their viability worsen. The plants, which grew from infected seeds, retard in growth, especially during the first period of vegetation, the yield decreases by 20%.

*B. subtilis* is the weak pathogen. Bacteria aren't capable to penetrate into the germ and the plant, which develops from it, therefore the disease isn't transmitted directly through the seeds to adult plant and from infected corn seeds to the healthy ones. The soil is the place of natural existence of *B. subtilis*. The leading role in the spread of infection is played by the capsid grain bug *Trygonotylus ruficornis*. The affection of ears by the causative agent of bacterial disease has direct correlation with the numbers of this insect. The especially dangerous is the number of capsid grain bug on the maize field in the stages of milky and the beginning of wax ripeness – the periods, most favorable for disease of plants.

**Bacterial spot of maize;** *pathogen name: Pseudomonas syringae* pv. *syringae* van Hall. Disease was newly diagnosed on the maize in Iowa State (USA) in 1916, later on sorghum and other sorghum plants (Gvozdyak *et al.*, 2011). Maize is differently affected by the causative agent of red bacterial spot in different years and in different climatic conditions. At high temperature and intermediate moisture the spot on the leaves of maize appears before the heading of panicles (June-July) and affects 30-50% of plants. In the years with the large amount of precipitation and low temperature it appears later (August-September) and affects 15-30% of plants. Red bacterial disease almost doesn't appear on the

maize in dry years. Stalk rot, which is caused by *P. syringae* pv. *syringae*, is characterized by great harmfulness, as the affected plants fail to produce. However, the extension of disease is small, as the single plants ( -7%) are sometimes affected (Paccola-Meirelles *et al.*, 2001).

*P. syringae* pv. *syringae* affects maize, sorghum and Sudan grass in all area of their cultivation. However, the Sudan grass and sorghum are more affected, than maize. The causative agent can be on the surface and in internal tissues of maize, sorghum and Sudan grass. The causative agent is spread by the seeds, and also by other cereals. In the case of artificial inoculation by *P. syringae* pv. *syringae* the symptoms of disease are observed on the millet, Siberian millet, foxtail, pale pigeon grass on the 4–5-th day (Ivashchenko, 2010).

**Bacterial leaf spot of maize;** *pathogen name: Pantoea agglomerans* (Beijerinck) Gavini *et al.* In cold summer months on leaf blades and sheaths of maize appear elongated spots, which at first are green and oily, and later they get light-brown coloring. Then the spots become roundish, oval or of irregular shape, with the yellowish aureole. Diameter of spot is 2-10 mm. The brown center and red-brown brim are formed in the spots over time, from what the leaf blade gets the "burnt" look. At severe damage the plants stop the growth, and new leaves are small and chlorotic (Gvozdyak *et al.*, 2011; Paccola-Meirelles *et al.*, 2001).

**Bacterial stalk rot of maize;** stalk rot is caused by several causative agents (Gvozdyak *et al.*, 2011):

**1) *Pectobacterium carotovorum* subsp. *carotovorum*** (Jones) Hauben *et al.*

Disease is characterized by full die-back of plants or their tops. The rot, which appears in the top part of the stalk in the place of initiation of panicles, can spread all over the stalk. The stalk has no signs of affection from external side. The rotting tissues of the stalk have very unpleasant smell. The affection of sprouts of maize by *P. carotovorum* subsp. *carotovorum* can reach 20%. The especially large number of plants of maize is affected upon conditions of irrigating method of cultivation. The appearance of stalk rot on lateral shoots starts in the middle of August, at the same time the stalk looks healthy from outside, the central leaf, which is rolled and turned yellow, draws attention.

**2) *Enterobacter dissolvens*** (Rosen) Brenner *et al.*

Disease was newly diagnosed in the USA in 1919. The bacterial disease does great damage to the plantings of maize in America in certain years. The stalk rot of maize, which is caused by *E. dissolvens*, is widespread on the fields of the number of areas of Ukraine, Krasnodar Krai, in the North Caucasus, in some regions of Russia (Ivashchenko, 2010). This bacterial disease occurs only in certain years in small amounts, in the size, which doesn't exceed the tenth part of the percent. According to other researchers, the prevalence of *E. dissolvens* reached 20% in the Saratov region. Disease occurs on young and adults plants. Symptoms are mainly manifested on the plants in the first half of vegetative period, before they reach the height of 60-70 cm. The temperature of 30–35°C and high moisture helps in development of disease. In the years, when the amount of precipitation is insignificant, the rot gains the small sizes. In dry years

the rot reaches 1 – 2%. Yield losses in the years with precipitation above the norm reached 10 – 30%.

*E. dissolvens* affects only maize. The causative agent gets into the plants through the water pores, stomata and various mechanical damages.

3) *Dickeya zea* (Burkholder, McFadden *et* Dimock) Samson *et al.*

The stalk rot of maize, caused by *D. zea*, was newly diagnosed by K. Sabet in Egypt in 1953. Later he described the similar disease in Southern Rhodesia and India. Soft rot of maize was also revealed in Hungary. The level of affection of maize was from 3 to 15%. *D. zea* is a major disease of maize in tropical and subtropical countries. It is particularly severe under conditions of high temperature and humidity. In temperate regions the disease is only a problem with overhead irrigation.

**Leaf spot of maize;** *pathogen name: Pantoea ananas* Mergaert, Verdonck *et* Kersters. Bacterial leaf spot of maize was newly diagnosed in Brazil in 1982. Nowadays the disease is widespread in all areas of cultivation of maize in Brazil and leads to substantial economic losses (Gvozdyak *et al.*, 2011).

Since corn seeds can be a place of conservation of pathogenic bacteria and in the future serve as a source of plant infection, the **aim** of our work is, the identification of pathogenic bacteria on the seeds of corn grown in Ukraine.

## MATERIAL AND METHODS

For analysis, the corn seeds that were grown in the Poltava and Kiev regions of Ukraine in 2016 – 2017 were inverted. The seeds were divided into two groups: Group 1- visually healthy seeds, which form in the field conditions shoots, are strongly affected by root rot, Group 2- visually healthy seeds, from which healthy corn seedlings are formed. Microbiological analysis of seeds was carried out by classical methods. The pathogenic properties of isolated bacterial isolates were determined by artificial inoculation of corn seedlings under laboratory conditions (Klement *et al.*, 1990; Patyka *et al.*, 2014).

To study the morphological and biochemical properties that are important for the identification of isolated bacteria, both the classical bacteriology methods (Klement *et al.* 1990; Patyka *et al.*, 2014) and the API 20E test system (BioMerieux) are used. API 20E test-system contain tests to identify gram-negative facultative anaerobic rods.

## RESULTS AND DISCUSSION

The investigated corn seeds were characterized by good germination (90%) and no signs of seed bacterial damage were detected by visual inspection. However, seedlings from the seeds of Group 1 on the first, second sheet have brown spots that increase along the veins of the leaf.

In microbiological analysis, it was found that the seeds of Group 1, visually healthy seeds, which form in the field conditions shoots, are strongly affected by root rot, are predominantly inhabited by bacteria that form white or gray translucent colonies. While the seeds of Group 2, visually healthy seeds,

from which healthy corn seedlings are formed, were dominated by bacteria that form yellow-pigmented colonies (Figure 1).

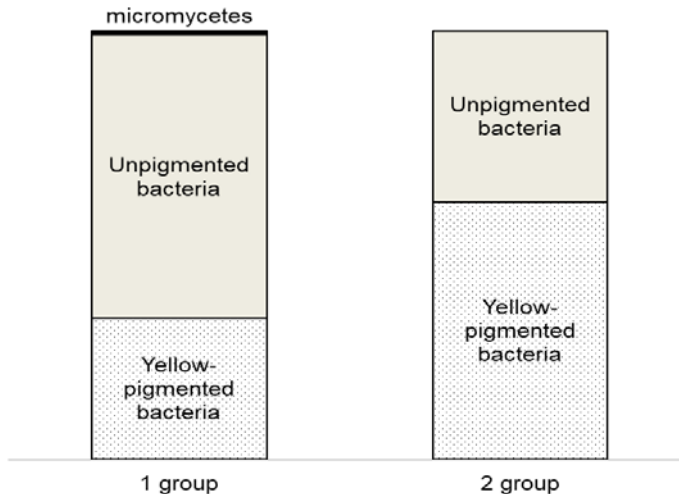


Figure 1. Bacterial biota of corn seeds

We selected all types of isolates from all the samples studied. A total of 70 isolates were selected. For the detection of phytopathogenic bacteria, pathogenic properties (artificial inoculation of maize seedlings in laboratory conditions) of all selected isolates were tested.

Among the unpigmented isolates that were isolated from the seeds of Group 1, five isolates were found that were pathogenic for maize. These isolates caused symptoms of bacterial damage on maize seedlings and maceration of tissues of potato tubers.

For identification of pathogenic isolates from seeds of Group 1 their morphological and physiological properties were studied. All isolates were gram-negative mobile aerobic rods (Table 1). Unpigmented pathogenic isolates from Group 1 maize seeds based on phenotypic properties were identified as *Pseudomonas fluorescens*. This type of bacteria is an opportunistic pathogen and is capable of causing soft rot of a number of crops.

Since one of the most dangerous causative agents of maize diseases belongs to the yellow-pigmented enterobacteria, we carefully analyzed all the yellow-pigmented isolates from both groups of seeds. 24 bacterial isolates on the basis of their morphological and biochemical properties were attributed to the genus *Pantoea* (Table 2).

Bacteria of the genus *Pantoea* are facultative anaerobic Gram-negative non-spore-forming oxidase-negative rods. Several species of bacteria which pathogenic for maize belong to this genus. First of all it is *Pantoea stewartii* subsp. *stewartii*. Also to the genus *Pantoea* belong species *P. stewartii* subsp. *indologenes*, *P. ananatis* and *P. agglomerans* (widespread plant epiphyte).

Table 1. Cultural and physiological properties of phytopathogenic isolates from seeds Group 1 (visually healthy seeds)

Test	Isolates from maize seeds	<i>Pseudomonas fluorescens</i> (Boore et Castenholz, 2005)
The form of cells	Rods	Rods
Gram's staining	-	-
Motility	Motile	Motile
OF-test	Aerob	Aerob
Pigment	-	-
Fluorescent pigment	+	+
Oxidase	+	+
HR	-	-
Pectinase	+	+
Artificial infection of corn seedlings	+	+
Arginine dihydrolase	+	+
Lysine decarboxylase	-	-
Formation of H <sub>2</sub> S and indole	-	-
Utilization:		
D-glucose, D-melibiose, L-arabinose	+	+
D-sorbitol, L-rhamnose, D-sucrose, D-mannitol, D-melibiose	-	-

Table 2. Cultural and physiological properties of yellow-pigmented isolates from maize seeds.

Isolates	Yellow pigment	The form of cells	Gram's staining	Spore formation	Oxidase	OF-test
5 isolates from Group 1 seeds	+	Rods	-	-	-	Facultative anaerobic
17 isolates from Group 2 seeds	+	Rods	-	-	-	Facultative anaerobic
Genus <i>Pantoea</i> (Boore et Castenholz, 2005)	+	Rods	-	-	-	Facultative anaerobic

In order to determine the species belonging of isolates, it was necessary to conduct additional tests to differentiate closely related species *P. stewartii* subsp. *stewartii*, *P. stewartii* subsp. *indologenes*, *P. ananatis*, *P. agglomerans* (Table 3) (Mergaert *et al.*, 1993; Boore et Castenholz, 2005). On the basis of differentiating

microbiological tests, isolates from maize seeds of the genus *Pantoea* were identified as *Pantoea agglomerans* and *Pantoea ananatis* (Table 4). All five isolates from the seeds of Group 1 belong to *P. agglomerans*. Three isolates from the seeds of Group 2 were identified as *P. ananatis*, 14 isolates from the seeds of Group 2 were identified as *P. agglomerans*.

Table 3. Tests for identification plant associated *Pantoea*(Mergaert *et al.*, 1993;Boore *et Castenholz*, 2005)

Test	<i>Pantoea stewartii</i> subsp. <i>stewartii</i>	<i>Pantoea stewartii</i> subsp. <i>indologenes</i>	<i>Pantoea ananatis</i>	<i>Pantoea agglomerans</i>
Motility	Non-motile	Motile (or non-motile)	Motile	Motile
Formation of indole	–	+	+	–
Nitrate reduction	–	–	–	+
Esculin hydrolysis	–	+	+/-	+
Acid production from:				
Maltose	–	+	+	+
Salicin	–	+	+	+
Utilization of citrate	–	+	+	–

Table 4. Differentially significant properties yellow-pigmented isolates from maize seeds.

Test	5 isolates from Group 1 seeds	3 isolates from Group 2 seeds	14 isolates from Group 2 seeds
Motility	Motile	Motile	Motile
Formation of indole	–	+	–
Nitrate reduction	+	–	+
Esculin hydrolysis	+	–	–
Acid production from:			
Maltose	+	+	+
Salicin	+	+	+
Utilization of citrate	–	+	–

Systematic examination of maize crops is necessary for the timely detection of *P. stewartii* subsp. *stewartii* and other phytopathogenic bacteria (Khan *et al.*, 1996). It must be remembered that the presence of phytopathogenic

bacteria in seeds has no visual manifestation, therefore laboratory analysis is necessary to exclude the possibility of the appearance of these pathogens.

Visual assessment of seed quality can not be reliable. Since from visually healthy corn seeds, we isolated the pathogenic strains of *P. fluorescens*, which were the reason of the damage of soft rot of seedlings in the field.

Also from the seeds of corn without visible signs of damage we isolated bacteria of the *Pantoea ananatis* species. *P. ananatis* causes disease symptoms in a wide range of economically important agricultural crops and forest tree species worldwide. It is regarded as an emerging pathogen based on the increasing number of reports of diseases occurring on previously unrecorded hosts in different parts of the world. Its unconventional nature lies in the fact that, unlike the majority of plant pathogenic microbes, *P. ananatis* is capable of infecting humans and occurs in diverse ecological niches, such as part of a bacterial community contaminating aviation jet fuel tanks and contributing to growth promotion in potato and pepper (Gvozdyak *et al.*, 2011).

*P. ananatis* is a common epiphyte; it also occurs endophytically in hosts where have been caused disease symptoms and in hosts where no such symptoms have been described (Coutinho *et Venter*, 2009).

## CONCLUSIONS

From visually healthy corn seeds that are grown in Ukraine, *Pantoea agglomerans*, *Pseudomonas fluorescens*, *Pantoea ananatis* were isolated. It has been established that *P. fluorescens* cause root rot of maize seedlings.

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**PHYTOCHEMICAL ANALYSIS OF *Gyrinops walla*  
AND COMPARISON WITH *Aquilaria malaccensis***

**SUMMARY**

Agarwood is a fragrant dark resinous wood formed in the heartwood of *Aquilaria* sp. (*Thymelaeaceae*), especially in *A. malaccensis*. The aromatic resin, agarwood, is used for the world's most expensive perfumes. *Gyrinops walla* (*Thymelaeaceae*) is an evergreen tree which grows in wet zone regions in Sri Lanka. Recently, it has been identified that *G. walla* possesses agarwood producing ability, which is similar to other species in family *Thymelaeaceae*. The objective of the present study was to preliminary identification of phytochemicals present in *G. walla* and to compare them with *A. malaccensis* which was the true agarwood resin forming species. Air dried coarse powders of different stem samples and leaf sample of *G. walla* and a stem sample of *A. malaccensis* were used and phytochemicals were extracted with each sample using soxhlet extractor with dichloromethane at 70 °C. After extraction, solvent was evaporated under reduced pressure and crude was re-dissolved in ethyl acetate for the phytochemical screening, using GC-MS. An external standard method was used to identify peaks and analyte concentrations in the chromatogram. From the stems, sixteen phytochemicals were identified in *A. malaccensis* and thirteen were found in *G. walla* all presented in agarwood resin. Out of those, nine compounds were found to be common in both species. Also, few important phytochemicals were identified from *G. walla* leaves. Therefore, *G. walla* could be confirmed as a species with significant influence on social, economic and natural environment in Sri Lanka and globally as an alternative of expensive agarwood resin for perfumery industry.

**Keywords:** Agarwood, GC-MS, Phytochemicals, *Thymelaeaceae*.

**INTRODUCTION**

*Gyrinops walla* Gaertn. (*Thymelaeaceae*) tree grows up to 15 m height with a straight, slender trunk and small, rounded crown (Dassanayake *et al.*, 1981). The "Sri Lankan agarwood", which is the common name used for *G. walla* is also called "walla patta" and "Sri Lanka agaru". *G. walla* is a vulnerable species found in the wet zone of Sri Lanka, where the elevation is below 1000 m and the average annual rainfall is above 2000 mm with an average temperature of 25 - 28 °C. Outside Sri Lanka, *G. walla* occurs only in the extreme Southwest of

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Notes: The authors declare that they have no conflicts of interest. Authorship Form signed online.

India, where it appears to be very rare (Subasinghe and Hettiarachchi, 2015). *Aquilaria malaccensis* Lamk. (*Thymelaeaceae*) tree, up to 50 m tall, with a bole up to 60 cm in diameter, usually straight, sometimes fluted or with thick buttresses. Commonly found in primary and secondary forest, mainly in plains but also on hillsides and ridges up to 1500 m altitude. *A. malaccensis* species scattered, in Peninsular Malaysia and north-eastern India and many South East Asian countries. It grows best with an annual rainfall of 1500-6500 mm, a mean annual maximum temperature of 22 - 28 °C (Mohamed, *et al.*, 2010).

*Aquilaria* species and also *Gyrinops* species in the family *Thymelaeaceae*, mainly produced agarwood which is the resinous, fragrant and highly valuable heartwood. The high demand occurred regarding *A. malaccensis* and *G. walla* in the recent past is due to cosmetics and medicines produced by the agarwood (Liyanage, 2014). There is a high demand from European countries, Middle East countries, Japan, Korea, China, etc. for agarwood products. Agarwood essential oil is a highly valued perfumery product in modern cosmetics and traditional Attar. *G. walla* is closely related to genus *Aquilaria* and in the past *G. walla* was considered to belong in genus *Aquilaria*. *G. walla*, together with genus *Aquilaria*, which is best known as the principal producer of the resin agarwood has high demand as economical plants (Barden, 2000).

Plants and its products are more reliable for its renewability and considered as a catalyst for human welfare. Therefore, in the past few decades, there was a growing research interest in plants as a source of phytochemical agents (Mahesh and Satish, 2008). *A. malaccensis* and *G. walla* have been investigated for the presence of phytochemically important substances which have biological, pharmacological and economic importance. This factor forced the scientists to develop methods of bioprocesses for the production and extraction of compounds from natural, renewable sources for their potential application in cosmetics and pharmaceutical industries (Velioglu *et al.*, 1998). Considering the importance of the bioactive compounds it has become a necessity to build up an integrated approach to extract, purify and characterize the active compounds. From the plant materials, the extraction of bioactive compounds is the first step in the utilization of phytochemicals. Bioactive compounds can be extracted from fresh, frozen or dried plant samples. Usually before extraction plant samples are size reduced by milling, grinding or homogenization, which may be preceded by air drying or freeze drying (Abascal *et al.*, 2005).

Selection of the type of solvent used in the extraction procedure, greatly depend on the successful determination of biologically active compounds from plant material and it will also depend on the targeted compounds to be extracted (Das *et al.*, 2010). The polarity of the targeted compound is the most important factor for solvent choice. Along with that molecular affinity between solvent and solute, mass transfer, low toxicity, ability to evaporate at low heat, rapid physiological absorption of the extract, inability to cause the extract to complex or dissociate are the main phenomena that should concern prior to the usage of

any solvent (Eloff, 1998). Solvent extraction techniques are the most commonly used methods to prepare agarwood extracts from plant materials due to their ease of use, wide applicability and efficiency. Usually, for extraction, solvents are used from nonpolar to polar. Solvents such as methanol, ethanol, acetone, ethyl acetate, acetic acid, petroleum ether, chloroform (Xu and Chang, 2007) and their combinations are widely used for solvent extractions. Gas chromatography-mass spectrometry (GC-MS) is one of the key techniques used for screening, identification and quantification of many groups of non-polar and polar chemical compounds or their derivatives in the agarwood. The highest feasible separation of GC in combination with different types of MS detectors engaging various detection principles to which it can be coupled makes GC-MS an important, recurrently irreplaceable tool in the analysis of trace levels of chemical compounds (Hajšlová and Cajka, 2007).

### MATERIAL AND METHODS

Two different stem samples and a leaf sample (L1) of *G. walla* and mature stem sample of *A. malaccensis* were selected for this experiment. In *G. walla*, one stem sample was dark in colour (G1) (assumed to be an agarwood bearing stem) and other sample was light straw colour (G2). Mean girth at breast height (GBH) of the trees from which the stem samples were collected was 30.0 - 40.0 cm. Samples were stored in a refrigerator at 4 °C prior to the experiment.

Samples were size reduced manually and air dried at room temperature. Then the dried samples were ground into a coarse powder. Phytochemicals were extracted using soxhlet extractor. About 10.0 g of each sample was extracted at a temperature of 70 °C for 20 - 30 extraction cycles over a period of 3 hours in dichloromethane (250.0 cm<sup>3</sup>).

After extraction, solvent was evaporated, using a rotary evaporator, yielding the crude extract under reduced pressure at 40 °C. Then the crude extracts were dissolved in ethyl acetate solvent. Following soxhlet extraction, 2.0 µL aliquots were screened for the presence of phytochemicals, using GC-MS. Agilent 7890A GC (5% Phenyl Methyl Siloxane) capillary column was used for the separation and 5975C inert XL EI/C1 MS detector identified the compounds present (Table 1). An external standard method was used by the GC-MS machine to identify peaks and to find out the relationship between peak areas and analyte concentration in the chromatogram. Each chromatogram obtained were compared.

Table 1: Column conditions for GC-MS analysis

	Rate (°C/min)	Value (°C)	Hold time (min)	Run time (min)
Initial		70	4	4
Ramp	10	280	4	30

## RESULTS AND DISCUSSION

It was observed that dry weight of crude obtained from dark colour stem sample (G1) of *G. walla* was the highest (48.27 mg/g), followed by dried leaf sample (L1) (10.92 mg/g) and straw colour stem sample (G2) (8.46 mg/g). That might indicate the presence of extra amount of phytochemicals in the G1, which believed agarwood bearing stem. L1 showed higher weight compared to G2. The crude obtained from *A. malaccensis* stem (A1) was 62.60 mg/g.

About twenty one phytochemicals were identified in dark colour stem samples (G1) of *G. walla* and some of them are constituents in commercially available agarwood oil. Some phytochemicals which are common in many aromatic plant species have been observed in G2. Meanwhile, eleven important phytochemical constituents were observed in mature leaf samples (L1). This indicates that mature leaves of *G. walla* also have the potential to be used as a source of agarwood oil (Table 02).

Different types of chemical constituents identified from the dark colour stem sample (G1) of *G. walla*, which believed that agarwood bearing stem are 4,7-diisopropenyldecan-3,8-diol; is an agarofuran, spiro[4.5]dec-6-en-8-one derivatives; a sesquiterpene, few naphthalene derivatives; eudesmane sesquiterpene which is a common structure found in plant derived flavours and fragrances, 2-cyclohexen-1-one derivative; a nootkatane sesquiterpene, 6-hydroxy-2-methyl-5-nitro-chromone; volatile chromone constituent responsible for the long lasting pleasant odour, aromatic compounds i.e. D-limonene, 2-fluorobenzoic acid ester derivatives and benzoic acid trimethylsilyl ester derivatives identified in commercially available agarwood oil.

Other compounds such as ledol; a sesquiterpene, myrtenyl acetate; naturally occurred fruit flavour, 2-octenoic acid methyl ester derivative; food and flavour ingredient, longifolene derivatives; abundant aroma constituent with good scent, 4- $\alpha$ -isopropenyl-2-carene; colourless liquid with sweet and pungent odour, andrographolide; a potential cancer therapeutic agent, quinazolinone derivative; shows wide spectrum of biological properties like antibacterial and antifungal, hexadecanoic acid ester derivatives, 2-butenylbenzene, 4,8-methanoazule-9-ol, undecanol derivative, cycloicosane and squalene present in the dark colour stem samples (G1).

There was no significantly important phytochemicals in agarwood oil has been observed in the chromatograms of straw colour stem samples (G2) of *G. walla*. However, as a mature stem of an aromatic plant, it showed many secondary metabolites in chromatogram such as phenolic compounds, alkanes, alkenes, cyclo compounds, benzene derivatives, naphthalene derivatives, pyridine derivatives, alcohols, etc. which can be found commonly.

Economically important phytochemicals present in *G. walla* and *A. malaccensis* by means of percentage similarity in dichloromethane solvent was identified and compared from the chromatograms obtained. Sixteen (16) agarwood phytochemicals were identified in *A. malaccensis* and only thirteen (13) were found in (G1) stems of *G. walla*. Out of those, only nine (09)

compounds were found to be common in both species. Present study revealed that, dichloromethane as a better solvent for extracting agarwood phytochemicals present in *A. malaccensis* and *G. walla*. Chromatograms obtained from GC-MS analysis (Figure 1) revealed that higher number of economically important phytochemicals with higher abundance, found in *A. malaccensis* than *G. walla* with dichloromethane solvent.

Table 2: Some important phytochemicals present in stem sample (G1) and leaf sample (L1) of *G. walla* extracted with dichloromethane

No.	Compound	Percentage similarity	
		Stem (G1)	Leaf (L1)
1	4,7-diisopropenyldecan-3,8-diol	88	-
2	spiro[4.5]dec-6-en-8-one derivatives	90	-
3	naphthalene derivatives	87	--
4	2-cyclohexen-1-one derivative	87	-
5	6-hydroxy-2-methyl-5-nitro-chromone	84	-
6	D-limonene	96	94
7	2-fluorobenzoic acid ester derivatives	85	-
8	benzoic acid trimethylsilyl ester derivatives	93	-
9	ledol	96	-
10	myrtenyl acetate	95	-
11	2-octenoic acid methyl ester derivative	86	-
12	longifolene derivatives	92	-
13	4- $\alpha$ -isopropenyl-2-carene	90	-
14	andrographolide	94	-
15	quinazolinone derivative	85	-
16	hexadecanoic acid ester derivatives	87	-
17	2-butenylbenzene	89	-
18	4,8-methanoazole-9-ol,	90	-
19	undecanol derivative	86	-
20	cycloicosane	95	-
21	squalene	87	-
22	sigmatadinene-3-one	-	96
23	$\beta$ -sitosterol	-	84
24	2-chloroethyl linoleate	-	85
25	phytol	-	90
26	$\alpha$ -tocopherol	-	89
27	pyrazine derivative	-	83
28	lup-20-en-3-one	-	95
29	pyrone,	-	83
30	azulene	-	85
31	olean-12-ene	-	90

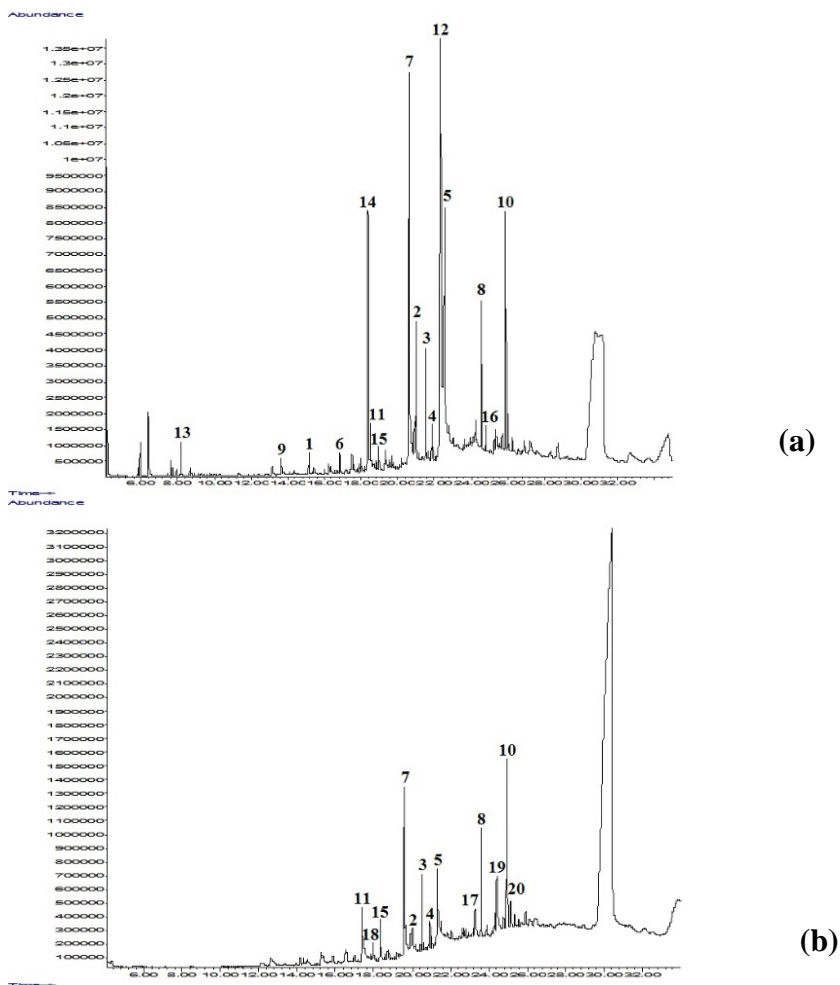


Figure 01: Chromatograms of stem samples of (a) *A. malaccensis* and (b) *G. walla* (G1) by GC-MS (Dichloromethane solvent)

Khalil *et al.* (2013) reported that, GC-MS analysis of the plant extracts led to the identification of 14 components from leaf extracts of two *Aquilaria* sp. Hexadecanoic acid was one of the major compounds in methanolic extracts. Other compounds were 1,4,7,10,13-pentaoxacyclopentadecane, acetic acid, 1,4,7,10,13,16-hexaoxacyclo octadecane, hexaethylene glycol monododecyl ether, 1,4,7,10,13-pentaoxacyclopenta decane, 2,6,10,14,18,22-tetracosahexaen and 2,6,10,15,19,23-hexamethyl. In the present study, wood samples of *A. malaccensis* contained pentadecanoic acid and octadecanoic acid. However other compounds were not observed in tested samples of *G. walla*. The presence of some volatile aromatic compounds that had been identified by Khalil *et al.*

(2013) were also found in tested *A. malaccensis* and *G. walla* samples of dichloromethane extracts.

Gas chromatography analysis revealed that the resin of *G. walla* contained aroma compounds such as sesquiterpenes of guaine and eudesmane which are commonly found in commercially available agarwood (Subasinghe *et al.*, 2012). Some phytochemicals identified from the present study which used for different industries at the present, such as 2-methoxy-4-vinylphenol, an aromatic substance used as a flavouring agent and also the natural aroma of buckwheat, 2,6-dimethoxy phenol (Syringol), a naturally occurring dimethyl ether of pyrogallol, dodecanoic acid (lauric acid) which gives a faint odour of bay oil and ethyl hexadecanoate, a volatile ethyl ester which gives the characteristic fragrance for vine was observed in both *A. malaccensis* and *G. walla*. However, hexadecanoic acid ethyl ester (ethyl palmitate), benzylacetone with a sweet, flowery smell that is considered to be the most prominent compound in flowers, which responsible for attractiveness and one of volatile components of cocoa was only found in *G. walla*.

Some fatty acids (tetradecanoic acid, pentadecanoic acid, etc.) and alkanes (heneicosane, heptacosane, etc.) that are reported to be present in commercially available agarwood (Naef, 2011) were also found in both *A. malaccensis* and *G. walla* samples analysed in the present study. Benzaldehyde and vanillin were common in samples collected from unwounded *A. malaccensis* and *G. walla* plants, revealing that those compounds are not only present in resin produced, but also in the natural heartwood too. Presence of  $\gamma$ -sitosterol, which is a phytochemical present in commercially available agarwood oil (Wetwitayaklung *et al.*, 2009) was observed in tested *G. walla* wood samples, but not detected in *A. malaccensis* samples. The present study revealed that although there are some common fragrance compounds present in *A. malaccensis* and *G. walla*, they do not share all fragrant compounds present in commercial agarwood samples. Therefore, felling *G. walla* trees to extract agarwood without knowing the exact mechanism behind resin induction is a waste of natural resources.

## CONCLUSIONS

Sixteen important agarwood phytochemicals from *A. malaccensis* and thirteen from *G. walla* identified from stems and using dichloromethane solvent with soxhelt extraction procedure. Also, eleven important phytochemical constituents observed in matured leaves indicates that *G. walla* leaves have the potential to be used as a source for agarwood. To optimize a screening procedure of agarwood chemical constituents, primarily, phytochemical extraction procedure shall optimize with different solvent systems and conditions that ensure higher fraction of agarwood oil to be extract. Then it should identify few important agarwood constitutes i.e. sesquiterpenes, agarofurans, eudesmanes, nootkatanes and chromones, that is essential for grading agarwood and should use the standards of those chemicals to optimize GC-MS analysis. These optimization further could be implement as an internationally recognized test for identification of agarwood bearing stems of *A. malaccensis* and *G. walla* without felling them unnecessarily.

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## **DESIGN OF LARGE SCALE ON-DEMAND IRRIGATION SYSTEM IN THE AGRICULTURAL AREA OF CORATO, APULIA, ITALY**

### **SUMMARY**

The objective of this project was to design a collective, on-demand irrigation network for an agricultural area near *Corato*, province of Bari, Apulia region, southern Italy. For this purpose, a study integrating agronomic, engineering, economic and environmental aspects has been conducted. The goal was to evaluate the ability to supply agricultural lands with irrigation water in order to cover the cropping pattern requirements in this district during the peak period of a dry year. After collecting and processing the climatic data for 30 years period, as well soil characteristics, crop water requirements have been determined. Besides that, modelling tool CROPWAT generated curves of yield response to different amounts of irrigation applied. The most optimal cropping pattern is chosen in socio-economic part through a predictive model, consisting of 3 annual (watermelon, tomato, lettuce) and 3 permanent crops (peach, grapevine, olive). Furthermore, sensitivity analysis have been done in order to avoid future risk and to predict farmers' behaviour. The irrigation network was designed based on the specific continuous discharge ( $0.422 \text{ ls}^{-1}\text{ha}^{-1}$ ) which was computed on GIR values for the peak month of the dry year. A layout of the distribution network is computed by Geographical Information System (GIS) including reservoir, main pipelines, secondary pipelines, nodes, 38 hydrants with a module of  $5 \text{ ls}^{-1}$  and 23 hydrants with a module of  $10 \text{ ls}^{-1}$ . Several tools (air and relief valves, control gates) are included in the network as well. Optimization model was used in order to calculate pipe diameter and their length (13271.7 m). In addition, storage reservoir was designed, as well as pumping station with 4 horizontal and 2 submerged pumps and pressurized regulating tank that controls opening and closure of the pumps. Cost and benefit analysis showed that the project is cost-effective on social basis and will fully recover its investment in two years, with an economic rate of return of 129%. An environmental impact assessment was conducted for the project and neighbouring area and it showed that they are not affected negatively. In addition, good management and monitoring practices to minimize the potential negative environmental impact are required and proposed for long-term sustainability of the irrigation project.

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**Keywords:** irrigation, hydrant, discharge, crop water requirements, precipitation, yield, groundwater.

## INTRODUCTION

Agricultural sector is the most water consuming sector in the world, reaching more than 70% of the withdrawal in many areas of the world and especially in the Southern Mediterranean countries.

In many countries, particularly those situated in the arid and semi-arid regions of the world, this dependency can be expected to intensify, due to the increasing demand on agricultural products. Thus, contribution of irrigated agriculture to food production is substantial and the expansion of irrigated agriculture will surely result in higher yield and production. According to it, irrigation is required to satisfy the water demand during the driest periods of the year, especially in semi-arid Mediterranean climate.

The objective of this project is to design a collective on demand irrigation network for an agricultural area *Corato*, in the province of Bari, Apulia region (Southern Italy).

The total area of *Corato* region is 280 ha, of which 235 ha are suitable for agriculture and irrigable. Water sources for irrigation in this region are provided by two wells with discharges of  $Q_1=80 \text{ ls}^{-1}$  and  $Q_2=50 \text{ ls}^{-1}$ .

For this purpose, a study integrated agronomic, engineering, economic and environmental aspects have been conducted as presented in the following steps:

Climatic, soil, water source, water quality and crop characteristics data collection and elaboration;

Economic analysis through cropping pattern optimization under certain constraints and for a specific objective function;

Determination of specific continuous discharge for optimal cropping pattern;

Irrigation network design and design of storage reservoir, pumping station, pressurized tank and all the other structures and equipment of the project;

Estimation of the project's implementation, operation and maintenance costs (cost of expropriation, excavation works, pipe cost, technical equipment cost, concrete works cost, electrical equipment cost, pump cost, etc.);

Economic evaluation of the project (Cost & Benefit Analysis) and

Environmental Impact Assessment (EIA) and propose of possible mitigation measures and the monitoring plan.

## MATERIAL AND METHODS

### Agronomic part

Obtained climatic data for *Corato* region is collected and elaborated for 30 years period, and information about minimum, maximum, average temperatures and rainfall are obtained and dry year precipitation is calculated. Available soil physical and chemical properties are used to determine soil texture, effective

depth, available water content, cation exchange capacity, electrical conductivity, exchangeable sodium percentage, organic matter, soil pH etc.

CROPWAT, software and modeling tool to support decision making for irrigation planning and management, was used in order to calculate reference evapotranspiration, crop water requirements as well as irrigation requirements. The data for twenty-one crops grown in the region were arranged using the FAO database and results of studies carried out in Apulia region. For the purposes of our project, after calculating the crop water requirements, CROPWAT model was used to generate the crop response curves that represent the yield response to different amounts of irrigation water applied. Different scenarios were introduced into the model, including different amounts of irrigation water from full irrigation to no irrigation (rainfed agriculture), under fixed management rules on evapotranspiration and effective rainfall estimation, as well as for irrigation timing, application and efficiency, (Smith et al., 1992).

#### **Socio - economic part**

The main objectives of the socio-economic analysis are to identify the optimal cropping pattern for the irrigation project area in *Corato* region that maximizes farmers utility, to assess the impact of different water pricing options on cropping pattern, on farm income and on water consumption and to choose the optimal water tariff system. To reach these objectives, a predictive model written in GAMS (General Algebraic Modeling System) programming language was used. It consists of a static and nonlinear optimization model aiming to represent farmer's behavior and to simulate his response to the introduction of irrigation. This is a constrained optimisation model that maximises an objective function subject to a set of constraints (land, water and crop constraints). Effectively, it assumes that farmers select the crop allocation that maximize the expected income and minimize the risk, which is measured by the risk aversion coefficient (the degree to which the farmer is averse to taking risks), (Hazell and Norton, 1986). Two sources of risks were considered in this model: market (i.e. price) and climate (i.e. yield) risks. Both prices and yields are assumed to be normally distributed and their means and standard deviations values are derived from regional statistical database (ISMEA – Istituto di Servizi per il Mercato Agricolo Alimentari, 2018).

#### **Engineering part**

A layout of the distribution network (reservoir, main pipelines, secondary pipelines, nodes and hydrants) are computed by Geographical Information System (GIS). In order to choose the proper diameters for each section of the irrigation network, it is necessary to compute the discharges in these sections which were calculated based on the peak month of the dry year. To avoid over-sizing the diameters of the pipelines, they were calculated using Clément first model probabilistic approach, in which, the probability that the flow discharge downstream of each section will not exceed Clément discharge is 95%. Selection of the most suitable pipe is done by an optimization process based on the peak discharge and network layout, (Lamadalena and Sagardoy, 2000). The optimization model that was used to calculate pipe diameters was developed by

Labye in 1981, and is called Labye's Iterative Discontinuous Model (LIDM). In order to facilitate the calculation of Clément discharges and LIDM's optimized diameters, a computer software package called COPAM was used, (Labye et al, 1988).

### Cost and Benefit Analysis

In order to assess if a project is profitable for general welfare or not, and what would be the best project alternative, useful economic model is CBA. Two types of analysis are done, financial and economic. The CBA method is conducted in the following phases: determination of the time frame (construction, management and dismantling phase), determination of costs and benefits (in financial analysis, costs are related to the total investment and maintenance costs, while the benefits are obtained from the water agency revenue, while for the economic analysis, costs and benefits of farmers are added considering the situation before and after the implementation of the project), choice of rate of interest according to the Italian standards (Arborea et al, 2017). In order to show project's feasibility, performance indexes are determined (the Net Present Value, the Benefit Cost Ratio, the Internal Rate of Return and the Payback Period).

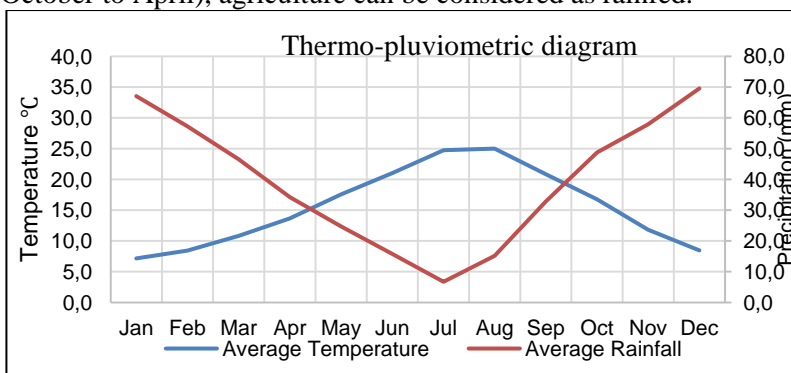
### Environmental part

The environmental impact assessment (EIA) of *Corato* irrigation scheme project was analyzed through five main categories: natural resources, biological life, socio-economic, political and economic impacts. For each category of impacts, a positive or negative score was given for both project area and nearby areas. The impacts were assessed and scored using the Environmental Impact Assessment Decision Support System (EIDASS) proposed by CEDARE (Abu-Zeid and Bayoumi, 1999).

## RESULTS AND DISCUSSION

### Agronomic part

The thermo-pluviometric, Bagnauls-Gaussen diagram, based on collected and analyzed 30 years historical data set, indicated the period when irrigation could be needed. According to the Figure 1, it can be seen that the period of drought is present from May to September, while in the period of wet season (from October to April), agriculture can be considered as rainfed.



**Figure 1:** Thermo-pluviometric Bagnauls-Gaussen diagram

In order to determine values of the dry year's rainfall and according to that, peak irrigation requirements, monthly rainfall of the dry year was calculated using the annual rainfall of the dry year, the average annual rainfall and monthly average rainfall (Table 1).

**Table 1:** Monthly precipitation values for the dry year

Months	Average Precipitation (mm)	Dry year precipitation (mm)
January	67.1	58.8
February	57.2	50.2
March	46.5	40.8
April	34.3	30.0
May	24.7	21.7
Jun	15.8	13.8
July	6.7	5.9
August	15.2	13.3
September	32.8	28.8
October	48.8	42.7
November	57.9	50.8
December	69.5	61.0
<b>Total</b>	<b>476.5</b>	<b>417.7</b>

According to the obtained soil properties in *Corato* region (Table 2), it can be concluded that land is suitable for agricultural activities. Soil has clay-loamy texture, three horizons with total effective depth of 88 cm, good capacity to hold cations, pH that is suitable for most of the crops and other positive properties. Furthermore, electrical conductivity and sodium percentage cannot have any negative impact on crop production.

**Table 2:** Soil physical and chemical characteristics

Physical characteristics										
Effective depth (cm)		Soil texture		Soil bulk density (g/cm <sup>3</sup> )		AWC (mm)				
Ap	25	Clay loam		1.23		34.5				
Bt	30			1.20		44.7				
B/C	33			1.22		39.3				
Chemical characteristics										
Effective depth (cm)		pH		CaCO <sub>3</sub>		Cations		CEC	OM	EC
				Tot.	Act.	Ca <sup>2+</sup>	29.0	(meq/100gr)	(%)	(dS/m)
Ap	25	7.13	10.5	0.53	Mg <sup>2+</sup>	2.8	34.63	4.6	0.658	
Bt	30	7.09	8.5	0.25	K <sup>+</sup>	0.7	31.93	3.7	0.611	
B/C	33	7.03	9.4	0.33	Na <sup>+</sup>	0.4	32.52	2.2	0.601	

The data for twenty-one crops grown in *Corato* region were arranged using the FAO database and results of studies carried out in *Apulia* region. They were used to estimate crop water requirements, irrigation requirements and crop response to water curves, which is presented in Table 3, (Allen et al. 1998).

Specific continuous discharge,  $q_s$ , expressed in  $l s^{-1} ha^{-1}$ , is used in the engineering part of the project in order to design the irrigation network in Corato region. For determination of value for specific continuous discharge, data about crop water requirements have been used for the peak period of the dry year and for the cropping pattern chosen by GAMS model (in economic part). In that case, enough amount of water will be provided even during the driest months.

$$q_s = \frac{GIR \left( \frac{mm}{month} \right) * 10 \left( \frac{m^3}{ha} \right) * 1000 \left( \frac{l}{m^3} \right)}{31(days) * 24(hr) * 60(min) * 60(s)} = 0.422 l/s/ha$$

Where GIR is weighted average gross water requirement for the chosen cropping pattern and irrigation technique during the peak period of the dry year (mm/month).

Implementing simplified salt balance in order to describe potential salinity hazard in *Corato* study area, it is concluded that salinity problems could possibly appear after about 6 years of irrigation (through potentially injurious salts). However, in reality, it is expected that some natural leaching process occurs during the winter season due to the precipitation (Ayers and Westcot, 1985).

**Table 3:** Seasonal ETC, Peff, NIR and yield percentage under different crop water requirements for the average year

Crop	Etc	IRR	Peff	Yield (%)				Avg yield t/ha
				0% NIR	50% NIR	75% NIR	100% NIR	
Citrus	712.1	549.9	162.2	52.8	76.7	88.7	100	20
Cherry	471.6	288.0	183.6	26.3	63.6	82.0	100	10
Pepper	502.5	422.2	80.3	21.4	63.6	84.1	100	25
Olive Tree	427.8	305.2	122.6	60.3	80.6	90.6	100	7
Sunflower	542.9	462.6	80.3	30.2	67.7	84.2	100	4.5
Tomato	659.8	569.7	90.1	22.5	61.2	80.6	100	75
Wheat	397.1	193.7	203.4	80.2	91.2	96.2	100	6
Peach	704.3	538.0	166.3	36.7	68.4	84.3	100	40
Grapevine	569.5	413.9	155.6	48	75.0	88.2	100	30
Watermelon	391.5	368.8	22.7	30.9	65.6	83.1	100	70
Soybean	506.0	424.2	81.8	43.8	72.9	87.1	100	3
S. sugar beet	587.4	476.1	111.3	31.5	66.4	83.5	100	70
A. sugar beet	634.7	455.0	179.7	48	73.4	86.3	100	80
Lettuce	145.2	2.6	142.6	99.9	100.0	100.0	100	45
Artichoke	801.1	532.4	268.7	50.8	75.7	88.0	100	20
Common Potato	372.2	272.0	100.2	38.2	71.7	87.9	100	40
Early potato	312.6	176.1	136.5	64.4	83.5	92.9	100	30
Eggplant	582.5	508.2	74.3	23.7	62.1	81.1	100	35
Broad bean	337.8	140.8	197.0	18.1	78.2	92.1	100	4.5
Carrots	378.1	225.5	152.6	48.9	76.1	89.3	100	45
Maize	606.2	536.7	69.5	6.9	54.2	77.9	100	7.5

### Economic part

After running the economic model, the optimal crop allocation for the base case scenario was chosen. As it can be seen from the Table 4, the highest percentage of area is dedicated to grapevine under full irrigation technique ( $T_1$ ), while the lowest percentage is devoted to rainfed lettuce ( $T_0$ ). Olive trees are present in almost 40 ha, and according to the current climatic conditions, there is no need for irrigation. Peach and tomato can be both grown with full and partial irrigation techniques ( $T_2$  and  $T_3$ ), while watermelon is suitable for full and complementary irrigation. All available crops that require irrigation in March (wheat, olives and cherry) can be grown only with rainfed technique due to water availability constraint (irrigation season from April to November).

**Table 4:** The optimal crop allocation for the base scenario

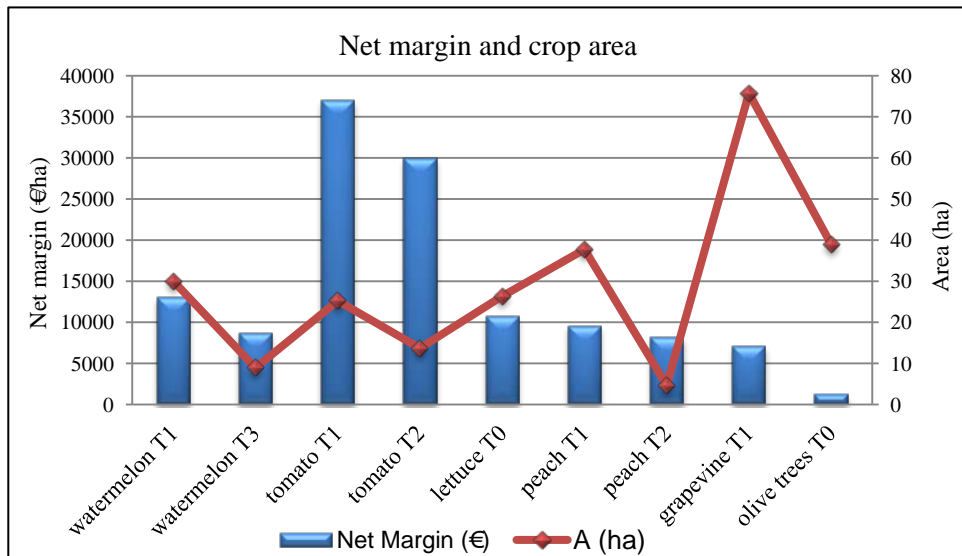
Crops	Irrigation Techniques				Area	
	T0	T1	T2	T3	ha	%
Watermelon		30.03		8.92	38.95	14.93
Tomato		25.24	13.71		38.95	14.93
Lettuce	26.35				26.35	10.10
Peach		37.73	4.67		42.40	16.24
Grapevine		75.74			75.74	29.02
Olive Tree	38.95				38.95	14.93

The Table 5 shows the effect of the risk aversion coefficient on the cropping pattern. It is noticed that increasing  $\Phi$  (from 0 to 1.65) increases the crop diversity and reduces the farm's profit and utility. A risk aversion coefficient of 1.6 was chosen by the model for the base scenario because it is the best prediction of the farmers' behavior. According to that, the expected income of this area is 13139 €/ha and the water consumption by this pattern is 4004 m<sup>3</sup>/ha.

**Table 5:** Sensitivity analysis for different risk aversion coefficient

Crops	T	$\Phi 1$	$\Phi 2$	$\Phi 3$	$\Phi 4$	$\Phi 5$	$\Phi 6$	$\Phi 7$	$\Phi 8$	$\Phi 9$
		0	0.25	0.5	0.75	1.0	1.25	1.5	1.6	1.65
watermelon	T1	78	78	78	78	78	66	41	30	26
watermelon	T3							2	9	11
tomato	T1	78	78	78	78	78	66	43	25	19
tomato	T2								14	18
lettuce	T0	235	235	235	157	93	57	32	26	24
peach	T1						38	44	38	35
peach	T2								5	7
grapevine	T1							62	76	80
olive trees	T0				78	78	66	43	39	38
Profit (ha)		27582	27582	27582	24417	21494	18536	14500	13139	12679
Annual water use (m <sup>3</sup> /ha)		3270	3270	3270	3270	3270	3753	4122	4004	3960

Figure 2 shows the net margin and the area per each crop for each technique in the optimal cropping pattern. For example, the highest net margin is recorded for tomato T<sub>1</sub> (37035 €). However, this crop occupies only 25.2 ha of the cultivated area, because, first, it is a risky crop, shown by the high price standard deviation, and, second, it is restricted by the crop rotation constraints implemented in the model. On the other hand, the net margin of olive trees is quite low (1247€), compared to other crops; however its area represents around 15% of the total farm land. This indicates that the net margin is not the only driver for selecting the optimal cropping pattern. Other key factors such as the farm constraints, the risk aversion coefficient and the standard deviation of yields and prices play a critical role.



**Figure 2:** Net margin and the area dedicated to each crop of the optimal pattern

To study the impact of water tariff on the crop allocation in the project area, 10 different water tariff scenarios were modeled (from  $S_{01}$  to  $S_{10}$ ). Water tariff is binomial and is based on fixed and variable tariff. The fixed tariff depends only on irrigable land (30 €/ha), while the variable tariff depends on the quantity of water consumed under each block. From  $S_{02}$  to  $S_{04}$ , water tariff is increasing proportionally for the three blocks. From  $S_{05}$  to  $S_{10}$ , only water tariffs of the second and third blocks are increasing. It can be noticed that increase of water tariff (water cost) leads to three effects:

- Increase in land occupied by rainfed crops (olive trees and lettuce) in detriment of irrigated crops (watermelon, peach and grapevine);
- Reduction of land cultivated by water intensive crops (such as peach);
- Switch from high water intensive techniques (T<sub>1</sub>) to water saving techniques (T<sub>3</sub>)



The choice of the optimal water tariff depends on compromising between the needs of: the farmers, the water agency, and all the society members. In order to choose the best scenario, the multi-criteria method, called Compromise Programming was used. In this method, a weight is given to each purpose according to its degree of importance. The highest weight was given to the minimization of water consumption, which has to be interest of all stakeholders, followed by the maximization of farm profit which is important for the economic viability of the irrigation project. And based on these assumptions, the best scenario was chosen as  $S_{05}$ , because it is the best in water conservation without significantly affecting the farmers' profit, (Romero and Rahman, 2003).

### Engineering part

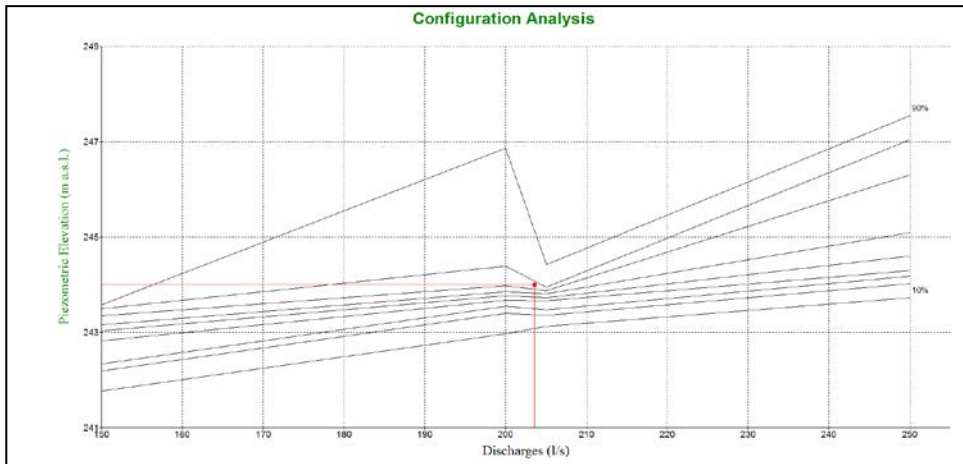
Water for the study area is supplied by two wells located at elevations of 215 m a.s.l. and 223,5 m a.s.l. The first well is located at a distance of 298,3 meters from the reservoir, while the second is at distance of 350,9 meters. Water is delivered by set of four pumps, designed to provide the required discharge of the network and satisfy a minimum pressure at the hydrant level (20 m). A pressurized tank is installed just after the pumping station in order to regulate and control the pressure of the pumps. In addition to the hydrants, the network consists of several tools: 2 air valves to remove air excess from the network, 3 relief valves to clean the network and remove all present sediments and 24 gates to control the operation and maintenance of the network. By combining three farms together or less, modules of  $5 \text{ ls}^{-1}$  and  $10 \text{ ls}^{-1}$  are used for hydrants, while the elasticity (farmers' freedom) is fixed at 3. A hydrant of  $5 \text{ ls}^{-1}$  can serve an area up to 4 ha, while a hydrant with the module of  $10 \text{ ls}^{-1}$  serve maximum of 7,65 ha. The total number of hydrants installed is 61, and 38 of them are with the module of  $5 \text{ ls}^{-1}$  and the rest, 23, have the module of  $10 \text{ ls}^{-1}$  (Figure 5).

The peak upstream discharge of the designed network is  $205 \text{ ls}^{-1}$ , obtained by COPAM, and the total price of optimized pipes is 208037 €. In the Table 6, the summary of the outputs is presented, considering pipe diameters and pipe length.

**Table 6:** Summary of optimization results for the network using COPAM

Diameter (mm)	Length (m)
110	3489.4
125	1248.8
140	1696
160	2107.8
180	435.7
200	1249.4
225	755.6
280	652.7
315	421.3
450	811
500	404

However, in order to avoid over-design of the system, network performance analysis is checked and indexed characteristic curve has been designed. The network is capable to satisfy only a percentage of the possible configurations by different values of upstream discharge and the corresponding piezometric elevation. The curve was drawn using 1000 random configurations of hydrants between  $150$  and  $250$   $\text{ls}^{-1}$ . The Figure 3 shows, that the point corresponding to a discharge of  $205$   $\text{ls}^{-1}$  and piezometric elevation of  $244,12$  m a.s.l. falls in the indexed curve of 75%, which means that the head of the hydrants is higher than the minimum head required (20m) in 75% of all the examined discharge configurations. Similar to that, hydrant reliability curve and hydrant deficit envelope curve have been designed, which showed that hydrants are reliable in more than 85% of the cases, which is more than satisfied result.



**Figure 3:** Index characteristic curve (configuration analysis)

In order to supply the network, two conveyance pipes are designed: the first one with a diameter of 280 mm and the second one with 315 mm. The total length includes both convey pipes, 452 meters from the bottom of the wells to the surface and 660 meters from the wells surface to the reservoir.

Using Darcy-Weisbach and Colebrook equation, the friction losses are calculated and used in order to determine the required pressure head for two submerged pumps. According to the discharges of the wells ( $50$  and  $80$   $\text{ls}^{-1}$ ) and pressure head of the pump, from *Caprari* catalog E12S42, two pumps have been chosen, 4A and 6A ( $P_{p1} = 157,1$  KW and  $P_{m1} = 174,5$  KW;  $P_{p2} = 260,4$  KW and  $P_{m2} = 289,3$  KW).

To reward the daily water deficit between the water source and the network demand through the peak period, storage reservoir is designed. Volume of the designed reservoir is  $4212$   $\text{m}^3$  for 9 hours and it is higher than the volume of water going out from the reservoir for a 15 hours working reservoir. It is assumed that the reservoir has a shape of truncated pyramid and dimensions of  $40 \times 33 \times 3.5$  meters.

The pumping station design has been made to supply the network with maximum upstream discharge ( $Q_{clé}=205 \text{ ls}^{-1}$ ), and at the same time maintaining an upstream pressure sufficiently enough to ensure a minimum pressure head in each hydrant. 4 pumps are selected, type (80-160) and 2900 rpm-50 Hz ( $P_p = 17,94 \text{ KW}$  and  $P_m = 19,94 \text{ KW}$ ). Pressurized regulating tank is designed in order to control the opening and the closing of the pump. The volume of the pressurized tank is obtained as  $12 \text{ m}^3$ , and the dimensions of: diameter of 2,2 m, the height of 3,15 m with an additional 50 cm for sensors in the tank to turn on and turn off the pumps. An important step in the project, more precisely, in engineering part is the total cost of the project, presented in the Table 7.

**Table 7:** Final cost of the project

Cost of Pipes	298037
Excavation Cost	226011
Technical Equipment	146015
Expropriation	104037
Concrete for reservoir and manholes	59108
Building cost of pumping station	102900
Electrical Equipment	25000
Protection works	2000
<b>TOTAL (1)</b>	<b>963,108</b>
Cost of Design (3%)	28893
Supervision of work (1%)	9631
Topographic Studies (1%)	9631
<b>TOTAL (2)</b>	<b>48155</b>
<b>TOTAL ==&gt; (1) + (2)</b>	<b>1,011,263</b>
Taxes (20%)	202253
<b>TOTAL (including taxes)</b>	<b>1,213,515</b>
Maintenance cost (8%)	97081
<b>FINAL COST</b>	<b>1,310,596</b>

### Cost and Benefit Analysis

From the table below (Table 8), it can be concluded that from financial, private, point of view, the project is not cost effective since the NPV is negative during the whole life cycle.

**Table 8:** Financial analysis indexes

Total Investment Cost (€)		1 213 515
Annual O&M Cost (8% of the total cost)		97 081
Annual Water Agency Income (€)		51 701
Rate of interest (%)		4
Indexes	NPV (€)	-1 932 073
	BCR	0.31
	IRR (%)	undetermined
	PBT (Years)	never

Furthermore, the income of water agency from chosen water tariff cannot cover the annual costs of operation and maintenance (O&M). When IRR is lower than discount rate, which is 4% in our case, it means that our investment costs cannot be returned as revenue.

In the economic analysis, social objectives are considered. Benefit of both, the water agency and farmers are taken into consideration. To obtain the costs and benefits of farmers, two situations are projected. The first one is to consider the cost and benefits before the implementation of the project (rainfed scenario), and the second after running the irrigation project. In the economic analysis (Table 9), it is proved that the project is justified, by having a positive NPV and satisfactory values of all of the rest economic indexes.

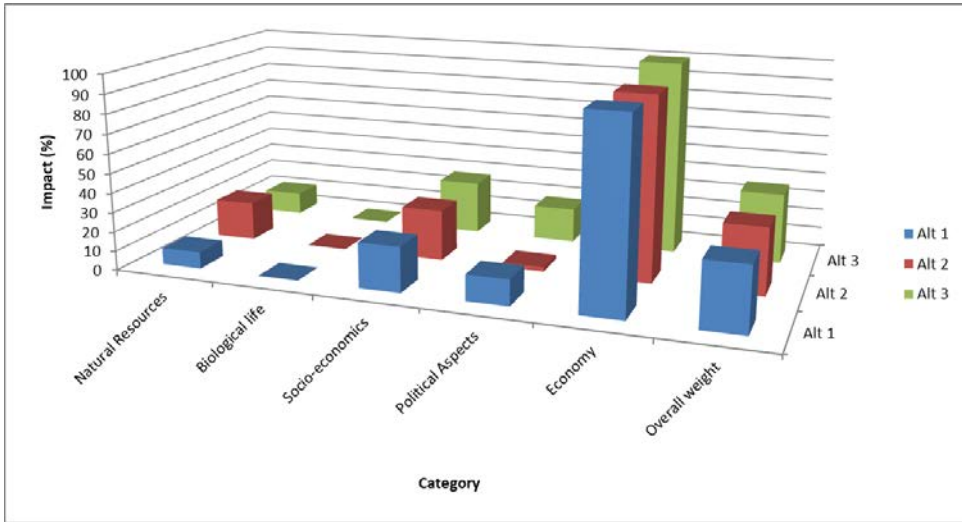
**Table 9:** Economic analysis indexes

Total Investment Cost (€)		1 213 515
Annual O&M Cost (8% of Total Cost) (€)		97 081
Annual Water Agency Income (€)		51 701
Discount Rate (%)		4
	Before Project	After Project
Annual Farmers Benefit (€)	537 910	3 703 360
Annual Farmers Cost (€)	295 880	1 277 666
Indexes	NPV (€)	34 195 245
	BCR	2.81
	IRR (%)	129%
	PBT (Years)	2

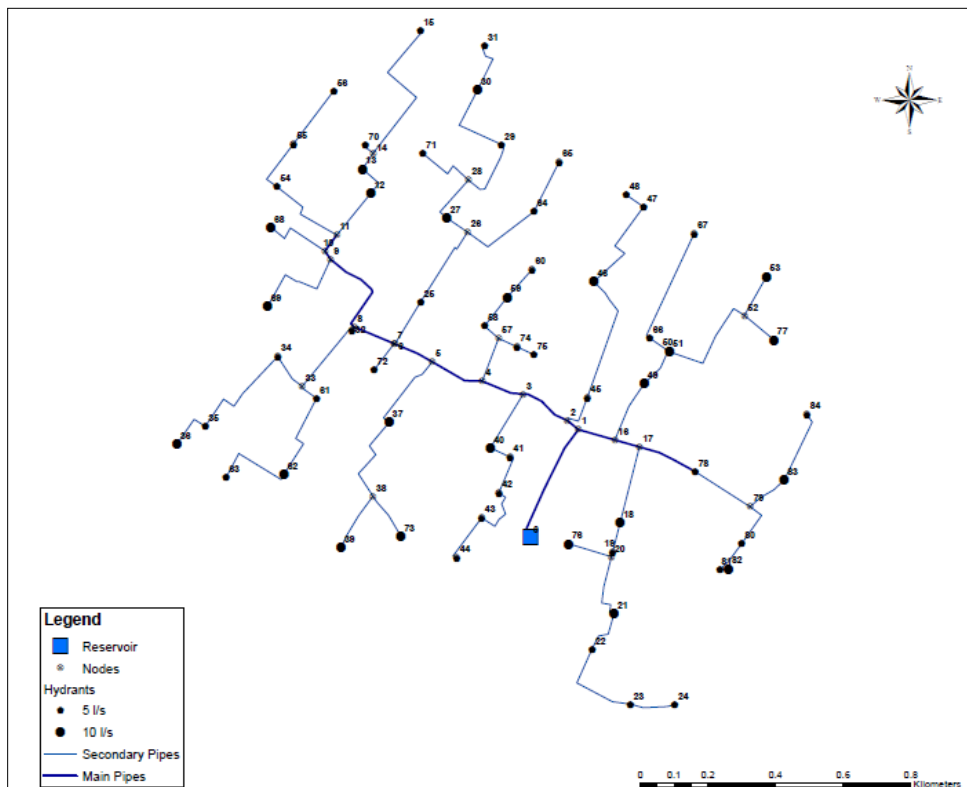
The used variables for different sensitivity analysis are discount rates, volumetric water tariff and fixed water tariff. The increment of the interest rate shows the decrease in the net present value significantly. Fixed water tariff does not have impact on economic analysis, since the increase of the agency benefit leads to the increment of the farmer's cost with the same amount. From financial point of view, increment of the fixed water tariff will not cover the total costs, and the net present value stays negative. Considering volumetric water tariff sensitivity analysis, from the economic point of view, NPV values decrease as water tariff increase due to the fact that as water price increases, farmers' income decreases. From the financial point of view, net present value is higher due to increased agency revenue, but in the scenario number 5, where the price of only the first water block is reduced to 0,09 €/m<sup>3</sup>, the benefit for the water agency cannot cover the costs.

### **Environmental Impact Assessment**

Figure 4 shows the difference between the three alternatives according to its impact on each of the environmental categories, as well as, the overall weighted impacts of the three alternatives.



**Figure 4:** The impacts of each alternative on each category and the overall weighted impact



**Figure 5:** Corato irrigation network scheme

## CONCLUSIONS

This research aimed to conduct a study for the design of an irrigation project in the agricultural region of *Corato*, in order to cover the cropping pattern requirements during the peak period of a dry year.

Historical climatic data were collected and analysed, and they represent suitable conditions for plant growth. Furthermore, the soil in the area is clay-loam with high fertility levels and insignificant salinity levels. The source of water for the project is groundwater, with good chemical composition, through two wells located within the study area.

After adopting cropping pattern that is possible to be grown even during the peak period of the dry year, the irrigation network was designed. Furthermore, simulations of several scenarios based on different water tariffs in order to determine the best water saving technique without a large decrease of farm income were conducted. The discharges of each section in the network were calculated based on a probabilistic approach and pipe diameters were optimized to minimize the network costs. In addition, a reservoir was designed to receive and store water from the wells by submerged pumps, and then deliver the stored water to the irrigation scheme system by a pumping station composed of 4 pumps and regulating tank.

Analysis results showed that the project was not convenient from private point of view, while it was cost-effective on social basis and will fully recover its initial investment in two years. The developed irrigation project has a great potential to improve the conditions of local community, considering the overall social benefit including the enhancement of farmers' income without having negative impact on the area.

## ACKNOWLEDGMENTS

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## **IN VITRO MICROPROPAGATION OF POTATO (*Solanum tuberosum* L). CULTIVARS**

### **SUMMARY**

*In vitro* micropropagation is an alternative to conventional (vegetative) propagation of potatoes whereas aseptically meristem cultures were used which gave pathogen free plants. Different sterilization protocols were used for disinfecting the isolates potato sprouts from two potato genotypes named Excuisita and Bergerac. After 35-40 days of culture shoot height, number of shoots per explants, and number of roots were measured. It was found that the suitable sterilization protocol giving high percentages of survived individuals was that of 1% HgCl<sub>2</sub>. The sterilized sprouts were cut to isolate apical meristems which were cultured on shoot induction medium containing solidified MS media with vitamins and exogenous plant growth regulators and incubated at optimized culture conditions in room culture. The cultivar Bergerac showed greater ability for *in vitro* propagation with 6.3 shoots per explants but Excuisita plantlets presented higher shoot length (72.5 mm). The aim of the study was the presentation of suitable protocol for *in vitro* induction of potato plantlets stocks free of pathogens.

**Key words:** *In vitro*, meristem cultures, cultivar, MS media, shoot length.

### **INTRODUCTION**

Potato (*Solanum tuberosum* L.) is a very important crop in agricultural production of our country and around the world. According to FAO data it is grown in 180 countries worldwide but mostly in Asia, then in Europe; South and Central America. The beginning of potato cultivation in Albania dates 100 years. Today in the Republic of Albania potatoes are propagated on over 9500 hectares and almost every year the area expand with an average yield of 20t/ha.

Micro propagation is the alternative to conventional propagation of potatoes. *In vitro* propagation methods using nodal cuttings, meristem tips and micro tubers are more reliable to maintain genetic integrity of the multiplied clones since differentiation and the subsequent organogenesis/embryo genesis with the accompanying genetic changes have been reported (Wang and Hu 1982). The potential value of tissue culture in potatoes production has been used for disease free seed production in many countries. Seed production technique of

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potato can be designed with *in vitro* multiplication through either plantlet regeneration or micro tuber production (Hossain M.J.2005).

The main goal of this research was to set up a culture of meristem tips cultures as initial explants of two potato cultivars Excuisita and Bergerac in *in vitro* conditions. Explants development, organogenesis stage of explants on MS media supplemented with growth regulators and possibilities for minituber production from potatoes plants were followed during this experimental work.

### MATERIAL AND METHODS

The experiment was conducted at the Institute of Plant Genetic Resources, (Agriculture University of Tirana). Potato cultivars Excuisita and Bergerac were used as plant material for evaluation on their response to *in vitro* regeneration. Clean tubers were treated with 2ppm GA<sub>3</sub> for rapid sprouting. One week old sprouts were used as initial explants.

#### *Sterilization of initial explants-sprouts:*

The sprouts of about 0.5-1cm were surface sterilized by washing under flow of tap water for 15-30 minutes. In this experiment four different sterilization protocols were used.

After washing the sprouts are surface sterilized by dipping in:

-70 % C<sub>2</sub>H<sub>5</sub>OH for 30 seconds

-mercuric chloride (HgCl<sub>2</sub>) solution 0.1%, 0.5% and 1%

-washed several times with autoclaved distilled water

Another chemical solution used for sprouts sterilization was sodium hypochlorite (10%). All the glassware and instruments were thoroughly washed and dried at 80°C. Distilled water and glassware used for explants were autoclaved for 20 minutes.

#### *In vitro culture conditions:*

The initial explants were cultured in Murashige and Skoog (Murashige and Skoog 1962) solid medium (pH=5.8) in test tubes supplemented with plant hormone. MS media was prepared with 3% sucrose, 0.6% agar, 2ppm Ca pantothenic acid and 0.25ppm GA<sub>3</sub>.

The cultures were placed in culture growth room under the following conditions:

-temperature 25 ±1°C

-relative humidity 50%

-photoperiod 16/8 hour light/dark and

-illumination of 50μmol·m<sup>2</sup>·s<sup>-1</sup>.

The observations were recorded regularly till to 30 days for the non-growing cultures, infected cultures and healthy cultures. Data recorded during proliferation and multiplication phases, of biometric parameters were statistically elaborated using ANOVA analyses.

## RESULTS AND DISCUSSION

The influence of gibberellin acid on stimulation of sprout formation was positive. The treatment with 2ppm GA<sub>3</sub> was efficient for the two potato cultivars. All treated tubers resulted with *de novo* a sprout, which shows effect of 100% in sprouts formation. A higher number of sprouts are formed from the cultivar Excuisita with an average of 8.21 sprouts per tuber (Figure.1).

**Table 1.** The effect of GA<sub>3</sub> treatments on *in vitro* sprout formation in potatoes tubers *in vivo*

Cultivar treated with 2ppm GA <sub>3</sub>	Number of treated tubers	Nr. of sprouts obtained per tuber	Sprout formation %	Length of sprouts ± mm	Sprout colour
Excuisita	20	8.21±0.5	100	9.07±1.0	Green
Bergerac	20	7.78±0.5	100	7.33±0.5	Pink



**Figure 1.** The effect of GA<sub>3</sub> for rapid sprout formation

### *Effect of sterilization treatment:*

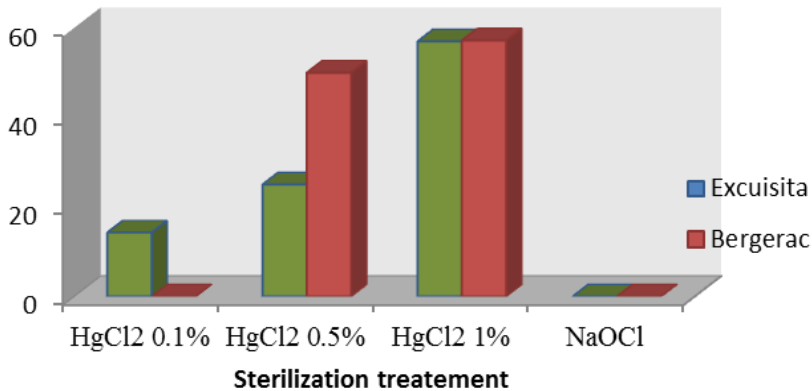
Results shows that between two chemical solutions HgCl<sub>2</sub> was found more effective than NaOCl. All the explants of two potato cultivars of our study, treated with sodium hypochlorite solution, resulted infected (100%). While mercuric solution gave positive results in Excuisita and Bergerac explants with a high survival rate of 57 %.

Our results were higher from those reported from other authors (Kanwal *et al.* 2006, Badoni *et al.* 2010, Liljana *et al.* 2012). During the first phase of *in vitro* propagation (proliferation), explants inoculated on MS Strong media supplemented with phytohormones, reacted positively giving high results on germination (Figure 1).



**Figure 2.** Explants proliferation in MS media

Between two cultivars used, Excuisita explants gave 96.34% of germination on agar media 0.6% and Bergerac cultivar 85.5% of explants germination. The germinated shoot tips produced plantlets with normal morphological development.

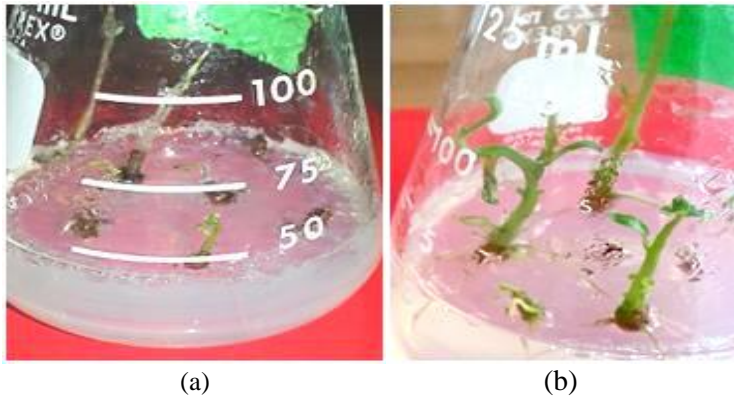


**Figure 3.** Effect of sterilization treatment on potato explants

The effect of MS media on *in vitro* shoots, roots and leaves formation from sprouts explants of two potato cultivars is shown in table 2.

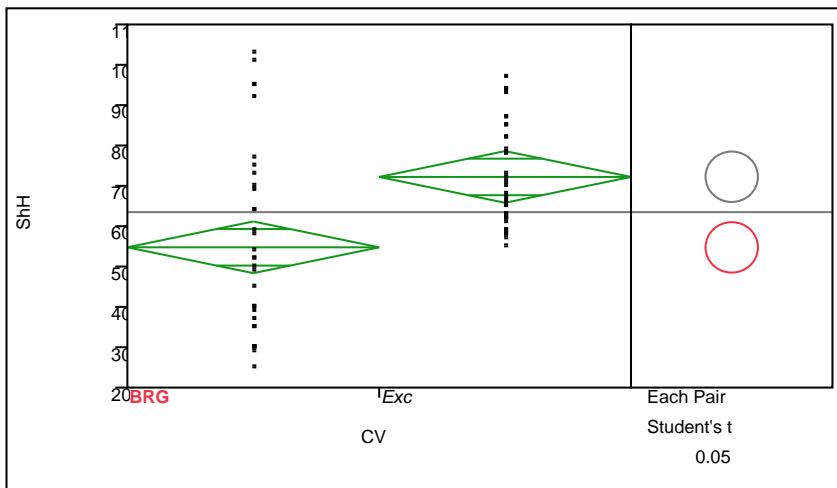
**Table 2.** Biometric parameters measured of Excuisita and Bergerac potato cultivars on MS media

Cultivar on MS media	Nr. of explants	Shoot length/m	Nr. of shoots /explants	Nr. of roots /explant	Nr. of leaves/ explant	% rootin g
Excuisita	34	72.2±0.5	3.7±0.7	4.6±0.8	4.0±0.5	32.35
Bergerac	34	54.7±0.7	6.3±0.9	3.8±0.9	3.7±0.7	100



**Figure 4.** Bergerac (a) and Excuisita (b) explants during proliferation phase

The combination of 0.25ppm GA<sub>3</sub> with BAP showed good results for improving shoot height during sub culturing phase. The results show that cultivar Excuisita has maximum potential for creating new plantlets with higher values on shoot height ( $\pm 72.2\text{mm}$ ) and leaf number in compare of Bergerac cultivar ( $\pm 54.76\text{mm}$ ). ANOVA analysis proved that the differences between two potatoes cultivars related shoot height trait were significantly different at the P0.05 and P0.01 level of the probability (Figure 5).



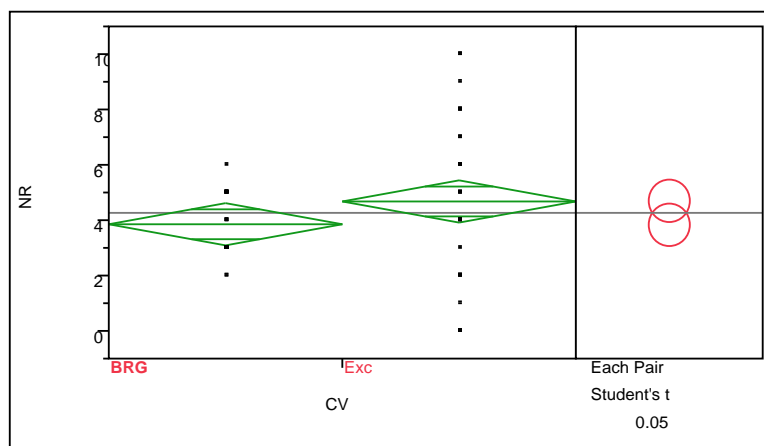
**Figure 5.** Comparison of shoot height data among two potatoes cultivars

Our results are comparable with those reported from Badoni *et al.*2009 and even better than the results of Liljana *et al.*2012. The percentage of rooting differs for the two potatoes cultivars where the maximum results (100%) are obtained for Bergerac *in vitro* plantlets.



**Figure 6.** Potatoes plantlets ( Excuisita & Bergerac) *in vitro* development

The combination of GA<sub>3</sub> with BAP was effective in improving other parameters of our study, as number of shoots/explants. Bergerac cultivar was the one with higher values on number of shoots per explants created ( $\pm 6.37$ ) in compare with 3.75 shoots/explants of Excuisita *in vitro* new plantlets. Our results goes within the limits of those reported from Badoni *et al.* 2009 (2.7 till 9.4 shoots). Our potatoes cultivar used in this study showed not significant differences for two other quantitative parameters as the number of leaves and number of roots created. This was proved statistically using anova analysis as it is shown (Figure 7).



**Figure 7.** Comparison of  $\pm$  number of root data among two potato cultivars

Earlier studies on *in vitro* potatoes regeneration have shown that the culture media, growth regulators and carbohydrate concentration affect micro tuberisation under *in vitro* conditions. El -Sawy *et al.* 2007 reported that sucrose is an important factor for micro tuber formation. Regarding this author the highest tuber formation was achieved when 12% sucrose was added to culture media. High sucrose concentrations may act as inducing signal leading to starch accumulation, so to increase the present of micro tuberisation the concentration

of sucrose must be higher. None of the cultivars used in our study was able for micro tuber formation on MS media supplemented with 3% sucrose. Same results are suggested from Hoque *et al.*2010.

Plantlets of two potatoes cultivars reacted differently on acclimatization process. Only the *Excusita in vitro* plants (Figure 8) survived after acclimatization on mixed pre-prepared composition (soil and sand).



**Figure 8.** *Excusita in vitro* plant acclimatization process

Three to four water sprays were given daily with a sprayer to keep the soil moist and maintain humidity for initial one week. Once the plants established and start growing normal irrigation was followed.

Minituber is an intermediate stage of potato seed production between laboratory micro propagation and field multiplication. We achieved the formation of minitubers with an average weight of 10-12 g from *Excusita* plants (Figure 8).



**Figure 9.** *Excusita* plant producing minitubers.

## CONCLUSIONS

Results obtained in the present study, suggest that the type of method used for explants disinfections, was the right one, indicating a high survival rate. Also the type of explant and nutrient medium used for the *in vitro* establishment and proliferation of *S.tuberosum* L. germplasm, effected the rate of proliferation giving satisfactory results for our two *potatoes* resources. The multiplication rate of two *potatoes* cultivars, object of the study, was highly effective on producing a satisfactory number of explants on MS media.

The combination of GA<sub>3</sub> with BAP was effective in improving the explants development, organogenesis stage and possibilities for minituber production from potatoes plants during this experimental work. The technique used in our study might be a possible one, for cloning of other potatoes cultivar plants.

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**INFLUENCE OF METEOROLOGICAL PARAMETERS ON THE YIELD  
AND CHEMICAL COMPOSITION OF COMMON BUCKWHEAT  
(*Fagopyrum esculentum* Moench)**

**SUMMARY**

Common buckwheat (*Fagopyrum esculentum* Moench) is an annual plant from Polygonaceae family which is well known as pseudocereal with high nutritional value. The yield and quality of the buckwheat's kernel depend on weather conditions, mainly due to variations in the air temperatures and precipitation during the growing period. The aim of this study was to determine the impact of some meteorological parameters on yields and chemical compositions in the kernel of buckwheat. Field experiments were conducted during three years (2011, 2012 and 2013) in the village Donje Selo, near Ilijaš. In this study grain yield, protein content, sludge, fats, mineral matter, cellulose and total phenols were determined. Experimental results suggested that the weather conditions in different years of the research have a significant impact on the yield and the chemical composition of the kernel. The yield of buckwheat varied from the year to year and ranged from 0.98 to 1.29 tons per hectare. Contents of protein and starch were also significantly dependent on the year of the research, as well as the content of phenol in the kernel. High total phenolic content was recorded in the year with the highest average monthly air temperature.

**Keywords:** buckwheat, meteorological parameters, yield, chemical composition

**INTRODUCTION**

Common buckwheat (*Fagopyrum esculentum* Moench) is an annual plant and belongs to botanical family *Poligonaceae*. It is a very old plant that is used as human or animal feed for more than 7.000 years (Gadžo et al., 2016). It originates from Asia and it was introduced to Europe in the 15th century. In agriculture and food technology common buckwheat is usually considered as a pseudocereal. In comparison with other cereals buckwheat has a low yield, however, there are some advantages. It is a short-season crop and it does not have

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particular soil or fertilization requirements. Also, buckwheat can grow at high altitudes (Khan *et al.*, 2013). In its grains buckwheat contains a variety of nutrients, main compounds are proteins, polysaccharides, dietary fibers, lipids, polyphenols, minerals and vitamins (Ahmed *et al.*, 2014). Thanks to the chemical composition, grain of buckwheat is primarily used to obtain high quality flour. Unlike flour of wheat, buckwheat's flour is gluten free (Gadžo *et al.*, 2016.), contains proteins with good balanced amino acids (Selimović *et al.*, 2014), and it is particularly rich in lysine and arginine (Christa and Soral-Šmietana, 2008). Compared to other cereals buckwheat kernel has a higher fiber content.

Buckwheat's flower and leaf in recent years become more popular due to their positive effect on human health. Healing properties of buckwheat are derived from the content of the phenolic compounds. It is believed that the consuming of buckwheat contributes to the alleviation or prevention of many diseases (Tomotake *et al.*, 2001; Kawa *et al.*, 2003; Li *et al.*, 2016).

According to FAO data (FAOStat, 2018), in 2016, it buckwheat was cultivated on an area of 2.37 million hectares globally. The main producers of buckwheat in the world are Russian Federation, China and Ukraine and they have 70% of the sown areas of the world (Gadžo *et al.*, 2016). In Bosnia and Herzegovina cultivation area for buckwheat is about 1056 ha with an average yield of 1.07 t ha<sup>-1</sup> (FAOStat, 2018).

Grain yield and chemical composition of buckwheat depends on a large number of factors, and some of them are variety of buckwheat (Gavrić and Gadžo, 2011; Golob *et al.*, 2016) and agrotechnical conditions of cultivation (Strakšas *et al.*, 2011; Rahimić and Gadžo, 2012). An important role in formation and quality of yield also have weather conditions (Gavrić *et al.*, 2018; Bavec *et al.*, 2002), mainly due to variations in the air temperatures (Strakšas *et al.*, 2011) and precipitation (Glamočlija *et al.*, 2012) during the growing period. Considering that meteorological parameters are changeable, unstable and unpredictable in certain areas for production of buckwheat, the aim of this study was to determine the impact of some metrological parameters on yield and chemical composition in the kernel of buckwheat.

## MATERIAL AND METHODS

Field experiments were conducted in 2011, 2012 and 2013 growing seasons in the village Donje Selo, near Ilijaš. Common buckwheat (variety Darja) originates from Slovenia and it was used in this research. Buckwheat was sown in three different sowing rates: 50 (S1), 80 (S2) and 100 (S2) kg per hectare. Experiment was set up by randomized block method in four repetitions. The size of basic plot was 4.8 m<sup>2</sup>. The seeds were sown when the soil temperature was 10 °C and the sowing was done manually. During the vegetation, weeds were removed manually. Yield of buckwheat was measured in the field. Total protein, starch, fat, ash, cellulose and phenol contents were determined at Laboratories of the Faculty of Agriculture and Food Science Sarajevo. Content of nitrogen in kernel was determined by kjeldahl method described by ISO 5983 and distillation

was made by Mikrokjeldahl apparatus (Foss Kjeltec 2200) to determine kernel nitrogen contents. After measurements, protein content was calculated by multiplying nitrogen contents in kernel with a constant factor of 6.25. Starch was determined by polarimetric method described by Ewers (ISO 6493), contents of lipids were determined by ISO 6492, contents of ash was determined by ISO 5984, and contents of cellulose was determined by Kürschner-Hanack (Kulić and Radojičić, 2011). The total phenolic contents of ethanolic extracts were measured by using of Folin Ciocalteu reagent as described by Bystrická et al. (2010). Statistical analyses were made using SPSS 22 software program.

## RESULTS AND DISCUSSION

In table 1 monthly average air temperature and precipitation amount for the period of the research (2011-2013) is presented. Analysis of weather conditions was done on the basis of data for the weather station Sokolac. During the research period, dry and above average warm weather was recorded. The year 2012 has been particularly contradictory comparing to the climate reference period (1961 – 1990). In 2012, air temperature was 4.4 °C higher compared to climate reference period (1961 – 1990). The temperature increase was accompanied by droughts. The minimum amount of precipitation during vegetation period was recorded in August of 2012 (0.6 mm) and 2011 (9.2 mm). The weather conditions in 2013 were more favorable compared to the other two years (2011 and 2012).

Table 1. Average monthly air temperature and amount of precipitation

Year	Month												Average
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
<b>Average monthly air temperature (°C)</b>													
<b>1961-1990</b>	-4.8	-2.2	1.6	6.4	11.4	14.3	16.0	15.5	12.0	7.2	2.1	-2.8	6.4
<b>2011.</b>	-2.5	-2.4	1.8	7.7	11.0	15.8	17.5	18.2	15.5	6.4	0.8	-1.0	7.4
<b>2012.</b>	-4.5	-7.2	3.6	7.4	10.9	18.7	20.4	19.7	15.1	9.5	6	-2.8	8.1
<b>2013.</b>	-0.7	0.0	2.3	8.9	12.5	15.6	18.1	18.5	12.3	9.4	4.7	-1.6	8.3
<b>Amount of precipitation(mm)</b>													
<b>1961-1990</b>	53	50	56	62	73	84	73	70	66	66	84	71	805
<b>2011.</b>	30.2	27.6	28.8	42.7	123	62.8	82.2	9.2	36.7	59.6	23.7	100.1	626
<b>2012.</b>	90.8	100.6	23.6	93.7	205.1	11.6	77.6	0.6	59.4	67	50.7	80.7	861
<b>2013.</b>	126.1	138.9	71.7	44	121.5	41.4	28.4	70.6	108	78.5	70.8	5.7	905

Results presented in Table 2. show that yield significantly depends on the year of research and the sowing rate. The results indicated that yield of buckwheat varied from the year to year and ranged from 0.98 (2012) to 1.29 (2013) t ha<sup>-1</sup>.

Yield in 2013 was relatively high compared to the other two years (2011 and 2012). Buckwheat is a plant which achieves better yields in humid regions and wet years (Milić *et al.*, 2013). Such conditions were not present during 2012 and it was one of the reasons why buckwheat achieved the lowest yields in this year (Table 1. and 2). During this three-year research, the highest monthly temperatures during the vegetation period were recorded in 2012 (Table 1.) and this fact is the second reason why the buckwheat had the low yield in this year. It is known that buckwheat has a long flowering period and that high temperatures have a negative impact on pollination and yield formation. Many studies found a close relationship between drought and yield of buckwheat (Gavrić and Gadžo, 2011; Glamočlija *et al.*, 2012; Popović *et al.*, 2014).

Table 2. Kernel Yield, t ha<sup>-1</sup>

Sowing rate	Year of the research			
	2011	2012	2013	Average
50 kg ha <sup>-1</sup> (S1)	0.57	0.83	1.04	0.81 <sup>c</sup>
80 kg ha <sup>-1</sup> (S2)	0.70	1.16	1.32	1.06 <sup>b</sup>
100 kg ha <sup>-1</sup> (S3)	1.82	0.95	1.52	1.43 <sup>a</sup>
Average	1.03 <sup>b</sup>	0.98 <sup>b</sup>	1.29 <sup>a</sup>	

Yield of buckwheat also significantly depended on sowing rate and they are in a positive relation. Therefore, the average grain yield in S1 variant was 0,81 t ha<sup>-1</sup>, and increased to 1,43 t ha<sup>-1</sup> in S3 variant. A similar effect of sowing rates was observed in all years of research except in the second year (Table 2). Influence of sowing rates on yield had been proved by many authors (Gavrić *et al.*, 2018; Thakuria and Gogo, 2000).

Buckwheat kernel contains a variety of nutrients and the main compounds are proteins, starch, cellulose, lipids, minerals and phenols. The total content of some components depends on the variety of buckwheat and environmental factors (Christa and Soral-Šmietana, 2008). In this research, it was found that the sowing rates had no significant effect on the chemical composition.

Table 3. Chemical compose of buckwheat´s kernel

Year of research	Crude protein, %	Starch, %	Cellulose, %	Fat, %	Ash, %	Total phenols, mg g <sup>-1</sup>
2011	10.94 <sup>c</sup>	63.78 <sup>a</sup>	9.86 <sup>ns</sup>	2.07 <sup>ns</sup>	2.20 <sup>ns</sup>	0.37 <sup>b</sup>
2012	14.26 <sup>a</sup>	60.71 <sup>b</sup>	10.55 <sup>ns</sup>	1.69 <sup>ns</sup>	2.18 <sup>ns</sup>	3.67 <sup>a</sup>
2013	12.08 <sup>b</sup>	62.34 <sup>ab</sup>	10.07 <sup>ns</sup>	1.97 <sup>ns</sup>	2.30 <sup>ns</sup>	0.35 <sup>b</sup>
Average	12.43	62.28	10.16	1.91	2.23	1.47

The results of chemical composition show that contents of crude protein and starch significantly depend on the research year. The average contents of crude protein ranged from minimal 10.94 % (2011) to maximal 14.26% (2012). During

second year of research (2012), the largest content of crude protein in kernel of buckwheat was detected (14.26%). Higher content of crude protein was probably caused by lower precipitation levels during vegetation (Table 1.). Increasing protein content in kernel can be explained by the reduction in the content of starch in kernel. If we compare starch content (Table 3) with precipitation levels, it can be concluded that the lowest content of starch (60.71%) is recorded in the year with smallest amounts of rain during vegetation. The highest starch contents (62.34% and 63.78%) were determined in the years with a larger amount of precipitation (2011 and 2013). These results are similar to Erekul and Köhn (2009). They found that lack of rain in some years led to high crude protein contents and low starch contents in kernel of wheat and triticale. Similar observations have been reported by many authors (Vafa et al., 2014; Pierre et al., 2008; Sial et al, 2005).

Observing the results from Table 3, it can be concluded that contents of cellulose, fats and ash did not depend on any of the research factors. Their content in kernel has been in the range as in the literature (Christa and Soral-Śmietana, 2008; Kreft and Germ, 2008).

Total phenols have a vital part in the protection of plants against high or low temperatures (Kreft et al., 2013), droughts (Lim et al. 2012), UV radiation (Kreft et al., 2003; Germ, 2004), pathogens and herbivores (Guo et al., 2011) and their high concentration in plants suggest to environmental stress factors (Stagnari, et al., 2017). The results in Table 3. suggest that concentrations of total phenols depend on the years of the research. During 2012 (3.67 mg GAE g<sup>-1</sup>), buckwheat had about ten times higher total phenol than in 2011 (0.37 mg GAE g<sup>-1</sup>) and 2013 (0.35 mg GAE g<sup>-1</sup>). Based on this fact, it can be concluded that buckwheat grew in stressful conditions during 2012, and it synthesized an additional amount of phenol to protect itself. High content was found in the years with a drought and higher average monthly air temperature, and it can be concluded that these factors are the main cause of differences. Kreft et al. (2013) and Lumingkewas et al. (2015) also found that temperature and humidity have effect on concentration of phenol in buckwheat.

## CONCLUSIONS

According to the presented results of the research, influence of meteorological parameters on the yield and chemical composition of common buckwheat during the seasons of 2011, 2012 and 2014, following conclusions can be reached:

- the yield of buckwheat significantly depended on sowing rate and meteorological parameters;
- Increasing sowing rate had positive effect on yield of kernel, but it had no effect on chemical compose of kernel;
- second year of research was with unfavorable weather conditions (drought and high temperature) which resulted in low average yield;
- contents of crude protein, starch and total phenol in kernel depended on meteorological parameters.

- high content of protein and total phenols was recorded in the year with relatively unfavorable weather conditions;
- drought at 2012 year significantly caused a reduction in contents of starch in kernel.

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## SOIL DISTRIBUTION IN STRUMICA RIVER BASIN AND ITS IMPORTANCE FOR AGRICULTURAL PRODUCTION

### SUMMARY

This paper is a result of many years of field and laboratory research of the soils in Strumica river basin, spread out on 148.124,02 ha, ranging from 150 to 1540 m above the sea level in order to gain a better understanding of the productive capacities of the soils and measures for their improvement. The filed research of the soils has been done according to methods described by (Markoski et al., 2014).

In laboratory, the following analyses have been carried out on the soil samples: hygroscopic moisture; mechanical composition; pH of the soil solution; humus content and total nitrogen; content of carbonates; available nutrients P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O. The mechanical composition and chemical properties of the soils have been determined by standard methods described by (Belić et al, 2014), (Mitrikeski & Mitkova, 2001); (Resulović et al., 1971), (Džamić et al., 1996). This area is very heterogeneous, with numerous relief forms, with different expositions and inclinations, and with great differences of altitude.

Additionally, there are several geological formations of a very heterogeneous petrographic-mineralogical composition and climate-vegetation zones. Long-term effects from human involvement should also be noted. The vast diversity of the factors required for soil formation in this area is the reason for the formation of many different soil types as well as the lower taxonomic units. These types of soils are characterized by different properties (chemical, physical, and physical-mechanical, productive). Therefore, they have varied effects on agricultural production.

There are 14 (fourteen) different soil types distributed in the Strumica river basin together with a considerable amount of subtypes, varieties and forms. Mountain soils are prevalent in this river basin: 79.337,6 ha, or (53.56%) of the whole. Soils of lake terraces and of undulated hilly relief cover 30.507,42 ha, (20.59%), whereas soils of colluvial fans occupy 18.739,6 ha (12.65%) of the area. Finally, soils of the plains occupy 17.784,4 ha (12.01%) and Urbisol 1.755,57 ha (1.18%).

**Key words:** soil types, Strumica river basin.

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## INTRODUCTION

The Strumica river basin is 1.649 km<sup>2</sup> that is 6.4% of the territory of the Republic of Macedonia. The major part of the total watershed (75%) is in Macedonia, while the remaining is in Bulgaria and Greece. River Strumica takes its source from the Plackovica Mountain at an altitude of 1.540 m asl running south in a deep valley and known as the Stara Reka (Popovska *et al.*, 2014). It then enters the Radoviš valley and runs through the eponymous Radoviš. Afterwards the river runs southeast through the Strumica valley passing through the town of Strumica and turning east to enter Bulgaria south of Zaltarevo. The main tributaries are Turija, Vodocnica, Radoviška and Podareška. The annual average discharges of Strumica River for the from both sides of the Strumica River there are a large number of tributaries mostly mountainous streams with permanent water flow.

River outflow Macedonian border southeast of Novo Selo and inflow Struma River in Bulgaria at an altitude of 186 m asl. It is the Struma's largest tributary. Total length of Strumica River is 114 km out of which 81 km in Macedonia and 33 km in Bulgaria.

This area (Strumica river basin) is very heterogeneous, with numerous relief forms, with different expositions and inclinations, and with great differences of altitude. Additionally, there are several geological formations of a very heterogeneous petrographic-mineralogical composition and climate-vegetation zones. Long-term effects from human involvement should also be noted. The vast diversity of the factors required for soil formation in this area is the reason for the formation of many different soil types as well as the lower taxonomic units.

The soils in the area also appear in the complexes that are presented on the soil (pedological) map. These types of soils are characterized by different properties (chemical, physical, and physical-mechanical, productive). Therefore, they have varied effects on agricultural production.

In this paper are presented the main aspects of the soil geography. The agro technical and meliorative measures are determined based on the properties and processes of the various soil types found in this area with the goal of improving their productive capacity to further increase agricultural production.

## MATERIAL AND METHODS

The filed research of the soils has been done according to methods described by (Mitrikeski and Mitkova, 2006). In laboratory, the following analyses have been carried out on the soil samples: hygroscopic moisture; mechanical composition; pH of the soil solution; humus content and total nitrogen; content of carbonates; available nutrients P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O.

The mechanical composition and chemical properties of the soils have been determined by standard methods described by: (Belić *et al.*, 2014); (Mitrikeski and Mitkova 2006); (Resulović *et al.*, 1971; Džamić *et al.*, 1996).



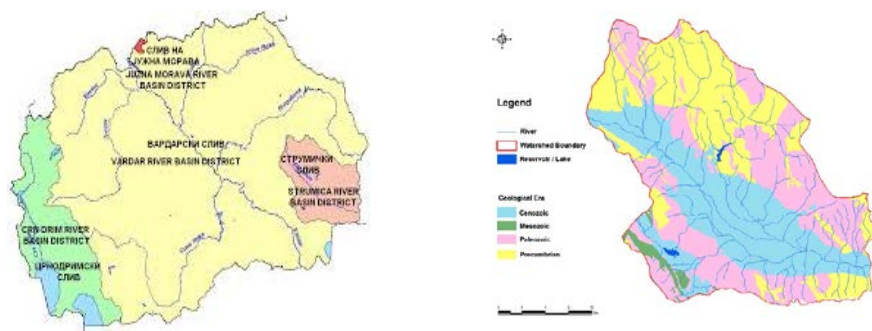


Chart 2. Geological eras of Strumica river basin

Table 1. Soil types and complexes distribution in the catchment area of the Strumica River (ha and %)

SOIL TYPES AND COMPLEXES (WRB Soil Classification)	Symbol	ha	%
<b>I. SOILS OF THE PLAINS</b>			
1. Fluvisol	J	17533.8	11.84
2. Gleysol	G	213.22	0.14
3. Solonetz+ Solonchak	Z/S	37.33	0.03
<b>Total</b>		<b>17.784,4</b>	<b>12.01</b>
<b>II. SOILS OF COLLUVIAL FANS</b>			
1. Fluvisol (Colluvial Soils)	K	<b>18.739,1</b>	<b>12.65</b>
<b>III. SOILS OF LAKE TERRACES AND OF UNDULATED HILLY RELIEF</b>			
1. Regosol	R	9896.73	6.68
2. Complex of Regosol and Leptosol	R/E	2870.31	1.94
3. Humic Calcaric Regosol	Rz	1818.39	1.28
4. Complex of Humic Calcaric Regosol, Regosol and Leptosol	Rz/R/E	647	0.44
5. Aric Regosol	ATa	1295.71	0.86
6. Chromic Luvisol on saprolite	Lc	3976.81	2.68
7. Complex of Chromic Luvisol on saprolite, Humic Calcaric Regosol and Regosol	Lc/Rz/R	571.21	0.39
8. Complex of Chromic Luvisol on saprolite and Regosol	Lc/R	7795.97	5.26
9. Vertisol	V	1246.88	0.84
10. Complex of Humic Calcaric Regosol and Regosol	Rz/R	264.32	0.18
11. Albic Luvisol	La	124.09	0.08
<b>Total</b>		<b>30.507,4</b>	<b>20.59</b>
<b>IV. MOUNTAIN SOILS</b>			
1. Leptosol	E	6724.81	4.54
2. Complex of Cambisol and Regosol	B/R	9255.01	6.25
3. Cambisol	B	51691.95	34.90
4. Complex of Humic Eutric and Umbric Regosol	Lm/R	9699.55	6.55
5. Chromic Leptic Luvisol on hard limestones	Lvd	71.79	0.04
6. Rendzic Leptosols	Eh	145.17	0.09
7. Complex of Humic Eutric and Umbric Regosol, Regosol and Leptosol	Lm/R/E	1529.38	1.03
8. Complex of Humic Eutric and Umbric Regosol and Regosol	Lm/R/R	219.92	0.14
<b>Total</b>		<b>79.337,6</b>	<b>53.56</b>
1. Urbisol	Urb	<b>1.755,57</b>	1.18
<b>Total area of this region</b>		<b>148.124,02</b>	<b>100</b>

Table 1 contains data on the soil types and complexes distribution according to the relief forms in the catchment area of the Strumica River in ha and %. It can be seen from the Table that the soils spread on mountainous terrains dominate in the catchment area and cover an area of 79.337,6 ha, or 53.56% of the area, followed by the soils spread on lake terraces and of undulated hilly relief with 30.507,4 ha, or 20.59%. The soils on sloping terrains (colluvial fans) and plains cover small areas (18.739,1 ha or 12.65% and 17.784,4 ha, or 12.01% of the area).

The plain terrains are mostly covered by Fluvisols (17.533,8 ha or 11.84%), while the percentage of other soils is under 1%. The sloping terrains are mostly covered by Fluvisols (Colluvial Soils) (18.739,1ha or 12.65%). The undulating-hilly terrains and the lake terraces are mostly covered by Regosols (9.896,73 ha or 6.68%), followed by Complex of Chromic Luvisol on saprolite and Regosol (5.26%), Chromic Luvisol on saprolite (2.68), Complex of Regosol and Leptosol (1.94%), as well as Humic Calcaric Regosol (1.28%). Most of the mountainous terrains are covered with Cambisols (51.691,95 ha or 34.90%) and Complex of Humic Eutric and Umbric Regosol (9.699,55 ha or 6.55%), and Complex of Cambisol and Regosol (9.255,01ha or 6.25%) as well as Leptosol with 6.724,81ha or 4.54%. The erosion processes, i.e. the human factor are strongly reflected in the geography of the soils in the area. The area of soils that occurred from erosion processes (Leptosols, Regosols, Fluvisols-Colluvial Soils and their complexes) is more than 25% of all areas and unfortunately, the spreading process for these areas is still active.

The individual terrain forms differ from each other by their terrain, geological structure, their climate – vegetation and hydrographic conditions and by the degree of anthropogenization. This is reflected on the geography of soils and their properties, as well as on the degree of their utilization and the measures that need to be undertaken in the agricultural production of the area.

#### **Common properties of the soils according to terrain (relief) forms**

*Common properties of the soils on the mountainous terrains.* The soils spread on mountainous terrains cover 79.337,6 ha, or 53.56% of the area. These are: Leptosols, Humic Eutric and Umbric Regosols, Cambisols, as well as their complexes, in combination with Regosols. Their common properties are: a) very pronounced erosion processes; b) weak chemical weathering resulting in shallow solum over some substrates, and lack of deep regolith and poor clay content; c) absence of carbonate, pronounced acidification (weaker in the soils over acidic rocks than in the basic rocks); d) absence or very poor textural differentiation of the solum; e) clearly expressed changes in the soil properties and the intensity of some processes as the altitude increases; f) clearly expressed dependence of the soil properties from the substrate: soils over acidic rocks contain less clay, they are more acidic and are less texturally differentiated, unlike the soils formed over basic rocks, g) absolute domination of the silicate over the carbonate substrate.

*Common properties of the soils from the lake terraces and of undulated hilly relief.* The following soil types are present on this terrain form: Regosols,

Humic Calcaric Regosols, Vertisols, and Chromic Luvisols on saprolite and Albic Luvisols. The occurrence of these types of soils is in close co-relation with the substrate, the relief, the climate-vegetation conditions and the degree of erosion. The soils on this terrain (relief) form have the following common properties: a) very pronounced erosion (occurrence of Regosols and erosion of horizon A or part thereof, in the soils with A-C, A-(B)-C and A-E-B-C profile type); b) absence of compact rocks as substrate and soil genesis over clastic sediments, resulting in deep solum and physiologically active profiles; c) greater presence of clay resulting from the substrate or the argilogenesis within the profile; d) presence of smectites in some soils (Vertisols) arising mainly from the substrate and partially from the soil genesis, and in relation to that, deterioration of the physical properties of the soils; e) occurrence of textural differentiation at some soils (Vertisols and Albic Luvisols), and in relation to that, deterioration of the physical properties; f) greater presence of the silicate – carbonate substrate in the soil genesis, in comparison to the substrate of the mountainous terrains; g) relative dryness of the soils (which is lower at the lake terraces), caused from insufficient quantity of rainfalls, surface water flow and very deep underground water; e) insufficient quantity of humus and nutrients (especially N and P); f) relatively good chemical properties (the high content of carbonates of Humic Calcaric Regosols and the acidity of the Albic Luvisols are an exception).

*Common properties of soils from sloping terrains.* These terrains are covered with Fluvisols (Colluvial Soils), which are characterized by: a) horizontal and vertical (according to depth of profile) heterogeneity in the mechanical and mineral-petrographic composition; b) low content of clay, drainage, dryness, good aeration; c) poor humus content, insufficient stability of the aggregates; d) good chemical properties with insufficient nutrient elements; e) increasing the finer particles by descending to the lower parts of the cones (“fans”); g) short duration of soil genesis (the youth of the soils); h) unregulated water regimen (floods and sedimentation of coarse material).

*Common properties of the soils of the plains.* Fluvisols with varying degrees of gleyzation are present in the flat bottom deep in the profile and the Gley soils (Gleysols and Mollic Vertic Gleysols) have the following characteristics: a) the appearance of non-saline underground waters at different depths; b) the appearance of a physiologically deep profile; c) gleyzation at the bottom of the profile; d) a significant amount of organic matter (especially in Gleysols), where it is of hydromorphic origin; e) occurrence of unregulated water regimen (floods, riverbad erosion, deposition of coarse sediments, regeneration of fertility by application of fine sediment); g) absence of texture differentiation of the profile (no occurrence of (B) or Bt horizons); h) favorable physical and chemical properties.

The occurrence of Halomorphic soils (Solonchak and Solonetz) on small areas (total 37.33 ha or 0.03%) is characteristic of these terrains in the area. Their formation is related to the presence of salts in the sediments, drier climate conditions and relief-topographical conditions in which there are shallow and

saline underground waters. Soil significance according to relief forms for agricultural production in the area

The properties (mechanical composition and chemical properties) of individual soils formed in the area are described in detail in the papers of: (Markoski et al., 2015; Filipovski, 2015 and Filipovski 2015a). Here, together and according to relief forms, we will explain their significance for agricultural production in the area, the measures for improving their productive ability will be explained in the conclusions.

In mountain reliefs, part of the Humic Eutric and Umbric Regosols is under summer pastures, a smaller part under forests, and a small part is cultivated. Fields are mostly abandoned, and some produce potato and seed material for it, then rye and oats, and at lower altitudes some fruit trees are cultivated. As for the use of Cambisols, it can be said that they have the greatest significance for forestry, because they produce and then exploit most of the wood mass in our country. By deforestation some of these soils are converted into pastures or into now abandoned fields. A very small part is cultivated, used as fields, and a smaller part as pastures. Potatoes are most commonly cultivated field crops, some forage field crops can be successfully grown, as well as crops for green fertilization. Some of these soils can be successfully turned into artificial grasslands. Several fruit crops can be successfully grown (chestnut, walnut, plums, apples, pears, raspberries, blackberries, ribes).

Among the soils formed on lake terraces and of undulated hilly relief, Vertisols and Humic Calcaric Regosols are characterized by greater productivity in comparison with Regosols, Chromic Luvisols on saprolite and Albic Luvisols. Depending on the conditions for irrigation these soils have heterogeneous use. Field crops, vegetable crops, forage crops, industrial crops, vineyards, orchards are cultivated on them. Fluvisols (Colluvial Soils) are significantly less productive than Fluvisols (with which they border. They are less sorted, do not have a flat relief, have higher impact from drought, contain less nutrients, and do not supply water from groundwater.

In the plain terrains of the area, Fluvisols are of the greatest significance for agricultural production. This is due to the favorable physical and chemical properties, the deep solum, the provided conditions for irrigation and the presence of available forms of  $P_2O_5$  and  $K_2O$ . They provide relatively high yields of all agricultural crops. Gleysols are potentially fertile. They have relatively good properties, but have shallow underground waters, occasional floods at some sites, anaerobic conditions and due to this, poor nitrification.

## CONCLUSIONS

There are 14 (fourteen) different soil types distributed in the Strumica river basin together with a considerable amount of subtypes, varieties and forms. They are formed on four relief forms (plain terrains, sloping terrains, mountain terrains and undulating-hilly terrains and lake terraces) that have different significance

for agricultural production. In order to increase their productive ability, the following joint measures should be undertaken according to relief forms:

- Joint measures for soils from mountain terrains: (protection from erosion, fertilization with organic and mineral fertilizers, proper tillage, liming if necessary);

- Joint measures for soils from lake terraces and undulating-hilly terrains: (deep tillage, humization: organic fertilizers and phytomeliorations, intensive use of mineral fertilizers N and P<sub>2</sub>O<sub>5</sub>, and for plants that need potassium during the entire year and for obtaining much higher yields and K-fertilizers, anti-erosion measures, proper irrigation method);

- Joint measures for soils from sloping terrains: (anti-erosion protection measures, irrigation, humization, intensive use of mineral fertilizers);

- Joint measures for soils from plain terrains: (regulation of the water regimen, lowering of the level of underground water-drainage, tillage and creating a deep fallow, fertilization with mineral and organic fertilizers with previous soil fertility control, proper irrigation).

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<http://www.maksoil.ukim.mk/masis/>



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## IMPACT OF CLIMATE CHANGE ON THE ANNUAL WATER BALANCE IN A HUMID CLIMATE

### SUMMARY

Lithuania and Bosnia and Herzegovina are considered as countries with high vulnerability and low adaptive capacity to cope with climate change. The entire territory of these countries is characterized by the warming trend, with positive trends in both the maximum and the minimum temperatures throughout the year. The increase in air temperature has resulted in change of evapotranspiration and mean annual water balance values. These countries are also frequently faced with an occurrence of severe droughts and heavy floods. The main purpose of this study was to determine and compare the severity of changes in mean annual water balance for two humid climatic zones, in order to understand how different areas of similar climate characteristics react to climate change and to analyze the significance of their influence. Monthly weather data from two weather stations, Kaunas (Lithuania) and Sarajevo (Bosnia and Herzegovina), for the time period of 30 years (1988 – 2017) were used to determine and analyze the mean annual water balance. The results indicate that climate change has a different effect on the water balance of these two humid areas. Both locations showed a positive trend of reference evapotranspiration, with an increase of 1.450 mm year<sup>-1</sup> to 1.503 mm year<sup>-1</sup>. However, the total runoff and soil moisture deficit are decreasing in Kaunas (-0.480 mm and -2.114 mm year<sup>-1</sup>, respectively), while they are increasing in Sarajevo (0.492 mm and 0.485 mm year<sup>-1</sup>, respectively).

**Keywords:** climate change, water balance, humid climate, Bosnia and Herzegovina, Lithuania

### INTRODUCTION

Human pressure on the natural environment resulted in the decrease in availability of freshwater supplies for consumption and agricultural production. Global climate change and associated impacts on water resources are the most urgent challenges facing mankind today and will have enduring societal implications for generations to come. Potential impacts may include the changes

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in watershed hydrologic processes including timing and magnitude of surface runoff, stream discharge, evapotranspiration, drought occurrence and flood events (Simonovic & Li, 2004). There is an essential need for effective management of limited water resources while increasing agricultural productivity (Sharma & Irmak, 2012).

Lithuania and Bosnia and Herzegovina (B&H) are considered as countries with high vulnerability and low adaptive capacity to cope with climate change (Easterling *et al.*, 2000; Žurovec *et al.*, 2017). The entire territory of these countries is characterized by the warming trend with positive trends in both the maximum and the minimum temperatures throughout the year (Radusin *et al.*, 2013; Radusin *et al.*, 2016; Merl, 2017; Popov *et al.*, 2018; Povilaitis *et al.*, 2018).

The average annual precipitation from 1977 to 2016 was 642 mm, or 1.4% higher than the climate normal (CN, defined as the three-decade 1981-2010 average) in Kaunas, Lithuania. The annual air temperatures in Kaunas for the 1977-2016 period (with an average of 7.0°C) showed increasingly variable patterns (Povilaitis *et al.*, 2018). According to Trbic *et al.* (2017) the entire territory of B&H is characterized by prominent and significant annual warming. The annual temperature increase was in the range of 0.2–0.5°C per decade (Radusin *et al.*, 2016; Trbic *et al.*, 2017). In addition, increase in the extreme precipitation is present over the entire territory of Bosnia and Herzegovina (Popov *et al.*, 2017).

The increase in air temperature and changes in precipitation amount has resulted in change of evapotranspiration ( $ET_0$ ) and mean annual water balance values (Giugliano *et al.*, 2013). Water balance is important for determination of water availability, crop irrigation requirement, flood risk assessment, regional water management decision-making, drought analyses, environmental studies, understanding the possibilities of organizing agricultural production in a given area and it is used to model climate change impacts and design effective adaptation and mitigation measures (Žurovec, 2012; Pereira *et al.*, 2015; Pandey *et al.*, 2016; Čadro S. *et al.*, 2017; Žurovec *et al.*, 2017).

Thornthwaite and Mather (1955) developed a simple heuristic model of water balance that estimates monthly actual evapotranspiration ( $AET$ ), runoff ( $RO$ ) and soil moisture deficit ( $SMD$ ) as a function of soil-moisture storage and potential evapotranspiration ( $PET$ ) via a “bookkeeping” procedure. Since then, many authors developed a large number of different versions of this water balance method (Thornthwaite & Mather, 1957; Alley, 1984; Dingman, 2002; Legates & McCabe, 2005; McCabe & Markstrom, 2007; Westenbroek *et al.*, 2010). Trends of main water balance components have been extensively analyzed globally. Gocic and Trajkovic (2014) analyzed data from 12 weather stations in a humid area of Serbia, for the period 1980 – 2010. They found significant increasing trends in  $ET_0$  for the majority of the stations. Tabari *et al.* (2011) analyzed the annual, seasonal and monthly trends of the  $ET_0$  series for 20 stations in the western half of Iran during 1966 – 2005. They concluded that the

increasing trends in all seasons. This condition is followed by increasing trends in soil moisture deficit and drought occurrence. The similar results are found in Spain (Espadafor *et al.*, 2011), Iran (Amirataee *et al.*, 2016), Montenegro (Knežević *et al.*, 2018), Slovenia (Zupanc & Pintar, 2004) Brazil (Silva *et al.*, 2016) and Togo (Djaman & Komla, 2015). Likewise, many authors reported the increased trend in the annual value of potential evapotranspiration (Čadro *et al.*, 2016), soil water deficit (Bukantis A. & Rimkus E., 2005; Žurovec & Čadro, 2010, 2015; Cammalleri *et al.*, 2016) and drought severity (Vlahinić *et al.*, 2001; Stankūnavičius, 2009; Žurovec *et al.*, 2011; Hodžić *et al.*, 2013; Taparauskienė & Lukševičiūtė, 2015; Čadro Sabrija *et al.*, 2017; Merl, 2017) in humid areas of Bosnia and Herzegovina and Lithuania.

The main objective of this study is to determine and compare the severity of changes in mean annual water balance for two humid climatic zones (Lithuania and B&H) using the linear regression methods, in order to understand how different areas of similar climate characteristics react to climate change and to analyze the severity of their influence.

## MATERIAL AND METHODS

Two countries have been the subject of this research, Lithuania and Bosnia and Herzegovina (B&H) (Figure 1). Lithuania is located in the Baltic region of northern-eastern Europe. The capital city is Vilnius, situated at latitude 54°41' N and longitude 25°19' E. Bosnia and Herzegovina is located in south-eastern Europe, with capital city Sarajevo situated at latitude 43°52' N and longitude 18°25' E. The basic characteristics of both countries are given in the Table 1.

**Table 1.** Lithuania and B&H country profiles

Characteristic	Lithuania	Bosnia and Herzegovina
Area (km <sup>2</sup> )	65,300	51,129
Water (%)	1.35	1.4
Coastline (km)	90	20
Mean elevation (m)	110	500
Highest point (m)	297	2,386
GDP per capita <sup>1</sup>	18,857	5,806
Population	2,823,859 <sup>2</sup>	3,531,159 <sup>3</sup>
Population density (per km <sup>2</sup> )	43.24	69.06
HDI <sup>4</sup> for 2017	0.858	0.768
BTI <sup>5</sup> for 2017	9.24	6.28
Köppen climate classification <sup>6</sup>	<i>Dfb</i>	<i>ET, Dfb, Cfa, Cfb, Csa</i>
CRI <sup>7</sup> for 1997–2016	121.83	72.00
CRI rank	136	69

<sup>1</sup>Gross domestic product, International Monetary Fund (2017).

<sup>2</sup>Estimation for 2017, Oficialiosios statistikos portalas.

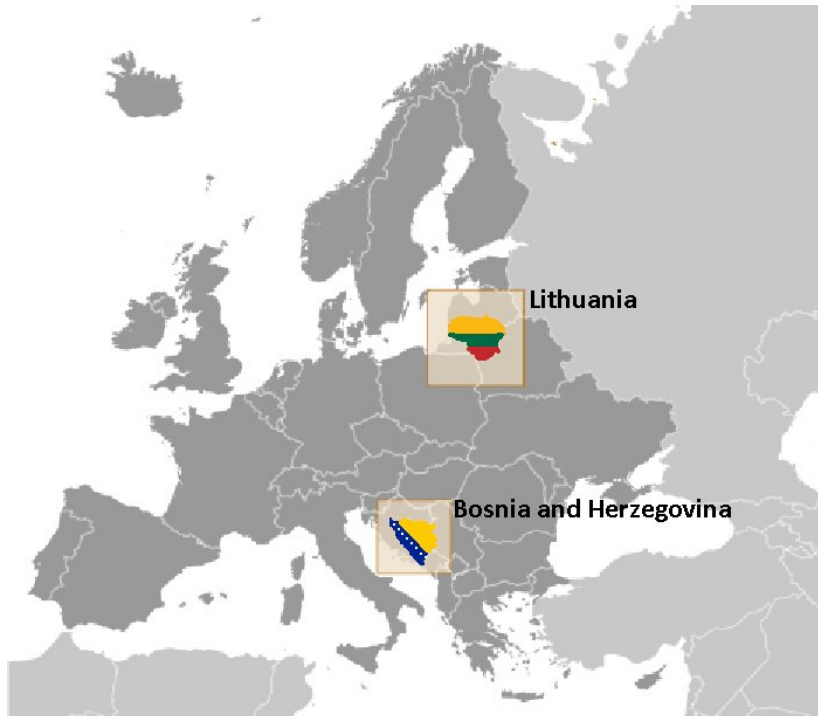
<sup>3</sup>Agency for Statistics of Bosnia and Herzegovina, Census 2013 (Jukić, 2016).

<sup>4</sup>Human development index, source: United Nations Development Programme.

<sup>5</sup>Bertelsmann Stiftung's Transformation Index (Stiftung, 2018a, 2018b).

<sup>6</sup>*Dfb* - humid continental climate, *Cfa, Cfb* - temperate warm and humid climates, *Csa* - mediterranean climate and *ET* - tundra climate.

<sup>7</sup>Global Climate Risk Index (Eckstein *et al.*, 2018).



**Figure 1:** Locations of the Bosnia and Herzegovina and Lithuania with in the Europe

Humid climate prevails in both countries. In Lithuania, it is mostly humid continental climate (*Dfb*), while in B&H in addition to *Dfb*, temperate warm and humid climates (*Cfa*, *Cfb*) and mediterranean climate (Giugliano *et al.*) are also present.

The Global Climate Risk Index 2018 (CRI) analyses to what extent countries have been affected by the impacts of weather-related loss events (storms, floods, heat waves etc.). Less developed countries are generally more affected than industrialized countries (Eckstein *et al.*, 2018). The most recent data available from 1997 to 2016, places B&H at 69<sup>th</sup> place, and Lithuania at 136<sup>th</sup>.

Monthly climatic data, including mean maximum and minimum air temperature ( $^{\circ}\text{C}$ ), minimum and maximum relative humidity (%), wind speed ( $\text{m s}^{-1}$ ) and sunshine hours (h) from 2 humid weather stations (WS), Kaunas in Lithuania and Sarajevo in B&H, for the period 1988 - 2017 (30 years) were obtained from state weather services. Before use, the data quality was checked with quality-control procedures recommended by Allen *et al.* (1998).

Reference evapotranspiration ( $ET_0$ ) was calculated using standard FAO-PM equation (Eq. 1) that is closely resembling the evapotranspiration of an extension surface of green grass of uniform height (0.12 m), actively growing

with enough water, with a fixed surface resistance ( $70 \text{ s m}^{-1}$ ) and an albedo of 0.23, given by (Allen *et al.*, 1998):

$$ET_0 = \frac{0.408\Delta(R_n - G) + \gamma \frac{900}{T_{mean} + 273} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34u_2)} \quad (1)$$

where  $ET_0$  is the reference evapotranspiration ( $\text{mm day}^{-1}$ ),  $R_n$  the net radiation at the crop surface ( $\text{MJ m}^{-2} \text{ day}^{-1}$ ),  $G$  the soil heat flux density ( $\text{MJ m}^{-2} \text{ day}^{-1}$ ),  $T_{mean}$  the mean daily air temperature at 2 m height ( $^{\circ}\text{C}$ ),  $u_2$  the wind speed at 2 m height ( $\text{m s}^{-1}$ ),  $e_s$  the saturation vapor pressure,  $e_a$  the actual vapor pressure,  $e_s - e_a$  the saturation vapor pressure deficit,  $\Delta$  the slope of the vapor pressure curve ( $\text{kPa } ^{\circ}\text{C}^{-1}$ ) and  $\gamma$  is the psychrometric constant ( $\text{kPa } ^{\circ}\text{C}^{-1}$ ).

All necessary parameters required for calculation of  $ET_0$  where computed following the procedure developed in FAO-56 (Allen *et al.*, 1998).

Since reflected solar radiation ( $R_s$ ) is required for  $R_n$  calculation and this parameter is not measured on WS in B&H, it was estimated from the measured sunshine hours data (The Campbell–Stokes sunshine recorder) with the Ångström (1924) equation:

$$R_s = \left( a_s + b_s \frac{n}{N} \right) R_a \quad (2)$$

where  $R_a$  is the extraterrestrial radiation ( $\text{MJ m}^{-2} \text{ day}^{-1}$ ) calculated for each day of the year and for different latitudes, from the solar constant ( $G_{sc} = 0.0820 \text{ MJ m}^{-2} \text{ min}^{-1}$ ), the solar declination ( $\delta$ ) and the time of the year ( $J$ ) and then by selecting the  $R_a$  for 15th day of each month converted to monthly values,  $n$  is the actual duration of sunshine (h),  $N$  is the maximum possible duration of sunshine or daylight hours (h), as is the regression constant, expressing the fraction of extraterrestrial radiation reaching the earth on overcast days ( $n = 0$ ) and  $a_s + b_s$  is the fraction of extraterrestrial radiation reaching the earth on clear days ( $n = N$ ).

In the absence of actual solar radiation ( $R_s$ ) measurements, the values  $a_s = 0.25$  and  $b_s = 0.5$  were used as suggested by Allen *et al.* (1998).

For the Kaunas WS measured solar radiation data ( $R_s$ ) or sunshine hours data were missing, so solar radiation was estimated using Hargreaves' formula (Hargreaves & Samani, 1985) (Eq. 3), as suggested in Allen *et al.* (1998):

$$R_s = k_{R_s} \sqrt{(T_{max} - T_{min})} \times R_a \quad (3)$$

where  $R_a$  is extra-terrestrial radiation ( $\text{MJ m}^{-2} \text{ d}^{-1}$ ),  $T_{max}$  maximum air temperature ( $^{\circ}\text{C}$ ),  $T_{min}$  minimum air temperature ( $^{\circ}\text{C}$ ),  $k_{R_s}$  adjustment coefficient (0.16 .. 0.19) ( $^{\circ}\text{C}^{-0.5}$ ).

In the case of Kaunas, since it is located inland ("interior" location) where land mass dominates and air masses are not strongly influenced by a large water body, value of  $k_{R_s} = 0.16$  was used (Čadro S. *et al.*, 2017).

Monthly water balance was calculated as described in Dingman (2002). Except data on monthly precipitation ( $P$ ) and evapotranspiration ( $ET_0$ ) applied water balance requires data on soil available water content ( $SOIL_{max}$ ). The value  $SOIL_{max} = 100$  mm was used (Mcbean *et al.*, 1995) since this is the most commonly used value for the types of soil that are found on the study locations.

To detect the trends within time series of water balance components (annual precipitation, reference evapotranspiration, actual evapotranspiration, soil moisture deficit, total runoff and snow) parametric method of linear regression was used, as shown in equation (4):

$$y = a + b \times x \quad (4)$$

where  $x$  is the explanatory variable,  $y$  the dependent variable,  $b$  the slope of the line and  $a$  the intercept.

The slope indicates the mean temporal change of the studied variable. Positive values of the slope show increasing trends, while negative values of the slope indicate decreasing trends (Gocic & Trajkovic, 2013, 2014).

## RESULTS AND DISCUSSION

Descriptive statistics (mean, standard deviation – SD, coefficient of variation – CV) for the air temperature ( $T$ ), precipitation ( $P$ ),  $ET_0$  and via water balance calculated actual evapotranspiration ( $AET$ ), soil moisture deficit ( $SMD$ ), total runoff ( $TRO$ ) and snow for the 2 WS for the period 1988 – 2017 are summarized in Table 2.

**Table 2:** Annual statistic for the climate and water balance components in Kaunas and Sarajevo WS during the period 1988-2017.

Climate and water balance components	Kaunas, Lithuania			Sarajevo, B&H		
	Mean	SD	CV	Mean	SD	CV
Air temperature ( $T$ )	7.27	0.81	11.19	10.41	0.69	6.61
Precipitation ( $P$ )	645	85.50	13.25	947	156.51	16.52
Reference $ET$ ( $ET_0$ )	636	25.40	3.99	805	45.17	5.61
Actual $ET$ ( $AET$ )	503	56.63	11.25	653	54.32	8.31
Total runoff ( $TRO$ )	141	44.50	31.49	295	86.78	29.47
Soil m. deficit ( $SMD$ )	133	62.06	46.63	151	85.69	56.70
$SNOW$	179	39.55	22.10	193	99.11	51.33

The averages of all analyzed and calculated climate and water balance characteristics are higher in Sarajevo than in Kaunas. The mean annual air temperature is 7.27 °C and 10.41 °C for Kaunas and Sarajevo, respectively. Annual sum of precipitation is for 302 mm higher in Sarajevo. These climatic differences have also caused the differences between water balance components. Mean  $ET_0$  for Kaunas is slightly lower than the annual sum of precipitation, while in Sarajevo this difference is much higher (142 mm). In Sarajevo, a big part of precipitation cannot be lost to the atmosphere by evapotranspiration and it

is retained by the soil or it appears in form of surface or subsurface runoff. Thus, total runoff (*TRO*) is much higher in Sarajevo than in Kaunas, 295 mm compared to 141 mm. *SMD* and amount of snow have similar values between analyzed locations. The highest coefficient of variation (*CV*) is for *SMD* ranging from 46.63 % in Kaunas to 56.70 % in Sarajevo. These high *SMD* variations are in line with many previous studies of water scarcity and high sensitivity of this locations to climatic extremes (Čustović & Vlahinić, 2004; Žurovec *et al.*, 2011; Hodžić *et al.*, 2013; Taparauskiene & Lukševičiute, 2015; Taparauskienė *et al.*, 2015; Radusin *et al.*, 2016; Čadro Sabrija *et al.*, 2017). Especially, this applies to agriculture production that depends on the soil moisture (Žurovec & Čadro, 2015; Miseckaite *et al.*, 2018). High variations were also obtained for total runoff (*TRO*) and snow ranging from 29.47 to 31.49 % and 22.10 to 51.33 % for Kaunas and Sarajevo, respectively.

Results of the regression analysis for annual air temperature (*T*), precipitation (*P*), *ET<sub>0</sub>* and via water balance calculated actual evapotranspiration (*AET*), soil moisture deficit (*SMD*), total runoff (*TRO*) and snow for the Kaunas and Sarajevo WS for the period 1988 – 2017 are presented in Table 3 and Fig. 2.

**Table 3.** Results for the statistical tests for the annual climate and water balance components in Kaunas and Sarajevo WS during the period 1988-2017.

Climate and water balance components	Kaunas, Lithuania				Sarajevo, B&H			
	P-value	R	R <sup>2</sup>	b	P-value	R	R <sup>2</sup>	b
<i>T</i>	0.001	0.562	0.316	0.052	0.000	0.607	0.368	0.047
<i>P</i>	0.044	0.369	0.136	3.582	0.610	0.097	0.009	1.724
<i>ET<sub>0</sub></i>	0.003	0.521	0.271	1.503	0.130	0.282	0.079	1.450
<i>AET</i>	0.003	0.521	0.272	3.355	0.409	0.156	0.024	0.965
<i>TRO</i>	0.617	0.094	0.009	-0.480	0.793	0.049	0.002	0.492
<i>SMD</i>	0.161	0.262	0.069	-1.851	0.794	0.050	0.002	0.485
<i>SNOW</i>	0.008	0.471	0.222	-2.114	0.759	0.058	0.003	-0.656

The results clearly show increasing trends in *T*, *P*, *ET<sub>0</sub>* and *AET* series and decreasing trend in the amount of the snow. The increasing trends of the air temperature (*T*) are similar for Kaunas and Sarajevo WS, ranging from 0.052 °C to 0.047 °C. Increasing trends were also detected for the annual amount of precipitation, ranging from 3.582 mm year<sup>-1</sup> in Kaunas to 1.724 mm year<sup>-1</sup> in Sarajevo. Annual sum of precipitation has increased by more than 100 mm in Kaunas for a period of 30 years (1988-2017). The observed air temperature and precipitation change patterns in Lithuania and B&H are consistent with the predominant trends in other areas of East Europe (Bukantis A. & Rimkus E., 2005; Bukantis Arūnas & Rimkus Egidijus, 2005; Jaagus *et al.*, 2009; Branković *et al.*, 2013; Burić *et al.*, 2013; Tripolskaja & Pirogovskaja, 2013; Unkasevic &

Tosic, 2013; Rutgersson *et al.*, 2014) and with trends observed globally (Kharin *et al.*, 2013; Trenberth *et al.*, 2013; Jacob *et al.*, 2018; Popov *et al.*, 2018).

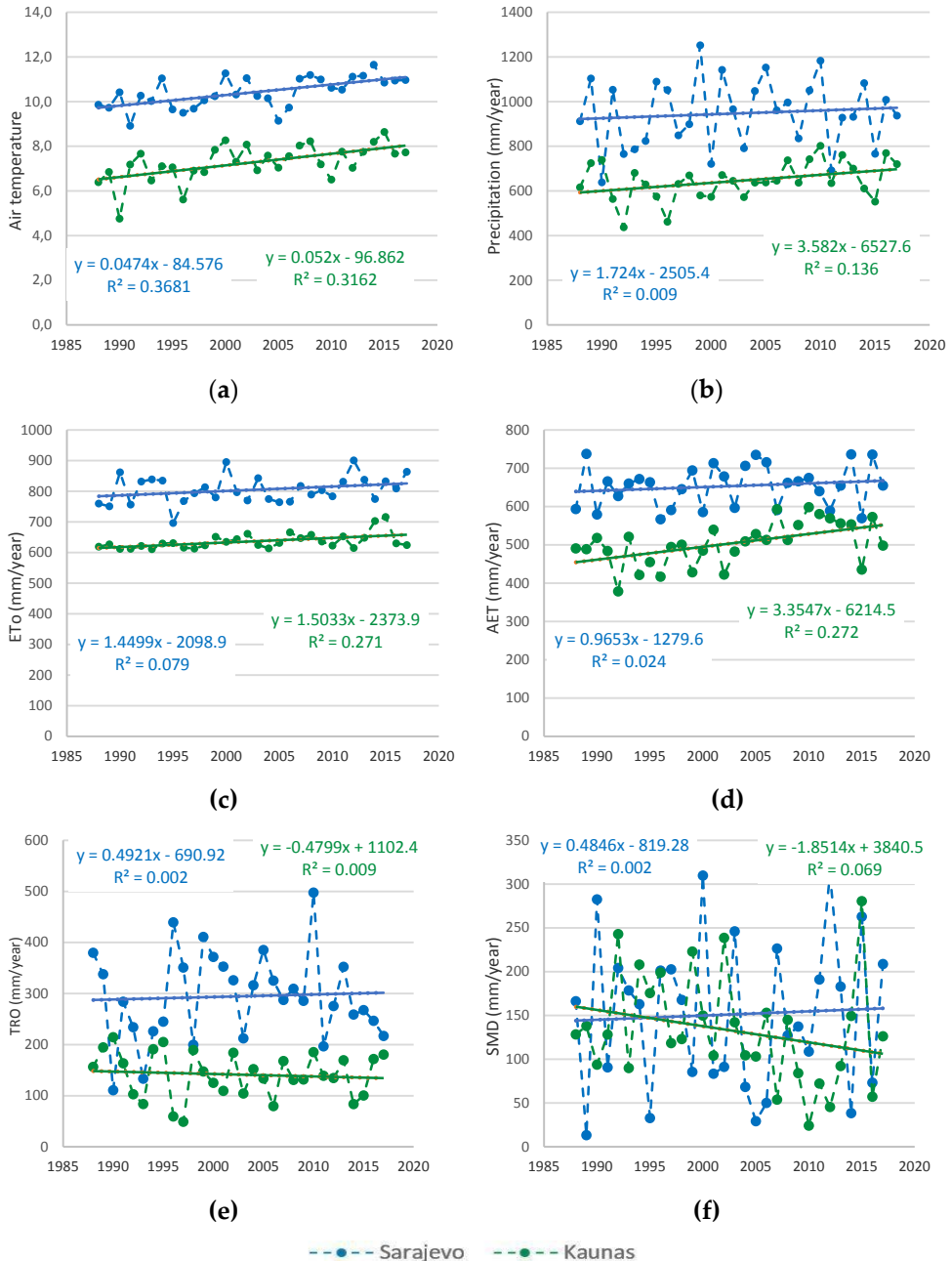
The annual increasing  $ET_0$  trends between Kaunas and Sarajevo are similar, they ranged from  $1.503 \text{ mm year}^{-1}$  to  $1.450 \text{ mm year}^{-1}$ . However, the annual increasing trend of  $AET$  has a lot higher rate in Kaunas than Sarajevo. The magnitude of increasing trends of annual  $AET$  varied from  $3.355 \text{ mm year}^{-1}$  in Kaunas to  $0.965 \text{ mm year}^{-1}$  in Sarajevo. Since  $AET$  can be considered as the measure of agricultural water productivity, obtained results indicate an improvement of general conditions for agricultural production. This is especially true for the study location in Lithuania (Kaunas). This indicates that the consequences of climate change are predominantly, but not exclusively negative and dependent on geographical location. Climate change may have a positive effect on the yield and quality of winter crops due to the extended growing period in northern Europe (Radusin *et al.*, 2016; Jacob *et al.*, 2018). However, the rest of Europe, especially the Mediterranean region, will mostly be negatively affected (Behrens *et al.*, 2010).

A negative trend in  $TRO$  was found for Kaunas, which decreased by  $0.480 \text{ mm}$  each year, and positive in Sarajevo, with an increase of  $0.492 \text{ mm}$  each year. Furthermore, there is a decreasing trend in the amount of snow in both locations, from  $-2.114 \text{ mm year}^{-1}$  to  $0.656 \text{ mm year}^{-1}$ . Such runoff and snow amount trends have already been recorded in Lithuania (Stonevičius *et al.*, 2014) and they are results of changes in influencing climatic elements - temperature and precipitation (Bukantis Arūnas & Rimkus Egidijus, 2005).

Soil moisture deficit occurs when the demand for water ( $ET_0$ ) exceeds that which is actually available from the precipitation ( $P$ ) or reserved in the soil (Žurovec & Čadro, 2015). In terms of  $SMD$ , two analyzed locations differ. The increasing trend in the  $SMD$  was found for Sarajevo ( $0.485 \text{ mm year}^{-1}$ ) and decreasing for Kaunas ( $-2.114 \text{ mm year}^{-1}$ ). Presence of positive trend in  $SMD$  causing more severe long-lasting droughts and yield reduction was found all over B&H (Vlahinić, 2000; Alagić, 2003; Žurovec & Čadro, 2010; Čadro Sabrija *et al.*, 2017).

Linear trends, correlation coefficient ( $R$ ) and coefficient of determination ( $R^2$ ) of annual air temperature ( $T$ ), precipitation ( $P$ ), reference evapotranspiration ( $ET_0$ ), actual evapotranspiration ( $AET$ ), soil moisture deficit ( $SMD$ ) and total runoff ( $TRO$ ) for the both analyzed locations are presented in Table 3. and Figure 2. The values of  $R$  and  $R^2$  for all analyzed parameters are generally lower for Sarajevo than Kaunas. This is the result of much higher variations in the annual values of all the obtained parameters in Sarajevo. Variations can be clearly seen in Figure 2, or by comparing the standard deviation ( $SD$ ) and coefficient of variation ( $CV$ ) between these two locations (Table 2). The annual amount of precipitation,  $TRO$ ,  $SMD$  and snow showed the highest variations. Such variations indicate extreme weather conditions, shifting between years with extremely high levels of precipitation, causing soil erosion, landslides and floods, and years with low precipitation causing prolonged droughts and serious yield declines. Agriculture is one of the most important socioeconomic sectors in B&H, but at same time a sector mostly affected by climate change (Žurovec *et al.*, 2015).





**Figure 2:** Linear trends, correlation coefficient ( $R$ ) and coefficient of determination ( $R^2$ ) of annual air temperature (a), precipitation (b), reference evapotranspiration (c), actual evapotranspiration (d), soil moisture deficit (e), and total runoff (f) are presented for the Kaunas and Sarajevo weather stations for the period 1988 – 2017.

There is an obvious need for planning and implementation of appropriate measures of adaptation to climate change (Zurovec *et al.*, 2017; Assan *et al.*, 2018). In the first place developing of the appropriate irrigation systems should be a preferred option, as well as the development and introduction of varieties resistant to dry climate conditions. Irrigation will certainly be one of the key mechanisms for adaptation. However, flood protection and drainage of excess waters from the plot, and in general regulation of water and air regime is a matter of priority for further development of the agricultural sector (Radusin *et al.*, 2016).

### CONCLUSIONS

Linear regression was applied to analyze annual trends in the air temperature, precipitation, FAO-56 PM reference evapotranspiration and water balance components (actual evapotranspiration, total runoff, soil moisture deficit and amount of snow). Monthly weather data from two humid weather stations, Kaunas (Lithuania) and Sarajevo (Bosnia and Herzegovina), for the time period of 30 years (1988 – 2017) were used. The main purpose was to determine and compare the severity of changes in mean annual water balance components for two humid climatic zones.

Results indicated that climate change differently affects the water balance of these two humid areas. Increasing trends in  $T$ ,  $P$ ,  $ET_0$  and  $AET$  series and decreasing trend in the amount of the snow were found. Both locations are showing a positive trend of reference evapotranspiration, with an increase of  $1.450 \text{ mm year}^{-1}$  to  $1.503 \text{ mm year}^{-1}$ . Increasing trends of annual  $AET$  varied from  $3.355 \text{ mm year}^{-1}$  in Kaunas to  $0.965 \text{ mm year}^{-1}$  in Sarajevo. However, total runoff and soil moisture deficit are decreasing in Kaunas ( $-0.480 \text{ mm}$  and  $-2.114 \text{ mm year}^{-1}$ , respectively) and increasing in Sarajevo ( $0.492 \text{ mm}$  and  $0.485 \text{ mm year}^{-1}$ , respectively). In general, results showed an increase in irrigation water demand for agricultural crops in Sarajevo (Bosnia and Herzegovina) and decrease in Kaunas (Lithuania).

Compared to Lithuania, location in B&H is highly vulnerable to climate change. High sensitivity is the result of large variations and positive trends of almost all analyzed parameters. The annual amount of precipitation, total runoff, soil moisture deficit and snow showed the highest variations (16.54 – 56.70 %). In comparison, same parameters for the area of Lithuania (Kaunas), indicate that climate change may have some positive effects on general conditions for agricultural production, such as increase of precipitation and actual evapotranspiration, and the decline in soil moisture deficit.

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## ALKALI AND ALKALINE EARTH METALS IN WATER – CASE STUDY OF THE BOJANA RIVER, MONTENEGRO

### SUMMARY

1) Background: Water is one of the most important natural resources as it is widely used in households, agriculture, industry and tourism. Therefore water conservation should be a basic human need. The goal of this investigation was to determine the content of alkali (*Na*, *K*) and alkaline earth metals (*Mg*, *Ca*, *Ba*, *Sr*) in real water samples from the Bojana River, Montenegro;

2) Methods: Samples were collected two locations: **L1** - where Bojana River forms a small delta, and **L2** - where it flows into the Adriatic Sea, and tested in August 2017. The contents of metals in samples were determined by inductively coupled plasma with optical emission spectrometry (ICP-OES);

3) Results: The concentrations of alkali and alkaline earth elements were higher in the sample **L2** when compared to the sample **L1**. Statistically significant differences were observed for contents of *Na* and *K* ( $p < 0.01$ ) as well as for *Mg*, *Sr* and *Ba* ( $p < 0.05$ ). As all alkali metal salts are well dissolved in water and due to the presence of a large amount of minerals in the sea, the obtained results for **L2** sample were expected. Due to the high popularity of tourist island – Ada Bojana, the preservation of the quality of the Bojana River is prerequisite.

**Keywords:** alkali metals; alkaline earth metals; ICP-OES analysis; the Bojana River; Montenegro

### INTRODUCTION

Water is one of the most important natural resources. It is an essential substance for life and also is considered as a universal solvent capable of dissolving almost all solutes. Additionally, water is widely used in households, agriculture, industry and tourism, and therefore water conservation should be a basic human need. The chemical characteristics of natural waters are the reflection of the soils and rocks which have been in contact with water (Pantelić *et al.* 2017).

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Skadar Lake catchment area represents one of the richest freshwater areas in the World due to the specific natural factors (Radulović *et al.*, 2015). The Bojana River arises from Lake Skadar and it is about 40 km long (Petković and Sekulić, 2015). The total outflow of water from the lake through the Bojana River is nearly 304 m<sup>3</sup>/s. After the Nile and the Po, the Bojana River is the third river on the Mediterranean by its water amount brought into the sea. The most distinctive feature of the Bojana River is that the bottom of its riverbed is under the sea level in the length of 36 km. At some places the bottom of the riverbed is between two and five meters under the sea level. In its riverbed there is both salty sea water and fresh river water. Although the Bojana River has a big flow, sea water rushes deeply upwards at the bottom of the riverbed, due to the fact that sea water is of higher density. Also, today there are two river flows with the same direction, aiming towards the sea; one is a surface water flow, while the other is about 10 to 15 meters below the ground water flow which contributes to the peculiarity of the Bojana River. Approaching the sea, the river splits into two sleeves which then separately flow into the Adriatic Sea close to the town of Ulcinj. The Bojana River Delta is a unique ecosystem in Europe. As a result of the deposition of sediment carried by the Bojana River to the Adriatic Sea, after the Drim River altered course to flow into the Bojana River, Ada Bojana island was created. This island, with about 5 km<sup>2</sup> of surface area, is situated in the Bojana River Delta, and now is one of the most important tourist resources of Montenegro (Petković and Sekulić, 2015).

The quality and quantity of the chemical elements in surface waters is affected by geo-chemical structure of surrounding area, land use, seasonal variations of weather conditions, vegetation and the atmospheric deposit (Potasznik and Szymczyk, 2015). By the extensive search of the literature no data on the chemical composition of the Bojana River was found.

In that respect, the aim of this study was to determine the content of alkali (*Na*, *K*) and alkaline earth metals (*Mg*, *Ca*, *Ba*, *Sr*) in real water samples from the Bojana River, Montenegro.

## MATERIAL AND METHODS

Water samples from the Bojana River, Montenegro, were taken at two locations; where Bojana River forms a small delta (L1), and - where it flows into the Adriatic Sea (L2), Figure 1. Samples were collected and tested in August 2017. The contents of metals (*Na*, *K*, *Mg*, *Ca*, *Sr* and *Ba*) in samples were determined by inductively coupled plasma with optical emission spectrometry (ICP-OES) as described in our previous work (Kostic *et al.* 2016).

For the analysis samples were collected in the glass flasks of 1 L which were previously washed with HNO<sub>3</sub> (1:1, v/v) and then thoroughly rinsed with ultra-pure water. For the purposes of element stabilization and reduction of the adsorption of metals on the glass surface, 1 mL of 65 % nitric acid was added on 1 dm<sup>3</sup> of water. The samples were stored in the refrigerator. The digestion was

performed on the Advanced Microwave Digestion System (ETHOS 1, Milestone, Italy) using HPR-1000/10S high pressure segmented rotor. ICP-OES analysis was performed using Thermo Scientific iCAP 6500 Duo ICP (Thermo Fisher Scientific, Cambridge, United Kingdom) spectrometer equipped with RACID86 Charge Injector Device (CID) detector, concentric type nebulizer, quartz torch and alumina injector. EPA Method 200.7 was applied (Cassap, 2010).

Total Hardness (TH) was calculated as described in Milojković *et al.* (2018):

$$TH \approx [Ca] + [Mg]$$

$$TH \text{ in } ^\circ d = 0.1339 \times [Ca \text{ in mg/L}] + 0.2307 \times [Mg \text{ in mg/L}]$$

The results of the study are the averages of triplicate measurements and are presented as means value and standard deviation (SD). The results obtained during the study were analyzed statistically, by the method of analysis of variance (F test) for single-factorial trials, and the significance of the differences between treatments was also tested by the LSD test at the same levels of significance (Stanković *et al.* 1989).



Figure 1. Study area

## RESULTS AND DISCUSSION

Water from the Bojana River was studied at two locations; one where it forms a small delta, and another one in the vicinity of tourist island – Ada Bojana where the river flows into the Adriatic Sea the obtained results are compared.

Elements that are dominant in water are alkali and alkaline earth metals (*Li, Na, K, Mg, Ca, Ba, Sr*). Their presence in the water is mainly attributed to natural processes (Kostić *et al.* 2016). Table 1 presents a literature overview of the contents of selected alkali and alkaline earth metals in different water sources.

**Table 1.** Content of selected alkali and alkaline earth metals (mg/L) in natural fresh waters

<i>Na</i>	<i>K</i>	<i>Mg</i>	<i>Ca</i>	<i>Sr</i>	<i>Ba</i>	Water source
1.4	1.2	7.2	40.7			The Reine River (Alps, Swiss) <sup>1</sup>
14.6	2.9	19.7	60.6	0.26	0.036	The Dunav River, Serbia <sup>2</sup>
3.3-4.3	0.9-1.8	4.3-7.2	25-62.5			The Božićka river, Serbia <sup>3</sup>
		10.9	56.1			The Symsarna River, Poland <sup>4</sup>
19.6	1.2	26.6	81.96	0.24	0.075	Well water <sup>2</sup>
				0.002-0.238	0.004-0.073	River water <sup>5</sup>
10500	380	1350	400	8.00	0.002-0.015	Sea/Ocean water <sup>1,5</sup>

<sup>1</sup><http://www.waterencyclopedia.com/En-Ge/Fresh-Water-Natural-Composition-of.html>;

<sup>2</sup>Kostić *et al.* 2016; <sup>3</sup>Presburger Ulniković *et al.* 2016; <sup>4</sup>Potasznik and Szymczyk, 2015; <sup>5</sup>Kabata-Pendias and Szeke, 2015.

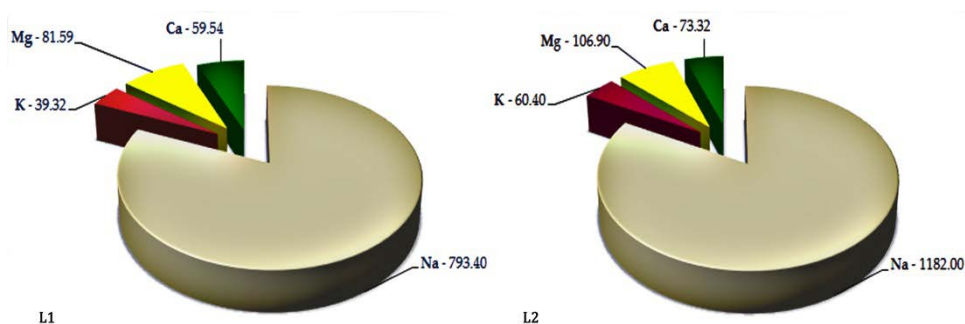
Concentrations of *Na* and *K* are nearly equal in the Earth's crust, but in natural waters *Na* is far more abundant compared to *K*, especially in sea water (Table 1). Potassium is an important element for aquatic animal and plant species, although the least abundant of four major elements ( $Ca > Mg > Na > K$ ) in natural waters. Nevertheless, the order of concentrations of major elements in average sea water is quite different,  $Na > Mg > Ca \approx K$ . It is reported in the literature that presence of *K* ions in some lake water is positively related to biological productivity (Talling, 2010).

In this study, *Na* and *Ba* were measured in the highest and lowest concentrations; 1182.00/0.011 mg/L in **L2** and **L1**, respectively (Figure 2). In general concentrations of *Na* and *K* in studied samples were much higher than reported in literature for river water (Table 1). Their content was significantly different between the samples ( $p < 0.01$ ), and higher concentrations of both elements were recorded in the sample **L2** originated from the location in the vicinity of Ada Bojana island (Figure 2, Table 1). As all alkali metal salts are

well dissolved in water and due to the presence of a large amount of minerals in the sea, the obtained values in **L2** sample could be expected.

Magnesium and calcium are naturally found in surface waters (Table 1), and they are the most widely available alkaline earth metals in the environment. Sedimentary rocks, usually limestone and chalk are the most common sources of *Ca* and *Mg* (WHO, 2011). Magnesium salts could be found naturally in high concentrations in surface and underground waters. The only other elements that occur on a larger scale are *Na* and *Ca*. Calcium compounds occur naturally in surface water, and their concentrations are mainly determined by the carbonate balance (Potasznik and Szymczyk, 2015).

Concentrations of *Mg* and *Ca* were higher in the sample **L2** (Figure 2), taken on the location where the Bojana River flows into the Adriatic Sea. Statistically significant difference between samples was observed only for the content of *Mg* ( $p < 0.05$ ). Having in mind that *Ca* and *Mg* are important nutrients for aquatic plants, the most effective *Ca/Mg* ratio should be 3:1 to 4:1 (Potasznik and Szymczyk, 2015). In the studied samples the ratio of *Ca/Mg* was much lower; 0.73:1 and 0.69:1 for **L1** and **L2**, respectively. Such ratios were attribute to higher concentrations of *Mg* in both water samples, 81.59 mg/L (**L1**) and 106.90 (**L2**) mg/L compared to concentrations of *Ca*, 59.54 mg/L (**L1**) and 73.72 mg/L (**L2**), which is specific for sea water (see Table 1).

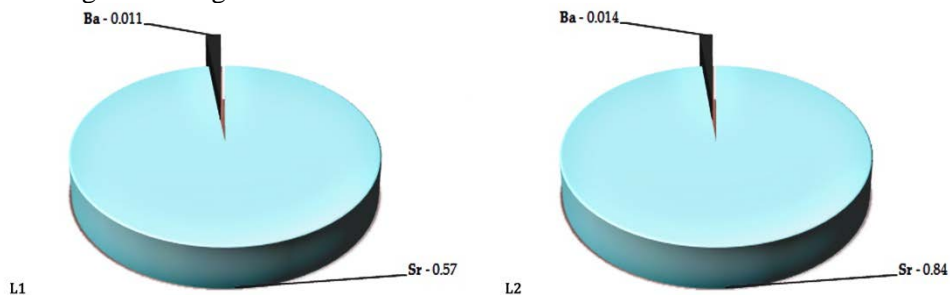


**Figure 2.** The content of major elements (mg/L) in water samples **L1** and **L2**

Total hardness is usually defined as the molar concentrations of all multi-valent cations in water with the exception of the monovalent cations. Calcium and magnesium are predominant cations, although other cations such as aluminum, barium, iron, manganese, strontium, and zinc also contribute. Nevertheless, in practice, the sum of two major fresh-water cations (*Ca* and *Mg*) usually determines TH (WHO, 2009).

In addition to performed analyses, total hardness (TH) of the Bojana River water was calculated based on the *Ca* and *Mg* concentrations. In samples **L1** and **L2**, TH expressed as German hardness degrees ( $d^{\circ}$ ), was 26.79 and 35.53,

respectively. The dissolved polyvalent metallic ions from sediments, leakage and swelling from the ground could be the main natural sources of hardness in water.



**Figure 3.** The content of *Sr* and *Ba* (mg/L) in water samples **L1** and **L2**

The results obtained for *Sr* and *Ba* are shown in Figure 3. As expected, these elements were detected in the lowest concentrations in both samples; 0.57/0.84 mg/L of *Sr* and 0.011/0.014 mg/L of *Ba* in **L1** and **L2**, respectively. According to surface water regulations of Environmental Protection Agency (EPA, 2001) imperative values for *Ba* are in the range of 0.10–1.0 mg/L. Results obtained in this study indicated much lower concentrations of *Ba* in both samples. On the other hand, results in this study for *Sr* were higher, whilst results for *Ba* were in the line with literature data for river water presented in Table 1 (Kabata-Pendias and Szeke, 2015).

Statistical analysis of results indicated significant differences between samples for contents of *Na* and *K* ( $p < 0.01$ ) as well as for *Mg*, *Sr* and *Ba* ( $p < 0.05$ ). Concentrations of *Ca* were not significantly different between samples (Table 2).

**Table 2.** Statistical analysis of elements content in water samples

	F	LSD	
		0.05	0.01
<i>Na</i>	431,654**	80,477	185,634
<i>K</i>	128,129**	8,013	18,483
<i>Mg</i>	26,901*	20,996	48,432
<i>Ca</i>	13,903	15,901	36,679
<i>Sr</i>	57,957*	0,151	0,349
<i>Ba</i>	23,822*	0,003	0,007

\*Statistically significant difference \*-  $p < 0.05$  and \*\*-  $p < 0.01$ .

## CONCLUSIONS

Results have shown that the concentrations of alkali metals (*Na* and *K*) and alkaline earth elements (*Mg*, *Ca*, *Sr* and *Ba*) were significantly higher in water sample originated from the location where the Bojana River flows into the Adriatic Sea (near Ada Bojana island). In both samples concentrations of elements were in the following order:  $Na > Mg > Ca > K > Sr > Ba$  which is, especially in the case of major elements, characteristic of sea water. These chemical

composition characteristics are more pronounced in the sample taken on the location where the Bojana River immerse into the Adriatic Sea, as the water from the sea flows back to the river.

The chemical composition of studied samples showed that water has specific characteristics and confirms the duality of the Bojana River water, due to the fact that its riverbed is a few meters under the sea level. Indeed, with all its characteristics the Bojana river represents a natural phenomenon.

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## **FORMATION OF FERTILITY AND PRODUCTIVITY INDICES OF RECLAIMED SOILS UNDER CONDITIONS OF REGIONAL CLIMATE CHANGE OF THE SOUTH OF UKRAINE**

### **SUMMARY**

The paper summarizes the results of research on the dynamics of changes in climatic parameters (air temperature and rainfall) over the 70-year period (the end of the XX and the XXI centuries) in the agro-soil zones of south of Ukraine; the main factors of the negative influence of elevated temperatures on properties of soils are revealed; the optimal parameters of agro-technical measures for the reduction of the risk of insufficient soil moisture and increase of agricultural stability are substantiated.

The represents the methodological approaches and the results of the evaluation of integrated space-time modelling of the heterogeneity of changes in agrochemical properties of soils in the steppe zone (on the example of Kherson region of Ukraine) under conditions of regional climate change. The paper evaluates the changes of soil-climatic potential of agricultural lands depending on climatic conditions and suggests their comparative estimation by fertility; it determines the total agrochemical potential of dry-steppe soils and develops the gradation according to their ability to maintain steady yields of grain crops.

The paper simulates the changes in energy expenditure on soil formation during the development of irrigated agriculture.

The study develops the models and the maps of productive moisture content in soils for demanding and less demanding crops for the development and implementation of reclamation measures aimed to increase soil fertility of the dry-steppe zone.

The paper suggests the system of nature protection measures for management of agricultural lands considering the qualitative estimation of soils (including irrigated lands) to determine the changes in the potential of reclaimed soils, increase informational content and objectivity of management decisions concerning the development of land reclamation measures and optimization of agricultural land use under conditions of regional climate change.

**Keywords:** climate, soils, fertility, productivity, irrigation, yield.

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## INTRODUCTION

The sensitivity of agricultural productivity to climate has not been sufficiently quantified (Liang, 2017). When adapting agricultural activity to the conditions of global and regional climate change in the dry steppe zone of Ukraine for the characterization of soil potential and the design of agricultural crops, the regularities of forming the soil formation process, fertility and productivity of reclaimed soils are topical issues.

In the context of regional climate change, the work of many scientists in Ukraine is devoted to irrigation: V.V. Medvedev, S.A. Balyuk, M.I. Romashchenko, V.O. Ushkarenka, V.V. Gamayunova, A.O. Limar, F.M. Lisetskogo, O.V. Morozova, V.V. Morozova V.I. Pichura and others (Medvedev, 2014; Morozov, 2013). Increasingly there is a need to develop a management system for reclaimed agricultural land (primarily irrigated), taking into account changes in the main fertility and productivity indicators of soils.

The *purpose* of the study is to determine the patterns and characteristics of the formation of fertility and productivity indicators of the reclaimed soils of the dry-steppe zone of Ukraine in conditions of regional climate change (for example, Kherson region).

*Research objectives:*

- to carry out a retrospective analysis of climate change over the past 70 years and to develop a classification of years by climatic indicators for the dry steppe zone;
- to carry out the zoning of the land suitability of the Kherson region for the cultivation of crops with average annual content of productive moisture;
- to study the formation of levels of crop yields by climatic characteristics of years;
- to develop a spatial model of the current state of suitability and potential of land for agrochemical properties of soils for cultivating and designing the level of crop yields.

*Objective of study* - processes of spatial-temporal formation of fertility and productivity of reclaimed soils of dry-steppe zone in conditions of regional climate change. Subject of research are soil-climatic indicators of fertility and productivity of agricultural land.

## MATERIAL AND METHODS

We used standardization of parameters of agroclimatic conditions for growing of agricultural cultures by the method of Medvedev (2014) and normalization of parameters of soil fertility indices in relation to the cultivation of agricultural crops by the method of Karmanova (1980).

To develop a modern classification of years by climatic indicators, spatio-temporal modelling of crop yield formation, transformation of fertility of irrigated and non-irrigated soils, conducting ground-climatic grounding of ground potentials, a database of statistical data was created and analysed: the analysis of the dynamics of climatic indicators (for the period 1945-2015 years); the dynamics of irrigation areas, weighted average irrigation rates, the efficiency

of water use (volumes of water supply and water intake), the dynamics of yield of major crops on irrigated lands; agrochemical indicators of soil fertility.

### RESULTS AND DISCUSSION

According to the analysis of changes in the main climatic indicators; air temperature, amount of atmospheric precipitation (Fig. 1, 2), the cyclic component of the average annual air temperature is 8 years. The average authenticity of the settlement data is 94%. The results of forecasting determined that in the period 2017-2022, a gradual cyclical increase in the average annual temperature of air with an average intensity of 0.08 °C per year is expected. In relation to the amount of annual precipitation, there is a stable tendency to increase the serene-periodic value in accordance with the multi-year norm. According to the results of generalization of long-term climatic data, a classification is proposed on the availability of atmospheric precipitation and air temperature in the dry steppe zone of Ukraine (Table 1).

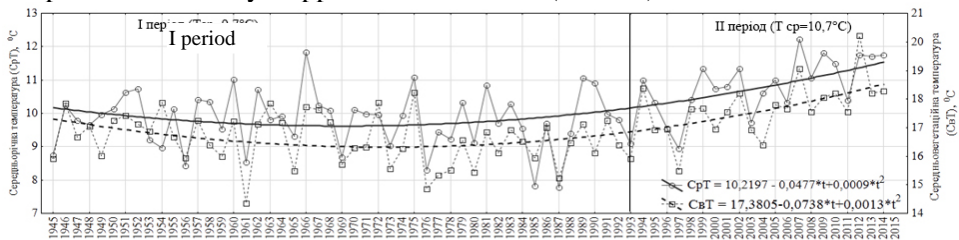


Figure 1. The long-term dynamics of air temperature (1945-2015), °C

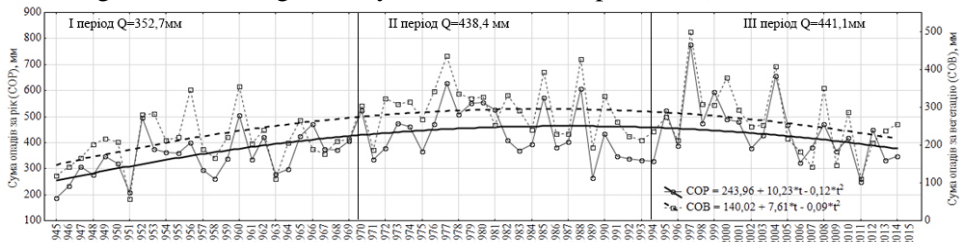


Figure 2. Long-term dynamics of atmospheric precipitation (1945-2015 years), mm

Table 1. Characteristics of years for the provision of atmospheric precipitation and air temperature in the dry steppe zone of Ukraine

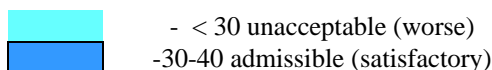
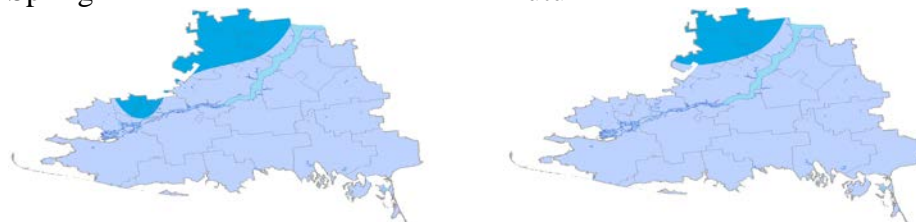
Characteristics of years on humidity	Atmospheric precipitation, mm		Characteristics of years by air temperature	Air temperature, 0C	
	multi-year rule 450 mm	during the growing season (long-term norm of 280 mm)		in a year	during the growing season
Dry	400	250	Cold	< 8,5-9,5	<15-16,0
Medium	401-499	251-309	Moderate	9,5–11,0	16,0-17,5
Moist	over 500	over 310	Warm	11,0-12,0>	17,5-18,0>

The zoning of the land suitability of the Kherson region by average annual content of productive moisture (Fig. 3) is proposed. As a result of the research, the zoning of land suitability for the cultivation of insignificant crops (winter wheat, spring barley) has also been developed, with an average annual content of productive moisture in a 0-20 cm layer.

Factors such as humus, nitrification nitrogen, exchangeable potassium, mobile phosphorus, exchangeable sodium, soil pH, amount of annual precipitation, average annual air temperature, water supply were used to form a winter wheat yield pattern on irrigated lands. In dry years (2007, 2011), the yield of winter wheat under irrigation in the region ranges from 1,9 to 4,79 t/ha.

Spring

Autumn



Class of land suitability	Area of arable land	
	%	mln.ha
<b>Spring</b>		
optimal conditions**	0	0
admissible (satisfactory)***	14,7	0,25
unacceptable (worse)****	85,3	1,45
Total	100	1,70
<b>Autumn</b>		
optimal conditions**	0	0
admissible (satisfactory)***	12,5	0,21
unacceptable (worse)****	87,5	1,49
Total	100	1,70

Areas of eligibility of arable land for the cultivation of demanding \* crops for the average annual content of productive moisture (layer 0-20 cm). \* - In demanding crops, the content of productive moisture include: corn for grain, sunflower, potatoes; \*\* - optimal conditions ensure implementation of adaptive potential of agricultural crops to the content of productive moisture; \*\*\* - acceptable conditions - reduction of potential yield by 20-30%; \*\*\*\* - Invalid conditions -30-50%.

Figure 3. Distribution of land suitability of the Kherson region for growing demanding crops on the criterion of average annual content of productive moisture in a layer 0-20 cm before sowing, mm.

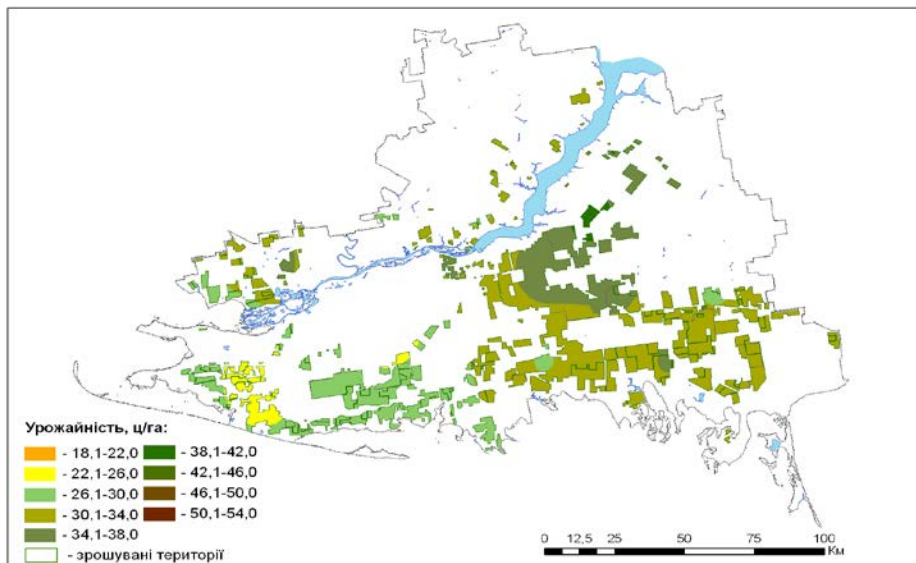
The multiple coefficient of correlation of the regression model ( $r=0,90$ ) indicates a close correlation between yield and investigated factors. The greatest influence in the dry years on the formation of winter wheat yields is the amount of precipitation, water supply and air temperature. Due to high dry air temperatures in irrigated lands, increased content of exchangeable sodium and changes in soil pH significantly reduce yields (Fig. 4).

In the middle years (2009, 2012), winter wheat yields in areas ranged from 2.15 to 3.76 t/ha ( $r=0,93$ ). In damp years (2008, 2010), the yield of winter wheat in the regions of the region varies from 2,47 to 4,72 t/ha ( $r=0,98$ ). In the formation of winter wheat yield in the presence of precipitation significantly increases the role of humus, nitrogen, potassium, phosphorus (Fig. 4).

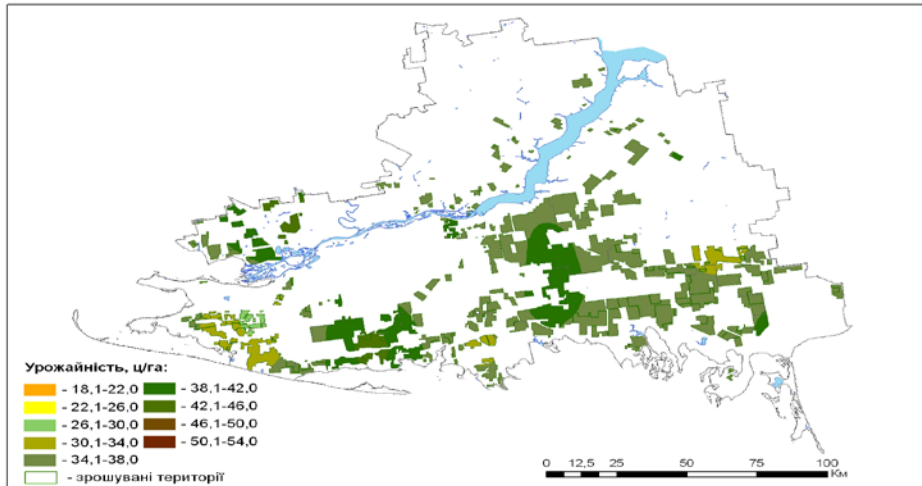
Long-term research has determined the probability of repeatability of years with weathering, which allows predicting the yield of winter wheat (Table 2).

Table 2. Probability of repetition of years on the availability of atmospheric precipitation

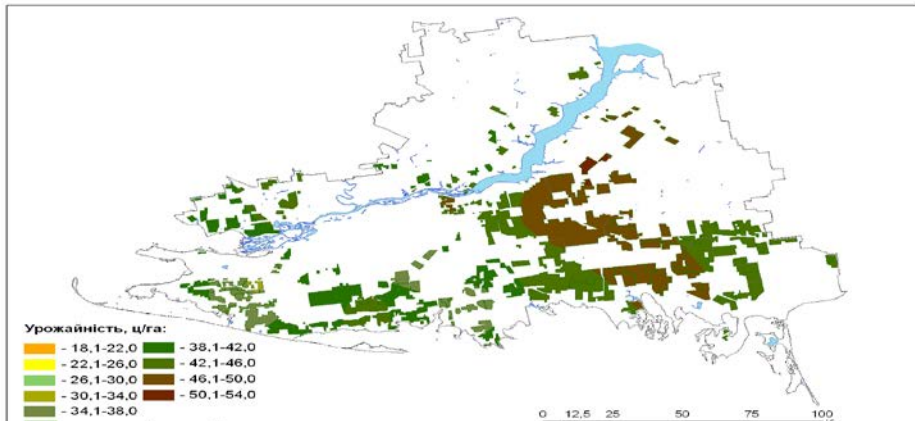
Characteristics of years on humidity	Probability of year in terms of humidity,%	Average yield of grain per region, t / ha	
		all the land	incl. irrigation
Dry	39	2,4	2,0-3,0
Medium	33	2,5	3,0-4,0
Moist	28	2,7	4,0-5,0



a) Dry years (annual rainfall up to 400 mm)



b) Mid-dry years (annual precipitation 401-499 mm)



c) Wet years (annual rainfall over 500 mm)

Figure 4. Cartogram of the yield of winter wheat grains on irrigated lands of the Kherson region, depending on the classification of years for the provision of atmospheric precipitation

A spatial analysis of the distribution of the region's lands according to a comprehensive assessment of the nutrient content has been carried out. It is determined that 75% of the lands located in the northwest and southeastern parts of the Kherson region have satisfactory, favorable and very favorable agrochemical conditions for the cultivation of grain crops, 25% of the land is predominantly in the southwestern part and the coastal zone of the Dnipro River, unsatisfactory (20,6%) and very unsatisfactory (4,4%) agrochemical properties of soils for cultivating grain crops (Figure 5).

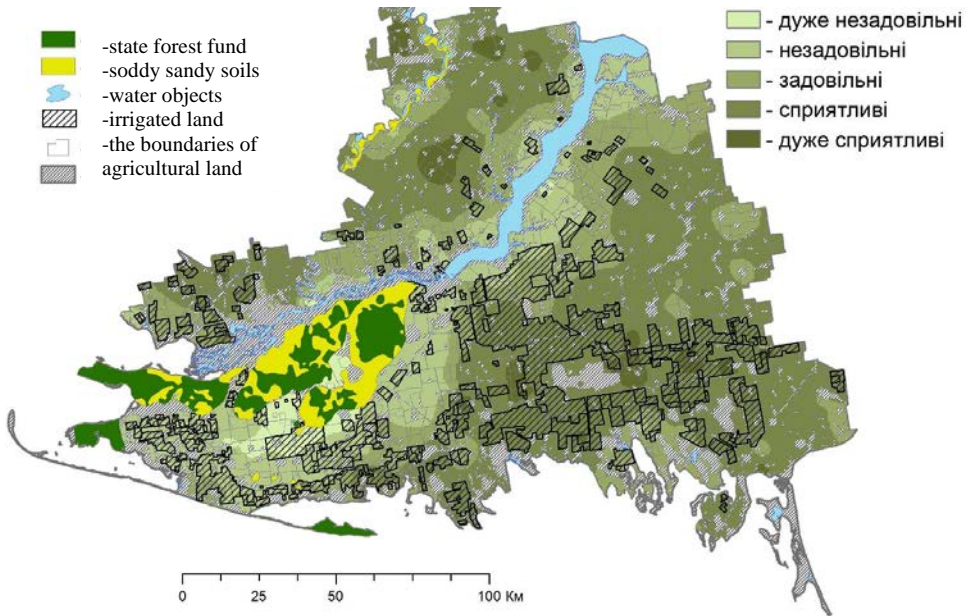


Figure 5. Agrochemical zoning of agricultural lands of the Kherson region on the suitability of cultivating grain crops

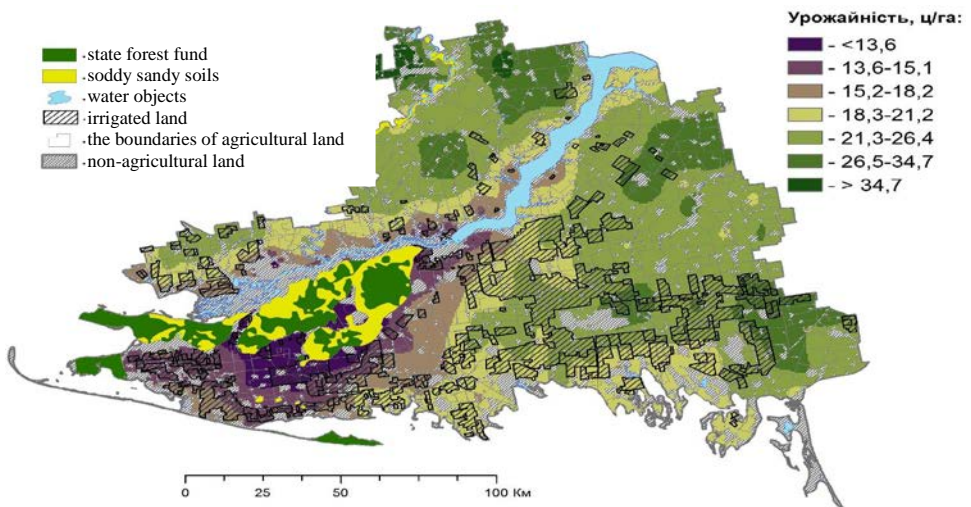


Figure 6. Cartogram of potential yield of grain crops depending on the content of humus (for example, Kherson region)

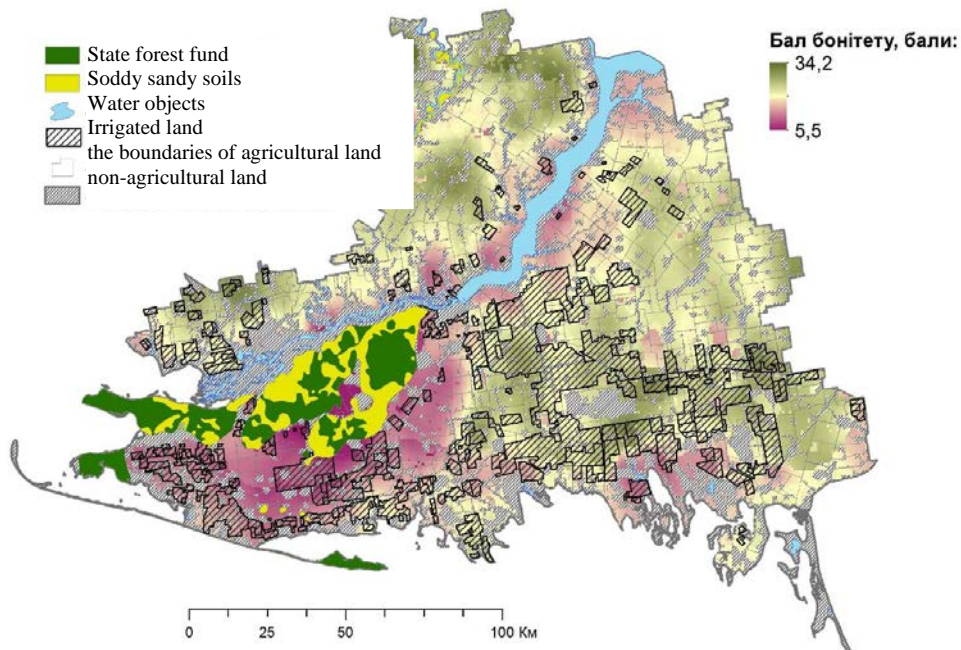


Figure 7. Soil-climatic quality of agricultural land for cultivating grain crops (according to Karmanova I.I.)

Accordingly, the classification proposed by Academician V.O. Ukhartan created a spatial model and determined the areas of formation of the design crop of grain crops depending on the content of humus. It was determined that 56,5% of the territory of the project crop yields is 1,8-2,6 t/ha; 29,77% within the limits of 1,3-1,8 t/ha and 13,74% -2,6-3,6 t/ha. The results of the research confirm that the land of the Kherson region is favorable for growing and obtaining stable yields of grain crops (Fig. 6).

As a result of the research, the ballooning point in the system of economic land valuation was determined and an energy assessment of the soil-forming process orientation under the conditions of regional climate change was carried out (Fig. 7).

The determined agitation of soils is a continuation of complex agrochemical research. As a result of GIS-modeling, calculation of Bonity points for growing of grain crops on agricultural lands of Kherson region was carried out. These data are typical for the soil and climatic conditions of southern Ukraine.

The correlation dependence of the grain crop yield on the irrigated ( $r = 0.81$ ) and non-irrigated lands ( $r = 0.88$ ) on the yield of grain crops was determined (Fig. 8). The methodology used in the bioenergy approach allows us to model scenarios of climatic influences (through heat and moisture provision),



expressed in energy equivalents, on the spatial and temporal trends of soil development. The correlation dependence of formation of irrigation regime on the amount of precipitation during the growing season is determined. An increase in rainfall contributes to a decrease in the weighted average irrigation rate.

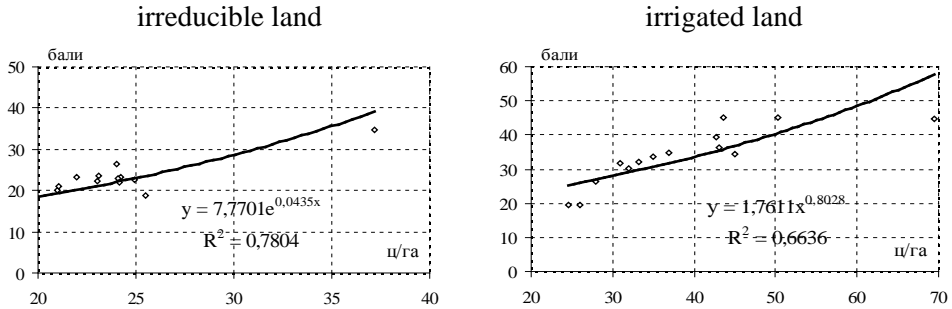
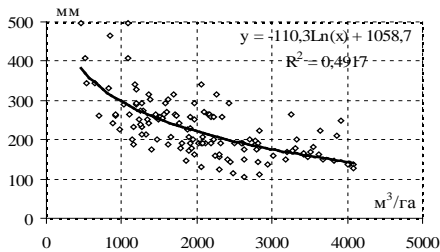
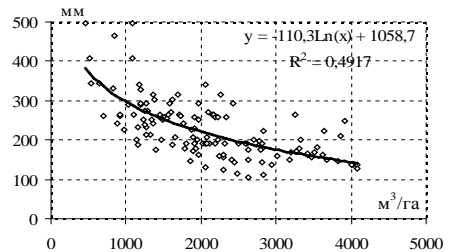


Figure 8. Dependence of the ground of the ground bonus on the soil-climatic potential of grain crop yields

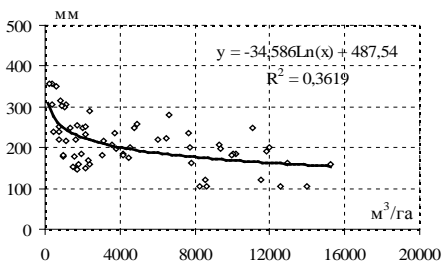
Kahovka irrigated array



Ingulets irrigated array



Krasnozamyansk Irrigation array



Right bank irrigated array

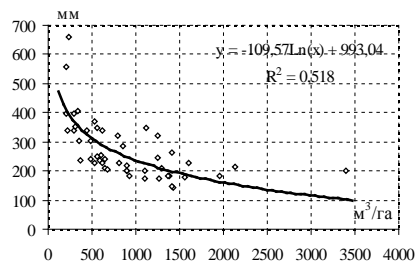


Figure 9. Dependence of the irrigation rate value on the amount of atmospheric precipitation during the growing season on the irrigated massifs of the Kherson region

## CONCLUSIONS

According to the results of the research, maps of the content of productive moisture in the soils of the Kherson region were created and the conditions of cultivation of agricultural crops were determined for the actual availability of productive moisture in the layer of soil 0-20 cm:

- For the cultivation of demanding crops: in the spring, in the western and north-western parts of the region, the average annual content of productive moisture is characterized as acceptable and covers an area of 0.25 million hectares (15% of the total area); in the direction to the southeast, the supply of available moisture is reduced and is characterized as inappropriate by an area of 1,45 million hectares (85%). Areas with optimal conditions for growing crops on the content of productive moisture in a layer of soil 0-20 cm - are absent.

- For the cultivation of insignificant crops: in the spring, in the western and north-western parts of the region, the average annual content of productive moisture is within optimal limits and covers an area of 0,25 million hectares (14,7% of the total area); To the southeast, the supply of available moisture is reduced and is characterized by a permissible area of 1,45 million hectares (85,3%). Areas with inadmissible parameters of cultivating crops on the content of productive moisture in a layer 0-20 cm-absent.

The potential yield of grain crops in agricultural land of Kherson region is determined. As a result of spatial modelling, it has been established that 56,5% of the land in the oblast can provide potential yield formation in the range of 1,8-2,6 t/ha; 29,77% within the limits of 1,3-1,8 t/ha and 13,74% -2,6-3,6 t/ha. According to agrochemical properties, agricultural lands of the region are quite favorable for growing and obtaining stable yields of grain crops.

It was established that the ground of the land for the cultivation of grain crops is in the range of 5,5-34,2. The highest potential is characterized by soils located in the central, central-eastern and north-western parts of the region with a ball of bonite 20.1-34.2, which occupy about 66% of the territory.

Investigations have determined the dependence of the irrigation rate on the amount of precipitation during the growing season on irrigated massifs of the region. The correlation dependence of formation of irrigation regime on the amount of precipitation during the growing season is determined. An increase in rainfall reduces the weighted average irrigation rate

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## ASSESSMENT OF OVERALL SUPPORT TO AGRICULTURE IN BOSNIA AND HERZEGOVINA

### SUMMARY

The previous analysis of support to agriculture in Bosnia and Herzegovina (BiH) implied only budgetary transfers without taking account of the support originating from market-price policy measures. The purpose of this paper was to provide, for the first time, an estimate of the total support to the agricultural sector in BiH as well as the level of protection of producers through the price of agricultural products. This estimate was done using relative support indicators (Ag incentives, Erjavec, OECD), Total transfers to producers (TTP), Nominal rate of protection (NRP) and Nominal rate of assistance (NRA). The computation of these indicators for Bosnia and Herzegovina was done using the original method (Erjavec et al, 2017) suitable for use in countries with underdeveloped agricultural statistics. Percental TTP represents the share of total budget and market-price support to agriculture in the total value of agricultural production, while NRP and NRA indicators show the level of protection of producers through domestic prices of agricultural products. The NRP is defined as the percentage ratio between domestic market price and the reference one, while the NRA represents the difference between gross revenue of agricultural producers calculated based on domestic prices and gross revenue calculated based on reference prices. In this paper, EU reference prices were used for comparison with BiH domestic prices. The results of the research have shown that there is a difference between BiH and EU reference prices indicating the significant protection of BiH agricultural producers which according to the NRP averages 9 %. On the other hand, the comparison of agricultural producers' revenues calculated based on BiH prices and revenues calculated based on EU reference prices shows a significantly lower total support – by approximately 4%. Finally, looking at the share of total transfers within the value of production (TTP), it can be seen that this indicator has almost the same trend as %NRA, which shows a more significant impact of market price transfer in comparison budget support.

**Keywords:** support, indicator, Bosnia and Herzegovina, reference price, domestic price

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## INTRODUCTION

By signing the General Framework Agreement for Peace in Bosnia and Herzegovina in late 1995 in Dayton, Bosnia and Herzegovina became a state with two Entities (Federation of BiH - FBiH and Republika Srpska – RS) and Brčko District of BiH (BD BiH). The complex administrative structure results in the agrarian policy being implemented at several levels. The highest state-level authorities of Bosnia and Herzegovina have limited power in the area of agrarian policy which is reduced to the foreign trade policy responsibilities. Strategic and program documents that are directly related to the programming and development of agricultural policy are the exclusive responsibility of the Entities and Brčko District. In addition to the complex state structure, there is the issue of lack of harmonization between the Entities, and each of the three territorial units has the authority to design, adopt and implement agricultural policy independently of other units, i.e. to implement their own agricultural policy, which ultimately leads to unequal and disadvantageous position of farmers. General assessment of agricultural policy in both BiH entities and Brčko District shows its inconsistency, inadequacy and absence of developed elements of modern public policies (Bajramović *et al.*, 2014, 2015).

Any public policy, including agricultural one, has to have an established policy cycle in order to be successfully implemented. The implementation of this cycle requires a strong analytical support, development of methods, constant monitoring of policy results, democratic discourse and discussion on problems, goals and measures of agricultural policy (OECD, 2010). Getting the effects of agricultural policy to be visible requires a continuous monitoring. Effectiveness of this process could be achieved only through the establishment of a data collection framework which will result in the indicators necessary for further research. The most commonly used method for monitoring the effects of agricultural policy is the one that measures the effect of the policy on the incentives to farmers and their ability to react to those incentives (Josling and Valdes, 2004). In order for these incentives to be comparable it is necessary to translate them into quantitative indicators with focus on prices. There are numerous indicators for monitoring the effects of the policy on the incentives through prices. The simplest and most commonly used is the Nominal Rate of Protection (NRP), which takes into account measures of market price policy and represents the percentage difference between domestic and world market prices. The more comprehensive indicator of the Nominal Rate of Assistance (NRA) implies all the agricultural policy measures that affect the prices of domestic products and is used to compute the level of protection of producers through price. It is defined as the percentage difference between farmer's gross income based on domestic prices relative to income at world prices. The previous analysis of agricultural policy in BiH relied on budgetary support to farmers (BP), without taking into account the market price support (MPS) as the second part of the overall support to agriculture. For this reason, the purpose of this paper is to provide, for the first time, an assessment of overall support to the

agricultural sector in BiH using the relative indicator TTP, and by computing the relative indicators NRP and NRA, to show the level of protection of producers through the price of agricultural products and thus provide a comprehensive insight into the agricultural policy of BiH.

### MATERIAL AND METHODS

With regard to the specification and systematization of policy that has impact on agriculture, there is no universal approach to be found in literature. Used in this paper, as an adequate method for assessing the overall agricultural policy, is the OECD PSE/CSE approach which is the standard global method and a sole database that regularly provides agricultural support information comparable for the entire observed period.

The Producer Support Estimate (PSE) is the most commonly used indicator for measuring overall support in agriculture since it has been recognized as a tool for comparing different agricultural policy measures. The OECD PSE approach divides measures into two main groups:

- Policy measures that affect domestic market prices MPS (Market Price Support) cause a gap between the domestic market price and the reference price of a particular product
- Budgetary support measures BP (Budgetary Transfers to Producers) may have a form of actual transfers to producers or a form of reduced budget revenues (e.g. tax incentives to agricultural producers)

Represented mathematically:

$$PSE = MPS + BP$$

Given that BiH, just like any other developing country, is faced with the problem of statistical data which do not ensure a sufficient level of reliability, for calculating indicators according to the OECD PSE / CSE approach<sup>2</sup> instead of MPS we used the nominal rate of protection %NRP to compute estimated rate of producer protection through prices, based on the following formula:

$$\%NRP_i = \frac{PP_i}{RP_i} * 100 - 100$$

$$\%NRPC = \frac{\sum PP_i * QP_i}{\sum RP_i * QP_i} * 100 - 100$$

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<sup>2</sup> Bosnia and Herzegovina does not have an Agricultural census as the basis for the establishment of the FADN system, the production-consumption balance-sheets of agricultural products, calculating agricultural economic accounts based on OECD methodology

*i* = individual product

*c* = aggregation level

% NRP = Nominal Rate of Protection

PP = Producer price

RP = Reference price

QP = volume of production

The quantitative estimate of price protection is based on the computation of the percentage ratio between domestic producer's price and reference price for selected product groups caused by measures such as direct measures within the sector or specific interventions by products (e.g. Prices or market regulation, import/export taxes or subsidies), disturbances resulting from macro-economic policies (e.g. Currency exchange rate), interventions in other sectors and non-political factors such as market failure (*Erjavec 2017*).

NRP is calculated only for representative products and then, based on the share of these products in the total production, it is extrapolated to the level of total production. Representative products are products that make up at least 70% of the total production value, where the minimum participation of any individual product can not be lower than 1 %.

The analysis includes data on producer prices and the total produced quantity for the following group of products: cereals, fruits, vegetables, meat, milk, eggs and honey. Most of the above-mentioned products (product categories) are monitored and there is data evidence registered by BH Agency for statistics, except for meat products. Lack of data in meat production was the main reason why this type of products was assessed.<sup>3</sup> For comparison with domestic prices, reference EU (world) prices were taken from the OECD website. In principle, the reference world market price should be selected based on the export-import status of a particular product. Simply said, for the products for which the country is a net exporter, average export prices are taken, while in the case of net import products it is the average import prices. Bosnia and Herzegovina has a small and underdeveloped market which makes it a net importer for most of the products that primarily come from the EU market, so this paper's analysis took the EU reference prices for they were considered as most relevant for comparison in the EU aspirant countries (*Erjavec, et al, 2003*).

In addition to the nominal rate of protection NRP, the indicator of more extensive rate of assistance to producers in the form of nominal rate of assistance - NRA was computed. The indicator represents the increase/decrease in gross income of producers incurred as a result of the implementation of policy measures, i.e. percentage ratio between domestic market price increased by subsidies and reference prices. The NRA actually measures the difference

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<sup>3</sup> The calculation was made according to the Eurostat method for calculating the meat balance, and on the basis of the data of the BiH Agency for Statistic on the number of live animals and data of the BiH Foreign Trade Chamber on the number of imported and exported live animals.



between "the incentive price of output" and the reference price (Cahill, Legg, 1990).

Represented mathematically:

For individual level:

$$\%NRA_i = \frac{PP_i * QP_i + PE_i}{RP_i * QP_i} * 100 - 100$$

For aggregation level:

$$\%NRA_c = \frac{\sum PP_i * QP_i + \sum PE_i}{\sum RP_i * QP_i} * 100 - 100$$

*% NRA – nominal rate of assistance*

*PE= PSE BOT budgetary transfer to producers*

Given that each Entity has its own agricultural policy, in the quantitative analysis of the budgetary support to agriculture in BiH we used the aggregated BiH database on agricultural policy measures (BiH APM database) in which all available information on agricultural policy measures, i.e. budget transfers made in a given year by the relevant entity ministries and agricultural department of the Brčko District Government, were collected. The BiH APM database covers a long period of time, 2002-2015, and data relating to the time series 2010-2015 were used in this paper.<sup>4</sup>

The single APM classification of agricultural budgetary support was created using the EU concept that is based on policy pillars as a basic starting point, combined with the OECD classification (*Rednak et al, 2013.*). Programming elements of the EU policy (pillars, axes) have been applied at higher aggregation levels, while the OECD criteria have been applied in the case of formation of groups and sub-groups under individual pillars and particularly in the case of defining the lowest level of classification (basic headings). Thus, the APM allows for a rough analysis of budgetary transfers for agriculture also according to the OECD PSE classification and vice versa (*Volk et al, 2015*).

According to the form of payment, i.e. the conditions for their provision, budgetary payments to the producers according the OECD PSE approach in the APM system, are divided into seven basic groups as follows:

A2 – payments based on output

B – payments based on input use

C- production required payments based on the current area, number of animals, revenue, income

D- production required payments based on historical rights

E- production not required payments based on historical rights

F – payment based on non-commodity criteria

<sup>4</sup> The APM database is the result of the project „Streamlining of agriculture and rural development policies of SEE countries for EU accession“, available at <http://seerural.org/>

G – other types of payment

Estimated market price support in terms of % NRP, i.e. market price differential MPD along with budgetary support represents total transfer to producers (%TTP)<sup>5</sup>, or

$$\%TTP_c = \%MPD_c + \%PSE\ BOT_c$$

$$\%MPD_c = \frac{\sum MPD_i}{\sum VP_i} * 100 = \frac{\%NRP_c * 100}{\%NRP_c + 100}$$

$$MPD_i = PP_i - RP_i$$

*%MPD*-share of the market price differential in the total production value

*%TTP*- share of total support in the total production value

By calculating %TTP we obtained the share of total support to producers which participates in the value of agricultural production.

## RESULTS AND DISCUSSION

Comparison of domestic producers' with EU reference prices shows a significant protection of domestic producers. The highest level of producer protection through price amounting 19.7% was recorded in 2012, and the lowest amounting 2.3% in 2014. The reason for this are huge natural disasters that BiH faced in 2014 and that caused significant damage to production, also reflecting in the total production value and, therefore, the calculation of NRP, NRA and TTP indicators. On average, the % NRP amounted 12%, but in recent years there is an evident decrease compared to the initial years of the observed period.

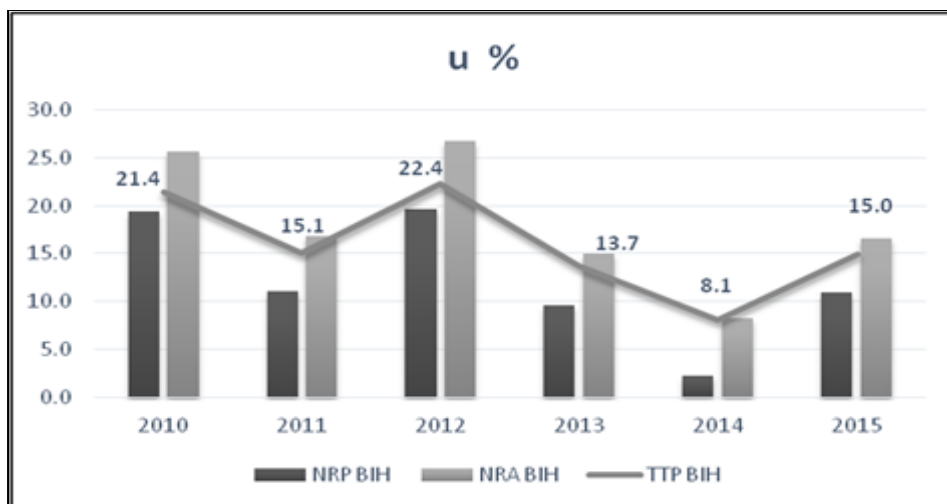
Nominal rate of assistance NRA, i.e. agricultural producer's gross income at domestic prices relative to the income at EU reference prices, varied during the observed period ranging from 26.8% in 2012 to 8.3% in 2014.

During the observed period, level of %NRA was higher relative to the NRP indicator, but with a considerably smaller share in the total amount of support which indicates a significantly lower protection of producers through budgetary support compared to the price-based one.

The assessment of the total support through % TTP indicator as a share of total support in the production value has nearly the same trend as %NRA, precisely because the total level of agricultural support is under the influence of positive/negative market price transfers, whereas budgetary support, due to its amounts, has no significant impact.

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<sup>5</sup> % TTP is an indicator that serves as an estimation of producer support aggregates; this is analogous to % PSE, but not identical. The reason for calculating this indicator using a simplified method instead of % PSE is poor quality or lack of certain data. Therefore, the numerical values of the two indicators can not be compared directly. However, compared to the PSE calculations performed by OECD similar trend values are shown in some countries.



**Figure1.** Estimated level of protection of agricultural producers in Bosnia and Herzegovina through price and total support to producers within the total value of agricultural production for the period 2010-2015 (own calculations)

## CONCLUSIONS

The estimate of protection of agricultural producers through price and total agricultural support in BiH through relative indicators represents a significant contribution to the analysis of agricultural policy because, for the first time the market price support was successfully calculated despite the poor quality of statistical data.

When it comes to the protection of agricultural producers, the EU Agricultural Policy is largely based on budgetary support, unlike BiH where, according to the computation of %NRP, %NRA and %TTP indicators, value of market price measures make up a large part of overall support, indicating that BiH agricultural policy is still not aligned with the European Union path.

Budget allocations for agricultural support in BiH as well as their structure are inadequate and insufficient and do not encourage farmers to invest in the production in which they would be more efficient. Measures should therefore be more oriented toward making producers more productive and strengthening their ability to recognize productions with less risky access to the market and ultimately making them more competitive.

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## PLANNING THE SOWING STRUCTURE APPLYING OPTIMIZATION MODELS

### SUMMARY

The selection of agricultural plants for sowing and planning the economic success in production is done in conditions of uncertainty and high risk. The factors, which cause the biggest uncertainty in achieving returns in agricultural production, can be quantified by the valuation method. The sense, value, manner and process of decision-making problems are determined by the cultural, social, temporal, value, as well as logical context. Fuzzy logic was developed more than five decades ago. T

he characteristics of fuzzy logic include operating by fuzzy notions, imprecise authentication tables, and fuzzy inference rules. All these characteristics of fuzzy logic are highly important, especially if we try to exchange or supplement the long-dominating approach of decision making in agricultural management with the descriptive one.

The criteria, limitations and performances of measures of alternatives bear in themselves some aspects of indefiniteness: in determinativeness, multiple aspects of meaning, incompleteness and fuzziness. Operational research offers optimization models aimed at finding an activity programme that will yield the best possible results. The models use precisely determined and known data.

Constraints are also precisely determined, and the goal function is clearly defined, so that it can be formulated easily and simply. Reality, especially in the agricultural production, however, is different: very often we lack precise information on the value of individual input parameters, or the values of coefficients in constraint and goal functions, and imprecise formulation of limitations themselves is possible as well.

This paper presents the valuation method at planning the structure of sowing: wheat, chamomile and mint.

**Keywords:** factors for achieving returns, valuation method, fuzzy systems.

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## INTRODUCTION

In the early 21<sup>st</sup> century the extensive use of the resources, fresh water scarcity, and sharp rises in the price of food have become causes for concern (Springer & Duchin, 2014). In agricultural production, land-use decisions are components of economic planning that result in the strategic allocation of fields (Reinmuth *et al.*, 2017; Peltonen-Sainio *et al.*, 2016). The farmer plans crops for sowing on the basis of available data and experiences. Yield in the next year is determined by quality and farmland size, the structure of husbandry production, machinery supply, and possibility to provide necessary working capital under favourable conditions, adequate labour and climatic conditions (Zhao *et al.*, 2018). The concrete yield of some crop depends on crop rotation, depth of ploughing, quantity of (mineral) fertilizer consumption, herbicide, crop-dusting, seed (grain) sort and quality (Derycke *et al.*, 2014). The cited factors can be quantified. Weighted indexes for some factors can be determined on the basis of data from the previous years. Starting from it, a general model can be designed to determine optimal conditions for husbandry production and expected yield (Hellemans *et al.*, 2018).

For centuries, various researchers have considered whether and how climatic conditions-such as temperature, rainfall, and violent storms-influence the nature of societies and the performance of economies (Carleton & Hsiang, 2016). Husbandry yields also depend on the climatic conditions as rainfall amount, temperature, the depth of the snow, storms, and so on. The cited factors cannot be easily predicted and quantify in advance. Decisions on crop sowing are made under conditions of uncertainty and high risk caused by:

- Many relevant factors that cannot be measured;
- Instability and nonlinearity of relevant factors;
- Lack of information for quantifying and measuring relevant factors influence;
- Insufficient exactness and information unavailability.

The method based on the unique evaluation of criteria will be applied for solving the cited uncertainties in husbandry. The evaluation method can be applied if the criterion values can be treated as estimates or it can be transformed into them.

The evaluation method is similar to R. Jain's method of arrangement (Jain, 1977) that is based on the weighted estimate aggregating. As estimate processing can be described with the help of many rules, the method forms the fuzzy set of extra estimates using aggregation based on the rules, and it can be also programmed as a fuzzy system (Jia *et al.*, 2018).

In the Republic of Serbia, the existing infrastructure and systems of knowledge transfer are not effective enough and they fail to meet the needs of dynamic development of the agricultural sector (Sedlak *et al.*, 2016).

## MATERIAL AND METHODS

### Characteristics of the Method for Evaluating Uncertainty Factors

To apply the method, it is necessary to arrange alternatives described by many criteria, where the values of criteria are fuzzy sets (Kosko, 1995). To treat the problem simpler, we chose the unique, five-degree way of criteria description. The same fuzzy set given by a triangular fuzzy number was associated to every estimate. This way of a unique criterion description enabled to evaluate variants in the way usual in education. This evaluation considers every criterion, with all possible values, and the result can be used as a basis for variant evaluating.

The characteristics of the method appear in the following way (Sedlak, *et al.*, 2005):

**1.** We describe different criteria by the same estimates, but the identical, gradual evaluation is not necessary in all criteria.

**2.** Estimates are defined by fuzzy sets where their belonging functions show the middle estimates of the estimates set in the belonging degree. Besides, they must point to the fact that the middle estimates and values, being in their immediate environment (for example, 1.5; 2.5; 3.5, and so on) belong to two neighbouring sets. The estimate set (supp) can be determined by the interval which is, for example, the estimate  $-0.6$ , the estimate  $+0.6$ . From the interval  $\mathbf{p}$ , the neighbouring ones have the mutual part. The belonging function of fuzzy estimate set is the symmetrical function which in the middle of the interval takes the value 1, and from the middle in both directions, it is monotonous falling. The choice of the appropriate function can be found on the basis of a poll or on the basis of looking for an approaching function. We made the choice on the basis of looking for an approximate function. So, to describe the belonging function, we chose the triangular fuzzy number (we chose the triangular for the belonging function for every estimate).

**3.** To realize the average which can be considered to be the estimate, the result is given to  $\mathbf{p}$ -estimates. The  $\mathbf{p}$  value determines the highest degree of evaluation, which is applied with criteria. Let's call this set, consisting of  $\mathbf{p}$  estimates, the set of results. In that case, the sum of weighted estimates will be some subset of the set of results (let's call it the set of extra estimates). The centre of gravity of this subset corresponds to the average.

**4.** To consider the centre of gravity the estimate, we have to determine the rule by which the estimates describing alternatives will be copied on the estimates in the set of results.

The following rules are necessary:

- One subset of the same estimate in the set of results is associated to every estimate. The belonging function of the subset is also the triangular fuzzy number.

- Every estimate exerts influence on the result to the degree corresponding to the gravitational value of criteria that belongs to it (maximal

gravitational value is 1). However, it should be noted that only criteria with higher gravitational values can exert influence on the final result (Yager's level set method assumes the same). We can attain it if we multiply the height of the subset by the square of the gravitational value.

–If more criteria get the same estimate, we associate different subsets in the set of results of the same estimate to the estimates multiplied by different weighted indexes.

–Aggregating obtained subsets with the estimate copying (t-konorma) in the set of results, we get the set of extra estimates. The centre of set gravity of extra estimates, i.e. the projection on the x-axis can be considered as an estimate.

The formal similarities between Jain's method and the mark-giving method are used for comparing (formerly applied signs are used in comparing).

Steps of Jain's method: (Jain, 1977)

1. One  $R_i$  fuzzy set is formed for every  $a_i$  alternative in the form:

$$R_i = \sum_{j=1}^m g_j \cdot r_{ij} \quad (1)$$

where  $g_j$  is the fuzzy set of weights,  $r_{ij}$  is the fuzzy value  $K_i$  of criteria in case of  $a_i$  alternative (signed operations mean the multiplication and addition of fuzzy sets).

2. A union of multiples of  $R_i$  sets is formed:

$$S = \bigcup_{i=1}^n \sup R_i \quad (2)$$

and one 'maximized'  $M$  fuzzy set is defined in the set  $S$ :

$$\mu_M(r) = [r/r_{\max}]^\beta \quad (3)$$

with the function of belonging, where  $r_{\max} = \sup S$  and  $\beta$  is a natural number (the set  $M$  gives the upper limit for the values  $\mu_{R_i}(r)$ ).

3. A fuzzy set  $R_{i0}$  is formed from  $M$  and  $R_i$  sets with the functions belonging to:

$$\mu_{R_{i0}}(r) = \min\{\mu_{R_i}(r), \mu_M(r)\}, (r \in S) \quad (4)$$

4. One  $Y_i$  value is assigned to every alternative:

$$y_i = \max \mu_{R_{i0}}(r), (r \in S) \quad (5)$$

Many have criticized Jain's method as it does not give any help in forming the set  $M$  (choice  $\beta$ ), and  $Y_i$ , which is assigned the alternative  $a_i$ , represents only one maximum value (the other ones are not taken into consideration in ordering).



Comparing to Jain's method, the steps of this method are the following:

1. Like in Jain's method, one  $R_i$  fuzzy set is formed for every  $a_i$  alternative in the form:

$$R_i = \sum_{j=1}^m g_j \cdot r_{ij} \quad (6)$$

where the values of the weight  $g_j$  can range within the interval (0,1) of real numbers, the values  $r_{ij}$  are special, and the fuzzy sets of marks are the same for every criterion (the degree of marks can be different depending on the criteria).

2-3. The method does not limit the values of functions of belonging to the sets  $R_i$ , it is not necessary to define  $M$ , nor form  $R_{i0}$  sets. Instead, the sets  $R_i$  are compared in the mutual  $E$  set.

4. The value  $y_i$ , which joins the alternative  $a_i$ , representing the centre of gravity, is formed taking into consideration all the values of criteria. The value  $y_i$ , shows the ordinal number of alternatives.

We can conclude that the mark-giving method, compared to Jain's method, represents a different principle of problem solving.

Taking into consideration every value of the "possibility of realization", Yager's method (Yager, 2005) assigns the value  $Y_i$  to the alternative  $a_i$ .

$$y_i = \max \min (\mu_k (a_i) t_j) \quad (7)$$

It also orders every  $K_j (\leq j \leq m)$ , as well as alternatives on the basis of the value  $Y_i$ . Yager's method does not always differentiate between alternatives with approximately the same weight, so it assigns the same numerical values to the groups of alternatives. With the mark-giving method we notice quite the opposite: it assigns a different numerical value to almost every alternative. According to this, the mark-giving method points more to the difference between alternatives than Yager's method (Zhao *et al.*, 2018; Sedlak and Ćirić, 2007). The application of certain methods we can find in the scientific papers of Yager and Zadeh (Yager and Zadeh, 2012). As we mentioned the yield of some crop depends on crop rotation, depth of plowing, quantity of (mineral) fertilizer consumption, herbicide, crop-dusting, seed (grain) sort and quality. In a given field a decision about selection of sowing was done on the basis of the conditions under consideration that it is best to choose: chamomile, mint and wheat.

## RESULTS AND DISCUSSION

### The Choice of Sowing Crop by the Evaluation Method

Three crops are planned for sowing: chamomile, mint and wheat. But, it is necessary to select only one taking into consideration factors exerting influence

on yield, therefore on our decision about the choice of crop for sowing. Based on the long-range research of experts, and farmers' working experiences (considered experts in this field), we chose 15 most important factors enabling yield planning in relation to immeasurable criteria. The experts are experts of field and plant production. They provide advisory services in the field of plant production, studying cultural plants and their cultivation. It is generally concerned with landscaping for high and high profitable production, composition of a herbal product and soil cultivation, and special cultivation deals with the reigning of crop cultures, explores their botanical, biological and ecological characteristics. The experts have expertise in agriculture and they task includes providing advisory services growing of vegetable plants.

These factors represent the limitations; therefore we called them the main criteria and relating to the strength of their possible influence, we added them the weighted indexes. Weighted indexes are determined on the basis experts' experiences. To their opinion, yield of some crops depends on different factors, in different extent. Table 1 includes criteria and their assumed weighted indexes. These factors were picked up by the experts, as their indexes also.

Table 1. Criteria and their weighted indexes

Criteria	Weighted index
1. Forming market prices of agricultural crops	1,00
2. Crop rotation	0,90
3. Condition of land	0,80
4. Work safety of machines and connecting machines	0,70
5. Choice of plant sort (sort or hybrid)	0,50
6. Seed germination and seedling quality	0,31
7. Prompt execution of technological operations	0,25
8. Quality of mineral and organic fertilizers	0,24
9. Quality of protective measures	0,22
10. Diseases of plants and pests	0,21
11. Air and land temperature	0,19
12. Reliability of weather forecasts	0,16
13. Precipitation (rain, snow, fog, hail, hoar frost, dew, freezing rain...)	0,15
14. Climatic conditions	0,14
15. Labour costs	0,11

Table 2 includes the description of yield of some crops with the help of estimates regarding to the criteria. The estimate points to the influence of some criteria (1-15) on crop yield; it ranges from five (big influence) to zero (no influence). For example, suppose that labour costs for soil cultivation (criterion 15) has no influence on any considered crop, while the policy of market pricing, as one of factors having influence on sowing, is evaluated by the highest estimate with all tree crops.

Table 2. Yield relation of some crops under influence of different criteria

Crop criterion	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.	14.	15.
Chamomile	5	5	4	4	4	4	4	3	3	1	3	3	3	4	0
Mint	5	5	3	4	4	5	4	3	3	2	3	3	3	4	0
Wheat	5	5	4	5	5	4	3	3	3	3	3	3	3	3	1

The obtained results are processed according to the advance established rules, which are cited in detail at the beginning of the work (according to the rules of the evaluating method). These results represent the estimate of crop uncertainty. The better result is, the bigger uncertainty in realizing the planned yield, and reversely.

Table 3. Result of arrangement

Crop	Results of the evaluation method
Chamomile	4,01
Mint	3,96
Wheat	3,51

According to this estimate, it can be concluded that (Table 3.) the influence of factors that are indefinite, immeasurable are at the same time the strongest on chamomile, and it points to the fact that its sowing is with the highest risk. Risk with wheat is the lowest (Jakovčević *et al.*, 2014).

However, if the decision on the choice of crop is made on the basis of economic indicators only (Table 4.), chamomile turns to be economically the most acceptable from the standpoint of realized profit per kg (Table 5.) and profit rate (Table 6).

Table 4. Average yield of crops and income, in €

Crops	Yield kg/ha	Redemption price	Income
1	2	3	4 (2 × 3)
1. Chamomile	8.000	0,17	1.330
1.1. flower	3.000	0,26	780
1.2. herb	5.000	0,11	550
2. Mint	25.000	0,11	2.729
2.1. first year leaf	5.100	0,17	867
2.2. first year herb	9.900	0,08	792
a) First year/total	15.000	0,11	1.659
2.3. second year leaf	3.000	0,17	510
2.4. second year herb	7.000	0,08	560
b) Second year/total	10.000	0,11	1.070
3. Wheat	5.500	0,18	990

Table 5. Direct production costs per hectare with farmers, in €

Elements	Chamomile	Mint		Wheat
		1 year	2 year	
1. Tilling and sowing	130,00	151,50	-	200,00
2. Seed – planting material	208,50	810,50	-	26,50
3. Mineral fertilizer	105,50	193,50	183,00	313,50
4. Care and crop protection	20,50	33,50	15,00	30,50
5. Crop - dusting	15,00	12,00	12,00	16,00
6. Combining (I+II)	165,50	80,00	80,00	112,50
7. Crop transport	80,00	96,00	65,00	130,00
8. Total	725,00	1.377,00	355,00	829,00
9. Average costs per kg.	0,09	0,07		0,15
10. Selling price	0,17	0,11		0,18
11. Profit per kg (10 – 9)	0,08	0,04		0,03

Note: Account in current prices

Table 6. Economic production indicators per hectare in crops, in €

Elem.	Income	Costs	Profit	Engaged capital	$K_e^2$	do' (%)
Crops						
1	2	3	4	5	6 (2 : 3)	7 (4: 5)
Chamomile	1.330	722	608	6.270	1,84	9,70
Mint First year	1.659	1.377	282	6.770	1,20	4,17
Mint Second year	1.070	353	717	5.760	3,03	12,45
Total	2.729	1.730	999	12.530	1,58	7,98
Average	1.365	865	500	6.265	1,58	7,98
Wheat	990	828	162	6.800	1,20	2,38

## CONCLUSIONS

The main conclusion that can be drawn is that the efficient economic sowing plan, at any level of investment, is providing the minimal realization of risk level and that the crop yields depend on many measurable and immeasurable factors.

The models for determining the optimal structure of agricultural production is possible to set with analysing the measurable factors. Here is very important to examine in all production phases the reliability of factors that are causing yields. The economic analysis does not quantify the influence of uncertainty which can have the decisive influence on the realized yields in agricultural production. The decision on the choice of the crop is made based on the economic indicators shown that only chamomile turns to be economically the most acceptable from the standpoint of realized profit.

<sup>2</sup>  $K_e$  – rate/coefficient of return; do' - profit rate.

From the point of the immeasurable factors, the fuzzy method includes many competitive and conflict criteria in a relatively simple way. Certain qualities of the applicants can't be measured accurately, and an expert's opinion is needed, although the expert might have doubts or hesitate to give a final position. In principle the method of evaluation can be generally used, and if the values of the criteria can be treated as estimates (or can be transformed into them), the method is applicable.

The applied fuzzy evaluating method that was based on giving of the marks allows the arrangement of such alternatives, where the fuzzy criteria can be described by estimates, or where the values of the criteria can be considered as estimates. The results are similar to the results achieved by other methods of evaluation. The "mark giving method evaluation method" criteria are treated as a fuzzy system with aggregates aggregation. The method in some of its points bears a resemblance to the method of editing an alternative to R. Jain. The weight-based editing associated alternatives, is with the different principle than one used by Jain. The weight-based editing associated alternatives, is with a different principle than that used by Jain. The method that ranks alternatives using fuzzy logic and fuzzy systems can be addressed in a relatively simple way with a large number of competitive and conflicting criteria. The main point of this study was in presenting a new approach in decision making and mark giving and this may constitute the object of future studies.

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**SATISFACTION OF HOTEL WORKERS AS A COMPONENT OF  
BUSINESS QUALITY IMPROVEMENT  
-A REPORT FROM RURAL AREAS IN SERBIA –**

**SUMMARY**

Satisfaction with the business is the current topic of global research, as one of the most important factors in the successful and quality business in tourist companies. The results of these surveys confirm that customer satisfaction and company profit can only be achieved through a quality service provided by satisfied employees. The authors of the research carried out research in accommodation in rural areas in Serbia in the period from March to May 2017, in order to examine the level of satisfaction of employees in these tourist accommodations. The results that the authors have come up with by survey research, undoubtedly indicate that there are segments in the business that the employees are really dissatisfied with, and that education does not have much connection with the chance of changing the job. Research is just a part of the entire research and aims at pointing out the employees' attitudes about working conditions and improving the quality of services.

**Keywords:** employees, accommodations, quality, satisfaction, rural Serbia

**INTRODUCTION**

The analysis of the success factor in services, especially in tourism and hotel industry, as specific and complex service activities, has shown that the importance of human resources for the success of the hotel organization is indisputable. It is precisely in the hands of management that driving lever is that it creates and drives entrepreneurial energy, connects and unifies the potentials and strengths of all employees (Baddar, 2005). The success of the hotel organization depends, therefore, on all employees and the degree to which they support one another in the process of providing the service. Many authors consider that pleasure is a pleasant or positive emotional condition for employees arising from fulfilled working conditions, and that the satisfaction with work is the general attitude of the employee to his job (Lam et al. 2003). The quality of services offered to the client depends on trained and qualified staff, which is vital for success and survival in the global competitive market. It starts from the

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assumption that a person is a resource, that is, a potential that can create an economic value for the organization by appropriate management practices and skills. Important changes in approach and understanding lie in the field of responsibility for human resources, which transfers from the functional area for the personnel directly to managers of all levels and emphasizes the strategic character of human resources (Poe, 2003). For the business of a hotel organization, it is crucial to identify the link between the performance of employees and the performance of the company, that is, the quality of employee performance management and the quality of the tourist service. In organizations, there is an increased awareness of the importance of human potential. The basic requirement for the development of new models for measuring the performance of a particular company is the flexibility and balance of quantitative and qualitative criteria, with the aim of more adequate and faster response and adjustment to a dynamic and market environment. At the same time, it is at the center of attention to all measurements in the company, measuring employee satisfaction. Employee satisfaction is usually measured by employee satisfaction surveys, including topics such as compensation, workload, work conditions, management perceptions, flexibility, teamwork, resources, etc (Nadiri et al, 2010). The authors of the paper conducted a survey of hotel workers in Serbia, in order to determine the level of their satisfaction. The obtained data were analyzed in the software program SPSS, version 23.0, and the authors started from the research several hypotheses and subhypotheses, in order to deepen the problems of employee satisfaction. The data presented in the paper are only part of a wider survey, and the goal is certainly to influence the quality of business in hotels with their analysis.

## **MATERIAL AND METHODS**

### **Background of study and hypothesis development**

#### *Employee satisfaction and quality of business*

Employee satisfaction is defined as the perception of employees about the degree of fulfillment of their requirements. For some organizations, satisfied employees are people with whom an organization could only be more successful and with the help of which it provides better quality services, moving only as an ascending path. Employee satisfaction is a good starting point in business, and will be expressed when the difference between expectations and experience is low, and vice versa. Factors that affect the level of job satisfaction are very different and depend on the type of work that is being done (Robbins et al. 2007). It starts primarily from the fact that human resources are not like other organizational resources. People have their own interests, goals, character and angle of observing reality. Employees are individuals with their own egos and are ready to invest their knowledge, abilities and energy for organizational goals and only thus achieve their own goals, that is, meet their needs (Anderson et al. 2000). The organization and individual are in a relationship of interdependence from which both parties can win the gain under certain conditions. The problem



with employee satisfaction is that management does not focus on essential things for talented staff. Some workers might be quite happy with work that requires very little effort, working out the minimum minimum needed to keep their jobs (Rotenberry et al. 2007). These employees are probably very satisfied with their jobs, or are not interested in further advancement, but are in some ways happy with what they have. They are unlikely to leave the company, but they do not necessarily add value (Keegan et al, 2005). Employee satisfaction, also known as satisfaction with work, is the degree to which an individual is satisfied with his work and the role he plays in his life. The degree to which employers give priority to employee satisfaction depends on the employer and industry (Homburg et al, 2005). Satisfaction researchers tend to distinguish affective satisfaction and satisfaction from cognitive work. Affective satisfaction is the sum of satisfied emotions and feelings related to the job and its place in the life of an individual, while cognitive satisfaction refers to rational satisfaction over certain aspects of work. There is a wide range of employee satisfaction theories (Testa, 2001). Dispositive theory, for example, claims that individuals are predisposed to a certain level of job satisfaction, regardless of work or industry. Satisfaction depends on the strength of what an employee expects from work and what he / she actually receives. In order to be successful in a competitive market, it is important that hotel managers know how their employees feel at work and what they want (Yang, 2010). How many employees are spent on achieving the goals of the hotel and achieving quality business depends on whether the employee believes that this job will lead to meeting their own needs and desires. Before the management of human resources, through theory and practice, a new task of transforming intellectual capital into additional value for the organization is set. Flexibility and quality of organization in conditions of rapid technological change are possible only if its employees are ready for continuous learning and professional development. The modern approach to human resources management contains levers that directly affect the performance of individuals and organizations (Baddar, 2005). Performance of the contemporary concept of human resources management stems from the following characteristics: strategic character that implies the connection between business strategy and human resources management, access to employees as human capital, connectivity and instrumentality of individuals' goals and organizational goals, or employee dedication, observing employees from management perspective and managerial responsibilities of all levels with the advisory and coordination role of human resources experts, dynamic and developmental character and interconnectedness of all processes in human resources management (Yeh, 2013). Successful organizations in the world look at employees as the primary development resource for achieving quality and survival on the market. Satisfied employees are the key to achieving quality. We can talk about more elements of satisfaction such as satisfaction with organization of work, prize-winning system, interpersonal relations, management, secured working conditions, own workplace, development opportunities, corporate communication (Zopiatis,

2007). Also, satisfaction factors vary according to their organization, according to their environment, according to the social system. In general, employees' satisfaction depends on the employee's expectations and the extent to which they are achieved. So then satisfaction is measured by the difference between the level of employee aspiration and the achieved results. The variety of jobs, years of life, education obviously present some general factors of employee satisfaction. Among the factors that determine satisfaction we can count on "personal adjustment" of the employee towards the company (Kuruuzum *et al.*, 2009).

### *Hypothesis development*

Based on authorial research, as well as available secondary documentation and existing literature, the authors proceeded from the relevant hypothesis to the research:

H1: employees are satisfied with working conditions;

H1a: there is a statistically significant difference in satisfaction with the number of employees;

H1 b: there is a statistically significant difference in the satisfaction of available equipment;

H1c: there is a statistically significant difference in the satisfaction of the work space;

H1d: there is a statistically significant difference in satisfaction with interpersonal relationships;

H1e: there is a statistically significant difference in satisfaction with adequate time for doing business;

H1f: there is a statistically significant difference in the satisfaction of the organization in work;

H2: employees are satisfied with their earnings;

H3: employees are satisfied with the existence of open possibilities for their own development;

H3a: there is a statistically significant difference in satisfaction with the conditions of further education;

H3b: there is a statistically significant difference in satisfaction with the conditions of further progress;

H4: employees are satisfied with the support of management;

H4a: there is a statistically significant difference in the satisfaction of the possibility of doing business with their own way of working;

H4b: there is a statistically significant difference in the satisfaction of the possibility of expressing one's own ideas;

H5: employees are fully committed to the business;

H5a: Employees consider that success is only related to a job;

H5b: Employees are fully accountable at work;

H5c: employees identify business problems with personal;

H6: Employees consider that they have the opportunity (chances) to change the job.

## Methodology

Measuring employee satisfaction is an indispensable part of the process of managing the organization for positioning the company as a whole and its organizational parts both in the part of the strategic analysis and in the strategic choice and the changes. The survey questionnaire is the most frequently used technique for measuring employee satisfaction. From a formal point of view, the advantage of applying the questionnaire is that it provides easy anonymity of the respondents and discretion of the data. The authors of the paper carried out a survey on the rural territory of Serbia. A total of 300 questionnaires were distributed, with constructed short answers with yes and no, but 238 sheets were taken in the analysis, in order to avoid the missing values. Distribution of the questionnaire was uniform in all rural areas. The test period covered March, April and May 2017. The authors used the SPSS software, version 23.0. The paper will only show a part of the research that relates to employee satisfaction, but the research of a broader scope and has included many variables related to the overall management of human resources in order to increase the quality of the services provided. A standardized questionnaire was used by Anastasios Zopiatis, Panayiotis Constanti, Antonis L. Theocharous (2014), with some modifications for this research.

## RESULTS AND DISCUSSION

Table 1. Demographic profile of respondents

Profile of respondents		Frequency	Percent
Gender	Male	148	62.2%
	Female	90	37.8%
Age	18-30	109	45.8%
	31-50	94	39.5%
	51+	35	14.7%
Education	Secondary	27	11.3%
	High	84	35.3%
	College	104	43.7%
	MSc, Phd	23	9.7%
Lenght of working	1-5	85	35.7%
	6-10	68	28.6%
	11-15	56	23.5%
	15+	29	12.2%
Earnings	<200 e	97	40.8
	200-400 e	105	44.1%
	>400 e	36	15.1%
Total		238	100%

Source: based on author's research

Table 1 clearly shows the profile structure of the respondents. The largest percentage of male respondents were 62.2% and women with 37.8%. When considering the age structure, it is noticeable that the largest participation in the

research is from 18 to 30 years of age (45.8%), then those aged up to 50 years, and the smallest respondents over 50 years of age (14.7%). The more educated structure of respondents with university education prevails (43.7%), followed by a three-year high school (35.3%), and the least number of respondents with MSc and PhD degrees. The employees who participated in the survey in the highest process were employed from 1 to 5 years of work (35.7%), and the rewards of those who work for more than 15 years. When looking at earnings, those with an average salary of up to 200 to 400 euros (44.1%) had the greatest share.

Table 2 shows the analysis of the results obtained by the survey of hotel employees.

Table 2. Frequency and Descriptive Statistics

Items: percentage frequency→	Yes (%)	No (%)	Min	Max	Mean	Std. Dev.
Number of employees	71.4	28.6	1	2	1,29	,453
Equipment	60.9	39.1	1	2	1,39	,489
Work space	66.8	33.2	1	2	1,33	,472
Relationships between colleagues	69.7	30.3	1	2	1,30	,460
Available time for work	68.1	31.9	1	2	1,32	,467
Organization of work	68.9	31.1	1	2	1,31	,464
Earning	35.7	64.3	1	2	1,64	,480
Education possibilities	31.1	68.9	1	2	1,69	,464
Progression possibilities	34.0	66.0	1	2	1,66	,475
Support of management	52.5	47.5	1	2	1,47	,500
Free mode	46.2	53.8	1	2	1,54	,500
Freedom in insinuation of ideas	26.9	73.1	1	2	1,73	,444
Complete possession of the work	68.5	31.5	1	2	1,32	,466
Living success exclusively related to the job	67.6	32.4	1	2	1,32	,469
Feel responsibility to the job	89.5	10.5	1	2	1,11	,307
Business problems are personal problems	73.1	26.9	1	2	1,27	,444
Chances for changing job	25.2	74.8	1	2	1,75	,435
Valid N (listwise)	238					

Source: based on author's research

About 71.4% expressed satisfaction with the number of employees in hotels (H1a+). The approximate number of employees is satisfied with the equipment available in the work (H1b+), and with the space in which they work (H1c+). When it comes to relations between colleagues, 69.7% are satisfied,

while 30.3% expressed dissatisfaction with interpersonal relations (H1d+). The way in which the processes of human resources management in the hotel organization are shaped essentially determines the attitude of employees towards the job, organization and its goals, and thus the overall organizational efficiency. The available time for work is sufficient for most respondents (68.1%; H1e+), and the organization of the process itself is also rated positively (68.9% satisfied; H1f+).

In general, observing the results of the set subhypotheses, the main H1 hypothesis is confirmed that the employees are satisfied with the working conditions. Observing the results for the wage item, the ratio is as follows: 35.7% satisfied, and 64.3% dissatisfied with the salary, which negates the H2 hypothesis. Salaries are in most cases small, and working hours are not always the best. For all this, the labor force flow is much higher here than is usual in other sectors. The key generic function of human resources management is the development of employees.

Tourism and hotel management are very specific activities that determine the number of jobs and their sustainability, and depend entirely on the work intensity of the employees. Their ability and work efficiency depends on good professional training, including lifelong learning. It is very important that the utmost attention is paid to education that enhances the ability of people to be flexible, professional, communicative and ready to satisfy different needs of service users in tourism. Therefore, the future of the hotel industry is in the hands of education. Development can include a whole range of designed and planned activities focused on new skills and abilities necessary for future work. The respondents also expressed dissatisfaction regarding the possibility of further education and training (68.9%), and the opinion on the impossibility of further promotion is more often represented (66%), which negates the H3 hypothesis, with its sub-codes H3a and H3b. A relatively equal number of respondents pleaded with contentment and dissatisfaction with the support of superiors (H4 can not be denied or fully confirmed). Freedom of business and free expression of attitudes and ideas are also negatively assessed, more precisely, more dissatisfied respondents (H4a and H4b-). A total commitment to work was indicated by 68.5% of respondents (H5+), and 67.6% of them think that their lifetime success is tied exclusively to the work they do (H5a+). Satisfied workers are those who are extremely loyal to their organization and are held even in the worst scenario. They do not work on any coercion, but because they dream to organize their organizations to a new level. Employees must be passionate about their work and passion comes only when employees are satisfied with their work and organization as a whole. All this affects the level of innovation, since it is unlikely that someone who is not committed to a company in the long term will have the motivation, knowledge or experience to contribute to the development process of the given company in tourism. Responsibility for the job is assessed in the following way: 89.5% stated that they absolutely feel the maximum degree of dependency (H5b+), and that business problems are exactly personal problems

(73.1%), which confirms the H5c subhypothesis. Satisfied employees are happy employees who will help their colleagues and cooperate with the organization even in emergency situations. Such employees do not think about leaving their jobs during the crisis, but they work together as a single unit to overcome challenges and get out of the situation as soon as possible. For them, their organization comes first, everything else later. They do not come to work simply because of money, but because they really feel about the organization and believe in their goals.

However, the opportunities, as well as the desire for a new job or job change, were not supported by a large number of respondents. Namely, their 74.8% think that this is currently not possible and the hypothesis H6 is denied. Ensuring permanent employment in tourism and hotel industry is very problematic, due to the existence of a pronounced seasonality of business activities.

On the other hand, this can be an advantage. The large turnover of the workforce leads to the exchange of personnel between firms in an area, which can increase knowledge transfer. This, of course, requires that executives in companies are ready to receive new ideas that their staff can present to them. Human abilities, motivation and engagement do not come to an end in themselves, so it is important for managers to find the right formula for discovering and using this valuable potential. Also, the done analysis of dependence varied education and opportunities or opportunities for change of work. The authors used the Crosstabulation - Chi Square test model, followed by the measurement of symmetry (Phi, Cramers and Contingency Coefficient) in order to reaffirm the probability of dependency. Individuals who have finished high school (22.2%) claim that they have chances and wishes To change the job, while 77.8% deny the answer. The situation is similar when the higher vocational education is concerned: 21.4% for the change of work, while 78.6% denies the answer.

The percentage of respondents who completed the faculties is a higher percentage of those who would change the job, but this feature is not very different, 25% for job change and 75% against. When it comes to human resources, the tourism sector has some special characteristics compared to other service companies. Most of the staff working in tourism companies in our country, but also in the world, are not sufficiently educated. Talented and more educated employees expect more from companies. For these employees, satisfying the job involves different criteria. They want to be engaged and give them the opportunity to progress further education. They want new work challenges, and that their personal and professional opportunities grow and improve their careers.

Respondents with the highest degree of education MSc and PhD degrees, are more interested in change and new chances of 43.5% for job change, while 56.5% are against.

Table 3. Education \* chances for changing job - Crosstabulation

			chances for changing job		Total
			yes	no	
education	Secondary school	Count	6	21	27
		Expected Count	6,8	20,2	27,0
		% within education	22,2%	77,8%	100,0%
		% within chances for changing job	10,0%	11,8%	11,3%
	High School	Count	18	66	84
		Expected Count	21,2	62,8	84,0
		% within education	21,4%	78,6%	100,0%
		% within chances for changing job	30,0%	37,1%	35,3%
	College	Count	26	78	104
		Expected Count	26,2	77,8	104,0
		% within education	25,0%	75,0%	100,0%
		% within chances for changing job	43,3%	43,8%	43,7%
	MSc, PhD	Count	10	13	23
		Expected Count	5,8	17,2	23,0
		% within education	43,5%	56,5%	100,0%
		% within chances for changing job	16,7%	7,3%	9,7%
Total		Count	60	178	238
		Expected Count	60,0	178,0	238,0
		% within education	25,2%	74,8%	100,0%
		% within chances for changing job	100,0%	100,0%	100,0%

Source: based on author's research

In this case it can be pointed out that more educated people are more inclined to change in their work, they are striving for new experiences, knowledge, additional education, but the approximate value of the answer for and against can be caused by the situation in the country and the poor supply of better jobs in the labor market.

In a further analysis of the correlation testing of these two variables, we established the values of the Chi Square test. The table shows that the Pearson value is 4,838, while the df or the number of degrees of freedom is equal to 3. Asymptotic Significance or the approximate two-sided probability is 0,184. Values are greater than 0.05, meaning that there is no significant connection between these variables. We can not claim that these two variables are in mutual association. In Symmetric Measures the values of Cramer's V, Phi I Contingency Coefficient are approximate, and the Approximat Significance for Cramer's V and for the Contingency Coefficient is identical probabilities to Pearson Chi Square statistics. On the basis of all the analyzes, it is again concluded that the values of significance are higher than 0.05, and that there is no significant dependence on education and the tendency to change the job.

Table 4. Chi-Square Tests

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	4,838 <sup>a</sup>	3	,184
Likelihood Ratio	4,413	3	,220
Linear-by-Linear Association	2,678	1	,102
N of Valid Cases	238		

Source: based on author's research

Table 5. Symmetric Measures

		Value	Approximate Significance
Nominal by Nominal	Phi	,143	,184
	Cramer's V	,143	,184
	Contingency Coefficient	,141	,184
N of Valid Cases		238	

Source: based on author's research

## CONCLUSIONS

The quality of tourist and hotel services depends on the employees in tourism. The hotel product is accessed as a chain of services created to meet the needs of increasingly demanding visitors. Services are provided in a specific area, at a specific time, environment, and other conditions. In order to fulfill the quality of the quality of the service, it is necessary, in all its segments, to fulfill the expectations of tourists, and to overcome them, and to offer products of defined quality to the market, today it is considered a condition of survival. The essence of this paper is that the human factor is key to success in tourism.



However, human resources are not in themselves a key factor in the success of a tourism organization, but it is necessary for management to create such conditions to develop the potentials of employees by certain methods and instruments, to motivate and identify in order to positively influence the organization's performance. We have long realized that only a satisfied worker can give the best results, regardless of what job he is doing. Therefore, to talk about quality is important, but to make a decision about its implementation and really do it, it is responsible and exemplary. Employee satisfaction is essential to ensure higher revenue for the organization. No training or motivation would help, unless and when individuals develop a sense of attachment and loyalty to their organization. Employees spend their half of their time fighting their counterpart or diluting problems with them. There are several factors that determine the quality of services in the service tourism industry, among which the most important human resource and satisfaction at work. The authors of the research carried out a survey for accommodations in rural areas of Serbia.

The aim of the research was to examine the level of satisfaction of the employees in hotels, and in that way to influence the improvement of working conditions and a higher level of business quality by corrective measures. Analyzed data in 238 subjects, and the results of confirmation of epoxy hypotheses and subhypothesis are presented in the following table. The employees were more favorable to the general conditions of work such as space, interpersonal relations. However, dissatisfaction was expressed most with earnings, as well as flexibility in business, to present their ideas, to further educate and advance.

The results show that respondents are mostly devoted to work and dedicated institutions in which they work, because failure and work problems identify with personal problems. However, there is a small percentage of employees who think that they might be able to change their jobs in the near future, and the authors of the work have determined the degree of dependence of education and greater chances for changing the job. It turned out that there is no great association between these variables.

Table 6. The state of the hypothesis after the analysis of the results

Confirmed hypotheses (+)	Denied hypotheses (-)
H1, H1a, H1b, H1c, H1d, H1e, H1f, H4, H5, H5a, H5b, H5c,	H2, H3, H3a, H3b, H4a, H4b, H6

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## FEMALE ENTREPRENEURSHIP IN MONTENEGRO: MOTIVES AND BARRIERS

### SUMMARY

This paper provides an overview of the socioeconomic and sociological context of entrepreneurship development in Montenegro. Having regard of the traditional social relations in Montenegro, female entrepreneurship is still emerging. In this respect, an empirical survey was carried out on a sample of 50 female entrepreneurs. The research should provide answers to the following questions: what motives were important for starting business; the barriers they encountered during business startup; the business activity they opted for, as well as to evaluate their cooperation with other business entities and their current social status. The survey was conducted in the period July-August 2017 in municipalities of Kolašin, Pljevlja and Podgorica.

**Keywords:** Female entrepreneurship, motives, barriers, Montenegro

### INTRODUCTION

Montenegro is situated in South Eastern Europe, in the Balkan Peninsula. Its area is 13,812 km<sup>2</sup> and population 620,029. Population density is 46 per m<sup>2</sup>. Compared to other countries of the region, the population density is lower than in Bosnia and Herzegovina (75), Croatia (56), Serbia (88), Slovenia (102), Macedonia (83). (World Bank, 2014) Podgorica is the capital city, where almost one third of the total population lives. Gross Domestic Product in 2016 amounted to €3,954 million EUR or €6,354 per capita. The real growth rate was 2.9 per cent compared to 2015. (Monstat, 2016)

According to the population census 2011, women account for 50.6% of the population in Montenegro. (Monstat, 2012) Higher share of women in the total population structure is recorded also in European states. (Šobot, 2012) However, male population is characterized by a significantly higher level of entrepreneurial activity. In Montenegro, 28.4% of employed men and 12.4% of employed women run their own business. (Monstat, 2016). In the structure of the total number of employees, women in Montenegro account for 45%. The situation in the region is similar: in Serbia 41%, Macedonia and Croatia 45%, Albania 38%, Slovenia and Romania 45%, Bulgaria 47%, while in Kosovo and Bosnia and

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Herzegovina the share of women ranges from 30 to 34% . (Ramadani, 2015) Analysis of differences in average earnings between employed women and men in Montenegro in the period 2008 – 2011 shows that the difference was in favour of men.

The gender gap in working hours was 16%. This gap indicates the presence of a “glass ceiling” for women. More specifically, women do not occupy positions that bring the highest monthly income (Unija poslodavaca CG, 2013)

According to population census 2011, there are 1,559 illiterate men and 6,590 illiterate women in Montenegro. Age of most of illiterate women is 70+. Average age of an illiterate woman is 66, and of a man 44. Historically, Montenegro has had a positive tradition in education of girls and young women. This is shown by the fact that in 1871/1872 Montenegro had 38 schools with 2000 pupils, 108 of whom were girls. The first female secondary school in Montenegro was founded in 1869. It was the Female Institute of Empress Maria Alexandrovna in Cetinje (Filipović, 2003).

Entrepreneurship in Montenegro has been a topical issue over the past ten years or so. Martin Ruef and Michael Lonsberry identified two dominant sociological perspectives in entrepreneurship research (Ruef et al, 2007). One is contextual and highlights the role of the social system, with special emphasis laid on issues concerning the reasons for starting an entrepreneurial business. The other perspective is behavioural and takes into account the structure and the process of entrepreneurial activity at the micro level.

The objective of the paper is to present the socio-economic and sociological context of entrepreneurship development in Montenegro. Female entrepreneurship is an important challenge in the processes of revitalization and further development of Montenegro’s economy. Developmental potential of female entrepreneurship is an important source for overall economic growth.

Empirical research presented in this paper was carried out on a sample of 50 female entrepreneurs. The results of the research should provide responses to the following questions: what motives were important for starting business, business barriers, barriers encountered when starting a business, business activity they opted for as well as assessment of cooperation with other business entities, their current social status, etc.

The paper is structured into the following chapters: introduction, reference overview, business climate in Montenegro, socio-economic context of entrepreneurship development, economic environment for female entrepreneurship development in Montenegro, methodology, survey results, conclusions and recommendations.

### **References overview**

A number of authors set quite strict conditions when defining the term “female entrepreneur”. This category includes only “women who own 50% or more of the capital of a formal enterprise and are actively involved in business operations and creating jobs for themselves and for others”(Avolio, 2011).

According to the OECD (*Organisation for Economic Co-operation and Development*) definition, female entrepreneurs are all women who are owners of an enterprise provided that simultaneously have the leading management role in that enterprise (OECD, 1998).

Women's entrepreneurship may also be positioned as a particular kind of opportunity for society. Caputo and Dolinsky provide an illustrative example. They advise governments to supply micro-loans to women, so they can contribute to the family income by starting a business that enables them to be flexible and work around the schedule of their husbands. They can take care of the children when the husband is at work, and run their business at other times, when the husband is available for childcare. The authors claim that this arrangement presents an opportunity for society since it will save taxpayer expenditure for public childcare (Caputo and Dolinsky, 1998). In the past, entrepreneurship was considered to be a male dominated sector. Research on women's entrepreneurship is marginal in the academic field, and it may take a tenured position to be allowed the opportunity to conduct such research (Baker et al, 1997). This research often demonstrates a sincere interest in giving women a more prominent place in science, as for example a study by Brush, Carter, Gatewood, Greene & Hart which aimed explicitly at dispelling myths about women entrepreneurs as being secondary to men (Brush et al, 2002).

As the number of women entrepreneurs increases, the number of studies conducted on this phenomenon increases as well. The majority of these studies are focused on the characteristics of women entrepreneurs (age, education, previous experience, motivation, problems and/or skills) and their businesses (sector, number of employees, financial results, growth rate, etc.) (Ramadani, 2015). Taylor and Newcomer's study of 402 women entrepreneurs found that the largest number of women entrepreneurs are aged from 30 to 40. In Poland, women entrepreneurs are aged from 35 to 50, in Canada from 31 to 45, in Asia from 30 to 40, in the USA from 36 to 55, etc. The age of women entrepreneurs in Ukraine varies from 19 to 61, where 42 per cent, the largest group, are in their 30s, while 19 per cent are under 30 years of age. If we analyse the age of women entrepreneurs when they established their enterprises, according to a survey conducted in the European Union (EU) in 2004, one can notice that over 70 per cent of women established their enterprises before the age of 35; whereas around 15 per cent started their businesses at the age of 36–40, while 14.4 per cent after the age of 40. The majority of women entrepreneurs have a formal education, which often plays an important and decisive role in solving problems that arise in business management (Sherrill et al, 2005). Taylor and Newcomer found that in general, women entrepreneurs completed their formal education, the largest number of them having graduated from university, while every fifth woman entrepreneur has finished their master's or doctorate studies. It can be noticed that from the total number of respondents, 20 per cent have a secondary school education, 35 per cent are university graduates, 10 per cent have two years of studies, 25 per cent have a master's degree and 10 per cent have a doctorate

degree in different fields (the majority in the field of business and economics). Another study shows that the majority of women entrepreneurs has post-secondary education; 35 per cent finished technical training/college; 41 per cent completed bachelor's degrees and 18 per cent completed graduate studies (Sherrill *et al.*, 2005). The most frequently mentioned university majors were: teaching (13 per cent), engineering (10 per cent) and business related fields (8 per cent). Most of the women's parents did not have university degrees (66 per cent of mothers did not; 60 per cent of fathers did not) and 29 per cent did not have a single parent who had more than a high school education (Hisrich and Brush, 1987). A study conducted in China shows that most of the 50 surveyed women entrepreneurs have higher education (44 per cent); 20 per cent have high school, 16 per cent secondary school, 10 per cent high professional school and another 10 per cent have primary, professional and technical school (Robert *et al.* 1991).

Considering the overall motives for starting their own businesses, researchers have not found any substantial differences between countries or between men and women (Carter and Marlow, 2007). Most of the literature dealing with the motivations of women entrepreneurs have placed the desire for autonomy and independence, as well as the desire for personal satisfaction and achievement, as key motivating factors (Goffe and Scase, 1985, Marlow, 1997, Moore and Buttner, 1997, Duche and Orhan, 2009). Dana has noted that motives for starting their own businesses are focused on three directions: the self (high need for achievement, innovativeness and ability to take calculated risks); the ethno-cultural milieu (culture, thrift, frugality, asceticism and ethnic resources) and the host society (stratification, social blockage, government assistance and occupational clustering) (Leo-Paul Dana, 1997). Since a greater proportion of women are financially dependent on their spouse/partner, entrepreneurship may also be a last resort solution following the death of a spouse/partner or divorce/separation (Campbell, 1994). Walker and Brown found that financial (profit and wealth creation) and non-financial (being one's own boss, having free time for personal activities, the flexibility to structure the business according to the entrepreneur's values and objectives) factors motivate women entrepreneurs. The most common motivations for starting their own business by women are: independence, job satisfaction, success, opportunities, status/image, money, power and economic needs and secure career (Walker and Brown, 2004).

Motivation that drives entrepreneurs is related to both positive and negative factors. As an example of a positive encouragement for starting business is the desire to be independent, to be one's own boss, successful in business, while negative motivation concern discrimination at the alternative job and discomfort. Some people are positively encouraged for starting business, while on the other side there are those whose existential needs forced them to establish a company (Ćeranić, 2013). Research carried out by Tominc and Rebernik shows that entrepreneurs with negative motivation cannot be expected to be the growth

entrepreneurs, as most of them are subsistence entrepreneurs (Tominc and Rebernik, 2006).

Despite the fact that most studies suggest no predominance of push factors among women, Hughes argues that this is largely due to the type of methodology employed (mainly quantitative surveys) and the interpretation of motivational factors. While she does not dispute the fact that pull factors appear to predominate, Hughes claims that the importance of push factors among women is more often than not underestimated (Hughes, 2003).

In the contemporary conditions of entrepreneurship, women entrepreneurs are increasingly participating and play a big part in the structure of the labour force, especially in the establishment of new businesses (Ramadani, 2015). Different research put forward that women entrepreneurs establish their businesses mostly in the following sectors: retail, consulting, educational services and public relations. For instance, Aidis et al. noticed that in Lithuania, women entrepreneurs operate in the following sectors: retail/wholesaling (37 per cent), services (37 per cent), manufacturing (22 per cent) and other sectors (4 per cent) (Ruta et al, 2007). The research conducted by Hisrich and Brush annotates that 90 per cent of businesses founded by women entrepreneurs are oriented toward services, 7 per cent to production and 3 per cent to finance (Hisrich and Brush, 1987). Research so far has elaborated some of the barriers or problems faced by women entrepreneurs in setting up their businesses. A study done by Gobagoba and Littrell found that women entrepreneurs faced a variety of barriers: lack of management skills, business skills, cash flow, technical skills and the ability to recruit qualified staff. In addition, the authors found that they have limited access to the appropriate technology and have difficulties in creating sustainable networks (Gobagoba and Littrell, 2003).

Dissatisfaction in the labour market is more prevalent among women, arguably due to circumstances specific to women such as reaching the “glass ceiling” (Moore and Buttner, 1997, Mattis, 2004), the predominance of “old boys” networks (Sia Group, 2001, McClelland, 2003) and the widespread use in employment of transactional leadership styles (Orhan and Scott, 2001). Women also have a need for increased flexibility arising from the fact that society assigns to them the role of primary caregiver for the young, elderly and sick people. Consequently, more women than men seek a more effective balance between their personal and professional lives (Orhan and Scott, 200, Marlow, 1997, Belle and Valle, 2003, Greer and Greene, 2003). To a lesser extent, women’s motivational factors also differ from men’s in relation to two further “push” factors. More women than men opt out of the labour force after having a child/children. Some of them choose not to re-enter. It is argued that the difficulties associated with re-entering the labour market can act as a positive motivational factor for women to engage in entrepreneurship (Sarri and Trihopoulou, 2004). Factors that affect the success of women entrepreneurs are different. According to Box et al., there are four factors which have a positive relationship with the business success of women entrepreneurs, such as: previous

experience as a member of an entrepreneurial management team, number of previous 'starts', entrepreneur's age and scanning intensity (Box et al, 1995).

Hisrich et al. enumerate the following factors: level of education, years of experience, business skills, personal goals and strategy to assess the performance of women entrepreneurs (Hisrich et al, 1999). Fielden and Davidson emphasize that the success of women in business mainly depends on two groups of factors. The first group consists of 'push' factors, while the second consists of 'pull' factors. 'Push' factors are those factors that produce pressure and force women to forgo their current work, which they do (control, independence, flexibility, dissatisfaction with work, opportunity to do more, etc.). 'Pull' factors are those factors that attract women into entrepreneurship and influence the decision to establish their own business (being the owner, financial independence, realization of personal growth, self-determination, etc.) (Fielden and Davidson 2005).

Management skills and approaches were recurrent matters within the researchers (Buttner, 2001). Women entrepreneurs, before they start their businesses, should evaluate their managerial skills which have relevant impact for the success or failure of their businesses. Therefore, managerial ability has to do with factors from which depends the success of the entrepreneurs' businesses in this case, of women entrepreneurs (Ramadani, 2015). In research conducted by Hisrich et al., when women were asked to evaluate their skills in several business areas, they rated themselves least capable in finance (Hisrich, 1987).

In earlier research, Hisrich and Brush concluded that the majority of women entrepreneurs show weaknesses in finance management; they have average skills in marketing and operations, while their great advantages are in generating ideas/ innovative products and establishing relations with people (Hisrich, 1987).

Women appear to draw heavily on cultural capital, for example in terms of educational level or experience, but these have not succeeded fully in providing an equalizer effect, due to high levels of structural segregation at the societal level (e.g. the type of education, type of work experience). Elam argues "gendered cultural capital results in different conceptualizations of and consequently different approaches to entrepreneurship". Finally, the importance of social capital is emphasised for women, who may disproportionately require it in order to become entrepreneurs (Elam, 2008).

## MATERIAL AND METHODS

### Methodology

With a questionnaire on female entrepreneurship in Montenegro the socio-demographic data were defined first (age, education, marital status and number of household members), followed by the data on views of the female respondents on motive and barriers affecting their entrepreneurial activity. The survey has 21 questions, with two two-choice questions while the rest are Likert-type scale questions. The data collected were analysed using the SPS software package. In addition to descriptive statistics indicators ( $\bar{X}$ ,  $S_{\bar{X}}$ , S,  $M_o$ ,  $M_e$ ,  $C_v$ ), appropriate



graphs were used to present the indicators. The analysis of causality of relations among variables was measured using non-parametric  $\chi^2$  test with the significance levels 5% and 1%. The correlation strength between variables that showed significance in the previous test was measured using Spearman correlation coefficient (rank-order correlation product), based on the following expression:

$$r_s = 1 - 6 \frac{\sum_{i=1}^n d_i^2}{n(n^2 - 1)}$$

with  $d$  being the difference in rank values between two variables observed, and  $n$  number of different series.

The survey was carried out in the period July-August 2017 in the municipalities Kolašin, Pljevlja and Podgorica. Out of the sample of 50 female respondents, 50% are based in Kolašin, 30% in Pljevlja and 20% in Podgorica.

### Business climate in Montenegro

Montenegro as an EU candidate country is preparing for participation in the single EU market by building its capacities within the EU development goals by 2020: smart growth, sustainable growth and inclusive growth. For small countries, such as Montenegro, activities contributing to the activation of all human resources are important. Creating conditions for entrepreneurship development is a priority task in the EU, in particular through the implementation of the principles set out in the SBA – Small Business Act. Montenegro's ambition is to become an entrepreneurial society with a high quality and flexible attitude towards small and medium-sized enterprises and entrepreneurs, both male and female (Nacrt strategije zenskog preduzetništva, 2017). According to the Global Competitiveness Report (*GEM*) 2012/2013, Montenegro took 72<sup>nd</sup> position by economy's competitiveness, tumbling 12 places compared to 2011/2012 (Table 1).

**Table 1.** Montenegro's position in global competitiveness, compared to countries of the region

Country /Year	GCI 2009-2010	GCI 2010-2011	GCI 2011-2012	GCI 2012-2013
Slovenia	37	45	57	56
Montenegro	62	49	60	72
Croatia	73	77	76	81
Serbia	93	79	95	95
BiH	109	96	100	88
Macedonia	84	88	79	80
Albania	96	102	78	89

\***Sources:** Assessment of environment for female entrepreneurship in Montenegro, Union of Employers of Montenegro

Compared to data for the period 2011-2012, Montenegro's decline in the period 2012-2013 was recorded in the following areas: institutions, infrastructure, macroeconomic stability, health and primary education, higher education and training, efficient goods market, efficient labour market, financial market, ability to harness benefits of existing technologies. In foreign investors' opinion, the best environment is in the sector of telecommunications and information technologies, tourism, followed by trade, transport and logistics (Unija poslodavaca CG, 2013).

Foreign investors in Montenegro evaluated also a number of specific areas of importance for business (on a 1 to 10 scale). The results show that the best rated were taxes with the score 6.3, followed by corporate management with 5.5. Property development and rule of law had a score of 4.8. Labour market and employment had the worst score of 4.3.

During an in-depth analysis, foreign investors identified a number of key areas that need to be improved in order to improve the business environment:

- *Labour market and employment*: still not sufficiently flexible due to mandatory permanent employment contracts and high severance pays.

- *Property development*: major development potential, burdened with inadequate regulatory framework and business barriers in the form of administrative procedures.

- *Taxes*: generally favourable tax regime at the national level. Local taxes and levies charged by municipalities give rise to concern, as well as ad hoc introduction of some levies.

- *Corporate management*: major changes needed in the fields of accountability, legislation and bankruptcy practice.

- *Rule of law*: issues such as lengthy and complicated court proceedings, lengthy administrative procedures and public service still present.

As Montenegro's legislation and market are being harmonized with the European, it is important also to present the problems identified in business operations of small and medium sized enterprises (SMEs) in the European Union.

*Bankruptcy issue*: In the EU, 15% of company closures is caused by bankruptcy. Almost 700,000 SMEs go bankrupt every year, resulting in a loss of 2.8 million jobs. Entrepreneurs not only have no desire to start a new business following the bankruptcy, they are also facing lengthy bankruptcy procedure that may take between 4 months and 9 years.

*Issue of excess regulatory and administrative burden*: SMEs are much more burdened and they spend up to ten times more than big companies for the same liability (e.g. if big companies pay €1 per employee, SMEs spend up to €10).

*Issue of complicated and lengthy administrative procedures*: Administrative burden is not only expensive, it is also time-consuming.

*Issue of insufficient participation of SMEs in public procurement*: Public procurement accounts for 16% of the European Union GDP, but SMEs do not participate sufficiently. Such situation is caused by poor information on existing

public procurement and lengthy and complicated procedures that discourage their participation.

*Issue of inadequate education and training of SMEs staff as well as management:* More than 60% of companies stated that entrepreneurs and their employees do not acquire the skills necessary in schools.

*Environmental protection issue:* Compared to big companies SMEs introduce energy and material efficiency to a much lesser extent, although prices of energy and raw materials are growing.

### **Socio-economic context of entrepreneurship development in Montenegro**

Historically, entrepreneurship in Montenegro does not have a long tradition. The initial stage of entrepreneurship development in the first half of 20<sup>th</sup> century was stopped with establishing of socialism. Socialism model in Montenegro was the model of “a political society”. The main socialism trait is strong dominance of ideology in all social spheres (Lazić, 1994, Pečujlić, 1980).

Over the past few years, entrepreneurship development in Montenegro has been followed primarily through development of small and medium sized enterprises. Economic life is closely connected with overall social relations. Economic actions are never driven by economic interests only, but social interests as well (Granoveter, 2000). All that points to existence of a significant relationship between economic and social factors that cause changes in economic and social life (Ćeranić, 2013). Montenegro’s economy is an economy with a relatively short history of entrepreneurial principle implementation.

Free market and private ownership did not exist in the socialist era. Independent business was considered a deviant business form. Post-socialism transformation in Montenegro began without the entrepreneurial class. In developed European countries, emergence of entrepreneurial elite and capitalist class had its own natural development cycle. In post-socialist countries, the state abolished the socialist system and created conditions for emergence of market economy. All countries that started capitalistic transformation late have developed “top-down” capitalism (Sekulić and Šporer, 2000).

The fall of socialism and beginning of post-socialist transformation was marked with blocking of reforms, armed conflicts and sanctions in former Yugoslavia. Citizens developed entrepreneurial skills in the informal economy zone, fighting for survival (Bolčić, 2008, 2006).

After 1990s, the process of economic restructuring and economic reforms begins. Development of entrepreneurship is characterized by issues and weaknesses. Entrepreneurs are not a social class strong enough to support more intensively the socio-economic development. This period was the period of development of capitalism without capitalists (Bolčić, 1994, Lazić, 2011). In the transition period, the conditions for development of entrepreneurship were less favourable for women than for men. This was caused by deeply rooted patriarchal relations marked with gender inequality. In the period of socialism,

women achieved great progress in emancipation. Progress was achieved in the field of education and participation at the labour market. Patriarchal patterns of gender roles and relations were preserved in the sphere of privacy. Most of the responsibility for taking care of the family and household was laid on women (Milić, 1994, Blagojević, 2000). The traits related to a typical Montenegrin woman are: obedience, loyalty, diligence, altruism, passivity and patience (Vujačić, 1980). These values are inherent to her “female nature” and provide grounds for legitimacy of her gender (in)equality (Popović, 2013).

Following the restoration of independence and economic reforms implemented in Montenegro, the legal and institutional framework was put in place, which supports gender equality, development of women’s rights and economic empowerment of women. Formal preconditions have been provided, but practice shows that adequate incentive mechanisms are lacking. In economies as traditional as Montenegrin is, women are not sufficiently recognized as equal players in economic field. This results in a worse starting position when launching but also when developing their own business. Patriarchal behavioural patterns and opinions are still deeply rooted in Montenegrin society (Popović, 2013).

Economic conditions under which entrepreneurship has been developing in Montenegro in the past ten years are characterized by the following: proclamation of independence in 2006 was followed by a major inflow of foreign investments into Montenegrin economy. In this period, Montenegro’s ease of doing business was rated 5.8 of 10 by the Foreign Investors Council. According to the Starting a Business Index in 2013, Montenegro took 58<sup>th</sup> place out of 185 countries surveyed. It is the year in which the fall from 45<sup>th</sup> to 58<sup>th</sup> place was recorded. In this same period, worse rankings also applied to Croatia (fell from 72<sup>nd</sup> to 80<sup>th</sup> position), Slovenia (from 28<sup>th</sup> to 30<sup>th</sup>) and Bulgaria (from 48<sup>th</sup> to 57<sup>th</sup>), while Serbia climbed (from 91<sup>st</sup> to 42<sup>nd</sup>). From the viewpoint of the European integration process, in 2012 the European Council verified the decision on opening of accession negotiations with Montenegro. An important step for Montenegro’s integration into contemporary economic relations was its gaining the full membership in the WTO (Unija poslodavaca CG, 2013).

According to the *Global Entrepreneurship Monitor (GEM)* survey grouping countries by the stage of economic development, in 2010 Montenegro was placed in a group of efficiency-driven economies. The survey shows that Montenegro was above average when it comes to entrepreneurial activity, but it contributes to generation of the new value (measured by Gross Domestic Product) to a much lesser extent than it was the case with other countries in the same group (Nacrt ženskog preduzetništva u CG, 2016). The fact that the reason for starting a business undertaking in Montenegro is need, rather than opportunity, significantly affects the lower contribution to generation of new value. In almost all countries, the share of women in entrepreneurial activity is lower than of men. Montenegro belongs to the group of countries with the share

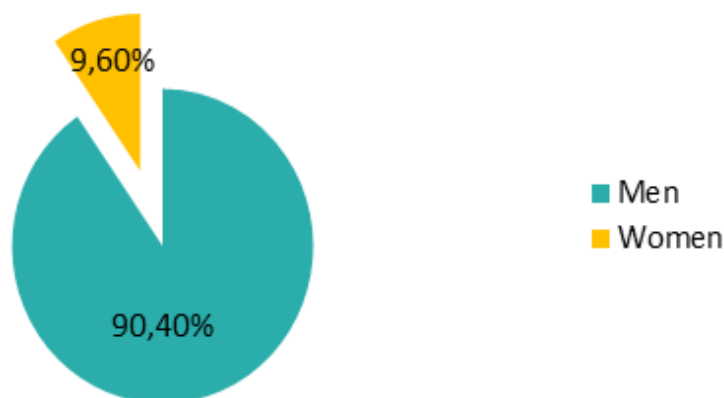
of women lower than the global average (Nacrt ženskog preduzetništva u CG, 2016).

The characteristics of socio-economic context of entrepreneurship development in Montenegro described above indicate the need for a stronger and more intensive institutional support. Complexity of legislation and regulations that entrepreneurs have to follow is an aggravating circumstance. It may pose an insolvable issue for a large number of small and micro entrepreneurs. Pronounced gender inequality in the sphere of economic participation and privacy contribute further to unfavourable conditions for female entrepreneurship. In Montenegrin society, women are primarily observed in the context of traditional role they have as wives and mothers. The attitude of the society towards female entrepreneurs is not negative, but they are expected to successfully perform their family duties. The support that women need in the private sphere is lacking both from men and from the state.

### **Economic environment for development of female entrepreneurship in Montenegro**

In the European Union, entrepreneurship is identified as the main driver of a modern and dynamic knowledge-based economy. Experiences of other countries show that in order to get to a higher level of development of female entrepreneurship, institutional support is needed. That is the only way in which entrepreneurial potential of women can give its full contribution to development of both the economy and the modern society in Montenegro.

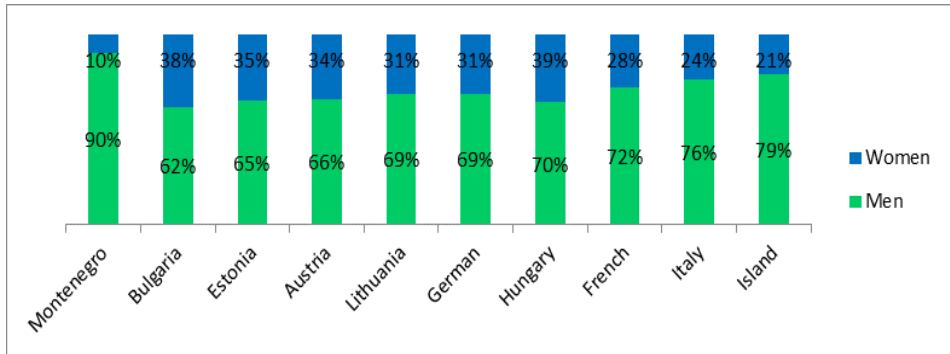
According to the population census 2011, the total number of business entities in Montenegro was 21127. Of that number, men are owners in 90.4% and women in 9.6% (Monstat, 2012) (Figure1).



\*Sources: Statistics Office of Montenegro, Monstat, pp.24

**Figure 1.** Owners of business entities in Montenegro, in 2011, by gender.

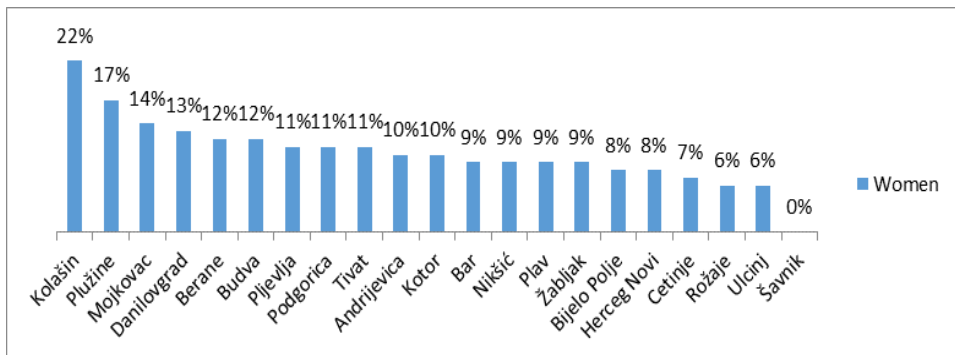
When data on Montenegro are compared to the data from other European countries, it can be noted that the share of women owners of business activities is the lowest (Figure 2).



\*Sources: Owners of business entities in Montenegro in 2011, by gender”, Monstat, 2011, pp.43

**Figure 2.** Owners of business entities by countries

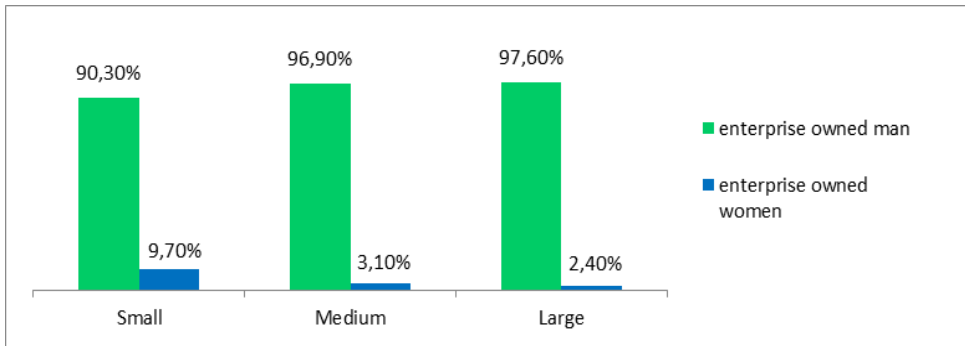
The overview of percentage of business entities owned by women by municipalities is given in Figure 3. The highest percentage of business entities owned by women is recorded in the Municipality of Kolašin, while there are no businesses owned by women in the Municipality of Šavnik.



\*Sources: Owners of business entities in Montenegro in 2011, by gender”, Monstat, 2011, pp. 43

**Figure 3.** Owners of business entities in Montenegro in 2011, by gender

Most of enterprises in Montenegro in 2011 belonged to small enterprises - 98.7% (or 20855). Women as owners of business activities are most represented in this group of enterprises (9.7%). Only one woman is the owner of an enterprise that belongs to the group of large enterprises (Figure 4).



\*Sources: Owners of business entities in Montenegro in 2011, by gender”, Monstat, 2011, pp. 43

**Figure 4.** Owners of business entities, by company size

Emergence and development of female entrepreneurship over the last 20 years is closely linked to the process of achieving equality in men’s and women’s rights and overall democratization of Montenegrin society. The transition period was marked with a lower production volume, faster privatization and transformation of business organizations. This caused an increase in unemployment, exacerbated poverty in some social groups and change in the lifestyle of men and women. Economic stagnation affects both genders. Statistical data show that women are in a more difficult position. This is reflected in higher unemployment and poverty rates. Such economic circumstances result in perceiving entrepreneurship as an important way to secure income through self-employment. Conditions for entrepreneurship development are still less favourable for women than for men. This situation is caused by deeply rooted patriarchal views of men, but also of women in Montenegro. Gender inequality is reflected also in: insufficient political participation of women, economic inequality, unequal distribution of family duties, differences in ownership rights, etc.

Female entrepreneurship in Montenegro today is at an early developmental stage that can be called “necessity entrepreneurship”. The entrepreneurship is characterized by the need to find an alternative to finding a job with an employer.

Global economic crisis effects were felt strongly in Montenegro. Around 60% of households were affected, while that percentage in the region amounted to 49%, with 31% in Europe.

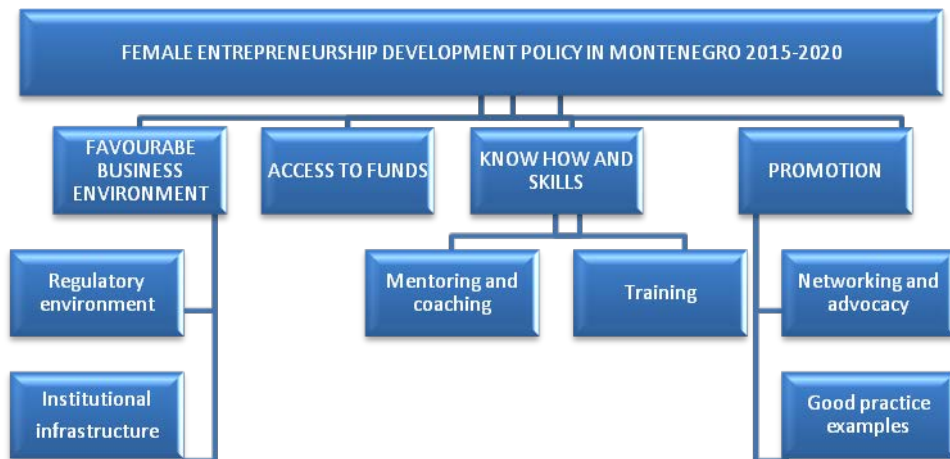
Unemployment was the result of economic crisis. In 2011, the unemployment rate in Montenegro was 19.7%. This is significantly above the EU average where 10.2% of active workforce was without a job (Unija poslodavaca CG, 2013). According to statistical data on workforce, unemployment rate in 2012 was higher among women. Women work in less profitable sectors. Managerial positions and entrepreneurial activities are more represented among male population. In the working age population (above 14 years of age) share of women is higher than men. There are more men in active workforce in

Montenegro than women, while women are more numerous in the inactive population category (Unija poslodavaca CG, 2013).

According to education level, on average, the number of employed women with college or university degree is higher than men. Around 33.2% of women graduated from a college or university, compared to 26.2% of employed men. Most of economically active men and women in Montenegro, regardless of their current labour status, have secondary education. Male population is characterized by a significantly higher level of entrepreneurial activity. Private business is owned by 19.2% of employed men, while the share of women in this employment form is 9.3%.

Existing constitutional setup and legislation in Montenegro provide a positive regulatory framework that enables addressing the gender equality issues. Deeply rooted patriarchal stances of men, but also women in Montenegro still pose a major barrier to female entrepreneurship development. Permanence of such a situation highlights the insufficient effect of various programmes, measures and initiatives. Current situation requires designing of a long-term vision and strategic policy for a gender balanced sustainable economic development. Intensification of entrepreneurial activity of women can generate new jobs, improve competitiveness and contribute to overall economic growth. On a long term, this process can assist in dismantling cultural, social, legal and political obstacles that women in Montenegro face. Diagram 1 below provides an overview of the structure of female entrepreneurship policy and key measures for its implementation by 2020.

**Diagram 1.** Female entrepreneurship policy structure in Montenegro 2015-2020



\*Sources: Draft Female Entrepreneurship Strategy of Montenegro 2015-2020, Directorate for Development of Small and Medium Enterprises of Montenegro



## RESULTS AND DISCUSSION

### Survey results

The Table 2 below provides an overview of socio-demographic characteristics of respondents. With regard to age structure, most of them are above 36, of which more than 40% belong to the age group 36-50. With regard to education, 62% of respondents have secondary education while the number of those holding university degree is much lower (only 20%). Most of them are married (72%) and live in a four-member (28%) or five-member (26%) household.

**Table 2.** Socio-demographic characteristics of female respondents

Element	Absolute frequency	Relative frequency
<b>Age</b>		
15-25	2	4.0
26-35	9	18.0
36-50	21	42.0
51-65	17	34.0
above 65	1	2.0
<b>Education</b>		
elementary	2	4.0
secondary	31	62.0
college	7	14.0
university	10	20.0
<b>Marital status</b>		
married	36	72.0
single	6	12.0
divorced	5	10.0
widow	3	6.0
<b>Household members</b>		
one	3	6.0
two	4	8.0
three	12	24.0
four	14	28.0
five	13	26.0
six and above	4	8.0

**Sources:** Survey results

### Business status of female entrepreneurs

In order to analyse the business status of female entrepreneurs prior to starting their own business, the respondents were asked to respond to the question: "had they been employed before". Out of 50 respondents, 56% answered they had been employed before they started their own business. Most of respondents (34%) were employed in industries (other) that were not included in the options given. Furthermore, a significant part of them had gained experience by working in the private sector (22%), as well as in public

enterprises (20%). Monthly salary had been up to EUR 200 for 78% of respondents. Most of the respondents opted for business within the service sector (38%) and trade (38%), followed by wholesale 6%, marketing 4% and consulting 2%. Most of them - 63% have been entrepreneurs for more than 9 years, a significant number of them for 1-3 years and in other time intervals their number is significantly lower and rather even.

### Motivation

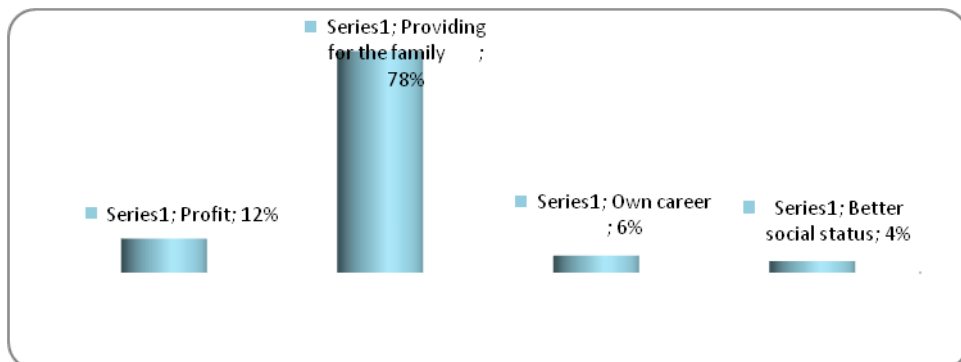
For further analysis, descriptive statistics indicators of characteristics analysed were defined, results of which are presented below (Table 3).

**Table 3.** Socio-demographic characteristics of respondents

Variable	$\bar{x} \pm s_x$	Median	Modus	St.dev.	Cv(%)
Motives	2,02±0,083	2	2	0,589	29,16
Family duties	2,46±0,183	2	2	1,297	52,72
Cooperation with men	3,72±0,134	4	4	0,948	25,48
Cooperation with women	3,86±0,114	4	4	0,808	20,93
Cooperation with banks	3,12±0,136	3	3	0,961	30,80
Cooperation with public institutions	3,64±0,110	4	3	0,776	21,32
Status in the society	3,84±0,129	4	4	0,912	23,75

\***Sources:** Author's calculation

On a scale of 1 to 5 (1=most important) by ranking the importance of specific motives that induced the respondents to engage in business, in majority of cases the score was two. Average value of the score was 2.02, with almost equal values of central tendency measures (Table 2). Analysis of the motives that induced the respondents to engage in business shows that the most frequent motive was **providing for the family** (78%), while profit (12%) or career building was significantly less frequent (6%), (Figure 5).



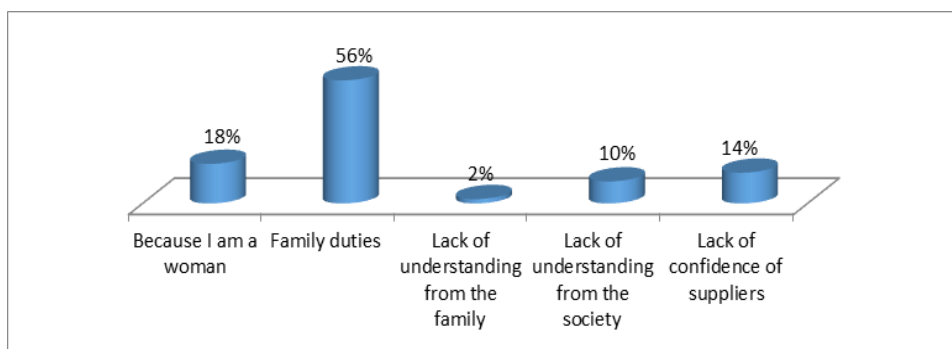
\***Sources:** Survey results

**Figure 5.** Main Motives for being a Woman Entrepreneur

### Problems and barriers in business startup

In answers to questions in the survey, the female entrepreneurs underline that the major barrier in business startup was lack of funds (66.7%) lack of confidence of credit institutions (20%). A significantly lower number of entrepreneurs stated lack of family support as the reason (2.7%), lack of support from the spouse (2.7%) and other factors. Due to lack of funds, they had to take loans (84%) and addressed banks in order to start their business (68%) and to a much lesser extent the investment-development funds (4%), micro-credit institutions (6%) or some other funding sources. For those needs, most of them took loans ranging from 1000€ to 5000€ (56%), and significantly less took loans above 5000€

Reasons limiting or preventing successful business vary: family duties, no understanding from the family or society, distrust of suppliers or the very fact that they were women. The survey showed that more than 56% of respondents state that family duties was the most important factor of limitation to successful business, although gender inequality (sex) was pronounced (18%). All other reasons are less significant (Figure 6).



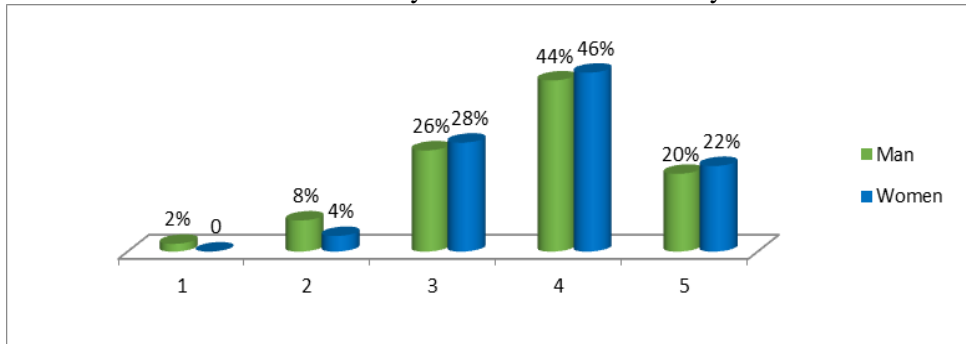
\*Sources: Survey results

**Figure 6.** Business limitation factors

By ranking all the options given for barriers to doing business on a scale of 1 to 5 (1-most important), the descriptive statistics indicators showed that family duties as the main barrier got the average score of 2.46, while most of respondents selected 2 for this factor of limitation, as an important turning point in work ( $Mo=2$ ), Tab. 2.

In order to analyse cooperation with business entities owned by men, the respondents were asked to give scores ranging from 1 to 5 (1-least efficient, 5-most efficient). Average score was 3.72, which can be considered satisfactory. Most of respondents (44%) gave 4 to cooperation, and not so small number of them gave it also 3 (26%), as well as 5 (20%), (Figure 7). Also, one of the objectives of the survey was to hear the experiences in cooperation with business entities owned by women. Is there any difference to the previous conclusion where owners were men? The conclusion is similar. Namely, on a 1 to 5 scale (1-least efficient, 5-most efficient) most of respondents (46%) evaluated the

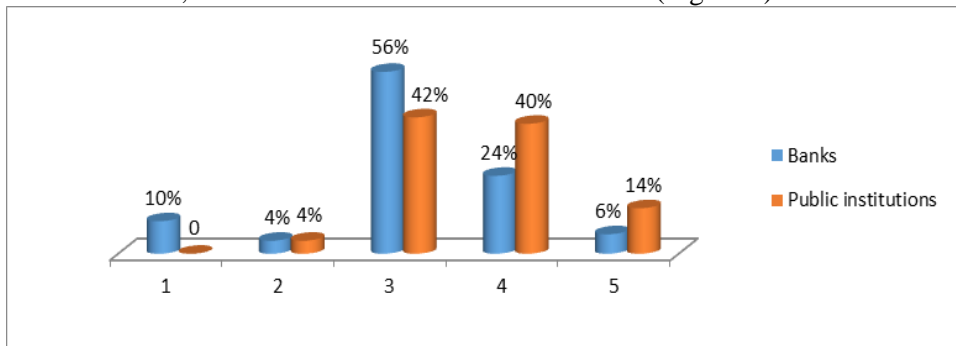
cooperation with 4, so average value of the score at the sample level was 3.86. The structure of scores given to this question is illustrated in Fig. 4 below. The graph clearly shows there are no major differences in assessment of cooperation between business entities owned by men and those owned by women.



\*Sources: Survey results

**Figure 7.** Assessment of cooperation with businesses owned by men and women

Cooperation with banks got the average score of 3.12 (Tab. 2), while on a 1 to 5 scale (1-least efficient -5 most efficient) most of respondents (56%) opted for 3 when it comes for efficiency in cooperation with banks. It should also be noted that a not so small number of respondents chose 4 (24%) and 5 (6%), to evaluate the cooperation with the bank sector. In that regard, this cooperation could be better, but the current situation is not that bad (Figure 8).

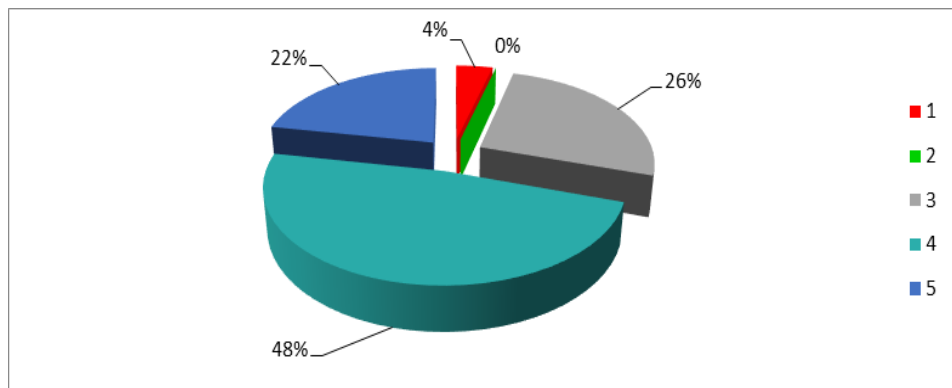


\*Sources: Survey results

**Figure 8.** Assessment of cooperation with banks and public institutions

The survey also covered the satisfaction with cooperation with public institutions. Most of respondents (82%) assessed the cooperation with 3 or 4 (42%, and 40%, respectively). Therefore, this satisfaction scored slightly better than cooperation with the bank sector (Figure 8). It is quite interesting to note that none of the respondents gave the worst mark (one). Average score of this cooperation is 3.64 (Table 2).

Current social status of the respondents, i.e. their status as a woman in the society was assessed as very good. Namely, 48% stated that their status was very good and that it could be given the mark 4. Also, a not so small percentage of them (22%) evaluated their status with the highest mark 5 (Figure 9). That is why the average score at the sample level is 3.84 (Table 3).



\*Sources: Survey results

**Figure 9.** Structure of satisfaction with the status in the society

When it comes to property ownership, 24% of respondents are owners of agricultural land, 18% house owners, 8% holiday house owners, apartment owners 6%, office space owners 4% and other property 40%.

#### **Relationship between socio-demographic characteristics of female entrepreneurs and motives for engaging in business**

Further research focused on identifying the relationship between socio-demographic characteristics of female entrepreneurs and motives for engaging in business. As an instrument to measure the strength of that relationship  $\chi^2$  test was used. First, existence of a relationship between age and motives for engaging in business was checked. By testing the null hypothesis (no relationship) and calculation of the coefficient  $\chi^2=6.611^{NS}$  ( $p=0.882$ ), it was shown that the coefficient is not statistically significant and the null hypothesis is accepted. Therefore, age is not an important factor as a motive to start a business. More precisely, there is no relationship between age and starting business.

The next question is whether education determines starting a business. Null hypothesis was set up (there is no relationship between education level and starting a business) and checked with  $\chi^2$  test. The calculated value of the coefficient  $\chi^2=9.638^{NS}$  ( $p=0.381$ ) shows that the hypothesis can be accepted, because Pearson coefficient has no statistical significance. Therefore, not even the education level is significant as a motive for starting a business. There is no statistically significant relationship between them.

Can marital status be important for doing business? This is a very interesting question that was checked using the same nonparametric  $\chi^2$  test. Pearson coefficient value was  $\chi^2=32.888^{**}$  ( $p=0.000$ ), which shows that the null

hypothesis (there is no relationship between the marital status and motives for engaging in business) is rejected at the level of 0.01. Marital status is a very important motive for starting a business. This is understandable, since the most important motivation for doing business is to provide for the family. Value of Spearman test coefficient shows that the relationship has a moderate strength ( $r=0.695^*$ ).

In further analysis, the question was whether number of family members is an important motive for doing business. Null hypothesis (there is no relationship between these sociological characteristics) was checked with  $\chi^2$  test. The calculated coefficient value was  $\chi^2=35.923^{**}$  ( $p=0.002$ ), showing that the hypothesis set up on independence of the variables is rejected at the level of 0.01. The conclusion is that the number of household members is a very significant motive for doing business. The strength of that relationship expressed with Spearman coefficient is moderate ( $r=0.585^*$ ).

Can salary level that the entrepreneurs covered by the survey had received when they had been employed by other employers be one of motives for starting one's own business? Null hypothesis: there is no relationship between the two variables. Coefficient value of  $\chi^2=22,899^{NS}$  ( $p=0.195$ ) shows that the hypothesis set up can be accepted and that there is no statistical significance between the two variables. The salary that the respondents had received in the previous business environment had not induced them to abandon the job and start their own business.

Also, dependence between motives for doing business and activities that the female entrepreneurs opted for was observed. Since Pearson coefficient value amounts to  $\chi^2=34.801^{**}$  ( $p=0.01$ ) is very significant, it shows that the starting hypothesis is rejected. More specifically, there is a significant relationship between the motives for doing business and activity that the female entrepreneurs had opted for. Correlation coefficient shows that the strength of the relationship was moderate ( $0.699^*$ ).

Dependence between variables education and status in the society was analysed as well. The assumption was checked using the nonparametric test ( $\chi^2$ ). The coefficient value determined ( $\chi^2=14.840^{NS}$ ,  $p=0.095$ ) shows that there is no causality and statistically significant relationship between these variables, so the starting hypothesis has to be accepted. Therefore, there is no relationship between the education level and assessment of the current social status of the respondents.

## CONCLUSIONS

Existing legislation in Montenegro provides a positive framework for addressing the gender equality issues. Deeply rooted patriarchal views of men, but women as well, pose a major barrier to female entrepreneurship development.

Gender inequality is reflected through uneven distribution of family duties, economic inequality, property ownership, etc.

Characteristics of female entrepreneurs in Montenegro were identified based on the survey carried out in the period July-August 2017. The survey was

carried out in Municipalities of Kolašin, Pljevlja and Podgorica. According to results of this study, most of the female entrepreneurs surveyed have secondary education and are aged 36-50. When it comes to marital status, most of them are married and live in a four-member household. Most of them have been entrepreneurs for more than 9 years and a significant number of them gained experience in the private sector.

Most of the entrepreneurs opted for business in service and trade. The main motive for engaging in business for most of the entrepreneurs was to provide for the family. They face various barriers in their business, most significant being the lack of funds and lack of confidence of credit institutions. Because they lacked funds for business startup, in majority of cases they addressed the banks. For those needs, most of them took loans from 1000-5000€ Family duties are the most important factor of limitation for successful business. The entrepreneurs have good cooperation with business entities owned by men. There is no major difference with regard to assessment of cooperation between entities owned by men and those owned by women. Cooperation with public institutions is assessed as better than cooperation with banks. The entrepreneurs assessed their current social status as very good.

The results of the survey show that the major problem women face is striking a balance between family and business. Female entrepreneurs need adequate institutional support. In order to make progress in the field of female entrepreneurship, we are giving a number of recommendations:

- *Creating a better business environment for female entrepreneurship development.* Further improvement of institutional infrastructure is necessary, which will ensure full provision of non-financial support, mentoring and coaching services and provision of business related information to female entrepreneurs. In initial stages, access to business premises should be made easier.

- *Better access to funding.* In this area, further improvement of current as well as development of new instruments is needed, in order to enable female entrepreneurs a simpler access to funding.

- *Provision of know-how and skills necessary.* Various forms of training courses assist potential and existing entrepreneurs to acquire the know-how and skills for business development. Specialized training is necessary in the first years, and is aimed at further improvement and acquiring of specialized entrepreneurial skills.

- *Promotion, networking and advocacy for female interests.* Female entrepreneurship promotion should be given a priority in further development of Montenegro. By changing the mindset with regard to the significance and role of the female entrepreneurship, creation of a critical mass is possible, which will place female entrepreneurship in the centre of economic development. Association of female entrepreneurs and putting in place the process of advocating their interests before public sector is strategically important.

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## EFFECTS OF DRYING AND EXTRACTION METHODS ON ANTIOXIDANT PROPERTIES OF HORSEMINT (*Mentha longifolia* L.)

### SUMMARY

*Mentha longifolia* has the widest natural geographic distribution of any menthe species, from Western Europe to central Asia and in southern Africa. The current study was conducted to determine the antioxidant capacity of extracts and essential oil taken from aerial portions of *M. longifolia* L. The plant materials were dried by three different techniques (shade, sun and oven drying). Collected horsemint shoots were grinded then for evaluating effect of different extraction methods (maceration by aqueous, ethanolic and hydroalcoholic solvents, soxhlet and essential oil by Clevenger) and drying technique (shade drying, sun drying and oven drying) were used. Total phenol content (TPC) and total flavonoid content (TFC) of the samples were measured. Furthermore, total antioxidant capacity (TAC) was evaluated by both phosphomolybdenum and DPPH% assays.

Soxhlet extraction of shade drying had highest TPC and TFC among other extracts a drying methods. In terms of antioxidant activity, essential oil obtained from Clevenger displayed high antioxidant capacity, resulting in a higher radical scavenging ability, which can be attributed to the lower temperatures in shade dried plant material and the higher stability of the extracted compounds. But by exposure the shoots to sun or high temperature (oven) decreasing of bioactive compounds amounts present in plant material were observed. There was a significantly positive correlation ( $P < 0.01$ ,  $r > 0.7$ ) between TPC and TFC in all drying techniques. Also a significant correlation was observed between TAC and %inhibition ( $P < 0.01$ ,  $r > 0.8$ ) in sun drying extracts. Extraction and drying the herbal material influences the bioactive compounds and antioxidant properties.

**Keywords:** *Mentha longifolia* L., extraction, antioxidant capacity, drying, total phenol content

### INTRODUCTION

*Mentha longifolia* L. Hudson as a member of lamiaceae (mint family) is a perennial and aromatic herb that most of the time is observed in moist and nearly shaded places (Sher and Khan, 2007; Shinwari et al., 2011). The aerial parts and leaves alone are added as condiment in salads and cooked foods (Facciola, 1990). In Iranian folk medicine has been introduced as carminative, sedative of stomach pains and antispasmodic (Zargari, 1990). In herbal medicine assumed to be especially useful in construction of immune system and protecting against

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secondary infections. The plant is consumed for cure of cough, cold and gripe. Extraneously, the usage of wild mint is for curing of sores and inflammation of glands (VanWyk et al., 1997). Also, horsemint is usually used in Iran in the form of dried that is added to Iranian conventional cheese or as herbal tea. Lamiaceae herbs are very rich sources of antioxidant compounds (Shan et al., 2005) which could be beneficial in prevention of oxidative reactions in foods and plants organs.

The antioxidants are chemical compounds that have the capability to counteract free radicals by reducing and scavenging these radicals or their activities (Pisoschi and Negulescu, 2011). The key role of antioxidant compounds is demonstrated in many reports in dietary plants such as flavonoids, carotenoids, proantocyanidins, benzoic acids and derivatives and phenolic compounds (Capecka et al., 2005). The drying process can lead to degradation of bioactive compounds (Chan et al., 2009), however it can also release the bound bioactive compounds to result in high recovery yield (Do et al., 2014). The effects of different drying methods on the chemical content and biological activities of essential oils of *Salvia officinalis* and *Juniperus phoenicea* has been established (Do et al., 2014; Ennajar et al., 2010). Aromatic herbs and spices are most sensitive to drying techniques and this raised biological deterioration. Therefore a high quality product is achieved when the drying process is carried out carefully.

Drying techniques are necessary in conservation and post-harvest of herbs. By decreasing the moisture content less than 15%, drying inhibits from any microbial reaction. Factually, effective drying methods will increase the quality of dried material such as aroma and appearance by preventing any biochemical changes (Facciola, 1990). The most drying methods in medicinal herbs that have been used is drying in shade, then oven or low temperature drying. The ratio of varied ingredient is also will affected by drying techniques (Gulluce et al., 2007). Many reports have showed the effect of sample preparation, processing and extraction methods on the phenolics efficiency (Hajlaoui et al., 2009; Handa et al., 2016; Horwitz, 1984). It was also expressed that the drying methods affects chemical composition and antioxidant properties of yam flours to various limits (Hossain et al., 2010). Therefore, it is necessary to identify a suitable thermal drying method to not only retain the bioactive compounds, but also minimize the cost to producers.

The hot air drying is widely used, but it lead to thermal harm and can strongly change the volatile compound and color of herbs (Hsu et al., 2003). Ambience temperatures and temperatures less than 50 °C are the best to preserve aromatic compounds (Ibáñez et al., 1999). However, the loss of phenolic compounds and antioxidant activity in hot air drying achieved up to 60% in comparison with freeze drying (Ji et al., 2012). It is recorded that oil yield in *Mentha longifolia* in dried state was three fold high than fresh state (Katalinic et al., 2006).

Extraction is an important step in the phytochemical processing for the discovery of bioactive constituents from plant materials. Also it is necessary to find the most effective solvent for the extraction of chemical compounds from a targeted material as previous studies have revealed that extraction solvents have a significant impact on extraction efficiency of bioactive compounds from plant materials (Katsube et al., 2004). It is necessary to consider that even though the maximum extraction yield is always procured to meet the requirements for functional activities, especially when they are sought to be applied in food or medicinal industries, the fitness and environmental risks must be taken into consideration when a solvent is chosen for extracting of herbs (Khan et al., 2011; Lim and Murtijaya, 2007). The polarity of the solvents of extraction influences the solubility of chemical constituents in the samples and therefore their extraction efficiency. Usage of water with other organic solvents by creating a polar medium is useful for extraction of polyphenols because the contact surface area between plant matrix and solvent and turgidity of herb substances will enhance the extraction efficiency (Lindsay and Astley, 2002).

In present study we evaluated the effects of some extraction methods and three drying types on antioxidant content and free radical scavenging activity of *M. longifolia* L. shoots. Also total phenol and flavonoid contents of various extracts and essential oil were evaluated.

## MATERIAL AND METHODS

### Plant material

The aerial parts of *M. longifolia* L. were gathered from the wild nature toward Hervi (Paveh county, Kermanshah province with 46° 14' Longitude, 35° 7' Latitude and 650 m Altitude) at March 2016. Plant material was disported into three portion. One portion was dried in front of direct sun; another portion was located in in a dark room, without radiating of direct sun to be dried and, while third portion was put in the oven to be dried (Mors, Model: LX-5340, Turkey) which was kept at 70 °C, 1 hour.

### Extraction of non-volatile compounds of the samples

The shade dried aerial parts of *M. longifolia* were powdered by a blender and then macerated in solvents that was included: Water, Hydro alcoholic (70% ethanol/ water) and ethanolic solvents according to 5% dry weight. Samples were kept in a dark room about 24 hours, then were sat on a shaker about 6 hours. Afterwards for the filtering of the extracts were used from a whatman filter paper No.1 and finally all samples were kept in sterile vessels impervious to air and light, in a refrigerator for further analysis.

### Extraction of volatile compounds of the samples

About 20 g each of the dried shoots were separately hydro distilled for 3 h, and for this purpose was helped from a Clevenger-type apparatus. Also for complete isolation of process about 20 ml ethanol was added to apparatus. Because of sensitivity of oils to light, oxygen and temperature and changing of

their components in this conditions, so the isolated oils were kept immediately into a sealed dark vials for further analysis.

#### **Extraction with soxhlet**

About 20 g each of the dried shoots were separately combined with chloroform or n- hexan for 30 min to separate the non-polar compounds such as lipids. Then were filtered and plant materials were placed into a whatman filter paper in the apparatus. Isolation process was done with 150 ml ethanol 96% in 90 °C.

#### **Total phenol content (TPC)**

For measuring of TPC was used from Folin-Ciocalteu method (Martysiak-Żurowska and Wenta, 2012). About 0.5 ml of the sample extract was admixed with 1ml of 7.5% (w/v)  $\text{Na}_2\text{CO}_3$ . Then 1 ml of Folin-Ciocalteu reagent and 1 ml of deionized water was added. Afterwards, incubating of the mixture was carried out at room temperature for 30 min. Its absorbance was recorded using spectrophotometer. Gallic acid was used as a reference standard and TPC being reported as mg Gallic acid equivalents per gram of dried weight (mg GA/g DW).

#### **Total flavonoid content (TFC)**

For measuring of TFC was used from aluminum chloride method (Nguyen et al., 2016). To brief, 0.2 ml of the sample extract was added to 0.8 ml of deionized water, then were admixed with 1 ml of 2% (w/v) aluminum chloride solution (5% Acetic acid solution into methanol). Eventually, related mixture was kept 30 min. The absorbance of samples was measured at 430 nm using spectrophotometer. The standard curve of various concentration of quercetin (0, 1, 2, 4, 6, 8 and 10mg/l) were prepared. TFC was reported as milligram quercetin equivalent per gram of dried weight (mg QU/g DW) was recorded.

#### **Antioxidant capacity**

##### **Reducing Power Assay**

The phosphomolybdenum method was used for evaluating the total antioxidant capacity of the extracts (Nickavar et al., 2010). 0.3 ml of extract solution (1 mg/ml) from each extraction sample was picked up and then 3 mL of reagent solution (6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added. The obtained mixture was placed at 95 °C for 90 min. Finally, the absorbance of the solution was measured at 695 nm against a blank. Ascorbic acid was applied for drawing of standard curve. Total antioxidant capacity of the extracts was evaluated as  $\mu\text{g}$  ascorbic acid equivalents per gram of dried weight ( $\mu\text{g}$  AA/g DW).

##### **DPPH assay**

The free radical scavenging activity of the plant extracts was assessed using 1, 1- diphenyl-2- picrylhydrazyl (DPPH) (Omidbaigi et al., 2004). 1 ml of the plant extract was admixed with 1 ml of methanolic DPPH 0.2 mM and left for 30 min in 25 °C. The absorbance of admixture was recorded in 520 nm. Methanol without extract was applied as control and free radicals scavenging activity was measured using following formula:

$$\% \text{inhibition} = \left[ \frac{(\text{OD control} - \text{OD sample})}{\text{OD control}} \right] \times 100$$

Where, %inhibition was free radical inhibition percent (antioxidant inhibition percent against free radical), OD control was absorbance value for control and OD sample was absorbance value for sample.

### Statistical analysis

Results were represented as the means  $\pm$  standard deviation of three replicate. The results statistically and difference between groups was accomplished by SPSS, version 19.0. The graphs was drew with the same software. For evaluating existence or non-existence significant difference between means were used from One-way analysis of variance (ANOVA) followed by Tukey HSD (high significant difference) multiple range test.

## RESULTS

The extraction effects in terms of TPC, TFC, TAC and DPPH radical scavenging activity, obtained by Shade, Sun and Oven drying methods, were compared to each other.

Obtained results from analysis of variance table (Table 1) showed that the difference among various extracts and drying methods separately on Total phenol content, total flavonoid content, total antioxidant content and % inhibition was significant on 5% level. Furthermore, interaction between extracts and drying methods in Total phenol and flavonoid content wasn't significant ( $P < 0.05$ ).

Table 1. Analysis of variance (mean squares) of extraction and drying effects on some phytochemical characteristics of *Mentha longifolia* L.

	df	Total phenol content	Total flavonoid content	Total antioxidant capacity	% inhibition
Extract	4	37.326*	0.120*	18621.432*	610.442*
Drying	2	19.497*	0.016*	7363.588*	1310.766*
Extract $\times$ Drying	8	0.533 <sup>ns</sup>	0.001 <sup>ns</sup>	1493.288*	36.250*
Error	30	0.270	0.000	59.613	0.941

<sup>ns</sup> Non significant and \* Significant difference at 5% level

### Total phenol content determination

Sample plants are affected various extraction and drying methods. As shown in Figure 1, the extraction method had significant effect on drying method, so that the highest total phenols content (TPC) was identified in

extraction with soxhlet in shade drying that it's amount was  $10.33 \pm 0.58$  mg GA/g DW and the lowest TPC was in Clevenger at oven drying amount of  $3 \pm 0.25$  mg GA/g DW. Among the drying methods content of total phenol in shade drying was high in comparison of other methods. It is clear that in each drying methods, extraction with soxhlet had high TPC, so this method was most effective for extraction of TPC. The Clevenger method in shade drying and maceration of ethenolic solvent in oven drying had significant difference ( $P < 0.05$ ) with other methods; in sun drying maceration with hydro alcoholic solvent and ethanolic solvent didn't indicate significant difference ( $P < 0.05$ ). Analysis of variance table indicated difference in total phenol content between extracts and dryings was significant ( $P < 0.05$ ) level but between extract  $\times$  drying was not significant.

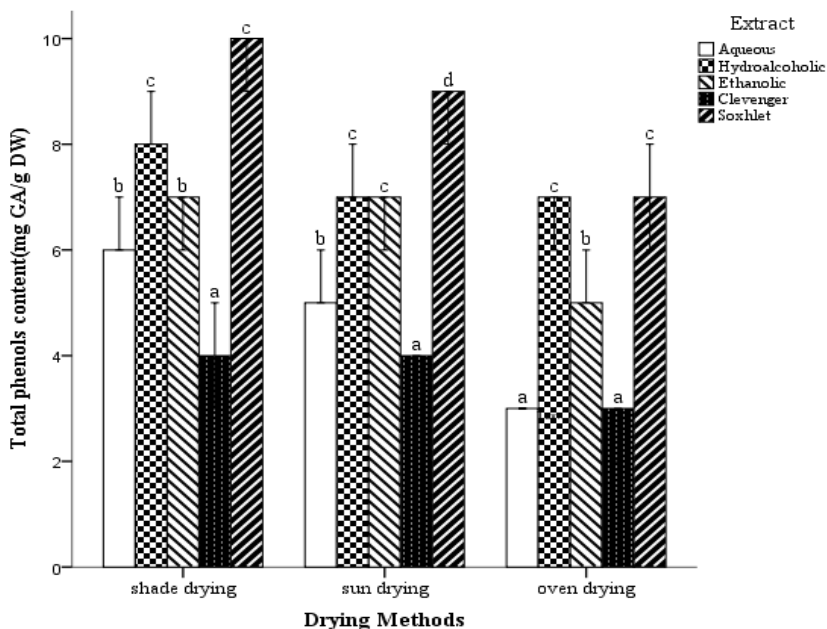


Figure 1. The effect of extraction and drying methods on total phenols content (TPC) of dried horsemint. Different letters over the columns showed significant difference ( $P < 0.05$ ) according to Tukey analysis.

### Total flavonoid determination

As shown in Figure 2, the extraction of soxhlet was most suitable method for highest total flavonoid content (TFC). The soxhlet method in shade drying gave highest flavonoid content that was  $0.59 \pm 0.28$  mg QU/g DW while the maceration using aqueous solvent had lowest amount of TFC was about  $0.22 \pm 0.1$  mg QU/g DW. There was no significant difference ( $P < 0.05$ ) between maceration using hydroalcoholic solvent with ethanolic solvent in all drying tests. Similar to phenol amounts, flavonoid content in extraction with soxhlet had beneficial efficiency compare to other drying methods. Analysis of variance table



indicated difference in total phenol content between extracts and dryings was significant in 5% level but between extract  $\times$  drying was not significant.

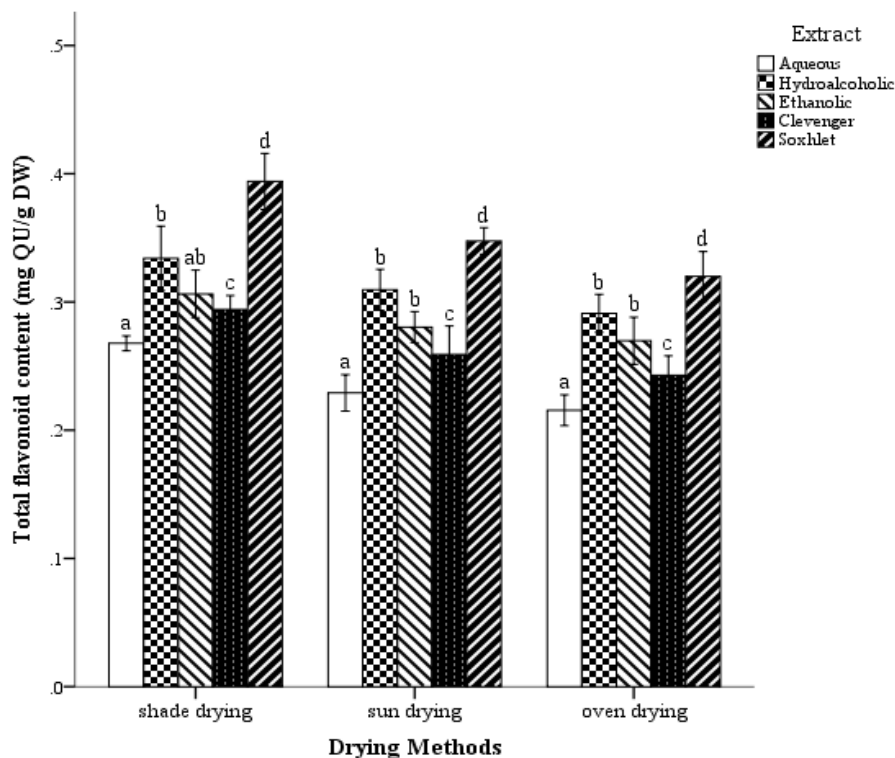


Figure 2. The effect of extraction and drying methods on total flavonoid content (TFC) of dried horsemint. Different letters over the columns showed significant difference ( $P < 0.05$ ) according to Tukey analysis.

### Determination of total antioxidant capacity

#### Reducing power assay

The results in Figure 3 indicated superiority of clevenger in three drying method. Total antioxidant capacity (TAC) in clevenger of shade drying was highest ( $222.15 \pm 10.08 \mu\text{g AA/g DW}$ ) although the lowest TAC was  $75.19 \pm 4.5 \mu\text{g AA/g DW}$  in maceration with ethanolic solvent of oven drying.

In all drying types there were no significant difference ( $P < 0.05$ ) between three maceration using various solvents, moreover clevenger and soxhlet extraction in drying sample using shadesignificantly hadn't difference. Analysis of variance table demonstrated difference in total phenol content between extracts, drying and extract  $\times$  drying was significant ( $P < 0.05$ ).

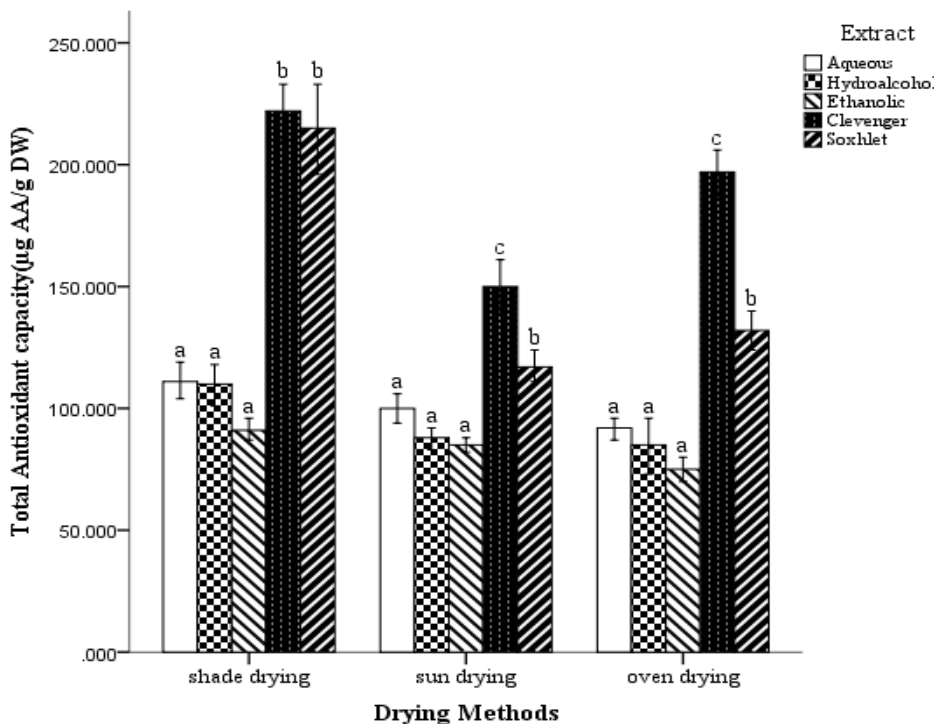


Figure 3. The effect of extraction and drying methods on total antioxidant capacity (TAC) of dried horsemint. Different letters over the columns showed significant difference ( $P < 0.05$ ) according to Tukey analysis.

### The scavenging activity of DPPH radicals

As shown in Figure 4, similar results to antioxidant capacity, inhibition percent in essential oil obtained from Clevenger had high efficiency. Average antioxidant capacity for horsemint samples in this study were determined using DPPH free-radical scavenging assay. Among all drying and extraction that tested in this article Clevenger method of shade drying gave highest free radical inhibition percent ( $56.3 \pm 0.56\%$ ) but soxhlet in oven drying produced lowest ( $14.55 \pm 1.32\%$ ) inhibition percent.

No significant difference ( $P < 0.05$ ) were observed between maceration with ethanolic solvent and soxhlet in sun drying, maceration with aqueous and Clevenger; other extraction hadn't difference with each other. Surprisingly, in oven drying maceration method with aqueous had highest inhibition percent other than extraction methods of oven drying, also hadn't significant difference with Clevenger.

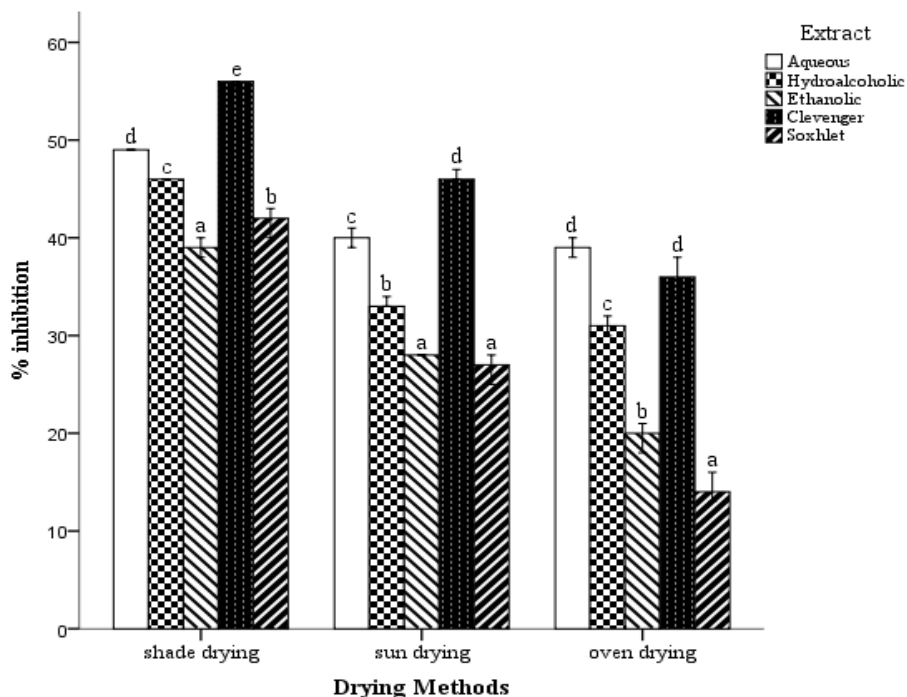


Figure 4. The effect of extraction and drying methods on free radical inhibition percent (DPPH) of dried horsemint. Different letters over the columns showed significant difference ( $P < 0.05$ ) according to Tukey analysis.

### Correlation between measured factors

In order to better evaluation the relationship between total phenol and flavonoid contents, antioxidant capacity and DPPH radical scavenging activity Pearson correlation under extraction and drying conditions were done (Table 2).

In the case of the variable "Extract" for shade drying (Data not shown), correlation between extract, TFC and antioxidant capacity were positive significant ( $P < 0.01$ ). This showed that most of the flavonoid compounds extracted, contributed to the antioxidant capacity of shade drying extracts. The pair of extract-DPPH correlation was non-significant and negative that meaning antioxidant capacity assays of plant samples by phosphomolybdat method had efficiency better than DPPH method.

Under the "Extract" parameter of sun drying (Data not shown), there was a positive significant correlation ( $P < 0.05$ ) between extract and TFC. This can be resulted for the effect of sun on the antioxidant capacity of dried samples or extraction conditions.

In the case of "extract" in oven drying method (Data not shown), TFC positively and significant ( $P < 0.05$ ), DPPH radical scavenging activity significantly negative ( $P < 0.01$ ) were correlated.

Concerning the "TPC", it was positively significant ( $P < 0.01$ ) correlated with TFC for all three methods (shade, sun and oven drying extracts), while "TFC" was negative and mostly significant correlated with TAC and DPPH assays in all three drying types. Therefore, it could be concluded that there are other phenolic compounds different from flavonoids that contributed to the antioxidant capacity of extracts.

Table 2. Pearson correlation coefficients between different assays <sup>a</sup> under effect of extraction and drying conditions <sup>b</sup>.

	Shade drying extracts				Sun drying extracts				Oven drying extracts			
	TPC	TFC	TAC	DPPH	TPC	TFC	TAC	DPPH	TPC	TFC	TAC	DPPH
TPC	1	0.744**	-0.057 <sup>ns</sup>	-0.686**	1	0.793**	-0.810**	-0.890**	1	0.885**	-0.624*	-0.766**
TFC		1	0.347 <sup>ns</sup>	-0.494 <sup>ns</sup>		1	-0.808**	-0.692**		1	-0.365 <sup>ns</sup>	-0.814**
TAC			1	0.511 <sup>ns</sup>			1	0.863**			1	0.523*
DPPH				1				1				1

<sup>a</sup> TPC, total phenol content, TFC, total flavonoid content, DPPH radical scavenging activity, TAC total antioxidant activity.

<sup>b</sup> Number of replicates

<sup>ns</sup> Non significant

\* Significant at  $P < 0.05$

\*\* Significant at  $P < 0.01$

Hence, this results is useful to clarify the relationship between the extraction technique and drying methods based on TPC, TFC, TAC and DPPH (% inhibition). Pearson correlation showed that TPC was only one technique that had a significantly high correlation with the concentration of TFC.

## DISCUSSION

Herbs with strong antioxidative properties are a complete supply of phytochemicals (Orphanides et al., 2013). It is important that should be attentioned to antioxidative performance of extracts and antioxidant activity depend not only on the extraction techniques, but also on the processing prior to extraction, the harvesting time, its geographic origin, the quality of initial plant, its storage conditions (Papageorgiou et al., 2008; Pham et al., 2015).

Although by performing of drying microbial activities and biochemical changes will be prevented, simultaneously, it is possible that losses of aromas or forming of new aromas in effect of oxidation and esterification processes increase. This alterations will affect on appearance, aroma content and herb quality. Furthermore, drying techniques destroys bioactive compounds may be antioxidant or had other health-improving properties (Pirbalouti et al., 2013). It is

noteworthy that the flavonoid content was lower than the respective Total phenol content in all the cases in our study, This is probably due to flavonoids are considered to be restricted in distribution and composition in different plant materials in comparison with the overall phenolic compounds (Pisoschi and Negulescu, 2011) that confirms our results. As the major groups of compounds acting as initial antioxidant free radical annihilator, phenolic compounds playing a key role (Pulido et al., 2000). It was reported that obtained essential oil yields (v/w on dry weight basis) were highest in both landraces that dried in shade, afterwards the freeze dried of purple landrace and the fresh green landrace that confirmed the results of present study, the Clevenger showed high essential oil yield especially in total antioxidant capacity and %inhibition. It is worth noting that different plant substances used in these experiments were related to plants may their physiology had been different, which is determine by their genetic makeup (Quispe-Condori et al., 2008). Thus drying method can affect the chemical composition of essential oils but it depends on plant material used.

The results of present study were showed that extraction with various methods in each three drying methods had phenol and flavonoid content and antioxidant capacity but highest amount among different drying method was in samples that dried in shade and the best extraction method in each drying methods was determined. The usage of heating deactivates enzymes fastly and at the same time, they may destroy heat-sensitive phenolic compounds (Rabeta and Lai, 2013). The reduction of TPC value in our study after exposure the plant materials under sun may be possibly caused by the enzymatic reaction during the process (Rabeta and Lai, 2013). Moreover, it also causes enzymes degradation and loss of antioxidant enzyme activities. New works also exhibited that the stability of phenolic compounds in herbal infusions will affected by temperature (Rafiee et al., 2011) that is in lined with our results that total phenol content of plant material had been dried with oven was lower than other methods, similar results was observed for total flavonoid content.

In addition to chemical content, the biological activity of the extracted substances was change by changing of method of drying herbal material; As well as the essential oil of *Mentha longifolia* L. Hudson dried in shade has exhibited the highest antioxidant activity and dried samples in laboratory in the oven has the lowest antioxidant property (Riehle et al., 2013) that similar results was obtained in our study. It was showed that most oil content in savory was in temperature of 45 °C oven, shade and sun drying methods, respectively (Rocha and Melo, 2011); this results is disagree with the results of our study that the most oil content was in shade, sun and 70 °C oven, respectively. In present study by enhancing drying temperature certainly reduced the essential oil content of all samples. The essential oil content of sage and thyme (*Thymus vulgaris*) dried in oven at 60 °C were reduced in higher temperature (Sefidkon et al., 2006). Furthermore, similarly results with our results was reported (Riehle et al., 2013; Sellami et al., 2015) that the extracts of herbs dried in the laboratory oven ( $1.13 \pm 0.11$  m/mol Fe<sup>2+</sup>/mg of the dry extract and  $EC_{50} = 0.033 \pm 0.001$  mg/mL) has

shown the lowest antioxidant capacity. Such results reveals value of methods that plant is dried prior of preparation. DPPH is a deep-purple colored stable free radical, in the hydrogen or electron donation process its color changes from purple to yellow and becomes a stable diamagnetic molecule (Señoráns et al., 2000). Because antioxidant has the electron and hydrogen donating ability, the discoloration degree of mixture indicates the scavenging power of the antioxidant determines by degree of mixture discoloration (Serra Bonvehí et al., 2001). Loss of other bioactive properties causes frequently in reduction of antioxidant properties (Hajlaoui et al., 2009). It was reported that IC<sub>50</sub>, defined as the concentration of sample extract necessary to obtain an activity of 50%, from lowest to highest was as follows: *M. piperita*, *M. pulegium*, *M. rotundifolia*, *M. longifolia*, *M. spicata*, respectively (Shan et al., 2005). When are able to obtain extracts that have high efficiency along with least changes in active properties of the extracts, it's said to be an extraction technique (Sher and Khan, 2007). According to accomplished investigation in present study, extraction with soxhlet had highest TPC and TFC but the most total antioxidant capacity and %inhibition was in essential oils obtained by Clevenger.

The antioxidant properties of the herbal material varies by application of different methods of extraction. For example, in one study because of highest content of total phenols ( $113.8 \pm 2.0$  mg of gallic acid/g of the dry extract) and flavonoids in dried herbs by shade was showed highest antioxidant capacity measured by two methods the ferric decreasing antioxidant property (FRAP) and DPPH assays ( $2.76 \pm 0.15$  m/mol Fe<sup>2+</sup>/Mg of the dry extract and EC<sub>50</sub> =  $0.022 \pm 0.001$  mg/ml). These differences on ABTS and DPPH data for the same samples can be attributed to differences in the polarity of the solvents that affect the main mechanisms of electron transfer involved in both assays. This means generally the electron transfer reaction is the base of DPPH assay, and the interactions between antioxidants-DPPH% radicals are also determined by the structural form of the antioxidants. Thus, decreasing the number of DPPH% molecules in correspondence to the number of accessible hydroxyl groups in the antioxidant compound is result of very fastly reaction of some substances with DPPH% (Shinwari et al., 2011). A study to evaluate the antioxidant activities of the essential oil in methanol extract of *M. Longifolia* was reported (Stanisavljević et al., 2012). In both applied assays (inhibition of free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and  $\beta$  carotene/ linoleic acid systems), the extract showed significant activity in comparison with the essential oil. Other studies explained that the chief cause of more antioxidant influence of methanol extract than the essential oil is the phenolic compounds (Suja and Mohanasundari, 2016) that confirmed our study. In another study, for finding a newly potential resource of natural antioxidants the free radical-scavenging potential (1, 1-diphenyl-2-picrylhydrazyl scavenging activity) of nine *Mentha* spp. was evaluated. The methanolic extracts of *M. longifolia* exhibited high antioxidant activity (79%) (Sulieman et al., 2011).

A significantly high correlation ( $r=0.7$ ,  $P<0.01$ ) between TPC and TFC; between TAC and %inhibition ( $r=0.8$ ;  $P<0.01$ ) was obtained in present study. Earlier studies found no agreement in correlation between total phenolics and antioxidant activity. Therefore some reports showed a strong positive correlation (Van Wyk et al., 1997; Venskutonis, 1997), other studies (Wong et al., 2006; Xiangyang et al., 2010) reported a poor correlation. In our study also there was a negative correlation between TPC and total antioxidant capacity. In another study (Zargari, 1997), the quantitative analysis of phenol and flavonoid content of the extracts highly correlated that was in lined with observed correlation in our results.

### CONCLUSION

The current study found that Shade drying showed superiority over the other drying methods. Soxhlet extract from dried samples in shade demonstrated to had high total phenol and flavonoid content and good antioxidant capacity, also in terms of total antioxidant capacity and %inhibition assays essential oil that obtained by Clevenger in the plant material dried in shade had highest TAC and DPPH radical scavenging activity. DPPH was the best assay for estimating amount of free radical scavenging activity in comparison with TAC assay by phosphomolybdenum method. Our results demonstrated a significantly positive correlation ( $r=0.7$ ,  $P<0.01$ ) between total phenol and flavonoid content. Furthermore, total antioxidant capacity and DPPH radical scavenging activity showed positive correlation ( $r=0.8$ ,  $P<0.01$ ).

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## **THE FLORISTIC COMPOSITION OF THE NATURAL PASTURES IN MASSIVE OF NOVOBËRDA**

### **SUMMARY**

This article presents data of the research in 2017 on the floristic composition of the pastures in the Novobërda area, in the eastern part of Kosovo plain, which is characterized by a medium continental climate. This study gives an outline of the floristic composition of this massive, and also an idea on the producing capacity of the wet and dry mass of the grass in these pastures and their quality. The aim of the investigations was to examine the floristic composition and green fodder yield and quality at a number of representative sites, having in mind the distribution of natural meadows and pastures. From these studies we can survey the pasture capacity, the ratio of the edible versus non-edible vegetation, poisonous plants, and the necessity of pasture upgrading. The data of the study show the presence of variation related to the floristic composition and plant cohabitation. The average presence of grasses was higher, 48.51% compared with legumes of 25.36% and other species 26.13%. The presence of legumes was relatively higher compared with investigation data presented from other authors in the region. Differences of species composition between localities were highly significant at level of  $LSD_{0.01}$  of probability.

**Keywords:** Floristic composition, yield, quality, Kosovo plain.

### **INTRODUCTION**

Meadows (14%) and pastures (30.2%) occupy a significant part of the land in Kosovo and they are very important source of fodder for the livestock and for the soil protection against erosion (Rusinovci, 2016a, Rusinovci, 2016b; Avdiu, 2003). As such, they raise interest in studying their floristic composition and their values. There is interest from the point of view of knowing the vegetation biodiversity as well as how it could be intervened to improve their production potential and nutritive values. Meadows and pastures are secondary vegetation with different floristic composition and reflections of the ecological conditions, which define their physiognomy. Average meadow and pasture yield is relatively low and range from that is due to absence of essential cultural practices and sometimes from improper utilization (Rusinovci, 2015; Rexhepi, 1988; Demiri, 1981 and Mirić, 1975). There is a need for improvement of meadows and

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pastures through cleaning, systematization, and protection against erosion, but also to cultivate improved types of plants in order to increase their production capacities. The similar methods have been used in Western Europe and presented through the articles of many authors such as: Talamaci *et al.* (1990); Arigno *et al.* (1990); Rusinovci *et al.* (2015). Parameters that show the quality of meadows and pastures are the chemical content of grass such as proteins, carbohydrates and fat content. The evaluation of germplasm resources has been considered of prime importance, especially on those species that have a further economic interest. (Aliu *et al.*, 2012)

This study was focused on the need to know the capacity and value of meadows and pastures in this region. Also the yield of wet, and dry mass production will be researched that will show the quantity potential of meadows and pastures. Based on the varieties of species which were found, a strategy for genetically improvement in the field of forage crops will be developed and also this research will notify the values of floristic diversity.

### MATERIAL AND METHODS

Investigations of the floristic composition were carried out over the 2017 growing season. Sampling of plant species and green matter yield determination was carried out at the optimum plant growth and developmental stage.

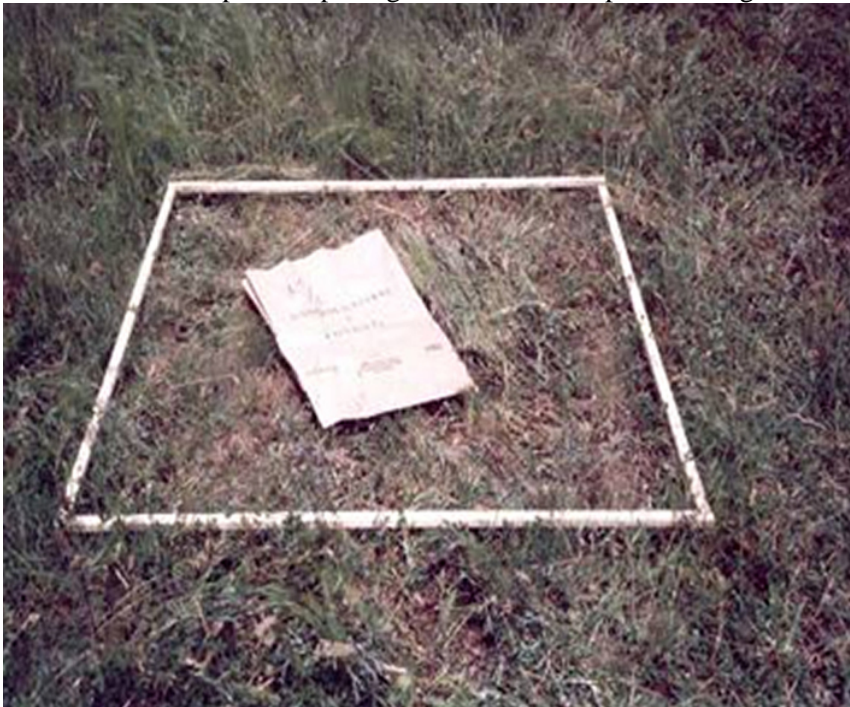


Figure 1. The quadrat for determination the number of plant species.

Samples were collected at 36 locations of natural pastures in region of Novobërda. From each location were taken by 3 samples which were used to calculate the average of grasses, legumes and other species.

Samples were taken depending on the altitude from 700 to 1200 m a.s.l., and different expositions (northern, southern, eastern, and western expositions) in order to determine their composition. Components have been determined for each sample as well as the embranchments presented by each of them. Then, the wet and dry mass of each species found was measured and calculated.

The initial sample represents plants in 1 m<sup>2</sup>. For this reason was prepared a quadrat (Figure 1.) of 1 m<sup>2</sup>. This quadrat was put in to the ground and all the plants inside the quadrat were harvested and selected by the species they belong.

Samples for determination of the floristic composition were classified in three main groups: grasses, legumes and other species. Samples were tested for the content of protein, fat and dry matter at the testing lab at Kosovo Institute of Agriculture in Peja.

Dry matter yield determination was made after sample drying at 65°C. Standard methods were used to establish the dry matter quality: contents of crude proteins (CP), crude fiber (CF) and crude fats (CF). For statistical analyses were used SPSS 22, Minitab 18 and Excell.

## RESULTS AND DISCUSSION

The floristic composition of Novobërda grasslands - Vegetation is characteristic of dry climate areas, which coincides with the studies made by other authors (Rusinovci et al, 2014a, Rusinovci et al, 2014b; Paparisto, 2000; Pardini and Talamaci, 1990).

The results showed relatively rich vegetation diversity composed in totally of 71 species include: 24 grasses, 16 leguminous, and 31 other species (table 1). Even the diversity of grasses species in our samples was almost double compare to leguminous species, the frequency of plants per square meter was quite different (table 2).

Based on our research grasses are most frequent in number per square meter, followed by similar number of other species and leguminous. The distribution of plants per square meter was different, grasses ranged from 23 to 720, legumes from 5 to 320 and other species from 17 to 345 plants per square meter.

The distribution of grasses species was 48.51%, compared to legumes 25.36% and other species 26.13% (Figure 2). This shows that Novobërda massive conditions favour the growth of grasses, which have to be considered for the purpose of upgrading.

Moreover, since the samples collected from different locations, results were analysed for their statistical significance, and according to the one-way ANOVA we found that between the locations for the plants frequency per square meter there was high statistical significance at level of LSD<sub>00,1</sub> (table 2).

Table 1. *Determination of species at Novobërda massive*

No.	Scientific name	No.	Scientific name
1	<i>Achilea millefolium</i>	37	<i>Podosperma laciata</i>
2	<i>Anthoxanthum odoratum</i>	38	<i>Polygala vulgaris</i>
3	<i>Aegilops triuncialis</i>	39	<i>Phleum pratense</i>
4	<i>Astragalus onobrychis</i>	40	<i>Plantago lanceolata</i>
5	<i>Ajuga laxmannii</i>	41	<i>Rumex acetosella</i>
6	<i>Arrhenatherum elatius</i>	42	<i>Rinanthus minor</i>
7	<i>Anthyllis vulneraria</i>	43	<i>Ranunculus psilostachys</i>
8	<i>Alopecurus myosinoides</i>	44	<i>Rinanthus major</i>
9	<i>Allium flavum</i>	45	<i>Medicago rigidula</i>
10	<i>Asperula cynanchina</i>	46	<i>Marrubium vulgare</i>
11	<i>Agropyron repens</i>	47	<i>Medicago falcata</i>
12	<i>Bromus erectus</i>	48	<i>Festuca ovina</i>
13	<i>Bromus sterilis</i>	49	<i>Festuca arundinaca</i>
14	<i>Convolvulus arvensis</i>	50	<i>Fragaria vesca</i>
15	<i>Capsella bursa-pastoris</i>	51	<i>Trifolium dalmaticum</i>
16	<i>Cerinthe minor</i>	52	<i>Trifolium incarnatum</i>
17	<i>Cruciata glabra</i>	53	<i>Trifolium pratense</i>
18	<i>Cirsium sp</i>	54	<i>Trifolium campestre</i>
19	<i>Chrysopogon gryllus</i>	55	<i>Trisetum flavescense</i>
20	<i>Dactylus glomerata</i>	56	<i>Tragopogon pratense</i>
21	<i>Dorycnium herbaceum</i>	57	<i>Thymus serpyllum</i>
22	<i>Euphorbia cyparissias</i>	58	<i>Teucrium chamedrys</i>
23	<i>Erodium cicutarium</i>	59	<i>Salvia verticillata</i>
24	<i>Galium verum</i>	60	<i>Serratula tinctoria</i>
25	<i>Genista sagitalis</i>	61	<i>Silena conica</i>
26	<i>Koeleria pyramidata</i>	62	<i>Sanguisorba minor</i>
27	<i>Lotus corniculatus</i>	63	<i>Succisa pratensis</i>
28	<i>Haynaldia villosa</i>	64	<i>Vicia cracca</i>
29	<i>Hypohoris radiata</i>	65	<i>Veronica sp</i>
30	<i>Hypericum perforatum</i>	66	<i>Viola tricolor</i>
31	<i>Hieracium pilosum</i>	67	<i>Helloborus sp.</i>
32	<i>Onobrychis viciifolia</i>	68	<i>Trifolium arvense</i>
33	<i>Potentilla hirta</i>	69	<i>Lathyrus aphaca</i>
34	<i>Poa pratensis</i>	70	<i>Daucus carota</i>
35	<i>Poa trivialis</i>	71	<i>Cyhorium sp.</i>
36	<i>Poa bulbosa</i>		

Table 2. Frequency of grasses, legumes and other species at Novo Berda natural pastures and one-way ANOVA.

Loc.	Grasses	Leguminous	Others	Loc.	Grasses	Leguminous	Others
1	172 <sup>t</sup>	89 <sup>pq</sup>	17 <sup>v</sup>	19	188 <sup>s</sup>	47 <sup>t</sup>	120 <sup>op</sup>
2	314 <sup>fgh</sup>	90 <sup>opq</sup>	180 <sup>jk</sup>	20	23 <sup>y</sup>	107 <sup>lmn</sup>	220 <sup>efg</sup>
3	299 <sup>ijk</sup>	90 <sup>opq</sup>	22 <sup>v</sup>	21	69 <sup>x</sup>	72 <sup>rs</sup>	24 <sup>uv</sup>
4	323 <sup>fgh</sup>	230 <sup>cd</sup>	345 <sup>a</sup>	22	101 <sup>v</sup>	5 <sup>v</sup>	37 <sup>t</sup>
5	290 <sup>kl</sup>	102 <sup>mno</sup>	141 <sup>mn</sup>	23	310 <sup>hij</sup>	80 <sup>qr</sup>	114 <sup>p</sup>
6	318 <sup>fgh</sup>	275 <sup>b</sup>	206 <sup>h</sup>	24	520 <sup>c</sup>	280 <sup>b</sup>	146 <sup>lm</sup>
7	210 <sup>r</sup>	241 <sup>c</sup>	136 <sup>mn</sup>	25	310 <sup>hij</sup>	124 <sup>jk</sup>	193 <sup>i</sup>
8	267 <sup>no</sup>	171 <sup>f</sup>	35 <sup>tu</sup>	26	273 <sup>mn</sup>	157 <sup>g</sup>	144 <sup>lm</sup>
9	298 <sup>ik</sup>	218 <sup>d</sup>	190 <sup>ij</sup>	27	255 <sup>op</sup>	88 <sup>pq</sup>	175 <sup>k</sup>
10	406 <sup>d</sup>	280 <sup>b</sup>	316 <sup>b</sup>	28	188 <sup>s</sup>	109 <sup>lm</sup>	213 <sup>gh</sup>
11	24 <sup>y</sup>	139 <sup>hi</sup>	130 <sup>no</sup>	29	327 <sup>f</sup>	151 <sup>gh</sup>	155 <sup>l</sup>
12	80 <sup>wx</sup>	320 <sup>a</sup>	22 <sup>v</sup>	30	126 <sup>u</sup>	117 <sup>kl</sup>	235 <sup>d</sup>
13	720 <sup>a</sup>	137 <sup>i</sup>	120 <sup>op</sup>	31	284 <sup>lm</sup>	122 <sup>jk</sup>	274 <sup>c</sup>
14	696 <sup>b</sup>	130 <sup>ij</sup>	226 <sup>def</sup>	32	87 <sup>w</sup>	95 <sup>nop</sup>	236 <sup>d</sup>
15	246 <sup>pq</sup>	240 <sup>c</sup>	25 <sup>tuv</sup>	33	219 <sup>r</sup>	162 <sup>fg</sup>	187 <sup>ijk</sup>
16	192 <sup>s</sup>	161 <sup>fg</sup>	20 <sup>v</sup>	34	365 <sup>e</sup>	129 <sup>ijk</sup>	231 <sup>df</sup>
17	412 <sup>d</sup>	79 <sup>qrs</sup>	51 <sup>s</sup>	35	235 <sup>q</sup>	184 <sup>e</sup>	214 <sup>fgh</sup>
18	325 <sup>fg</sup>	26 <sup>u</sup>	101 <sup>d</sup>	36	312 <sup>ghi</sup>	67 <sup>s</sup>	69 <sup>r</sup>
Mean (m <sup>2</sup> )					<b>271.78</b>	<b>142.06</b>	<b>146.39</b>
F					1936.42**	520.97**	764.82**
LSD <sub>0.05</sub>					9.72	9.24	8.92
LSD <sub>0.01</sub>					12.78	12.14	11.73

Means that don't share a letter are significantly different at level of  $p < 0.01$ .

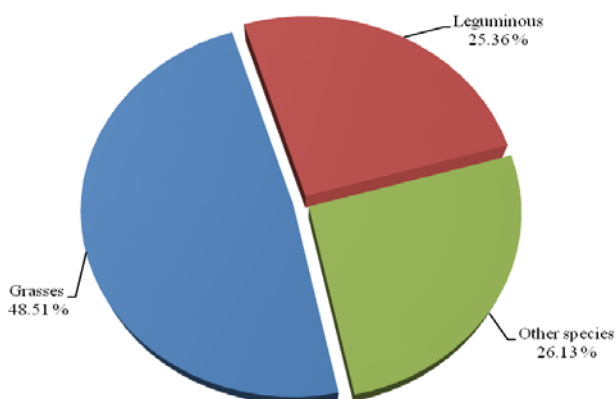


Figure 2. Distribution of plant species at natural pastures.

It's valuable to mention that grasses species occurred with higher diversity and frequency in north-west exposure and higher altitude, whereas leguminous species had a slight dominance in lower altitude and south-east exposure. Also, other grasses species with some exceptions, had the same extension like leguminous species. These exceptions may attribute to the soil bonity.

The higher number of grasses and legumes is an indicator of high quality of pastures (Cowan, 2011). One-way ANOVA, in our research, shows significant results at level  $LSD_{0.01}$  of plant species richness groups in different location of Novobërda region (table 3).

Table 3. ANOVA and average weight in grams per square meter of the wet and dry mass of plant species in the Novobërda natural pastures

Plant species	Wet mass	Dry mass
Grasses	237.3 <sup>a</sup>	93.2 <sup>a</sup>
Leguminous	143.5 <sup>b</sup>	42.2 <sup>b</sup>
Other species	104.2 <sup>c</sup>	32.9 <sup>b</sup>
<b>Total weight g/m<sup>2</sup></b>	<b>485</b>	<b>168.3</b>
F	166.10**	71.50**
LSD <sub>005</sub>	18.36	13.29
LSD <sub>001</sub>	27.82	20.13

Means that don't share a latter are significantly different at level of  $p < 0.01$ .

Data from the above table show that grasses has the biggest weight for wet and dry mass, which is almost double the weight of the leguminous and other families. The percentage of dry mass compare to the wet mass appears to be higher for grasses for approximately 40%. It appears to be lower for the other species. The content of the dry matter, mineral matter, proteins, fats, fibbers, and nitrogen free extracts of natural pastures are presented on table 4.

Table 3. Chemical composition % of natural pastures and one-way ANOVA.

Plant species	Moisture	Dry Matter	Mineral content	Crude Proteins	Crude Fats	Crude Fibber	Nitrogen Free Extracts
Grasses	8.59 <sup>a</sup>	91.59 <sup>a</sup>	6.00 <sup>a</sup>	10.00 <sup>ab</sup>	1.61 <sup>a</sup>	34.50 <sup>a</sup>	39.90 <sup>a</sup>
Legumes	8.50 <sup>a</sup>	91.50 <sup>a</sup>	6.98 <sup>a</sup>	13.31 <sup>a</sup>	1.73 <sup>a</sup>	30.38 <sup>a</sup>	39.06 <sup>a</sup>
Other species	8.69 <sup>a</sup>	91.31 <sup>a</sup>	6.28 <sup>a</sup>	11.07 <sup>ab</sup>	1.85 <sup>a</sup>	33.00 <sup>a</sup>	39.12 <sup>a</sup>
<b>Mean</b>	<b>8.59</b>	<b>91.41</b>	<b>6.42</b>	<b>11.46</b>	<b>1.73</b>	<b>32.63</b>	<b>39.36</b>
F	0.09	0.09	2.41	13.65**	1.43	4.40	0.11
LSD <sub>005</sub>	1.11	1.11	1.12	1.58	0.36	3.44	4.83
LSD <sub>001</sub>	1.68	1.68	1.70	2.40	0.54	5.21	7.32

Means that don't share a latter are significantly different at level of  $p < 0.01$ .



Data show that there is a significant difference at level of  $LSD_{001}$  between legumes compare to the grasses and other species on crude protein content. The pastures with large legumes specie or their frequency per unit were characterized with high content of crude proteins.

### CONCLUSIONS

The following conclusions derive from the study of the floristic and grazing composition of Novobërda pasture massive: Pastures of Novobërda massive consist of a rich diversity of vegetation, which is shows cohabitation of 71 plant species. The distribution of plants species and their frequency in different locations of the study was different. This seems to be related to the altitude, exposition and the composition of the soil characters of this massive. The average presence of grasses was higher, 48.51% compared with legumes of 25.36% and other species 26.13%. The wet and dry weight of the grass per unit of area varies significantly from one sampling location to the other. This is related to the environmental factors such as exposition, solid composition, altitude, which affect the composition of the grass.

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## BIPLOT ANALYSIS OF SEED YIELD OF FABA BEAN GENOTYPES AT DIFFERENT PLANTING DATES

### SUMMARY

A split plot experiment was carried out during 2012-13 in Iran to appraise the effects of genotypes and planting dates, as well as their interactions on seed yield in faba bean, based on randomized complete block design with three replications. The dates of planting and six genotypes were arranged in main and split plots, respectively. Analysis of variance revealed significant effects of genotype for all of the traits except plant height. The interaction effects of two factors were significant on seed yield, number of pods per plant and pod length. The polygon view of treatment by trait (TT) biplot indicated the autochthonous landrace from north of Iran (G1) were as a genotype with high mean productivity, but their stability in four planting date is low. This genotype was also the best for first, second and forth sowing dates. The breeding variety (France) was as a more stable variety in all of the planting times. The polygon view of TT biplot is also indicated the best genotypes with respect to each of planting date. The results of present study can help to planning a crosses program for achieving the heterosis, since heterosis only can occur between genetically divergent germplasms.

**Keywords:** Graphic Analysis, Faba bean, Sowing Date, Seed yield

### INTRODUCTION

Faba bean (*Vicia faba* L.;  $2n = 12$ ), which used as a food for its high nutrient components in seeds, is a major legume (Duc, 1997). It is consumer in the Middle East, Mediterranean, China and Ethiopia as a vegetable green or fresh seed (FAO, 2016). Faba bean is divided to four botanical varieties including *paucijuga*, *major*, *equine* and *minor* according to their differences in some of the seed characters such as shape, weight and size (Duc, 1997).

Planting date is crucial in faba bean, because early or late sowing expose the crop to drought, adverse temperature, pests and diseases attack. Some of researchers indicated that sowing date significantly influenced the seed yield and growing traits in faba bean and late sowing increased the severity of insect and disease attack and reduced days to flowering, green pod length, seeds per pod and seed yield (Yusufali *et al.*, 2007; Kawochar *et al.*, 2010; Khalil *et al.*, 2010). Abdelmula and Abuanja (2007) used three sowing dates to evaluate the heat stress on faba bean genotypes and indicated the high temperature stress

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significantly reduced seed yield and most of the studied traits. They were also indicated significant differences between genotypes. Alghamdi (2007) were also indicated the significant differences between studied genotypes for yield and yield components. Crossing system in faba bean is intermediate between autogamy and allogamy, and therefore the genetic variability of this crop is very large (Hanelt and Mettin 1989). Knowledge of the extent of genetic variability for quantitative and quality traits in faba bean would facilitate the breeding improvement of faba bean.

Genotype main effects and genotype  $\times$  environment interaction (GGE) biplot analysis were proposed for graphic interpretation of the genotype  $\times$  environment interactions (Yan *et al.*, 2000). Although agronomic traits represent the combined effects of genotype (G), environment (E), and genotype  $\times$  environment (G $\times$ E) interaction, the GGE Biplot analysis considers that only the G and G $\times$ E effects are relevant and they need to be considered simultaneously when evaluating cultivars.

The graphic axes of biplot are the first two principal components of multivariate analysis and identify the superior cultivars, classify the environments in mega-environments and determined the superior genotype in any of environments (Yan *et al.*, 2000). Balalić *et al.* (2010) used biplot method to assess the effects of hybrids and planting dates as well as their interaction on oil yield in sunflower and indicated the higher values of oil yield in earlier planting dates in comparison to later. They also facilitate the choice of stable hybrids and planting dates for desired characters in sunflower by graphical presentation of biplot. Genotype by trait (GT) biplot were also used in rye (Yari *et al.*, 2017), Spinach (Sabaghnia *et al.*, 2016), rice (Sharifi and Ebadi. 2016). Sabaghnia and Janmohammadi (2014) used treatment by trait (TT) biplot for studying the various nano-silicon dioxide treatments on seed germination of lentil under various NaCl concentrations.

The aim of this study was to appraise the effects of genotypes and planting dates, as well as their interaction on yield in faba bean by biplot graphic method.

## MATERIALS AND METHODS

### Experimental field area

This study was carried out during 2012-13 in Shanderman, Guilan province, Iran (longitude, 49° 55' E; latitude, 37° 27' N; altitude, 71 m above sea level; climate, wet). The experiment comprised of 24 treatment combinations, consisting of six genotypes (Table 1) and four date of sowing (1 December 2012, 22 December 2012, 5 January 2013 and 2 March 2013). The experiment was laid out in a split plot design with three replications based on randomized complete block design. The dates of planting and genotypes were arranged in main and split plots, respectively. Each plot consisted of four rows with 6 m long and distance between rows was 50 cm. The seeding rate was 15 plants per m<sup>2</sup>. Routine cultural operations were attended to keep the plots free from weeds.

Table 1. Information of studied faba bean genotypes.

Genotype	Genotype name	Origin	Breeding status	Seed structure
1	-	North of Iran (Guilan)	Autochthonous Landrace	Large
2	Barrakat	Iran/ Gurgaon	Breeding variety	Large
3	France	France	Breeding variety	Intermediate
4	-	Lorestan (Borujerd1)	autochthonous Landrace	Small
5	FILIP3	Syria	Breeding variety	Small
6	FILIP5	Syria	Breeding variety	Small

### Estimated characters

The characters containing pod length (PL), seeds per pod (NSP), stems per plant (NStPl), pods per plant (NPoPl), hundred seed weight (HSW) and dry seed length (LS) and width (SW) were measured on fifteen plants of each plot. Seed yield per plot was measured after removal of the marginal effect and reported as dry seed yield per m<sup>2</sup> (SY) and used for treatment by trait (TT) biplot analysis.

### Statistical analysis

The statistical model was adopted for this experimental design is:

$$Y_{ijk} = \mu + \rho_k + (\alpha\rho)_{ik} + \alpha_i + \beta_j + \alpha\beta_{ij} + e_{ijk}$$

Where,

$\mu$ : general mean;  $\alpha_i$ : effect of i<sup>th</sup> factor, genotype, (i = 1, 2, ..., 6);  $\beta_j$ : effect of j<sup>th</sup> factor, sowing date, (j = 1, 2);  $\rho_k$ : effect of k<sup>th</sup> replication (k = 1, 2, 3);  $(\alpha\rho)_{ik}$ : main plot error;  $\alpha\beta_{ij}$ : the interaction effect of i<sup>th</sup> genotype with j<sup>th</sup> sowing date;  $e_{ijk}$ : experimental error.

Genotype  $\times$  planting date interaction was evaluated according to Gauch and Zobel (1996). GGE biplot software according to Yan (2001) was used for plotting the biplot.

## RESULTS AND DISCUSSION

### Analysis of variances

Analysis of variance indicated significant effects of genotype on all of the traits except of plant height. These results are in agreement with the findings of Alghamdi (2007) which revealed the faba bean genotypes were significantly differed for yield and yield components. The results were also indicated the significant effect of planting date on all of the studied traits. In agreement to this result, many of researchers revealed that sowing date had significant effect on

yield and yield components and late sowing reduced the number of days to flowering, green pod length, number of seeds per pod and seed yield (Abdelmula and Abuanja, 2007; Yusufali *et al.*, 2007; Kawochar *et al.*, 2010; Khalil *et al.*, 2010). The interaction effect of planting date and genotype were significant on seed yield, number of pods per plant and pod length. The magnitude of interaction indicates the influence of planting date on adaptability and stability, which is a desired character only when it is connected with yield above average (Yan and Hunt, 2003). Therefore, the genotype  $\times$  planting date interactions were studied on seed yield by biplot method.

Table 2. Analysis of variance for some of yield and yield components of faba bean genotypes

Source	df	Mean Square						
		SY	HSW	NStPl	NPoPl	PL	NSePo	PH
Replication	2	126349.81*	921	0.71**	0.37	4.24	0.55*	1857.79**
Planting date	1	46991710.5**	16469.44**	21**	601.06**	93.18**	3.61**	26292.62**
Genotype	5	704319**	39327.73**	0.39*	46.07**	79.34**	4.03**	142.92
Planting date * Genotype	5	295130.82**	576.71	0.22	26.16**	5.12*	0.27	153.55
Error	22	26673.46	369.69	0.11	2.09	1.36	0.12	167.62
CV		11.22	17.19	17.19	21.64	13.00	10.15	13.73

ns, not significant; \* and \*\*, significant at the 0.05 and 0.01 probability level, respectively.

PL: pod length, NSP: number of seeds per pod, NStPl: number of stems per plant, NPoPl: number of pods per plant, LS: dry seed length, SW: dry seed width, HSW: hundred seed, SY: seed yield.

### Treatment by trait (TT) Biplot analysis

The graphic TT biplot analysis of six genotypes in four planting date indicated in Figure 1 A. The genotypes farthest from the biplot origin give most to increase the genotype  $\times$  planting date interaction, such as G1, G2, G4 and G6. Whereas, G3 and G5 that were closer to the center of origin of the axes, contributed least to the genotype  $\times$  planting date interaction.

In the biplot method, the small angles between the genotype vectors within the same quadrant indicated the similarity of the genotypes. According this view of biplot the genotypes such as G2 and G3 and G4 and G5 had similarity behavior. These results are logical, since Barrakat (G2) and France (G3) are breeding varieties that were selected in the same environment, while small seed genotype from Lorestan (G4) and FILIP3 (G4) are genotypes that have similar agronomic performance.

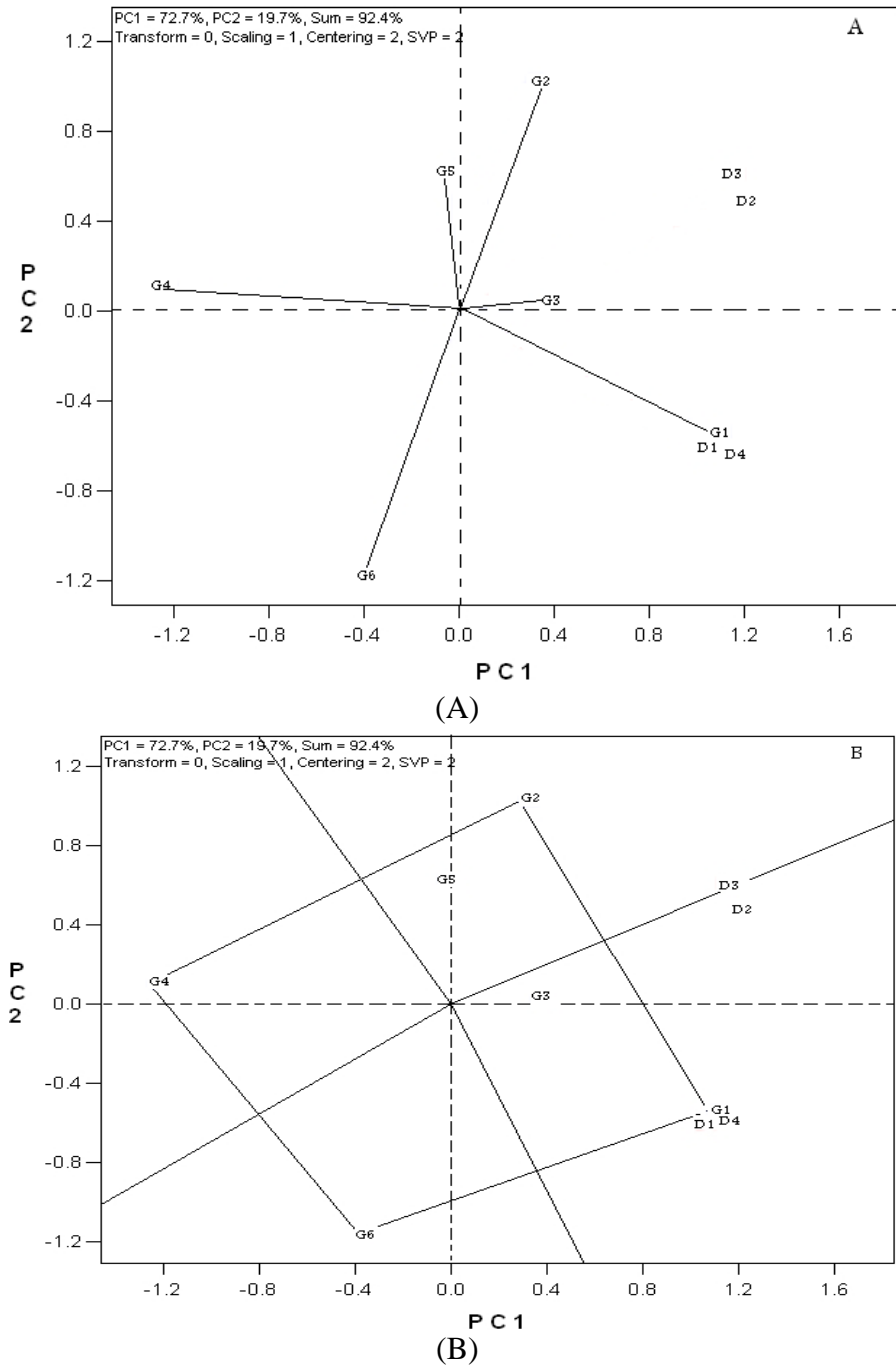


Figure 1. Graphic analysis of vector (A) and polygon (B) view of biplot of faba bean genotypes in four planting date: 1 December 2012 (D1), 22 December 2012 (D2), 5 January 2013 (D3) and 2 March 2013 (D4).

The Autochthonous landrace from north of Iran (G1) is an adapted genotype in Guilan, the region that there were carried out this experiment. The places of the genotype in contrasting quadrants indicate their dissimilar genetic performance, as can be observed for G1 and G5, G1 and G4, G3 and G6, G2 and G6, and G1 and G2. These results can help to planning a diallelic crosses for achieving to heterosis, since heterosis only can occur between genetically divergent germplasm.

Since the second principal component (PC2) score play a significant role (19.7%) in explaining the genotype  $\times$  environment (planting date) interactions (GEI), the first principal component (PC1) score were plotted against PC2 score, to further explore adaptation (Figure 1 B.). The polygon view of biplot of the six faba bean genotypes in four planting date is displayed in Figure 1 B. The first two principal components explained 92.4% of the total variability. In the graphic analysis, PC1 and PC2 represent genotype productivity and stability, respectively (Yan *et al.*, 2000). However, Burgueño *et al.* (2000) stated that such properties tend to occur when the cultivars' PC1 is highly correlated with cultivar effects.

The TT Biplot indicated that the ideal genotype must have a high PC1 value (high mean productivity) and a PC2 value next to zero (more stable). Thus, based on the graphic interpretation, the genotypes with the highest PC1 values were G1, G3 and G2, respectively. The most stable genotypes were G3 and G4. It was not possible to identify the ideal genotype by measuring just productivity and stability. According to this polygon view of biplot, the autochthonous landrace from north of Iran (G1) were as a genotype with high mean productivity, but their stability in four planting date is low. The breeding variety (France) was as a more stable variety in all of the planting time and intermediate productivity.

The polygon is formed by joining the markers of the genotypes that are furthest away from the biplot origin such that all other genotypes are contain in the polygon. The vertex genotypes (G1, G2, G4 and G6) have the longest vectors, in their respective direction, which is a measure of responsiveness to planting time. The vertex genotypes are, therefore, among the most responsive genotypes; all others are less responsive in their respective direction. These genotypes that located in the vertex are an unstable. Genotypes located near the plot origin (G3 and G5) were less responsive than the vertex genotypes. G3 is closer to the center of biplot, showing to be more stable (Purchase, 2000).

The polygon view biplot is also indicated the best genotypes with respect to each of planting date (Yan and Hunt, 2003). The Autochthonous landrace from north of Iran (G1) were the best for first, second and forth date of faba bean sowing. Barrakat (a breeding variety) was the best genotype for third planting date.

## CONCLUSION

On the basis of the obtained results can be concluded all main effects (genotype, planting date), as well as interaction showed highly significant values



for seed yield. Biplot analysis showed that both PC axes (PC1 and PC2) were highly significant. The major part of variation belonged to PC1 (72.7%). G3 did not differ in the mean values for seed yield, which were above the general average and indicated high stability for seed yield response to four planting dates. Seed yield was higher in earlier planting dates than in later planting dates. Graphical performance of TT biplot could promote the choice of stable genotypes and planting dates for desired traits in faba bean.

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## UPTAKE OF HEAVY METALS BY TOMATO PLANTS (*Lycopersicum esculentum* Mill.) AND THEIR DISTRIBUTION INSIDE THE PLANT

### SUMMARY

The aim of this study was to examine the concentration of total and available forms of heavy metals (Ni, Cr, Cu, Zn, Pb and Mn) in the greenhouse soils as well as the distribution of these metals in the different parts of tomato plants grown on these soils. Atomic Absorption Spectroscopy (AAS) was used to determine heavy metals concentration. The concentration of available forms of all examined heavy metals in the soils and in tomato fruits were low, although the total concentration of hazardous heavy metals Ni and Cr in soils exceeded the maximum permissible values, prescribed by legislative rules in Bosnia and Herzegovina.

The reasons for the low uptake of heavy metals by tomato plants are mainly related to the chemical properties of soil which are not favorable for heavy metal availability. In addition, the results of this study also showed that the accumulation of all examined heavy metals especially Cr and Ni were much higher in the roots than in the fruits. The low accumulation of heavy metals in tomato fruits is the result of synergy of different plant defense mechanisms that limiting or reducing heavy metal transport from root to fruits.

**Key words:** transport, root, leaves, fruit, greenhouse soil

### INTRODUCTION

The intensification of agricultural soil use, and changes in farming practice, characterized by intensive application of fertilizers and pesticides may cause soil pollution by heavy metals in greenhouses (Nouri *et al.*, 2008). Namely, many chemical products used in agriculture, especially nitrogen and phosphate fertilizers, contain certain amounts of heavy

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metals, as result of their production from minerals used as a source of these elements.

Although the low level of heavy metals in fertilizers and pesticides in one-time use do not pose any hazard of soil or plant contamination, their long-term use in food crops production, especially in greenhouse vegetable production, contributed to the accumulation of heavy metals in the soils and consequently in food crops (Huang and Jin, 2008).

Cadmium (Cd), chromium (Cr) and lead (Pb) are the heavy metals of most concern because they can affect human health even in small quantities. Wa'ngstrand *et al.* (2007) reported that application of nitrogen fertilizers containing ammonium-N may increase Cd concentrations in plants, even if the fertilizers do not contain significant levels of heavy metals. In addition, Atafar *et al.* (2010) were also observed that application of some non-heavy metal fertilizers can potentially increase concentrations of Cd, Cr, and Pb in plants.

The conclusion of their work was that the application of acidic fertilizers in soils dramatically decreases soil pH resulting in desorption of heavy metals from the soil matrix and their higher bioavailability by plants.

Accordingly, determining the available forms of heavy metals in soils is an important approach to the soil contamination assessment, especially in greenhouse areas which have great impact on environment and human health due to intensive use of fertilizers and pesticides containing heavy metals.

However, up to now, heavy metal concentrations of greenhouse soil in Bosnia and Herzegovina and its pollution problem have been studied very little, especially there is no systematical investigation of the dynamics of heavy metals in the greenhouse soil-plant system in intensive tomato production. Since the heavy metals have a long residence time in soils, it is very important to study the status of heavy metals in soil, and their influence on vegetable safety, and consequently human health.

The objective of this study was to examine the concentration of total and available forms of heavy metals (Ni, Cr, Cu, Zn, Pb and Mn) in the greenhouse soils as well as the distribution of heavy metals in the different parts of tomato plants (*Lycopersicum esculentum* Mill. 'Berberana F1') grown on these soils. Tomato was selected as the subject of this study, primarily because the global production of this vegetable is consistently increasing in our country and therefore, any attempt to evaluate the health risks of consumption of tomato or other vegetable crops grown on greenhouse soils is of great interest to producers and consumers.

## MATERIAL AND METHODS

### Study area

The experiment was carried out from March to July 2018 in a multi-span plastic greenhouse with natural ventilation at Srebrenik, north eastern part of Bosnia and Herzegovina. The geometrical characteristics of the greenhouse were as follows: eaves height of 2.2 m; ridge height of 3.6 m; total width of 8 m; total length of 20 m; ground area of 160 m<sup>2</sup>, and total volume of 480 m<sup>3</sup>. The greenhouse was polyethylene covered and equipped with two side roll-up vents. Shade cloth was used to prevent excessive light intensity during warm day.

Fertilizer application in greenhouse studied area was based on use of different chemical fertilizers: slow release NPK (nitrogen-phosphorus-potassium) compound fertilizers applied before vegetable growth, and quick release fertilizers (urea, potassium fertilizers, foliar fertilizers) applied during intensive vegetable growth and development. Average amount of applied chemical fertilizers was approximately 1000 kg ha<sup>-1</sup> year<sup>-1</sup>. Application of fertilizers on the studied greenhouse area was managed in this way for eight years from the time of establishment.

According to FAO Soil Classification (FAO, 1998), the soil on which the greenhouse is placed belongs to eutric cambisol. Pedological profile A - Bv - C and base saturation degree higher than 50%, at least in the upper part of the Bv horizon, is a typical characteristic of this type of soil. Furthermore, eutric cambisols have relatively good structure and chemical properties, and therefore moderate sensitivity to yield decline (Husnjak, 2014).

### Soil sampling

Experimental ground area in greenhouse was divided into three equal plots. The soil sample from every plot were collected in March 2018, few weeks before tomatoes planting, at a depth of 0 - 30 cm using stainless steel shovel. Each plot sample was obtained from five individual soil cores that were thoroughly mixed to make one sample. These three plot samples were mixed to form the average greenhouse soil sample.

### Soil analysis

The soil samples were air-dried at room temperature, and then crushed and grinded using soil porcelain mortar and pestle to achieve homogeneity. After, samples were passed through sieves (2 and 1 mm) and then stored until analysis.

Following parameters were subject of soil chemical analysis: soil reaction (pH), soil organic matter, available forms of phosphorus and potassium, and concentration of total and available forms of heavy metals (Ni, Cr, Cu, Zn, Pb and Mn).

Soil reaction (pH) in H<sub>2</sub>O and 1 mol dm<sup>3</sup> KCl was determined according to ISO 10390 method (ISO, 2005), organic matter (OM) according to ISO 14235 method (ISO, 1998), available forms of phosphorus and potassium by ammonium-lactate extraction (Egner *et al.*, 1960), and total and available forms of heavy metals by atomic absorption spectrophotometer (AA-7000, Shimadzu, Japan) according to the instructions specified in the ISO 11047 method (ISO, 1998). Measurements were made in triplicate to check the precision of the results.

Previous extraction of total Ni, Cr, Cu, Zn, Pb and Mn from the soil was conducted using *aqua regia* solution (ISO, 1995) as follows: 3 grams of air-dried soil (fraction smaller than 1 mm) was placed in 250 ml flat bottom flask, then 28 ml of *aqua regia* was added (21 ml HCl and 7 ml HNO<sub>3</sub>). The flask was covered with a watch glass, allowed to stand 16 h (overnight) at room temperature, and then was heated on hotplate under reflux for 2 h. After cooling down to room temperature, solution was filtered through quantitative filter paper into 100 ml flask and diluted to the mark with deionized water.

Extraction of available forms of heavy metals from the soil was performed using EDTA solution (Trierweiler and Lindsay, 1969) as follows: 10 g of air-dried soil was placed into 100 ml plastic bottle, then 20 ml EDTA solution (0.01 mol dm<sup>3</sup> ethylenediaminetetraacetic acid (EDTA) and 1M (NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>, adjusted to pH 8.6) was added. The bottle has been shaken 30 min at 180 rpm in an orbital shaker, then extract was filtered through quantitative filter paper into 25 ml flask and diluted to the mark with deionized water.

### **Plant sampling**

Five tomato plants (whole plant with root) from each examined plot were carefully collected at the stage of commercial maturity. Leaves, root, and fruits of every plants were separated, dried at room temperature, grinded and then stored in little paper bags until analyses.

### **Plant analysis**

The concentration of heavy metals (Ni, Cr, Cu, Zn, Pb and Mn) in the plant samples was also determined by atomic absorption spectrophotometer (AA-7000, Shimadzu, Japan) according to ISO 11047 method. Measurements were made in triplicate for each plant sample to check the precision of the results.

Previous extraction of heavy metals from the plant material was performed using HNO<sub>3</sub>-H<sub>2</sub>SO<sub>4</sub> solution (Lisjak *et al.*, 2009) as follows: 1 g of dry matter was placed in 100 ml flat bottom flask, and 10 ml HNO<sub>3</sub> and 4 ml H<sub>2</sub>SO<sub>4</sub> were added. The flask was covered with a watch glass, allowed to stand for few hours at room temperature and then heated gently

on a hot plate for thirty minutes. After cooling down to room temperature, the extract was filtered through quantitative filter paper into 50 ml flask and diluted with deionized water to the mark.

### Statistical analysis

All results were presented as mean  $\pm$  standard deviation and were processed by one-way analysis of variance (ANOVA). The differences between means were tested using the least significance difference (LSD) test at  $P < 0.05$ .

## RESULTS

### Chemical properties of the greenhouse soil

The analysis of basic parameters of soil fertility showed that the examined soil had a slightly acid reaction, moderate level of organic matter (OM), and high content of available forms of phosphorus ( $P_2O_5$ ) and potassium ( $K_2O$ ), indicating that the examined greenhouse soil is suitable for tomato cultivation (Table 1).

Table 1. Results of soil chemical analysis

Parameter	unit	measured value	recommended values for vegetable production*
pH H <sub>2</sub> O	pH unit	6.9	5.7-7.2
pH KCl	pH unit	6.1	5.2-6.7
OM	%	3.83	3-5
P <sub>2</sub> O <sub>5</sub>	mg 100 g <sup>-1</sup>	43.06	12-16
K <sub>2</sub> O	mg 100 g <sup>-1</sup>	110	25-35

\* values reported by Vukadinovic and Vukadinovic (2011)

### Heavy metal concentrations in soil

Total and available heavy metals concentration (Ni, Cr, Cu, Zn, Pb, Mn) in the average greenhouse soil sample are listed in Table 2.

Table 2. The heavy metal concentrations in the average soil sample

greenhouse soil	Concentration (mg kg <sup>-1</sup> dry mass)					
	Ni	Cr	Cu	Zn	Pb	Mn
total	216.58	127.56	22.57	38.39	15.4	256.18
limit value	40*	100*	80*	100*	100*	850**
available forms	3.99	0.13	2.23	1.54	0.46	6.33

\* limit value prescribed by B & H legislation;

\*\* toxic level of Mn in soils reported by Pais and Jones (1997)

The highest total concentration was recorded for Mn, followed by Ni, Cr, Zn, Cu, and Pb content being the lowest one. The concentration of heavy metal Ni and Cr exceeds the limit value in soils prescribed by the legislation in Bosnia and Herzegovina (Official Gazette of FBiH, 2009), while the concentration of Zn, Cu and Pb did not exceed the limit value prescribed by the same legislation.

Limit value of Mn in soils is not in the legislative rules, because Mn is not direct contaminant of soil. However, the concentration of Mn in examined soil did not exceed the toxic level of Mn in soils ( $850 \text{ mg kg}^{-1}$ ) reported by Pais and Jones (1997).

### Heavy metal concentrations in tomato plants

Heavy metals concentration (Ni, Cr, Cu, Zn, Pb, Mn) in different parts of tomato plants are presented in Table 3.

Table 3. Concentration of heavy metals in tomato plants

Part of the plant	Concentration ( $\text{mg kg}^{-1}$ dry mass)					
	Ni	Cr	Cu	Zn	Pb	Mn
root	$32.4 \pm 3.00$ a	$12.92 \pm 5.27$ a	$10.24 \pm 0.79$ a	$30.95 \pm 3.03$ a	n.d.	$94.35 \pm 29.9$ a
stem	$2.81 \pm 0.13$ b	$0.97 \pm 0.04^b$	$1.85 \pm 0.51^c$	$14.23 \pm 1.92$ c	n.d.	$9.59 \pm 1.17^c$
leaves	$3.23 \pm 0.29$ b	$1.02 \pm 0.13^b$	$2.65 \pm 0.39^c$	$14.72 \pm 0.36$ c	n.d.	$43.83 \pm 1.71$ b
fruit	$2.16 \pm 0.11$ b	$1.11 \pm 0.22^b$	$4.83 \pm 1.66^b$	$19.51 \pm 1.44$ b	n.d.	$10.05 \pm 5.11$ c
Lsd <sub>0,05</sub>	1.23	2.21	0.85	1.64	-	12.13

\* significant; n.d. - not determined

Presented data have shown that the concentration of all tested heavy metals in tomato fruits was below the maximum permissible value of heavy metals in food crops reported by FAO/WHO (2001). Accordingly, the maximum permissible value for Ni is  $4 \text{ mg kg}^{-1}$ , for Cr  $2.3 \text{ mg kg}^{-1}$ , for Cu  $40 \text{ mg kg}^{-1}$ , for Zn  $100 \text{ mg kg}^{-1}$ , and for Pb  $0.3 \text{ mg kg}^{-1}$ . The maximum permissible value for Mn is not reported by FAO/WHO since the Mn is not considered a health hazard.



## DISCUSSION

Contamination of greenhouse soils by heavy metals as a result of long-term use of fertilizers and pesticides in food crops production becomes more and more intense, resulting in harmful effects on human health through consumption of food crops grown on these soils (Liu *et al.*, 2014). Thus, it is imperative to reduce heavy metal contamination in greenhouse soils, and in order to achieve this goal, legislation of some developed countries has set tolerance limits on heavy-metal additions (fertilizers and biosolids) to soils. Unfortunately, Bosnia and Herzegovina (B & H) has no legislative rules associated with this issue. In our country only maximum permissible concentrations of hazardous heavy metals (Cr, Cd, Pb, Ni, Cu, Zn) in agricultural soils were prescribed by legislation. If the concentration of any of the above-mentioned heavy metals in the soil is higher than limit value, such soil can be considered as polluted by heavy metals and not suitable for agriculture.

In this study, it was determined that the total concentration of Ni and Cr exceeded the maximum permissible value for agricultural soil prescribed by legislation in B & H. These results lead to the conclusion that the examined soil is polluted by Ni and Cr. Nevertheless, the available forms of these elements in the same soil were very low. Moreover, the amount of available Cr in examined soil was less than 0.1% of total Cr concentration in soil, and from these results it is evident that the total concentration does not provide reliable information on the mobility, availability and toxicity of the heavy metals, especially Ni and Cr. Many scientists agree with our observation that the total amounts of heavy metals in soils are not suitable for estimating the solubility and mobility and consequently the toxicity of heavy metals (Abollino *et al.*, 2002; Pueyo *et al.*, 2004; Nunes *et al.*, 2014). The results of heavy metals analysis in tomato fruits also confirm that observation. Namely, the average Cr and Ni concentration in tomato fruits was 1.11 and 2.16 mg kg<sup>-1</sup> respectively, which is significantly lower in comparison with the maximum permissible value of Cr (2.3 mg kg<sup>-1</sup>) and Ni (4 mg kg<sup>-1</sup>) in food crops reported by FAO/WHO (2001).

One of the main reasons for the low mobility of Ni and Cr in examined soils and consequently for their relatively low accumulation in tomato fruits are closely related to the chemical properties of soils, primarily to soil reaction (Nadgórska-Socha *et al.*, 2013). Our examined greenhouse soil had a slightly acid reaction, which is not the most favorable for the mobility and availability of Cr and Ni to plant roots. Mobility and availability of these elements is much higher in strongly

acidic soils, and results of many studies confirm that fact (Kukier *et al.*, 2004; Adamczyk-Szabela *et al.*, 2015).

The total concentration of other tested heavy metals (Cu, Zn, Pb, Mn) in examined soils did not exceed the maximum permissible value for agricultural soil prescribed by B & H legislation, indicating that investigate soil is not polluted with these heavy metals. The concentration of these elements in tomato fruits were also lower than the maximum permissible value of heavy metals in food crops reported by FAO/WHO (2001). Moreover, the presence of Pb was not determined in any parts of tomato plants. This data is highly desirable, since the Pb is extremely harmful to human health through consumption of food crops even in small quantities.

The results of this study also showed that all the examined heavy metals especially Cr and Ni accumulated in higher amounts in the roots than in the above-ground parts of a plant. These results agree with results of other authors who examined this issue (Adki *et al.*, 2013; Wu *et al.*, 2013).

Gomes *et al.* (2017) reported that plants possess several different strategies to neutralize the negative impact of heavy metals on plants. One of first strategies is related to limit their uptake from the soil through complexing metals with organic compounds produced and exuded from the roots. If hazardous heavy metal enters the root, plants may activate different tolerance mechanisms such as metal compartmentalization in different intracellular compartments, or biosynthesis and accumulation of several compounds aimed at metal complexation, thus prevention of their transport from root to other part of plants. Selection of strategy primarily depends of plant genetic background and growth conditions. The results of this study related to the availability of heavy metals in soils and their accumulation in the plant strongly support above-mentioned hypotheses.

Interesting finding of this study was that the concentrations of hazardous heavy metal Cr and Ni were even 10 to 15-fold higher in the root than in other parts of tomato plants, while the difference in distribution within the plant was much lower for Mn, Zn and Cu. Taking into account that these elements (Mn, Zn and Cu) are necessary for plant metabolism, mainly in photosynthesis and as an enzyme antioxidant-cofactor (Nguyen-Deroche *et al.*, 2012; Farzadfar *et al.*, 2017), the results of this part of study lead to the conclusion that mechanisms for reducing or preventing transport of these elements from root to other parts of tomato plants are not fully activated.

On the other hand, for highly hazardous heavy metals such as Cd, Cr, and Pb, these mechanisms are significantly more involved in the

tomato plant's defense system. It is certain that the plants have developed many types of mechanisms for identifying and involving heavy metals into their metabolism, but also for blocking them if their presence is harmful to the plant. Understanding these mechanisms creates a predisposition to write correct conclusions about the possibilities of tomato cultivation on soils contaminated by heavy metals.

## CONCLUSION

The general conclusions of this study were that the reaction of examined greenhouse soil was not favorable for the mobility and availability of heavy metals by plants, contributing to the lower accumulation of heavy metals in the plant. Furthermore, tomato plants accumulated heavy metals mainly in root, indicating that these plants possess different heavy metals tolerance mechanisms to limit or reduce the accumulation of hazardous heavy metals in fruits.

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## QUALITATIVE LAND SUITABILITY ASSESSMENT AND ESTIMATING LAND PRODUCTION POTENTIAL FOR MAIN IRRIGATED CROPS IN NORTHERN OF FARS PROVINCE

### SUMMARY

The aim of this research is land suitability evaluation (qualitative) and estimation of biomass net production (Bn) and yield potential (Y) for wheat and sugar beet in a 15000-hectare area in Fars province in southern Iran. Climate data for last 25 consecutive years were collected from the nearest synoptic meteorological Station. These soils were classified based on semi details studies with 5 physiographic units and were classified in four orders of Alfisols, Vertisols, Inceptisols and Entisols consisted of eleven soil families based on soil data and Keys to Soil Taxonomy. Qualitative evaluation was carried out using the square root of parametric (SRP) method and yield potential was calculated for the area based on FAO method. The results showed that the climatic class of the surveyed area is moderately suitable ( $S_2$ ) for wheat and very suitable ( $S_1$ ) for sugar beet due to limitations imposed by the relative humidity of the growing cycle. According to the parametric method, the studied area can be classified from (S2) to (N1) for the mentioned products. For different soil units, the parametric method (square root method) is better than limitation method. Finally, yield potential was calculated for sugar beet and wheat in the Aspas as 9307.49 and 7206.13kg DM per hectare, respectively. Regarding the parametric multi-criteria evaluation, the soil qualities characteristics including soil physical properties (s), topography (t), water table limitations (w) and soil fertility(F) are the most limiting factors for studied crops.

**Keywords:** land suitability, qualitative land evaluation, wheat, sugar beet, FAO method

### INTRODUCTION

The ability of the world's natural resources to provide the needs of its growing population is a fundamental issue for the international community. Productive capacity of land resources are limited by climate, soil and landform conditions as well as land use and management. Land is the ultimate source of wealth and the foundation upon which many civilizations were constructed. Land evaluation may be defined as 'process of assessment of land performance when

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used for specified purposes' (FAO 1985). Sustainability of ecosystem productivity and biodiversity imply the quality and quantity of natural resources and their suitability for a range of land-use planning processes in future rural, urban and industrial activities (Kilic et al. 2005). Land evaluation plays a major role in comparing various types of land for different uses, and provides information for subsequent activities such as optimum land-use planning or increasing the area per unit with respect to land-suitability evaluation (FAO 1976). Determination of land suitability for various productivity is not only a way to prevent the destruction of agricultural lands, but is also one of the most important and basic methods to combat this issue. Agro ecological land evaluation predicts land behavior for each particular use; soil quality evaluation predicts the natural ability of each soil to function. However, land evaluation is not the same as soil quality assessment, because biological parameters of the soil are not considered in land evaluation (Braimoh and Vlek 2008). In agricultural context, finding optimal locations for crops can increase the economic benefits, as well as reducing the negative environmental consequences (Ashraf et al., 2010). Numerous studies have addressed various aspects of land suitability for crop cultivation on the basis of FAO framework in different countries (Chinene and Situmbanauma 1988; Embrechts et al. 1988; Oise 1993; Habrurema and Steiner 1997). Zang et al. (2004) introduced a system for the quantitative evaluation of soil productivity developed and deployed in Gaoyou County, China. The objective of their study was to develop a new quantitative method within the framework of a GIS. Results of this study showed that the soils with a bleached layer in their profile located in sloping areas were not suitable for rice and wheat, but they were however suitable for tea plantations, fruit trees or other kinds of cash crops. In several parts of Iran, land suitability evaluation has been done for some of the crops by Movahhedi Naenui (1993) Ghasemi Dehkordi (1994), Givi (1996, 1997) Sarvari and Mahmoudi (2001), Seyed Jalali (2001), Shahbazi and Jafarzadeh (2004), Shahabi (2005), Akef (2005), Jafarzadeh and Abbasi (2006), Jafarzadeh et al. (2008), Rahimi Lake et al. (2009), Behzad et al. (2009).

The calculation of radiation-thermal potential by FAO model allows estimating net biomass production based on climate and crop data. The predicted production is obtained by taking the impacts of soil and water management and the radiation-thermal potential into account (Givi, 2000). The method is based on some simple assumptions which allow estimating the biomass and economical yield of most annual plants provided that they are protected against pests and diseases and enjoy optimum moisture and nutritional conditions (Sys et al., 1991a). For this purpose, enormous efforts have been devoted in Iran for a variety of products. For instance, Farajnia (2002) computed production potential of irrigated wheat as 6700 kg.ha<sup>-1</sup> in Tabriz Plain, Iran. Also, Farajnia (2002) assessed 100000 hectares of Yekanat plain of Marand, Iran and calculated the production potential of sugar beet as 77127 kg.ha<sup>-1</sup>. Sohrabi et al. (2003) calculated production potential of sugar beet in Silakhoor Plain of Lorestan, Iran



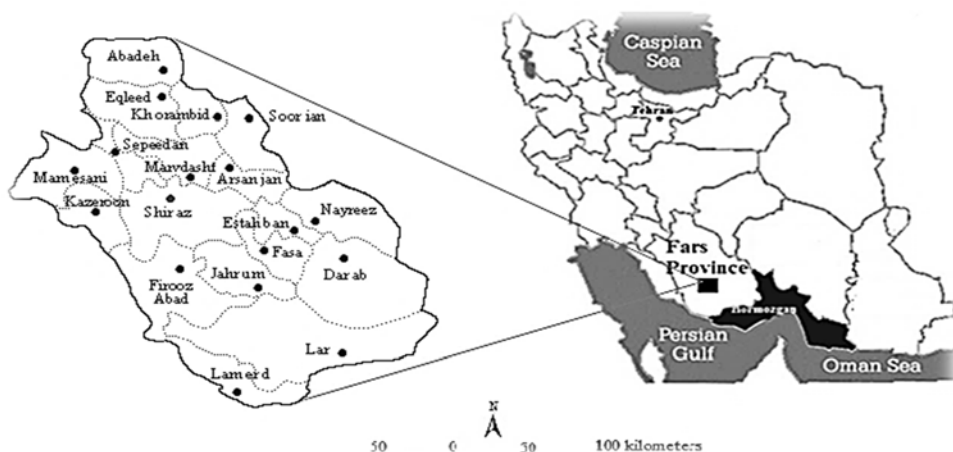
as  $68628 \text{ kg}\cdot\text{ha}^{-1}$ . Seiedjalali (2000) studied the production potential and land suitability in 36205 hectares of Mian-ab lands of Shushtar, Iran for rain fed and irrigated wheat cultivation and estimated wheat biomass yield as  $13284 \text{ kg}\cdot\text{ha}^{-1}$ .

The main objective of this research is then evaluation and comparison of land suitability (qualitative) for principal crops based on the simple limitation and parametric evaluation systems and estimation the biomass net production and yield potential for main irrigated crops in Aspas region, northern of Fars Province, as wheat and sugar beet crops are among the important and commercial products in the most parts of Fars province.

## MATERIALS AND METHODS

### Study site

This study was carried out in Fars province, southern Iran. The study area is about 15000 ha located in Aspas, northern of Shiraz city ( $30^{\circ}27'02''\text{N}$ ,  $52^{\circ}42'29''\text{E}$ , Fars Province, Iran) (Figure. 1).



**Figure 1.** Study area in South of Iran (Fars province)

The study area is 2300 m above sea level with mean annual temperature of  $10.2^{\circ}\text{C}$ . According to Banai (5), the soil moisture and temperature regime of the study area are "xeric" and "mesic", respectively. Climatic data indicate mean annual rainfall of about 177 mm, the precipitation mostly occurred from December to April with minimum rainfall in summer. Annual mean temperature is  $10.2^{\circ}\text{C}$  with temperature range of  $-1.04$ - $3.33^{\circ}\text{C}$  during winter and  $9.12$ - $21.28^{\circ}\text{C}$  during the summer. Mean annual potential evapotranspiration (thornthwaite method) is about 1746 mm. The native vegetation in the region is thinly scattered consisting of *Gundelia sp.*, *Astragalus sp.*, *Akgagu camalar sp.*, *Gundelia tourenf.* and annual grasses. The soil survey reports were identified from the profiles inspected and 5 physiographic units consisted of 5 physiographic units, namely gravelly colluvial fans, Piedmont plains, low lands, mountains and hills. Then several profiles were dug and 8 of them were selected

as representative profiles for different experiments within different land units where mostly parent materials in pedons were calcareous. A brief morphological characteristic of horizons for the selected profiles (Schoeneberger et al. 2002) is presented in Table 1.

**Table 1:** Results of chemical and physical analysis of some soil parameters in the studied area.

Profile no	Depth (cm)	pH	EC (dSm <sup>-1</sup> )	OM	CCE	Gypsum %	Sand	Silt	Clay
<b><u>Typic Xerorthents</u></b>									
1	0-15	7.74	0.39	1.21	4.62	0.045	22.92	39.44	37.64
1	15-50	7.81	0.34	0.32	24.98	0.046	28	38.72	33.28
<b><u>Aquic Haploxerepts</u></b>									
2	0-12	7.86	0.73	0.61	24.24	0.042	33.28	41.08	25.64
2	12-50	8.2	0.61	0.32	21.93	0.361	16	68.72	15.28
<b><u>Typic Calcixererts</u></b>									
3	0-25	7.54	0.54	2.24	30.93	0.078	16	40.72	43.28
3	25-45	7.67	0.4	0.65	30.01	0.109	14	44.72	41.28
3	45-65	7.84	0.5	0.002	25.39	0.085	15.28	43.08	41.64
3	65-115	7.96	0.27	0.32	34.62	0.166	14.92	53.44	31.64
<b><u>Petrocalcic Calcixererts</u></b>									
4	0-20	7.47	0.45	0.42	4.62	0.031	8	34.72	57.28
4	20-70	7.97	0.22	0.29	13.85	0.066	24.92	43.44	31.64
4	70-120	7.87	0.25	1.62	60.01	0.062	36.92	47.44	15.64
<b><u>Typic Xerorthents</u></b>									
5	0-20	7.78	1.05	0.9	25.38	0.032	24	45.3	30.7
5	20-55	7.81	0.98	0.33	31.2	0.028	25	45.5	29.5
<b><u>Typic Xerorthents</u></b>									
6	0-15	7.71	0.52	2.28	25.39	0.047	23.28	29.08	47.64
6	15-45	7.63	0.49	0.94	24.33	0.037	20.61	33.44	45.95
<b><u>Calcic Haploxeralfs</u></b>									
7	0-15	7.72	0.42	0.002	13.85	0.041	32.92	23.44	43.64
7	15-50	7.87	0.21	0.58	27.35	0.066	26.92	37.44	35.64
7	50-95	8.01	0.2	0.002	21.93	0.059	20.92	43.44	35.64
<b><u>Typic Fluvaquents</u></b>									
8	0-20	7.62	1.2	1.4	53	0.028	33	37	30
8	20-45	7.93	1.51	1.1	52	0.09	24	32	44
8	45-70	7.86	1.36	0.7	55	0.078	41	33	26

### Soil sampling and chemical analyses

Based on topographic maps, satellite images and field visits were used for several pedons studies in the area. For further studies, eight representative pedons on

different landscapes were selected and sampled (Schoeneberger et al. 1998); then, air-dried soil samples were meshed through a 2-mm sieve for routine physicochemical soil properties. In this way particle size distribution was analyzed using hydrometer method described by Bouyoucos (1962), organic matter content was assessed by wet combustion (Nelson 1996), cation exchange capacity (CEC) was measured using sodium acetate 1 N at pH 8.2 (Sumner et al. 1996), percentage of gypsum was determined by acetone precipitation (Soil Conservation Service, 1972) and calcium carbonate equivalent (CCE) was tested by titration (Loppert et al. 1996), electrical conductivity (EC), and pH in saturated paste were also extracted according to methods of analysis for soils in arid and semi-arid regions handbook (Bashour and Sayegh, 2007). Soils with cambic, calcic, argillic horizons and ochric epipedon were classified as Entisols, Inceptisols, Vertisols and Alfisols, respectively (Soil survey staff, 2014).

### Land evaluation studies

A wide range of limiting physical, economic and social factors can restrict suitability of the land for different applications (FAO 2007). For qualitative land suitability investigation, simple limitation and parametric methods (Storie and square root) were employed. Simple limitation method compares the plant requirements with its corresponding qualitative land and climatic characteristics and the most limiting characteristics define land suitability class. The parametric land evaluation involves numerical rating of different limitation levels of land characteristics according to a numerical scale ranging between climatic index, as well as the land index which can be calculated from these individual ratings. The calculation of these indices can be carried out by two procedures (Eq. 1 and Eq. 2);

$$1. \text{ Storie method (Storie 1976): } I = A \times \frac{B}{100} \times \frac{C}{100} \times \dots \quad (\text{Eq. 1})$$

Where:  $I$  = index (%)     $A, B, C$  etc. = ratings (%)

$$2. \text{ Square root method (Khiddir 1986): } I = R_{min} \sqrt{\frac{A}{100} \times \frac{B}{100} \times \dots} \quad (\text{Eq. 2})$$

where:  $I$  = index (%)     $R_{min}$  = minimum rating (%)     $A, B, C$  etc. = remaining ratings (%)

Application of these methods necessitates providing the requirement tables for each land utilization type. We compared the land characteristics with the plant requirements tables introduced by Sys et al. (1993). For determination, the limits of land classes we used pattern introduced by Sys et al. (1991). The land suitability classes are defined as follows:

- S1 (very suitable) class: Lands having indexes >75.
- S2 (moderately suitable) class: Lands having indexes ranging in 50-75.
- S3 (marginally suitable) class: Lands having indexes ranging in 25-50.
- N (non-suitable) class: Lands having indexes < 25.

Fall growth of irrigated wheat and irrigated sugar beet are among the common agricultures in the studied region. The agriculture in the area uses

traditional to semi-mechanized techniques and equipment. According to the available data, the growth periods and development stages for the wheat and sugar beet crops in the studied region including growth period, type of plant, data of planting according to information in connection with plant wheat planting stage, germination, vegetative into the anatomy, pollen and deal were determined (Table 2).

**Table 2.** Study of wheat and sugar beet growth cycle in the region

Plant	Planting stability	Vegetative stage	Flowering stage	Ripening stage	Harvest	Growing cycle
Wheat	17-30 Oct	5-20 Nov	15 May-5 June	1-25 July	25July - 1Aug	197 days
Sugar beet	20 Apr-15 May	15 June-15July	30July-5Sep	5 Sep-15 Oct	15Oct-16 Nov	211 days

### The Method of Estimating Biomass Net Production Rate (Bn) and Yield Potential (Y)

Irrigated wheat and sugar beet production potential were determined for Aspas region by radiation-thermal production potential (RPP) method. This model estimates net production of a living crop and its yield for the best cultivar under the optimum conditions in terms of water and nutrients availability and pests and diseases control. For calculating biomass net production, we used Eq. 3 (Sys et al., 1991):

$$Bn=(0.36*bgm*KLAI)/((1/L)+0.25*ct) \text{ (Eq. 3)}$$

Where, Bn is the net production rate of biomass (kg/ha), ct denotes the respiratory rate calculated from:

$$ct=C30(0.044+0.0019t+0.001t^2) \text{ (Eq. 4)}$$

In which Bgm is the maximum net production of biomass (Kilogram CH<sub>2</sub>O in hectare per hour), KLAI shows the correction factor for LAI <5 and L represents the number of days required for crop maturity. C30 represents the respiratory rate for plants except the legume which equals 0.0108, T stands for the average temperature (°C);

Crop production can be computed by:

$$Y=Bn*Hi \text{ (Eq. 5)}$$

where, Y is crop production (kg.ha<sup>-1</sup>) and HI is harvest index

## RESULTS AND DISCUSSION

Climatic data were derived based on nearest synoptic station (Eghlid). General information such as main crops, alternation condition and yield as well as socioeconomic information was collected from farmers and existing services centers. Two land utilization types including irrigated wheat and sugar beet were selected for qualifying and evaluation.

Regarding the results obtained from climatic properties and climatic data and climatic suitability evaluation given by Sys et al. (1991), the climatic characteristics of region are Suitable (S<sub>2</sub>) for wheat and very suitable (S<sub>1</sub>) for sugar beet plantation (Table 4).

**Table 3:** Rating of climatic factors for wheat crop in studied region

Climatic factors	Properties	Parametric Rating	Climatic Suitability Class
Mean temp of growth (°c)	11.61	80	S <sub>2</sub>
Mean temp of vegetative stage (°c)	5.42	65	S <sub>2</sub>
Mean temp of flowering stage (°c)	15.24	96	S <sub>1</sub>
Mean temp of ripening stage (°c)	21.26	98	S <sub>1</sub>
Mean daily min temp coldest month (°c)	-6.8	100	S <sub>1</sub>
Mean daily max temp coldest month (°c)	4.72	100	S <sub>1</sub>
Climatic index	Storie/ Square root	65	S <sub>2</sub>
		65	S <sub>2</sub>
Climatic rating	Storie/Square root Climatic rating= 16.67+0.9Cl	75.1	S <sub>2</sub>
Climatic classes		S <sub>2</sub>	S <sub>2</sub>

**Table 4:** Rating of climatic factors for sugar beet crop in studied region

Climatic factors	Properties	Parametric Rating	Climatic Suitability Class
Growth period in terms of thermal suitability (day)	211	100	S <sub>1</sub>
Minimum absolute temperature at early growth period(°c)	-6	95	S <sub>1</sub>
Average maximum temperature at the coldest month during sugar beet growth cycle(°c)	19.5	90	S <sub>1</sub>
Average minimum temperature at the coldest month during sugar beet growth cycle(°c)	6.1	85	S <sub>1</sub>
Climatic index	Storie Square root	85	S <sub>1</sub>
		85	S <sub>1</sub>
Climatic rating	Storie/Square root Climatic rating= 16.67+0.9Cl	93.2	S <sub>1</sub>
Climatic classes		S <sub>1</sub>	S <sub>1</sub>

The results also showed that on the basis of soil taxonomy system, the soils can be classified as *Typic Xerorthents*, *Aquic Haploxerepts*, *Typic Calcixererts*, *Lithic Xerorthents*, *Petrocalcic Calcixerpts*, *Haploxeralfs* (Soil Survey Staff 2014) and Calcisols, Luvisols, Leptosols and Regosols in WRB system (IUSS Working Group WRB 2014). Calcite content was higher in profiles with calcareous parent mater. The most important feature was the clay illuviation process shown as Bt horizon in profiles. The calculation results (Table 3 and 4) revealed that climatic suitability classes in study area and all land units had moderate limitation levels ( $S_2$ ) for wheat and very suitable ( $S_1$ ) limitation levels for sugar beet. According to simple limitation method, 3.1 land unit had moderate suitability ( $S_2$ ) for wheat and marginal suitability ( $S_3$ ) for sugar beet, the most important limiting factors in whole land units were topography and physical soil characteristics limitations. Furthermore, 8.1 land units had pH-induced fertility limitations. Also, 1.1, 2.1, 4.1, 5.1 and 6.1 land units had physical soil characteristics limitation due to coarse fragments (high gravel percentage). High gravel percentage limitations comprised physical, chemical and fertility limitations which decreased organic matter retention, number and intensity of microorganism activity, cations and anions in soil.

Results obtained by parametric methods (Storie) for irrigated wheat showed unsuitable condition for this cultivation ( $N_2$ ) for 1.1, 2.1, 4.1 and 6.1 land units; 5.1 land unit had non-suitable but correctable ( $N_1$ ) land classes. 7.1 and 8.1 land units had marginally suitable ( $S_3$ ) land classes. Only 3.1 land unit possessed moderate limitation levels ( $S_2$ ). Results of square root method showed non-suitable but correctable ( $N_1$ ) condition for 1.1, 2.1, 4.1 and 6.1 land units. For 5.1 and 8.1 land units, marginally suitable ( $S_3$ ) land classes were obtained. Only 3.1 land unit had moderate limitation levels ( $S_2$ ) (Table 5). Results obtained by parametric methods (Storie) for irrigated sugar beet (Table 6) showed unsuitable condition for this cultivation ( $N_2$ ) for 4.1 and 6.1 land units; 1.1, 2.1 and 5.1 land unit had non-suitable but correctable ( $N_1$ ) land classes; while 3.1, 7.1 and 8.1 land units had marginally suitable ( $S_3$ ) land classes. Based on square root method, 4.1 and 5.1 land units had non-suitable but correctable ( $N_1$ ) condition. For 1.1, 2.1 and 8.1 land units, marginally suitable ( $S_3$ ) land classes were observed. Only 3.1 and 7.1 land unit had moderate limitation levels ( $S_2$ ) (Table 6). Comparing climate information and product requirements, the results of this study showed that climatic suitability classes (based on three methods) were  $S_2$  and  $S_1$  for irrigated wheat and sugar beet, respectively.

Regarding high annual rainfall in this region (>508 mm), at the first look, it seems that it is enough to fulfill wheat water requirement and no irrigation is needed. However, a detailed study of the rainfall showed its uneven distribution throughout the year with higher occurrence during non-cultivation months of the year (winter) when wheat is in hibernation period. Considering that most percent of wheat production occurs in summer, water balance in this season is negative and the cultivation of wheat and sugar beet in summer requires supplementary

irrigation. As the severe topography problem affects feasibility of effective irrigation system, reaching to a high yield is restricted.

**Table 5.** Qualitative land suitability and climatic suitability classes for irrigated wheat

Land unit	Simple Limitation	Qualitative suitability class				Climatic Suitability Class
		Parametric (Storie)		Parametric (Root square)		
		Land Index	Land Class	Land Index	Land Class	
1.1	N <sub>S</sub>	11.64	N <sub>2</sub>	22.88	N <sub>1</sub>	S <sub>2</sub>
2.1	N <sub>SW</sub>	11.87	N <sub>2</sub>	24.36	N <sub>1</sub>	S <sub>2</sub>
3.1	S <sub>2</sub>	51.13	S <sub>2</sub>	61.97	S <sub>2</sub>	S <sub>2</sub>
4.1	N <sub>TS</sub>	8.04	N <sub>2</sub>	19.02	N <sub>1</sub>	S <sub>2</sub>
5.1	S <sub>3TS</sub>	19.72	N <sub>1</sub>	32.93	S <sub>3</sub>	S <sub>2</sub>
6.1	N <sub>TS</sub>	5.76	N <sub>2</sub>	15.18	N <sub>1</sub>	S <sub>2</sub>
7.1	S <sub>3T</sub>	31.53	S <sub>3</sub>	62.48	S <sub>2</sub>	S <sub>2</sub>
8.1	S <sub>3F</sub>	31.85	S <sub>3</sub>	43.7	S <sub>3</sub>	S <sub>2</sub>

**Table 6.** Qualitative land suitability and climatic suitability classes for sugar beet

Land unit	Simple Limitation	Qualitative suitability class				Climatic Suitability Class
		Parametric (Storie)		Parametric (Root square)		
		Land Index	Land Class	Land Index	Land Class	
1.1	N <sub>S</sub>	12.86	N <sub>1</sub>	25.87	S <sub>3</sub>	S <sub>1</sub>
2.1	N <sub>SW</sub>	13.22	N <sub>1</sub>	25.16	S <sub>3</sub>	S <sub>1</sub>
3.1	S <sub>3</sub>	44.10	S <sub>3</sub>	52.07	S <sub>2</sub>	S <sub>1</sub>
4.1	N <sub>TS</sub>	9.64	N <sub>2</sub>	19.69	N <sub>1</sub>	S <sub>1</sub>
5.1	N <sub>TS</sub>	15.27	N <sub>1</sub>	22.97	N <sub>1</sub>	S <sub>1</sub>
6.1	N <sub>TS</sub>	7.36	N <sub>2</sub>	12.12	N <sub>2</sub>	S <sub>1</sub>
7.1	S <sub>3T</sub>	25.53	S <sub>3</sub>	57.58	S <sub>2</sub>	S <sub>1</sub>
8.1	S <sub>3F</sub>	33.15	S <sub>3</sub>	46.12	S <sub>3</sub>	S <sub>1</sub>

F: fertility limitations, t: Topography limitations, s: Physical soil characteristics limitations, W: water table limitations

The results showed close correlation the employed methods (simple limitation and parametric methods (Storie and Square root methods) but square root method had better outcomes. The big part of the studied area was classified as non-suitable for sugar beet and wheat crop due to physical and chemical soil parameters (Tables 5 and 6). Furthermore, the most limiting chemical factors considered in this area were soil alkalinity (pH). The qualitative land suitability evaluation assists decision makers to ensure land use according to their capacities to satisfy human needs for present and future generations thus, sustaining ecological and economic productivity of natural resources. One way to increase

the production rate and optimized usage of the lands is to determine their production capacity and choosing appropriate applications for them. In this method, the yield is computed regardless of limitation type such as soil, water and management. One important and applicable method to optimize the usage of soil resources is determination of land capacity and potential.

One of these methods involves estimation of the potential of the product yield in ideal and optimized conditions. In this research the net production of biomass (Bn) and yield potential (y) of the irrigated wheat and sugar beet were estimated in Aspas region of Eghlid, Fars province based on weather reports of synoptic station of Eghlid. The results showed that regardless of soil, water and management limitations, the yield of wheat and sugar beet equals 7206 and 9307 kg in dry matter hectare, and 7981 and 62049 kg in humid matter hectare, respectively (Tables 7 and 8).

**Table 7-**The estimated coefficients of yield potential for **sugar beet** , in Aspas region, based on FAO method

Quantity	Calculating the maximum net production Amount of Biomass(bgm)
25	Maximum leaf photo synthesis (Kilogram CH <sub>2</sub> O in hectare /hour ): Pm
411.7150	Maximum gross biomass production (kg.ha <sup>-1</sup> .d <sup>-1</sup> ): bc
222.9975	Maximum gross biomass production in cloudy day (kg.ha <sup>-1</sup> .d <sup>-1</sup> ): bo
0.3211	f : The ratio of days with unclear weather (1-n/N)
0.6790	1-f : The ratio of days that the weather is clear(n/N)
389.6678	The maximum net production of Biomass (kg CH <sub>2</sub> O/hectare/day time): bgm
Calculating the net production rate of Biomass(Bn)	
0.0108	Respiratory rate for all accept legume:C30
0.0051	Respiratory rate: Ct
211	The number of day to harvest: L
1	correction factor: KLAI
23268.74	Biomass net production rate: Bn
0.4	Harvest index: HI
9307.49	Sugar beet production potential (kg/hectare/dry matter)
85	Moisture percentage
62049.98	Sugar beet Yield: (kg fresh weight per ha)



**Table 8-**The estimated coefficients of yield potential for **wheat** , in Aspas region, based on FAO method

<b>Quantity</b>	<b>Calculating the maximum net production Amount of Biomass(bgm)</b>
20	Maximum leaf photo synthesis (Kilogram CH <sub>2</sub> O in hectare /hour ): Pm
408.2275	Maximum gross biomass production (kg.ha <sup>-1</sup> .d <sup>-1</sup> ): bc
220.9300	Maximum gross biomass production in cloudy day (kg.ha <sup>-1</sup> .d <sup>-1</sup> ):bo
0.3115	f : The ratio of days that the weather is not clear(1-n/N)
0.6885	1-f : The ratio of days that the weather is clear (n/N)
349.8908	Maximum net production of Biomass (kg CH <sub>2</sub> O/hectare/day time) bgm
<b>Calculating the net production rate of Biomass(Bn)</b>	
0.0108	Respiratory rate for all accept legume:C30
0.0048	Respiratory rate: Ct
197	The number of days to harvest: L
0.9	correction factor: KLAI
18015.33	Biomass net production rate: Bn
0.4	Harvest index: HI
7206.13	wheat production potential (kg/hectare/dry matter)
12	Moisture percentage
7981.19	wheat Yield: (kg fresh weight per ha)

Considering that the observed yield of sugar beet in this area (39 ton per hectare), we can increase it to 62049 kg fresh weight per hectare by applying proper management and eliminating the reparable limitations. Also, the qualitative assessment of the land proportion for wheat and sugar beet was done in Aspas region based on the simple and parametric methods.

The results of parametric method (Storie and square root) showed that for producing wheat and sugar beet in this area proportion class ranged from S2 to N2 and S2 to N1, respectively and the most limiting factors were slope, wetting, percentage of calcium carbonate, salinity and alkalinity. The results of this study indicated that most parts of sugar beet and some parts of wheat growth stages occur after May, as the growth period of this product is outside the growth period of the area (19 November to 30 April).

Therefore, moisture content was not sufficient for sugar beet and wheat growth and supplementary irrigation was required. These results are in agreement with those reported by Masihabadi et al. (2001) and Sohrabi (2003) who stated that there were no thermal limitations for sugar beet growth in Qazvin Plain, Iran, but supplementary irrigation was required.

## CONCLUSION

To evaluate the land suitability for irrigation, the parametric evaluation system was applied using soil and land characteristics. Results of climatic suitability classification based on different methods showed that climatic class of the studied area was moderately suitable (S2) for irrigated wheat and very suitable (S1) for sugar beet. The main limiting climate factor for irrigated wheat was the mean temperature during the growth cycle. Therefore, a modification of climatic suitability classes is suggested. Therefore, it was more suitable for sugar beet cultivation. Considering the existing limitations, the studied area was classified from moderately suitable (S2) to not suitable (N) for the selected crops based on the parametric method. However determination of the final class of this area depends on soil limitations such as surface and subsurface stone and gravel percentage, land slope and the amount of lime. Consequently, the most important limiting physical, fertility and topographical factors were soil parameters like texture, pH, coarse fragments, and land slope, CaCO<sub>3</sub> content and micro relief. Their effects can be evaluated alone or in combinations for different soil units. The parametric methods (square root method) performed better than limitation method. Finally, production potential was calculated for sugar beet and wheat in Aspas as 9307.49 and 7206.13kg DM per hectare for the studied area, respectively. Of course, reaching to such production is impossible due to soil and management limiting factors in irrigated farming. In the other words, the difference between the actual and potential yield can be attributed to these limitations. The results of this study indicated that most parts of sugar beet and some parts of wheat growth stages occur after May, as the growth period of these product falls outside the growth period of the area (19 November to 30 April). Therefore, moisture content was not sufficient for growth of sugar beet and wheat and supplementary irrigation was necessary. Therefore, farmers can enhance the productivity by removing the modifiable limitations and improving management.

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## IMPORTANCE OF GEOLOGY AND GEOMORPHOLOGY IN WATERSHED HEALTH ASSESSMENT

### SUMMARY

Watersheds constituted by different geology, geomorphology, climates, land uses, soils, ecological communities, and vegetation covers. The watershed landscapes have established over geologic time while being shaped by patterns of climate, vegetation, and lithology. However, understanding the nexus between watershed health, geology and geomorphology has been less considered and requires a deep knowledge of their spatiotemporal scales of evolution. The current article, therefore, attempts to provide a brief review of geology and geomorphology concepts, importance and applications in watershed health assessment. Additionally, a list of most important geologic and geomorphologic criteria for watershed management and assessment provided as well. The provided information provides useful insights for land managers and decision makers and thus helps in the identification of gaps in knowledge that need to be addressed on a priority basis. It is highly suggested that the future developments in watershed health studies have focused in the direction of the geological and geomorphological process, taking advantage of empirical observations, mathematical and conceptual modeling to attain a quantitative description of real watershed health conditions.

**Keywords:** health; geomorphic insights; geomorphic system; landform; land management

### INTRODUCTION

Geology includes elements such as lithology and structural settings (Wilson and Droste, 2000). Whereas, geomorphology processes incorporate the climate or hydrology sectors with parent materials, sediment, and vegetation cover to form a landform (Swanson et al., 2017). Geomorphology indeed comprises both quantitative and qualitative explanations of landscapes and landforms, as well as processes investigations and process interactions creating these forms in temporal and spatial scales (Renschler and Harbor, 2002). Geology and geomorphology sectors are fundamental elements in watershed landscape analysis (e.g., García-aguirre et al., 2014; Jain et al., 2012) due to their influence on terrain evaluation and life of existing plants and animal species. Physical processes and their linkages with ecosystem quality have become a

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priority within the context of watershed health (EPA, 2012) and one of the aspects that need to be considered in the monitoring and ecological health assessment.

The global increasing focus on the geological and geomorphological processes has highlighted their importance in watershed sustaining (Ahn and Kim, 2017a and b; EPA, 2014; 2013; 2012; 2011; Jat et al., 2008). For example, morphological characteristics of the river channel, floodplain, and valley sides and consequently the river biocoenoses arrangement along the river continuum determined by geomorphic processes functioning in particular river reaches (Rinaldi et al., 2013). Indeed, since the 1990s, increasing scientific studies have carried out concerning watershed health assessment. A progressive evolution toward process-based watersheds restoration has occurred (Hazbavi et al., 2018a and b), where the target is to restore natural geomorphic processes (Rinaldi et al., 2013). Thence, the essential need to consider process-oriented approaches and self-restoration strategies, persistently (Rinaldi et al., 2013; Wohl et al., 2005).

Increasing attention is being paid to the ecosystem health assessment and management on a watershed basis, necessitating a cross-disciplinary approach to data collection and analysis. Linking the geologic and geomorphologic data with the watershed health assessment applications can provide a simpler and more accurate way to understand the complex behavior of watersheds. Therefore, this review was made to highlight this important issue and giving new insights for developments of future watershed health assessment frameworks.

## **METHODS OF GEOLOGY AND GEOMORPHOLOGY VARIABLES MEASUREMENT**

### **Field surveying**

The main method to study geologic and geomorphologic data is field surveying. Indeed, fieldwork is the formative experience central, and the U.S. Geological Survey (USGS) is well suited to guide the upcoming workforce (Gundersen et al., 2011). Cianfrani et al. (2005) applied a rapid geomorphic assessment (RGA) following the Vermont Department of Conservation Protocols (VTDEC, 2002). This method characterizes the channel geomorphology based on the field data. Expect six conditions to identify viz., pre-modified, constructed, degradation, threshold, aggradation, and restabilization (For more information see Cianfrani et al. (2005); VTDEC (2002)). However, there are many methods to help the derivation of valuable and useful information as explained below.

### **Air photo and digital techniques application**

Air photo interpretation and digital techniques including GIS-based spatial analysis and modeling, and remote sensing provide high important information on the geologic origin and structure (Field, 2015; García-aguirre et al., 2014). The sandstone, shale and limestone rocks can be distinguished easily with help of remote-sensed documents interpretation. The geomorphology knowledge is an essential tool to recognize landform types, the strike and dip attitudes, the orientation of highlights and shadows drainage patterns, and the relative

susceptibility degree of the formations to flooding and other natural hazards (García-aguirre et al., 2014; Verstappen, 1988).

Watershed health researches provide ample evidence that morphological characteristic can exert a significant influence on the processes shaping other landscape patterns. Digital elevation models (DEM) is one of the important remote-sensed documents and as an elementary data used for watershed health assessment. DEM could be extracted from contour map through ArcGIS spatial analysis tool and based on two maps of slope and aspect (Kabite and Gessesse, 2018; Field, 2015; Ding et al., 2008). Simultaneous analysis of maps of non-biotic elements viz. geology, geomorphology, and biotic elements include land use/cover allow to generate synthetic and systematic information of watershed landscape in the form of the bio-geomorphic land unit (BGU) maps (Zonneveld, 1995) applicable for watershed health assessment.

It is further necessary to mention that the different geomorphological indices developed based on digital techniques to better understand the behavior of geologic and geomorphologic changes and process. The stream-gradient index, hypsometric integral, valley floor width–valley height ratio, drainage basin shape, drainage basin asymmetry, and mountain-front sinuosity are some examples of the applicable indices for geologic and geomorphologic characterization (Faghih et al., 2015).

### **Connectivity analysis**

Geomorphic connectivity in a watershed landscape affected by different Landforms or human disturbances (Poepl et al., 2018; 2017). In regards to the significant importance of connectivity to analyze the bio-physical fluxes movement in a large geomorphic system (Jain et al., 2012) introduced as an important determinant for watershed health assessment (Hazbavi et al., 2018b and EPA, 2012). Connectivity can be measured through flux interaction(s) (functional connectivity) and physical connectedness (structural connectivity) between Landforms (Jain et al., 2012). Geomorphological variables determine the potential for water and sediment to be transported through a geomorphic system (Keesstra et al., 2018). In addition, the connectivity could be investigated from three dimensions of longitudinal, lateral and vertical through geomorphic systems. In fact, geomorphic systems with low connectivity are less sensitive and thus more resilient to disturbances (Poepl et al., 2017). Understanding the reaction of the watersheds against to drivers the concept of connectivity thinking could be adapted as a useful approach in watershed health assessments.

### **Other Methods**

Rădoane et al. (2015) noted that for the geomorphologic characterization of the river beds in the established moments of time there is a morphometric database regarding the features of the entire alluvial lowland of the river, at the active stripe of flood-plain, at the stream channel itself. Strahler (1957) quantified the geomorphic methods in two general classes of (1) linear scale measurements and (2) dimensionless properties. The criteria measured by each method is described in Table 1.

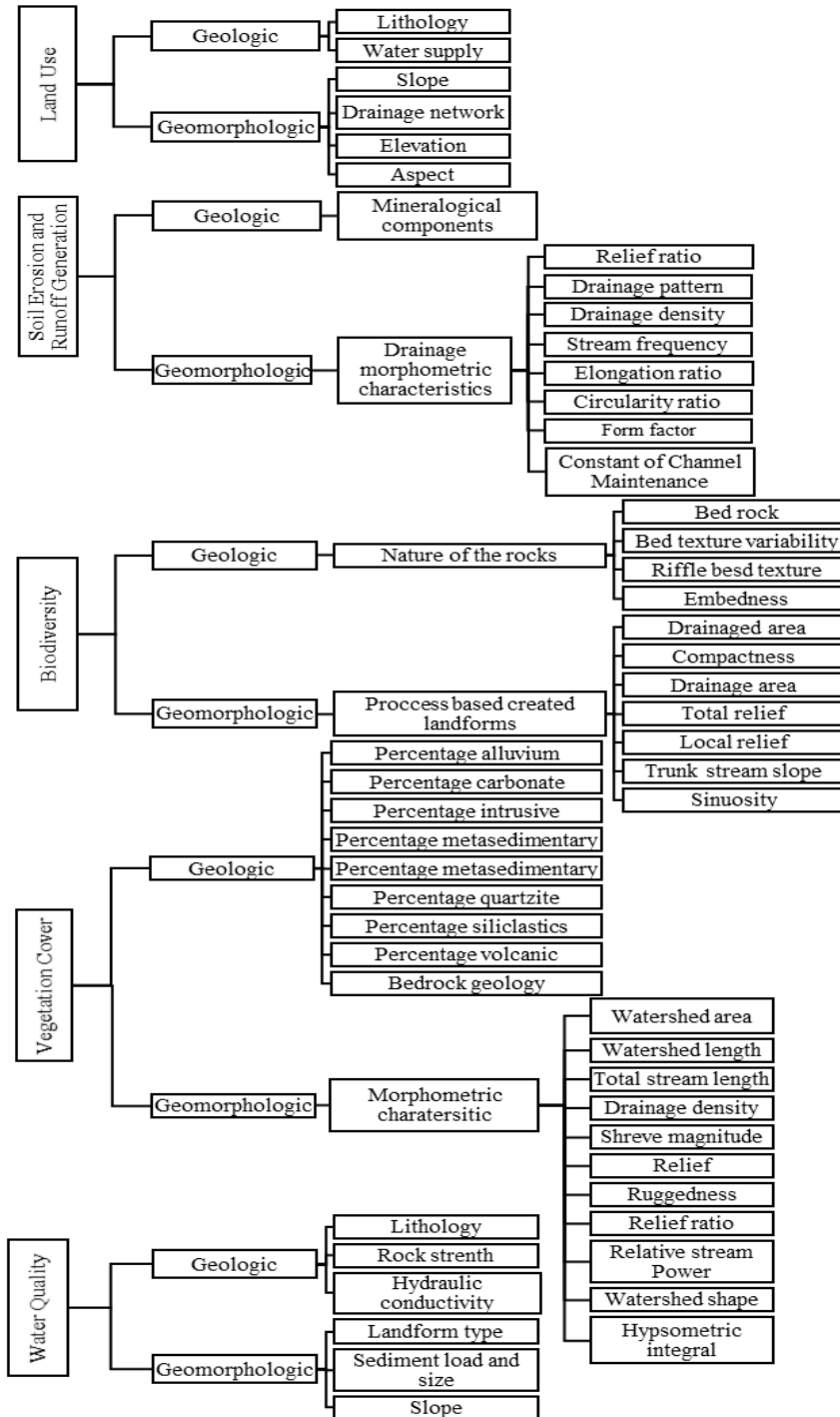


Figure 1: Geological and geomorphological criteria and interrelationship with other affecting criteria in watershed health assessment



Table 1. Quantitative methods of watershed geomorphology (Adapted from Strahler, 1957)

Linear Scale Measurements	Dimensionless Properties Measurements
<ul style="list-style-type: none"> <li>• length of stream channels of given order</li> <li>• drainage density</li> <li>• constant of channel maintenance</li> <li>• basin perimeter</li> <li>• relief</li> </ul>	<ul style="list-style-type: none"> <li>• stream order numbers</li> <li>• stream length and bifurcation ratios</li> <li>• junction angles</li> <li>• maximum valley-side slopes</li> <li>• mean slopes of watershed surfaces</li> <li>• channel gradients</li> <li>• relief ratios</li> <li>• hypsometric curve properties and integrals</li> </ul>

The U.S. Geological Survey (USGS) also provides digital and nationwide information through a publicly accessible data server for many parts of the world ([www.usgs.gov](http://www.usgs.gov)). Recently, GEOMORSIS, a semi-automatic geo-morphometric analysis package is released for quantitative analysis of watershed geomorphology using GIS. It has six basic modules i.e., AUDRALA, STMPARA, RELEIF PARAMETER, REPORT GENERATION and BASGEO. Win Basin is another watershed analysis system applicable for extraction of realistic drainage networks and calculation of geomorphological indices from DEMs. The Natural Resources Conservation Services (NRCS) Geo-Hydro as an Arc GIS application extension could compute geomorphological and hydrological elements (Khan et al., 2016).

After a very extensive literature review, the most important of geologic and geomorphologic criteria was then extracted and summarized in Figure 1.

### **WATERSHED HEALTH ASSESSMENT, GEOLOGY, AND GEOMORPHOLOGY RELATIONSHIP**

Watershed health assessment and restoration have been undertaken using a wide variety of approaches and techniques (Hazbavi et al., 2018; Sadeghi et al., 2018; Liao et al., 2018; Hazbavi and Sadeghi, 2017; Sadeghi and Hazbavi, 2017; Li et al., 2013; An et al., 2002) yet there is often a high rate of failure to improve watershed ecosystems health. Part of the problem is allocated to a failure to understand the fundamental principles of watershed-scale geology and geomorphology that control watershed responses to disturbance (Townsend, 2009). Notwithstanding the increasing experiences and evolving approaches in the field watershed health assessment, very limited researches have considered the geology and geomorphology sectors for the ecosystem (even include watershed) health assessment as given in Table 2.

Recently, Hazbavi (2018) provided a list of watershed health assessment tools as follows: 1) Thermodynamic analysis, 2) Network analysis, 3) Multi-metric approach, 4) Predictive model approach, 5) Healthy watersheds initiative (EPA Protocol), 6) Reliability-resilience-vulnerability (RRV), 7) Vitality (Vigor)-organization force-recovery force (VOR) and 8) Pressure-state-response (PSR) and its new versions.

Table 2. List of geological and geomorphological criteria used in watershed health assessment

<b>Criteria</b>	<b>Reference</b>
Stream order, stream length, drainage density, drainage texture Area, perimeter, length and width of watershed	Jat et al. (2008)
Water storage (aquifer storage capacity vs. unused capacity) Water storage (aquifer storage filled with “clean” water vs. contaminated water) Net recharge/withdrawals (water year) Detention/retention basin capacity vs. bio-retention capacity Extent and diversity of soft bottom conditions in rivers and streams. Water flow dynamics adequate to support diverse habitats (e.g., volume, rate, and seasonality). Sediment and material flows adequate to support diverse habitats. Per capital water use Local (native) vs. imported source for delivered	Vos et al. (2008) and Antos et al. (2011)
Drainage area, compactness, drainage density, total relief, local relief, trunk stream slope, map slope, erosion index	Walters et al. (2009)
Bedrock and surficial, soil resistance properties, geography - continental, mountain, valley, and coastal Landforms, geomorphic reaches, functional process zones, active river areas, ground water- dependent, springs, seeps, wetlands, lakes; Historical planform and floodplain modification, channel, floodplain, and valley geomorphology, sediment, and woody regimes; Geomorphic stability and stage of channel evolution, channel geometry and hydraulics, distribution and sorting of sediment and wood, boundary conditions and vegetation (soil erodibility testing, roughness elements and coefficients)	EPA (2011)
Percent of assessed stream miles in reference condition	EPA (2012)
Dominant Surface Geology	EPA (2013)
Stream habitat condition, rating and dam presence/absence	EPA (2014)
Substrate, habitat complexity, velocity/depth regimes, bank stability, channel alteration	Wu et al. (2015)
Ratio of stream length of reference condition in watershed to total stream length in watershed	Ahn and Kim (2017a)
Percentage of assessed stream length in the reference condition	Ahn and Kim (2017b)

Table 3. SWOT analysis of geology and geomorphology concepts in watershed health assessment (Adapted from Downs and Booth, 2011)

<p><b>Strengths (S)</b></p>	<ul style="list-style-type: none"> <li>- Directly concerned with the surface of the earth</li> <li>- Directly concerned with regional (e.g. watershed) functions that are the basis for maintaining healthy ecosystems and valued native biological populations</li> <li>- Long history of studying the role of human impact in system functioning</li> <li>- Well positioned to integrate biology, engineering and planning into practical solutions</li> <li>- Well positioned to practice design and management with nature to achieve truly sustainable designs</li> </ul>
<p><b>Weaknesses (W)</b></p>	<ul style="list-style-type: none"> <li>- Poor representation at policy levels</li> <li>- Poor representation on funding bodies to ensure adequate research funds Lack of standard methods</li> <li>- Lack of routine monitoring of geomorphic systems</li> <li>- Viewed as a sub-set of engineering, especially in more quiescent landscapes</li> <li>- Lack of a professional group and professional accreditation</li> </ul>
<p><b>Opportunities (O)</b></p>	<ul style="list-style-type: none"> <li>- Directly concerned with the surface of the earth</li> <li>- Directly concerned with regional (e.g. catchment) functions that are the basis for maintaining healthy ecosystems and valued native biological populations</li> <li>- Long history of studying the role of human impact in system functioning</li> <li>- Well positioned to integrate biology, engineering and planning into practical solutions</li> <li>- Well positioned to practice design and management with nature to achieve truly sustainable designs</li> </ul>
<p><b>Threats (T)</b></p>	<ul style="list-style-type: none"> <li>- Geomorphology practiced by others with little or insufficient training, and so lacking in broad areas of necessary skills</li> <li>- Perception of simplistic geomorphological descriptions of system functioning that do not apply in all cases</li> </ul>

According to the widely used procedure for watershed health assessment i.e., United States Environmental Protection Agency (EPA, 2012), six essential indicators of (1) the landscape condition, (2) geomorphology, (3) hydrology, (4) water quality, (5) habitat, and (6) biological condition are fundamental to the assessment of watershed health. It is understood from the literature that the geology and geomorphology play a critical role in upland function (Stringham and Repp 2010) as it dictates soil and vegetation characteristics found within the watershed system (Hecker, 2017). However, the watershed geomorphology (e.g., valley type) could not be altered by the human, but the land management decisions could influence the stream morphology and etc. (Hecker, 2017). As a result of human-induced infrastructure, the terrestrial and aquatic habitat fragmented throughout a watershed and could affect the natural stream geomorphology, significantly (EPA, 2012).

Towards above-mentioned notes, the geomorphological application in watershed health assessment like environmental management as stated by Downs and Booth (2011) could be analyzed by prospects of strengths, weaknesses, opportunities, and threats (SWOT) as explained in Table 3.

### CONCLUSIONS

Interdisciplinary research is still needed in the areas of geology and geomorphology to appropriately implement watershed assessment and monitoring procedures within the scope of the watershed health. Watersheds should be considered as basic landscape units for understanding natural resources and environmental issues. This review has focused on issues surrounding the geologic and geomorphologic application to watershed problem-solving. Despite applied geomorphology long but largely unrepresented history within the watershed health discipline, escalating environmental awareness and better technical expertise have brought increased opportunities to contribute to integrated watershed management. Linking the geologic and geomorphologic characteristics with the health concept of the watershed can provide a scientific basis and more accurate and simpler way to apply effective and fruitful strategies to identify particular areas within the watershed that should receive a higher priority for management. In addition, it needs to learn more about related terminologies to geology and geomorphology concepts like hydro-geomorphologic, eco-geomorphologic, geochemistry and other similar ones and obtain more comprehensive information. It does not forget to give more attention to know more about the nexus of geological and geomorphological sectors with other like ecology, hydrology, climate and etc.

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**FURTHER DATA ON PECULIAR SUBTERRANEAN FAUNA OF  
NIPHARGIDAE FAMILY IN GREECE  
(CONTRIBUTION TO THE KNOWLEDGE OF THE AMPHIPODA 306)**

**SUMMARY**

The subterranean species *Niphargus karkabounasi* Ntakos et al., 2015 (Amphipoda, fam. Niphargidae) was known from type-locality only (AgiioTheodoroi, Korinthos, Peloponnese, Greece). This species is established now in some other localities in Greece, partially redescribed, figured and variability of some of its taxonomical characters are presented.

The new peculiar species, *Niphargus pararhodi*, sp. n. is described from the spring-brook along Gadouras River (Apolona region, Greece) based on one adult male and its taxonomical position regarding other species from Greece is discussed.

**Keywords:** Amphipoda, *Niphargus karkabounasi*, *pararhodi*, taxonomy, subterranean waters, Greece.

**INTRODUCTION**

The fauna of the subterranean family Niphargidae in Greece has been studied by various scientists and numerous new taxa were discovered and described. Our recent study of this family in Greece show the astonishing variety of taxa: 3 genera and over 20 species and subspecies, despite only partially investigated this fauna in Greece. During present investigation of the material collected in Greece by several scientists from Italy and Holland, new localities and variability of one known endemic species are presented and description of one peculiar new species of the genus *Niphargus* Schiödte, 1849 is described and figured.

**MATERIAL AND METHODS**

The collected specimens were preserved in 70% ethanol and sent us for determination. The specimens were dissected using a WILD M20 microscope and drawn using camera lucida attachment. All appendages were temporarily submersed in the mixture of glycerin and water for study and drawing. The appendages were transferred to Liquid of Faure on permanent slides. All illustrations were inked manually. Some morphological terminology and setal formulae follow G. Karaman's terminology (Karaman, G., 1969; 2012) regarding the last mandibular palpus article [A= setae on outer face; B= setae on inner face;

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Notes: The author declare that they have no conflicts of interest. Authorship Form signed online.

D= lateral marginal setae; E= distal long setae] and propodus of gnathopods 1 and 2 [S= corner S-spine; L= lateral slender serrate L-spines; M= facial M-setae; R= subcorner R-spine on inner face]. Terms “setae” and “spines” are used based on its shape, not origin. Some ecological data of samples-localities of Greece have been published by Pesce & Maggi (1983).

The research in this work is based on the classic morphological, ecological and zoogeographical studies.

## TAXONOMICAL PART

### Family NIPHARGIDAE

#### *NIPHARGUS KARKABOUNASI* Ntakis, Anastasiadou, Zakšek & Fišer 2015 Figures 1-3

*Niphargus karkabounasi* Ntakis, Anastasiadou, Zakšek & Fišer 2015: 37, figs. 2B, 7-10.

#### MATERIAL EXAMINED: GREECE

G-78= Attica, road Korinthos-Athens near Megara, water temp. 18.5°C, pH 6.8, 2 exp. 3.3 mm; 9.5.1977 (leg. Pesce, Maggi & Miranda);

G-93= Attica, road Korinthos-Athens, nearly 4 km from Korinthos, well, water temp. 17.4°C, pH 6.9; 17 small exp., 9.4.1978 (leg. Pesce & Maggi);

G-94=Attica, road Korinthos-Athens, Agioi Theodoroi, water temp. 19.9°C; pH 6.9; 6 exp., 9.4.1978 (leg. Pesce & Maggi) (probably toptotypic locality);

G-95 (S-6374) = Attica, road Korinthos-Athens, Megara, 2 wells, water temp. 16.9°C, pH 6.9; 2 exp. 3 mm, 9.4.1978 (leg. Pesce & Maggi);

G-96= Attica, road Korinthos- Athens, Megara, 2 wells, water temp. 16.9°C, pH 6.9; one exp 3.4 mm; 9.4.1978 (leg. Pesce, Maggi & Silverii);

G-97=Nafplio (=Nauplion) (Argos), Peloponnese, 2 wells, water temp. 21.5°C, pH 6.8; one exp. 2.8 mm, 9.4.1978, (leg. Pesce, Maggi & Silverii);

G-232= Skopelos island, Panormos (cca 250 m from the sea), 2 wells, water temp. 14.2°C, pH 7.1, 6.7.1980, one damaged exp. 3.6 mm (leg. G. Pesce).

#### DESCRIPTION

Ntakis et al. (2015) described this species based on specimens from one locality [Agioi Theodoroi in Korinthos region, Greece]. The specimens from our new localities agree mainly with description of this species from type-locality, with some minor differences.

**Female 4.0 mm with setose oostegites from G-93 (near Korinthos):**  
Metasomal segments 1-3 with 2-3 dorsoposterior setae each (fig. 2B).

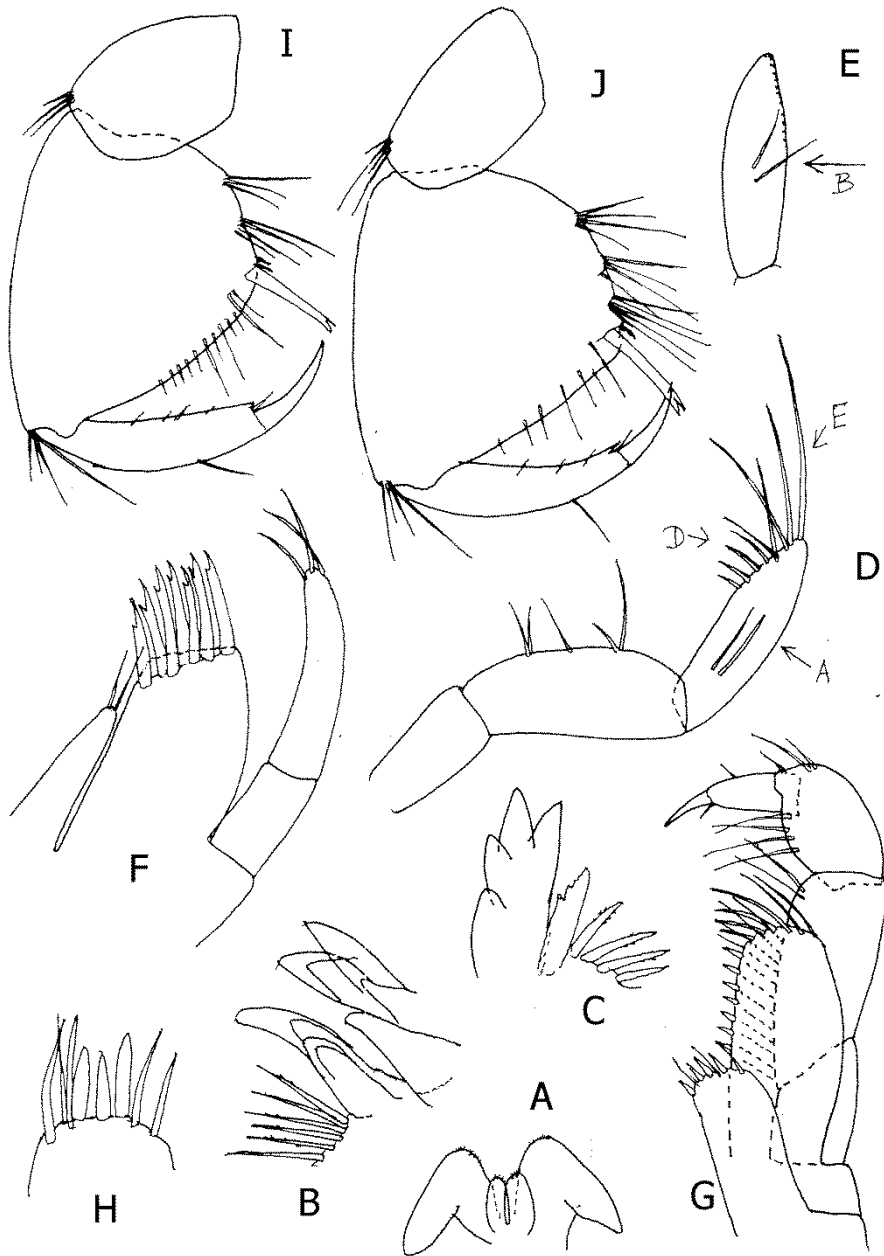


Fig. 1. *Niphargus karkabounasi* Ntakos et al., 2015, G-93, Korinthos, female 4.0 mm: A= labium; B= right mandible, incisor and lacinia mobilis; C= left mandible: incisor and lacinia mobilis; D= mandibular palp, outer face with A, D, E setae; E= last article of mandibular palp, inner face with B-setae; F= maxilla 1; G= maxilliped; H= inner plate of maxilliped; I= gnathopod 1 propodus, outer face; J= gnathopod 2 propodus, outer face.

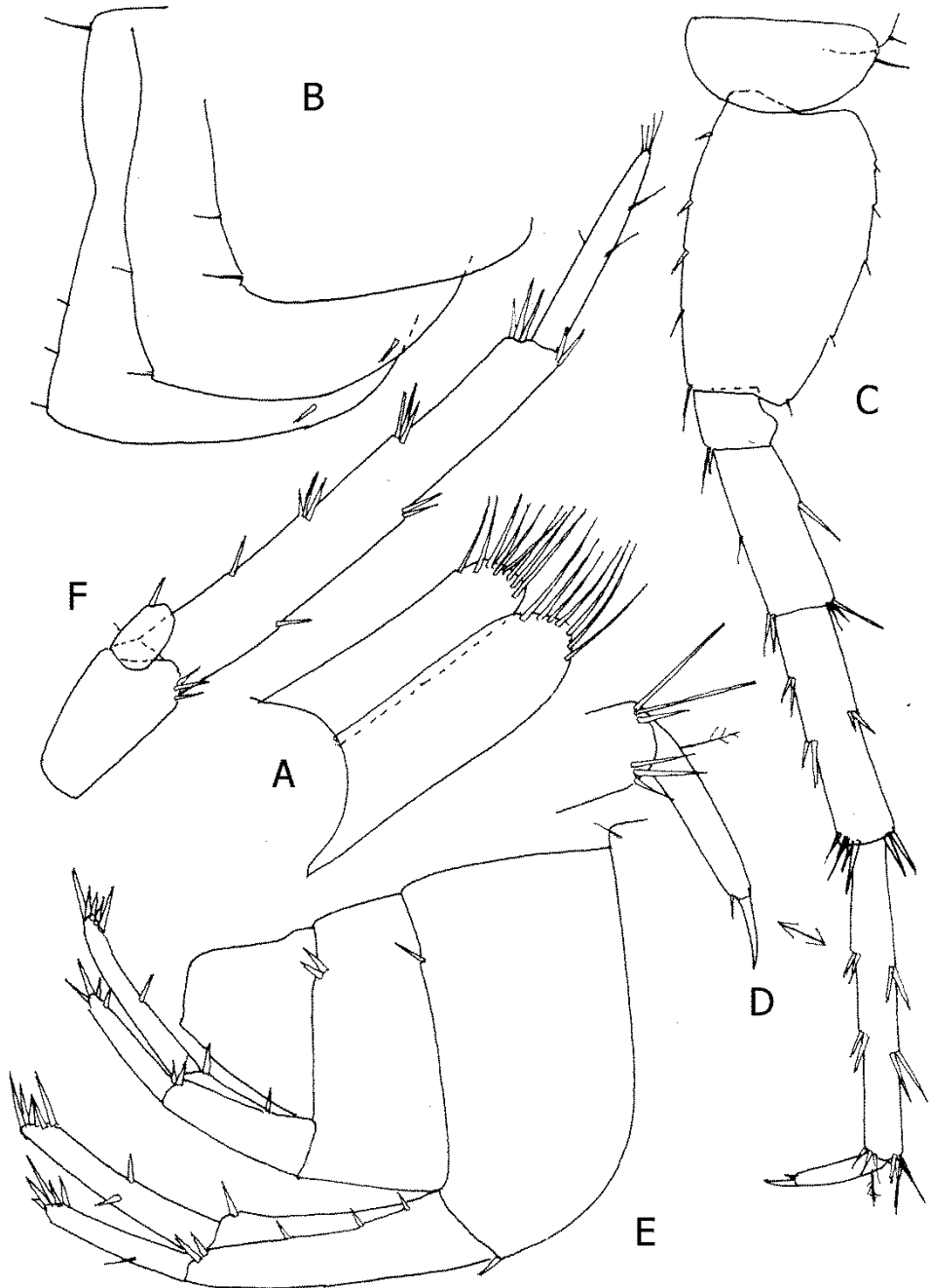


Fig. 2. *Niphargus karkabounasi* Ntakis et al., 2015, G-93, Korinthos, female 4.0 mm: A= maxilla 2; B= epimeral plates 1-3; C= pereopod 7; D= dactylus of pereopod 7; E= urosome with uropods 1-2; F= uropod 3.

Urosomal segment 1 on each dorsolateral side with one seta; urosomal segment 2 on each dorsolateral side with 2 spines; urosomal segment 3 naked (fig. 2E). Urosomal segment 1 at each ventroposterior corner with one short spine near basis of uropod 1 peduncle (fig. 2E).

Epimeral plates 1 and 2 obtusely angular, with slightly convex posterior margin bearing 1-2 lateral setae; epimeral plate 3 angular, but not pointed, with inclined or poorly convex posterior margin provided with 1-2 lateral setae; one ventral slender spine appears on epimeral plates 2 and 3 (fig. 2B).

Lateral cephalic lobes short, subrounded. Antenna 1 reaching half of body-length, with progressively shorter peduncular articles 1-3; main flagellum consisting of 14 articles (most of them with one aesthetasc exceeding 2/3 of article itself (fig. 3C). Accessory flagellum short, 2-articulated, exceeding half of peduncular article 3 (fig. 3B).

Antenna 2 flagellum slender, longer than last peduncular article and consisting of 5 articles.

Labrum broader than long, convex distally (fig. 3A). Labium broader than long, inner lobes short, outer lobes subrounded (fig. 1A).

Mandibles with triturative molar. Left mandible: incisor with 5 teeth, lacinia mobilis with 4 teeth accompanied by 6 rakers (fig. 1B). Right mandible: incisor with 4 teeth, lacinia mobilis bifurcate, serrate, accompanied by 4 rakers (fig. 1C). Mandibular palpus 3-articulated: first article naked, second article with 5 strong setae (fig. 1D); third article subfalciform, as long as article 2 and provided with 5-6 strong D-setae and 3-4 long distal E-setae; 2 A-setae appear on outer face (fig. 1D) and 2 B-setae are present on inner face (fig. 1E).

Maxilla 1: inner plate with 2 setae, outer plate with 7 spines provided with one strong lateral tooth each (fig. 1F); palpus 2-articulated, reaching tip of outer plate-spines and provided with 3 distal strong setae.

Maxilla 2 with nearly subequal both plates bearing distomarginal setae (fig. 2A).

Maxilliped: inner plate short, with 3-4 distal spines mixed with single setae, outer plate exceeding half of palpus article 2 and provided with 7-8 distolateral spines (fig. 1G); palpus article 4 with nail, one short seta is attached at inner margin near basis of the nail.

Coxae are short. Coxa 1 broader than long (ratio: 58:36) with subrounded ventroanterior corner and poorly concave ventral margin provided with 3 marginal setae (fig. 3D). Coxa 2 poorly broader than long (ratio: 55:53), with 3 ventral setae (fig. 3E). Coxa 3 nearly as long as broad, at margin with 2 setae (fig. 3F). Coxa 4 hardly broader than long (ratio: 64:60), with 2 ventral setae and without ventroposterior lobe (fig. 3G).

Coxa 5 rather shorter than coxa 4, bilobed, broader than long (ratio: 74:46), with 2 setae at posterior margin (fig. 3H). Coxa 6 is smaller than coxa 5, bilobed, broader than long (ratio: 62:37), with one posterior seta (fig. 3I). Coxa 7 entire, broader than long (ratio: 63:30) with convex ventral margin and one posterior seta (fig. 3J).

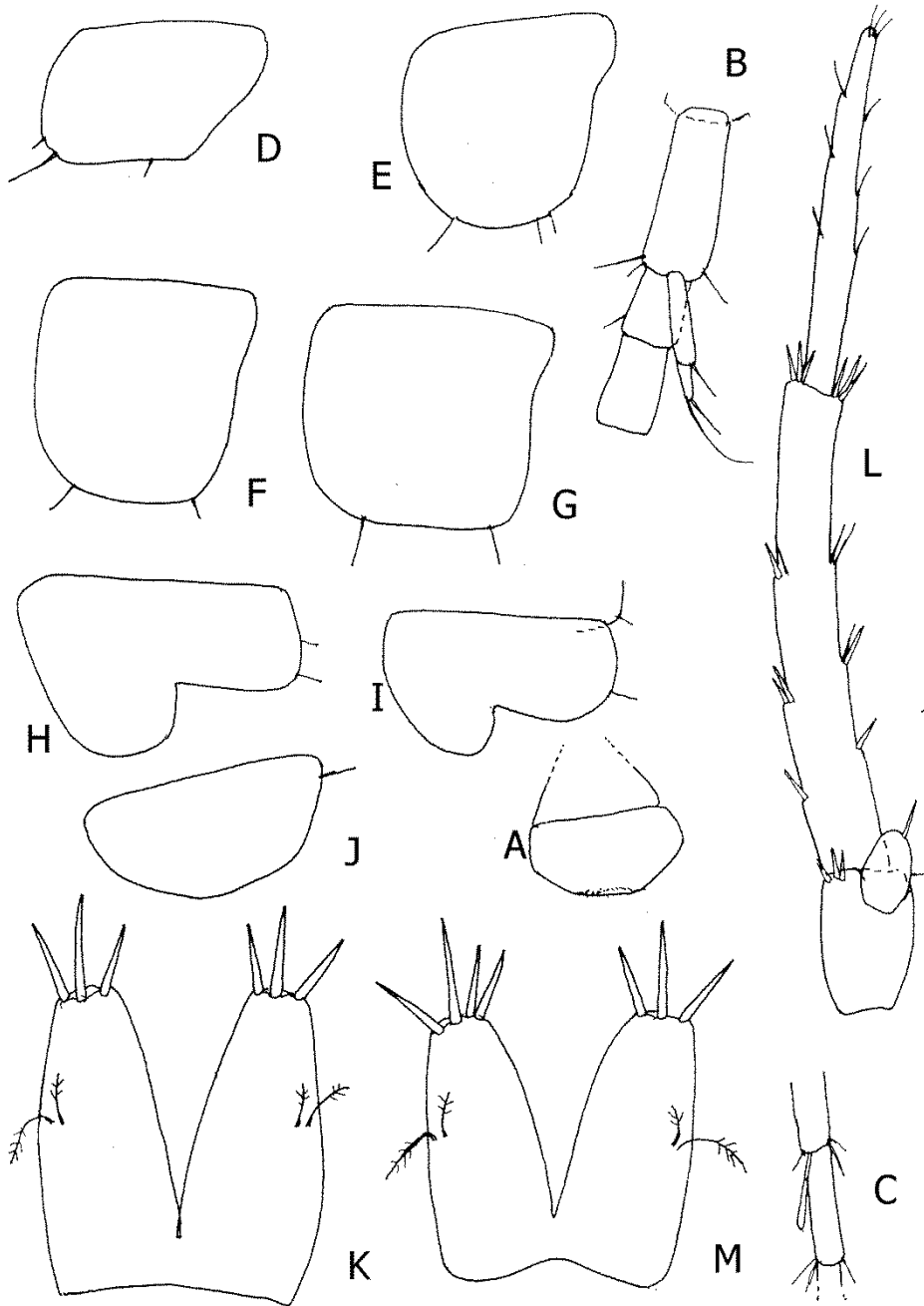


Fig. 3. *Niphargus karkabounasi* Ntakis et al., 2015, G-93, Korinthos, female 4.0 mm: A- labrum; B= accessory flagellum; C= aesthetasc on antenna 1; D= coxa 1; E= coxa 2; F- coxa 3; G= coxa 4; H= coxa 5; I= coxa 6; J= coxa 7; K= telson. **Male 3.9 mm, G-94, Agioi Theodoroi:** L= uropod 3; M= telson.

Gnathopods 1-2 of moderate size, with propodus slightly larger than corresponding coxa. Gnathopod 1: article 3 at posterior margin with one bunch of setae; article 5 much shorter than propodus (ratio: 50:85), with 3 distal setae (fig. 1 I). Propodus trapezoid, longer than broad (ratio: 86:66), along posterior margin with 2 transverse rows of setae (fig. 1 I). Palm slightly convex, inclined nearly half of propodus-length, defined on outer face by one corer S-spine accompanied laterally by 2 L-spines and 2 facial M-setae, on inner face by one subcorner R-spine. Palm provided with setae of unequal length sitting mainly in distal part of palm. Dactylus reaching posterior margin of propodus and provided with one median seta at outer margin and several setae at inner margin (fig. 1 I).

Gnathopod 2: article 3 at posterior margin with one bunch of setae; article 5 shorter than propodus (ratio: 50:82), with 4 distal setae (fig. 1 J). Propodus trapezoid, almost of the same size of that in gnathopod 1, trapezoid, poorly longer than broad (ratio: 82:72), along posterior margin with 3 transverse rows of setae. Palm convex, inclined nearly to the half of propodus-length, defined on outer face by one S-spine accompanied laterally by one L-spine and one facial M-seta, on inner face by one subcorner R-spine. Palm provided with scarce number of setae. Dactylus reaching posterior margin of propodus and provided with one median seta at outer margin and several setae at inner margin (fig. 1 J).

Pereopods 3-4 relatively slender; dactylus slender, at inner margin with one slender spine neat basis of the nail.

Pereopod 5 shorter than pereopod 6, both moderately slender, article 2 dilated but without ventroposterior lobe, along posterior margin appear several setae only; articles 4-6 along both margins with spines and setae; dactylus slender, at inner margin with slender spine near basis of the nail.

Pereopod 7: article 2 longer than broad (ratio: 80:48), at anterior margin with 5 single spine-like setae, along posterior convex margin with 5 short setae, ventroposterior lobe not distinctly developed. Articles 4-6 of unequal length (ratio: 46:64:83), along both margins with spines and single short setae (fig. 2C). Article 2 hardly shorter than article 6 (ratio: 80:83). Dactylus slender, remarkably shorter than article 6 (ratio: 33:83), at inner margin with one slender spine near basis of the nail, at outer margin with one median plumose seta (fig. 2D); nail shorter than pedestal (ratio: 18:50).

Pleopods 1-3 with 2 retinacula each; peduncles naked.

Uropod 1: peduncle with dorsoexternal row of spines, at dorsointernal margin appears distal spine only (fig. 2E); inner ramus shorter than peduncle, with 2 lateral and 5 distal short spines; outer ramus distinctly shorter than inner one, with one lateral short simple seta and 5 distal short spines.

Uropod 2: peduncle with lateral and distal spines; inner ramus with one lateral and 5 distal short spines (fig. 2E); outer ramus remarkably shorter than inner one, with 5 distal short spines.

Uropod 3 long and slender: peduncle rather longer than broad, with 3-4 distal spines; inner ramus very short, scale-like, much shorter than peduncle and provided with 0-1 lateral seta and one distal spine. Outer ramus 2-articulated:

first article at outer margin with 3 groups of short spines, at inner margin with 4 bunches of spines, setae absent (fig. 2F); second article slender, reaching up to half of first article and provided with 2-3 lateral and 4 short distal simple setae.

Telson slightly longer than broad (ratio: 80:74), deeply incised, each lobe with 3 distal spines much shorter than half of telson-length, lateral and facial spines absent; a pair of plumose setae attached near the middle of outer margin of lobes (fig. 3K).

Coxal gills ovoid, relatively short. Oostegites broad, with setae at margins.

**The males of locality G-93:** similar to females, including uropods 1-3. Ntakos et al (2015) mentioned that males differs from females by elongated distal article of uropod 3 outer ramus, but on the figured uropod 3 of male and female it seems to be similar to each other. We observed also large variability of length of distal article of uropod 3 in females and males (reaching 30-50% of first article).

**The male of G-94 (3.9 mm) from Korinthos, Agioi Theodoroi:** uropod 3 second article of outer ramus elongated, reaching nearly 70 percent of first article (fig. 3L). Uropod 3 in all other specimens of this locality like that in typical specimens (distal article reaching 1/3 to 1/2 of first article). Outer ramus of uropod 1 with 0-1 lateral simple seta. Uropod 2 outer ramus of variable length, but always distinctly shorter than inner one. Telson with 3-4 distal spines on each lobe, facial and lateral spines absent (fig. 3M).

**Specimen 3.5 mm from Scopelos island (Aegean Sea, Greece) (G-232):**

Partially damaged specimen (pereopods 5-7 are missing) is very similar to typical *karkabounasi*: telson with 3 distal spines on each lobe; epimeral plate 3 obtusely angular, uropods 1 and 2 with outer ramus much shorter than inner one; distal article of uropod 3 outer ramus reaching 2/5 of first article, propodus of gnathopods 1-2 of almost similar size and with strongly inclined palm.

## AFFINITY

*Niphargus karkabounasi* is relatively small species, belonging to the group of Greek species with one seta on gnathopod's dactylus. Within this group, partially reduced number of D-setae on mandible palpus is present in *Niphargus lakusici* G. Karaman 2017a, known from Crete Island.

*N. lakusici* is similar to *N. karkabounasi* also by shortened outer ramus of uropods 1 and 2, by similar shape of gnathopods and epimeral plates, pleopods, dactylus of pereopods 3-7, but differs from *N. karkabounasi* by short second article of uropod 3 outer ramus in males, by short inner plate of maxilliped provided with reduced number of distal spines, by short outer ramus of maxilliped, by lobed article 2 of pereopods 5-7, by presence of dorsointernal row of spine-like setae on uropod 1 peduncle, presence of lateral spines on lobes of telson.

As *N. lakusici* was described based on one male only, more detailed comparison with *N. karkabounasi* is not reasonable. Both taxa seems to be close to each other despite the geographical distances, and further study on new material of both taxa will show their real taxonomical relation.



**LOCUS TYPICUS:** Agioi Theodoroi, Korinthos, Peloponnese, Greece.

**DISTRIBUTION:** Greece, endemic.

***NIPHARGUS PARARHODI* sp. n.**

**Figures 4-8**

**MATERIAL EXAMINED:**

No-11A. Spring-brook along river Goudouras (Gaidouras), Apollona region, Rhodos Island, Greece, 12.3.1973, male 8.2 mm (leg. J. Stock).

**DIAGNOSIS (male only).**

Body strong, metasomal segments with marginal setae, urosomal segments 1-2 with spine. Lateral cephalic lobes subrounded, eyes absent; maxilla 1 inner plate with 1-2 setae, outer plate with 7 spines bearing 1-5 small lateral teeth; maxilla 2 inner plate is smaller than outer one; maxilliped inner plate with 2 spines. Coxae relatively short, coxa 4 unlobed, coxa 5 smaller than coxa 4; epimeral plates pointed. Gnathopods 1-2 strong, with dactylus bearing a row of setae along outer margin; dactylus of pereopods 3-7 with one spine at inner margin; article 2 of pereopods 5-7 unlobed. Pleopods with 2 retinacula; uropod 1 with dorsointernal row of setae, rami equally long; uropod 2 inner ramus longer than outer one. Uropod 3 outer ramus with distal article relatively short. Telson with distal and mesial marginal spines.

**DESCRIPTION: Male 8.2 mm (holotype):** Body moderately slender, metasomal segments 1-3 with 2 stronger distoposterior setae accompanied by 2 shorter setae each (fig. 8C). Urosomal segment 1 and urosomal segment 2 on each dorsolateral side with one spine; urosomal segment 3 naked (fig. 8D). Urosomal segment 1 on each ventroposterior corner with one spine near basis of uropod 1 peduncle (fig. 8D).

Epimeral plates 1 strongly angular, epimeral plates 2 and 3 slightly pointed, all with strong corner spine-like seta and several posterior lateral setae. Epimeral plates 2 and 3 with 3 subventral spines each (fig. 8C).

Head with short rostrum and subrounded lateral cephalic lobes, ventroanterior sinus developed; eyes absent (fig. 4E). Antenna 1 reaching half of body, peduncular articles 1-3 progressively shorter (ratio: 50:43:20), bearing several shorter setae each (fig. 4F). Main flagellum consisting of 21 articles (most of them with one short aesthetasc); accessory flagellum short, 2-articulated (fig. 4F). Antenna 2: peduncular article 3 with distal bunch of setae longer than diameter of article itself; article 4 slightly longer than article 5 (ratio: 75:65), along dorsal margin with short setae, along ventral margin with 2 bunches of setae (the longest setae remarkably exceeding diameter of article itself); article 5 along dorsal margin with 4 groups of short setae, along ventral margin with 3 bunches of long setae (fig. 4G); flagellum slender, consisting of 9 articles bearing short setae. Antennal gland cone relatively short (fig. 4G).

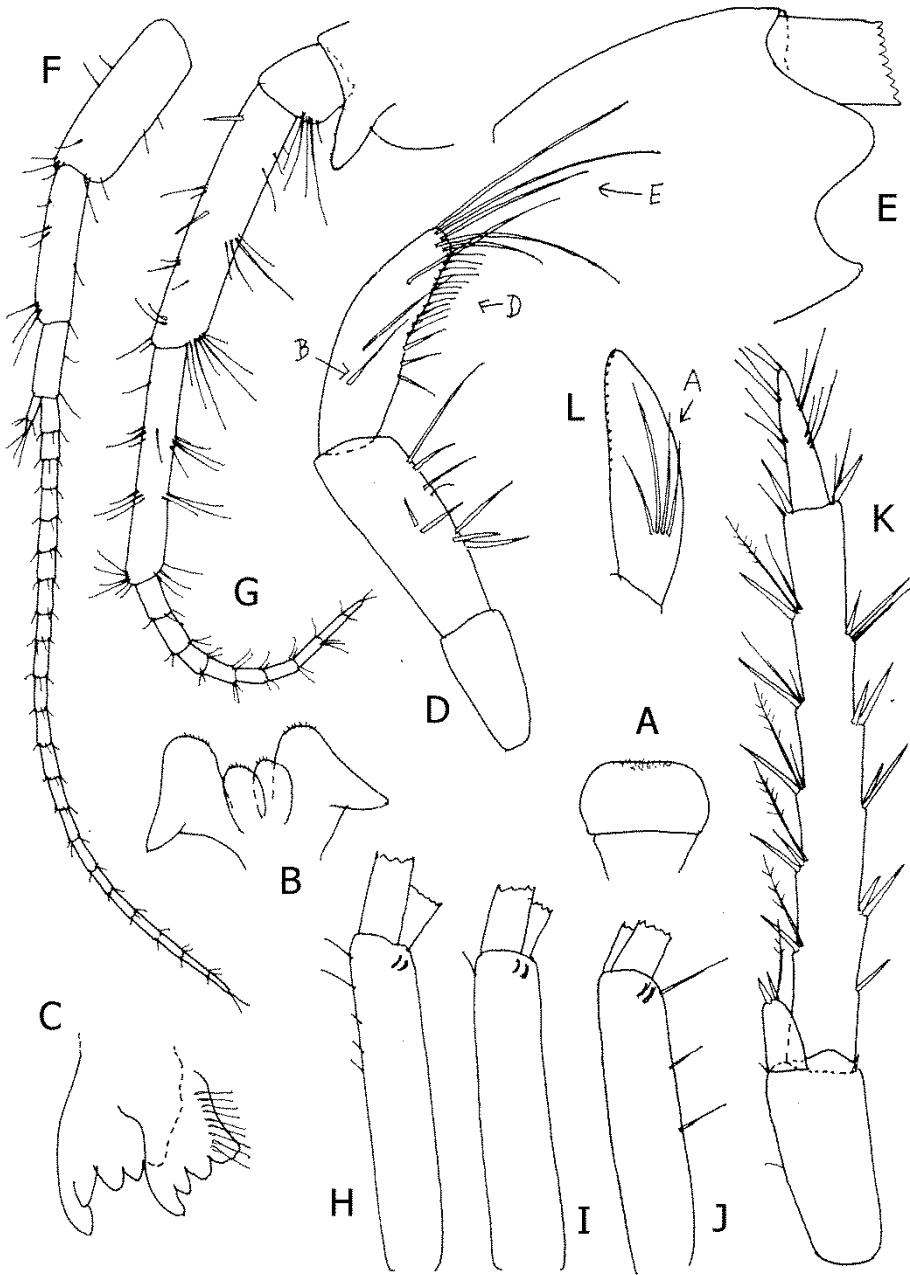


Fig. 4. *Niphargus pararhodi*, sp. n., Spring-brook along river Goudouras, male 8.2 mm: A= labrum; B= labium; C= left mandible: incisor and lacinia mobilis; D= mandibular palp, inner face, with B, D and E setae; E= head; F= antenna 1; G= antenna 2; H= pleopod 1 peduncle; I= pleopod 2 peduncle; J= pleopod 3 peduncle; K= uropod 3; L= last article of mandibular palp, outer face with A-setae.

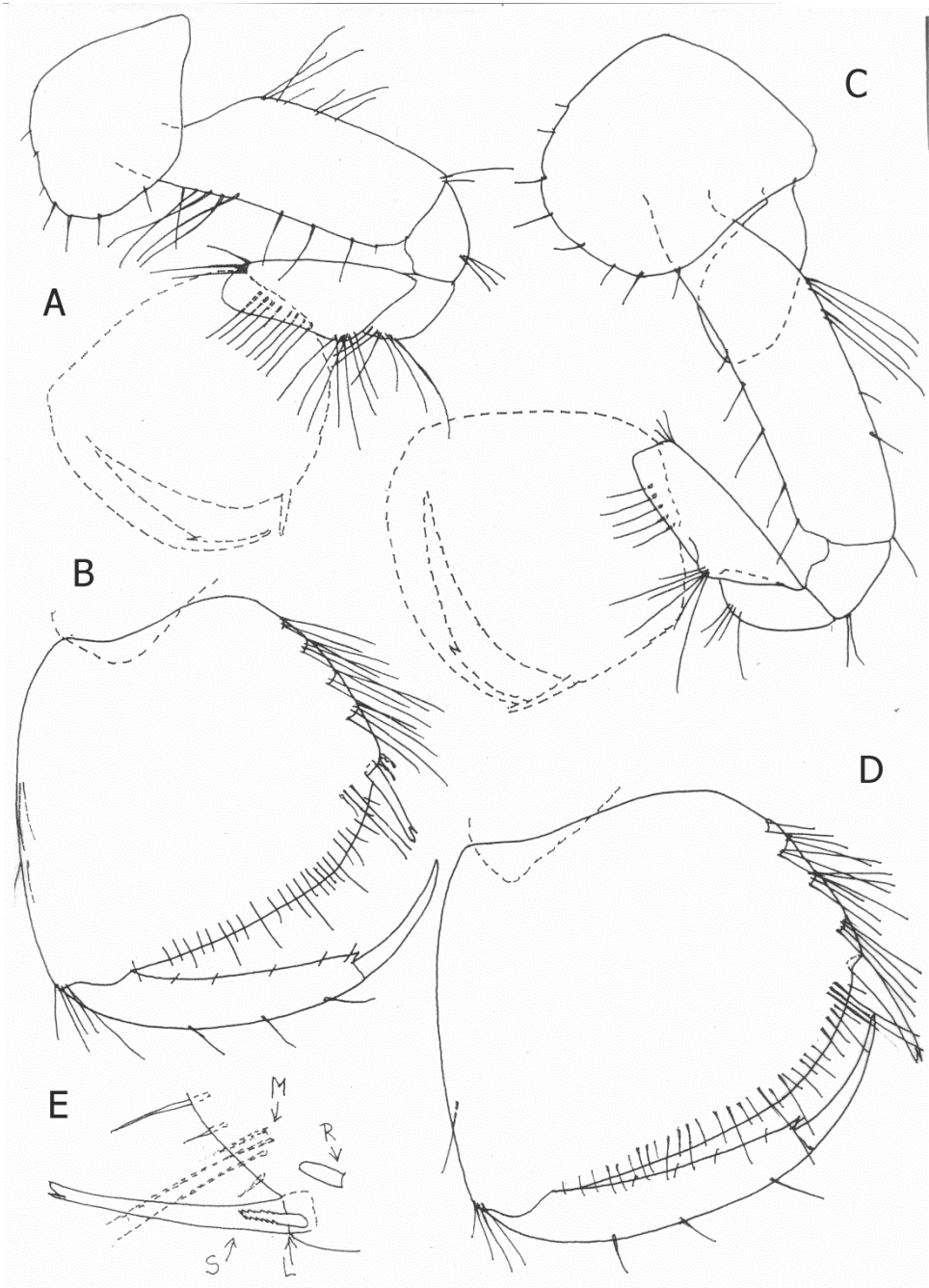


Fig. 5. *Niphargus pararhodi*, sp. n., Spring-brook along river Goudouras, male 8.2 mm: A-B= gnathopod 1; C-D= gnathopod 2; E= distal corner of gnathopod 2 propodus, inner face with S, R, L spines and M-setae.

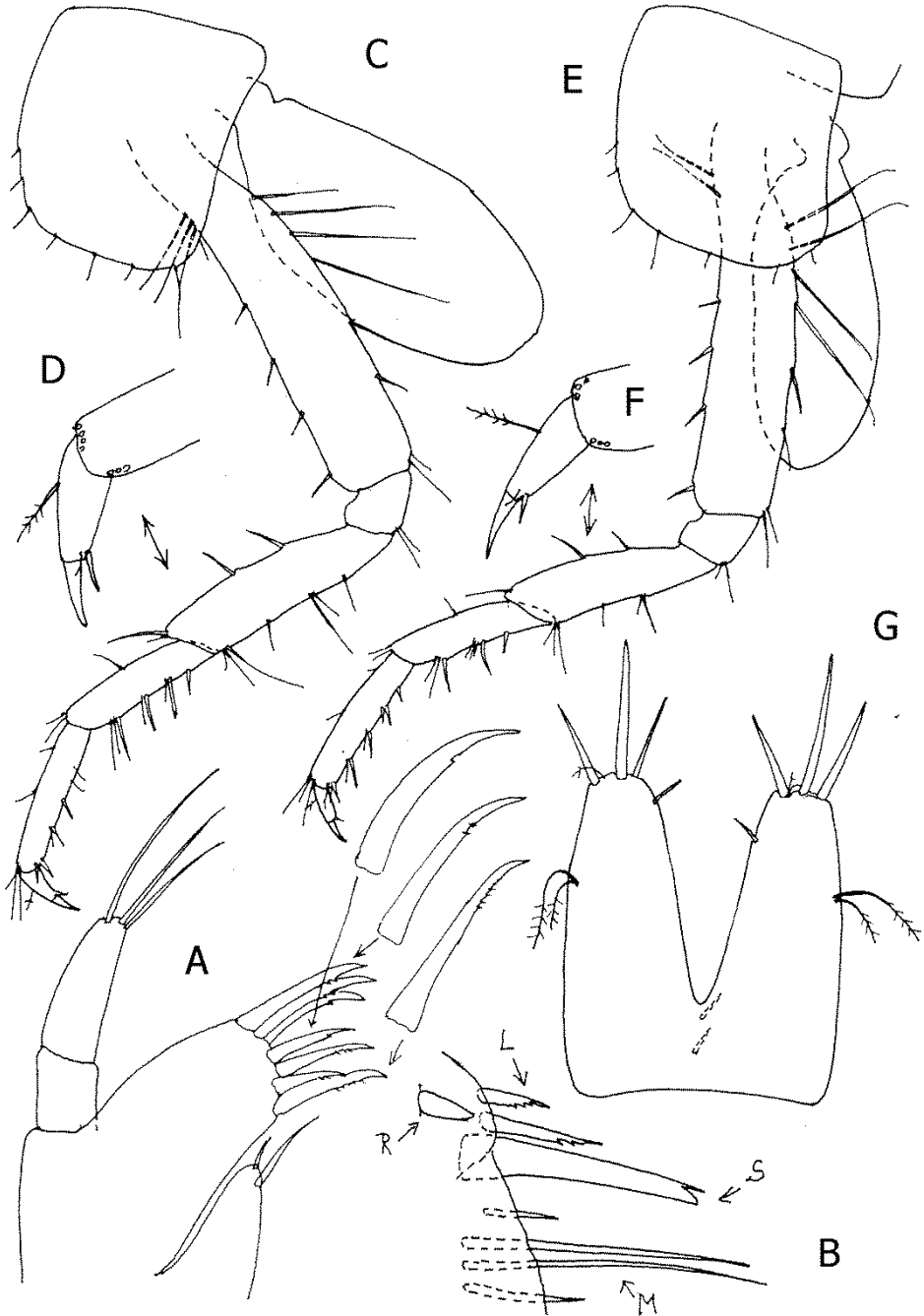


Fig. 6. *Niphargus pararhodi*, sp. n., Spring-brook along river Goudouras, male 8.2 mm: A= maxilla 1; B= distal corner of gnathopod 1 propodus, inner face, with R, S, L-spines and M-setae; C-D= pereopod 3, outer face; E-F= pereopod 4, outer face; G= telson.

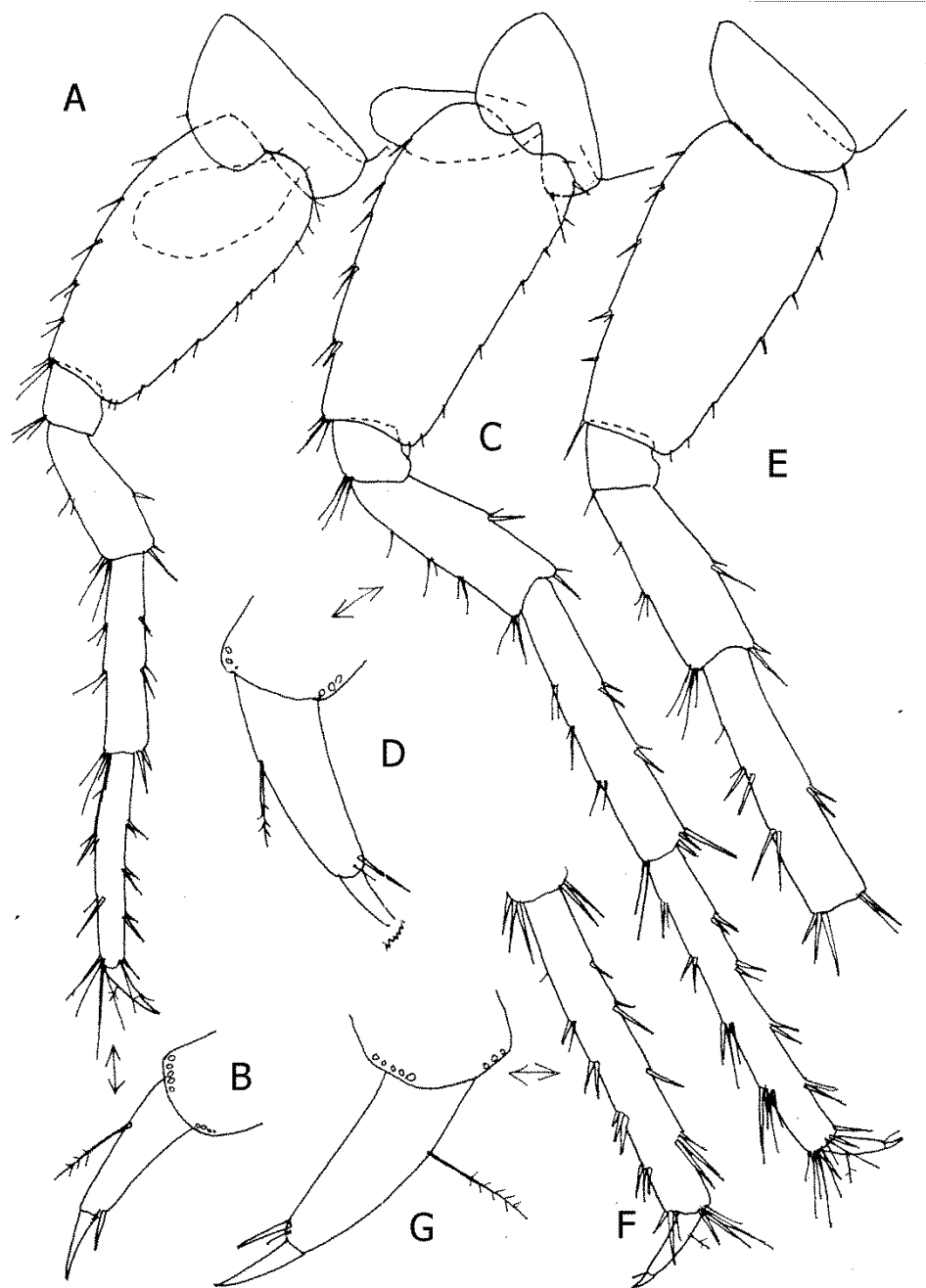


Fig. 7. *Niphargus pararhodi*, sp. n., Spring-brook along river Goudouras, male 8.2 mm: A-B= pereopod 5; C-D= pereopod 6; E-F-G= pereopod 7.

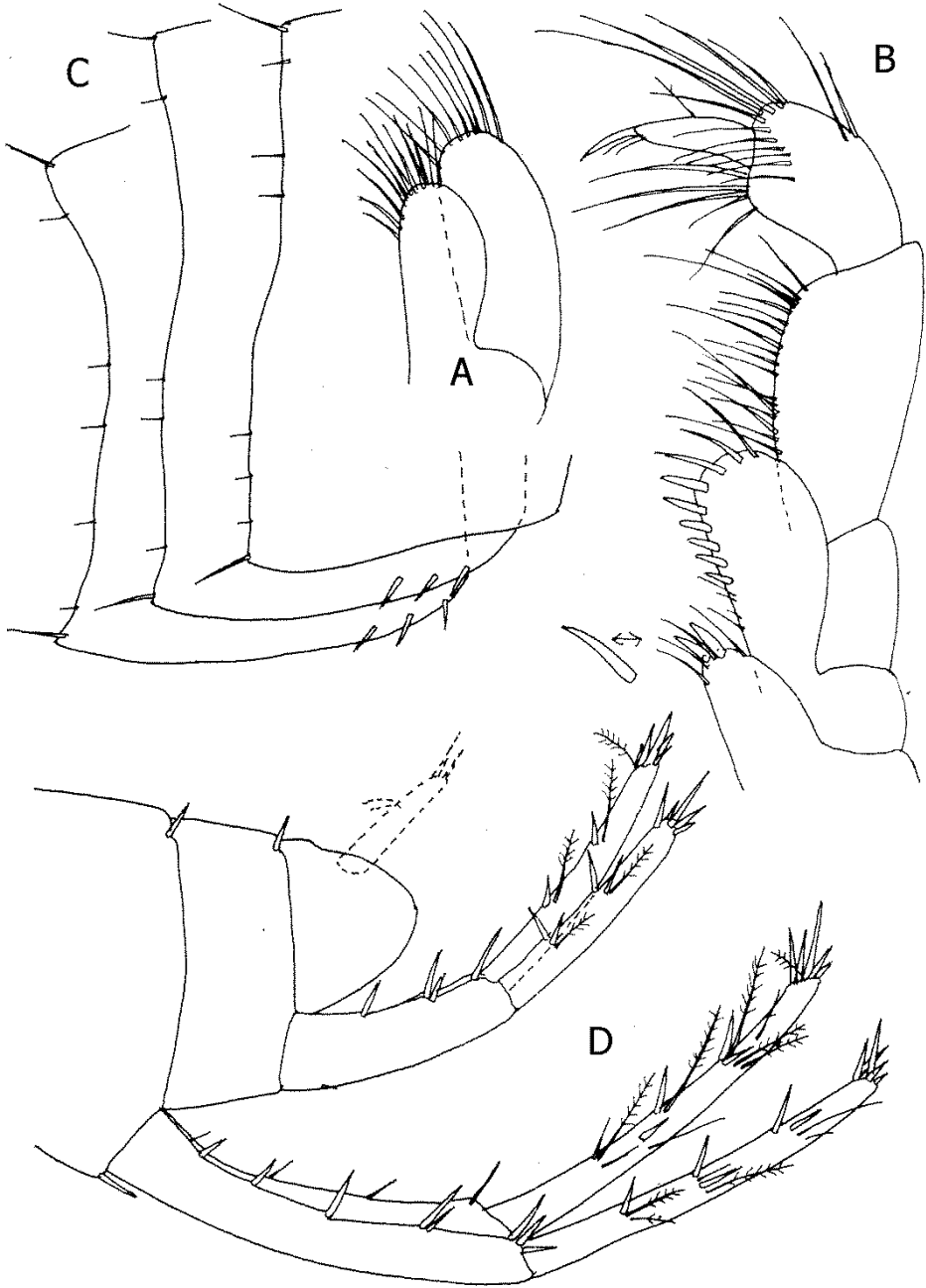


Fig. 8= *Niphargus pararhodi*, sp. n., Spring-brook along river Goudouras, male 8.2 mm: A= maxilla 2; B= maxilliped; C= epimeral plates 1-3; D= urosome with uropods 1-2.

Mouthparts well developed. Labrum much broader than long, with straight to poorly concave distal margin (fig. 4A).

Labium broader than long, with well developed subrounded outer lobes and small inner lobes (fig. 4B).

Mandible with triturative molar. Left mandible: incisor with 5 teeth, lacinia mobilis with 4 teeth and 9 rakers (fig. 4C). Right mandible: incisor with 4 teeth, lacinia mobilis bifurcate, with several teeth and 8 rakers. Mandibular palpus article 1 naked, article 2 with 8 strong spine-like setae (fig. 4D). Palpus article 3 subfalciform, slightly longer than article 2 (ratio: 68:60), provided with nearly 14 D-setae and 5 E-setae, on outer face with one bunch of 4 A-setae (fig. 4L), on inner face with 3 single B-setae (fig. 4D).

Maxilla 1: inner plate with 1-2 unequal setae; outer plate with 7 spines provided with very small, poorly visible lateral teeth each (5-2-3-1-1-2-2; or: 5-2-3-2-1-3-1); palpus 2-articulated, with 3 long distal setae (fig. 6A).

Maxilla 2: inner plate smaller than outer one, both plates with distomarginal setae (fig. 8A).

Maxilliped: inner plate very short, with 2 distal spines accompanied by several setae (fig. 8B); outer plate short, not reaching half of palpus article 2 and provided with 6-7 distolateral teeth and single setae; palpus article 3 at outer margin with one median and one distal bunch of setae; palpus article 4 at inner margin with 2 setae near basis of nail (fig. 8B).

Coxae relatively short. Coxa 1 hardly broader than long (ratio: 48:44), with broadly subrounded ventroanterior corner and provided with nearly 7 marginal setae (fig. 5A). Coxa 2 slightly longer than broad (ratio: 65:56), broadly subrounded and bearing nearly 8 marginal setae (fig. 5C). Coxa 3 longer than broad (ratio: 65:55), along margin with nearly 8 setae (fig. 6C). Coxa 4 slightly longer than broad (ratio: 60:54), along margin with nearly 7 setae, posterior margin concave, ventroposterior lobe absent (fig. 6E).

Coxae 5-7 are short. Coxa 5 shorter than coxa 4, much broader than long (ratio: 55:32), with one strong ventroposterior corner spine-like seta (fig. 7A). Article above coxa with naked ventroposterior corner (fig. 7A,C,E). Coxa 6 broader than long (ratio: 50:29), with strong ventroposterior spine-like seta (fig. 7C). Coxa 7 shallow, convex ventrally, with ventroposterior corner spine-like seta (fig. 7E).

Gnathopods 1 and 2 are relatively large, with propodus much larger than corresponding coxa (fig. 5A, C). Gnathopod 1: article 2 along anterior margin with long setae, along posterior margin with proximal bunch of longer setae. Article 3 at posterior margin with one distal bunch of setae (fig. 5A); article 5 shorter than propodus (ratio: 48:64), along anterior margin with one distal bunch of setae (fig. 5A). Propodus is only slightly smaller than that of gnathopod 2 (fig. 5B, D), obtusely trapezoid, slightly broader than long (ratio: 100:93), with 4 transverse rows of setae along posterior margin (fig. 5D). Palm convex, inclined nearly 3/5 of propodus-length, defined on outer face by one S-spine accompanied laterally by 2 serrate L-spines and 3 facial M-setae (fig. 5B), on inner face by one

subcorner R-spine (fig. 6B). Dactylus reaching posterior margin of propodus, along outer margin with 3 median setae, along inner margin with 5 short setae (fig. 5B).

Gnathopod 2: article 2 along anterior margin with row of shorter setae, along posterior margin with 3 long proximal setae and 2 short marginal setae; article 3 with one distal bunch of setae at posterior margin; article 5 slightly elongated, but shorter than propodus (ratio: 55:65) (fig. 5D), along anterior margin with one distal group of setae. Propodus broadly trapezoid, broader than long (ratio: 114:99), along posterior margin with 4 transverse rows of setae (fig. 5D). Palm convex, inclined 2/3 of propodus-length, defined on outer face by one corner S-spine accompanied by one L-spine sitting behind S-spine and with 3 facial M-setae (fig. 5E), on inner face by one subcorner R-spine. Dactylus reaching posterior margin of propodus, along outer margin with 3 median setae, along inner margin with nearly 5 short setae (fig. 5D).

Pereopods 3-4 moderately slender. Pereopod 3: article 2 along anterior margin with 3 proximal long setae and 4 distal short spine-like setae, along posterior margin are attached 4 long proximal setae and 3-4 setae in distal part. Articles 4-6 of unequal length (ratio: 57:38:40); article 4 at posterior margin with several setae (the longest setae exceeding diameter of article itself), along anterior margin with 4 short spine-like setae (fig. 6C); article 5 along posterior margin with 4 strong slender spines accompanied by 2 setae; article 6 along posterior margin with 5 groups of single or paired short spines and setae. Dactylus short and strong, much shorter than article 6 (ratio: 19:40), at inner margin with one spine near basis of the nail, along outer margin with one median longer plumose seta (fig. 6D), nail is much shorter than pedestal (ratio: 17:32).

Pereopod 4: article 2 along anterior margin with 2 long proximal setae and 4 short spine-like setae in distal part of article, along posterior margin are attached 4 long setae in proximal part and 4 strong spine-like setae in distal part of article. Articles 4-6 of unequal length (ratio: 46:38:40); article 2 at posterior margin with 3 groups of setae (the longest setae nearly reaching diameter of article). Article 5 along posterior margin with 3 spines mixed with single short setae; article 6 along posterior margin with 5 bunches of short spines and single setae (fig. 6E). Dactylus strong, much shorter than article 6 (ratio: 17:40), at inner margin with one spine near basis of the nail (fig. 6F), along outer margin with one median plumose seta; nail is shorter than pedestal (ratio: 18:30).

Pereopod 5 distinctly shorter than pereopods 6 and 7, with article 2 elongated, much longer than broad (ratio: 82:45), along anterior margin with row of 6 groups of spine-like setae, along posterior almost straight margin with nearly 11 setae, ventroposterior lobe not developed (fig. 7A). Articles 4-6 of unequal length (ratio: 40:53:56); article 5 along posterior margin with 2 groups of short spines; article 6 along posterior margin with 3 groups of short spines, along anterior margin with 3 groups of setae; article 6 along both margins with 4 bunches of spines mixed with single short setae and with distal bunch of longer spine and setae. Article 2 remarkably longer than article 6 (ratio: 82:56).



Dactylus much shorter than article 6 (ratio: 21:56), at inner margin with one slender spine near basis of the nail, along outer margin with one median plumose seta (fig. 7B); nail shorter than pedestal (ratio: 19:41).

Pereopod 6: article 2 narrowed, much longer than broad (ratio: 93:47), along anterior margin with 6 groups of spine-like setae, along posterior straight margin with nearly 9 slender and strong setae, ventroposterior lobe not developed (fig. 7C). Articles 4-6 of unequal length (ratio: 57:79:89); article 4 at anterior margin with 4 groups of short setae, along posterior margin with 2 spines; articles 5 and 6 along both margins with bunches of spines mixed with simple setae; article 2 only poorly longer than article 6 (ratio: 93:89). Dactylus much shorter than article 6 (ratio: 23:89), at inner margin with slender spine near basis of the nail, on outer margin with one median plumose seta; nail much shorter than pedestal (fig. 7D).

Pereopod 7: article 2 narrowed, much longer than broad (ratio: 89:45), along anterior poorly convex margin with 6 groups of spine-like setae, along posterior margin with 7 setae or spine-like setae, ventroposterior lobe not developed (fig. 7E). Articles 4-6 of unequal length (ratio: 55:73:94); article 2 with setae along anterior margin and spines along posterior margin (fig. 7F); articles 5 and 6 along both margins with bunches of short spines mixed sometimes with single short seta. Article 2 slightly shorter than article 6 (89:94). Dactylus much shorter than article 6 (ratio: 22:94), at inner margin with slender spine near basis of the nail, at outer margin with one longer median plumose seta (fig. 7G); nail much shorter than pedestal (ratio: 25:65).

Pleopods 1-3 with 2 retinacula each. Peduncle of pleopod 1 along anterior margin with 5 slender setae (fig. 4H); peduncle of pleopod 2 naked (fig. 4 I); peduncle of pleopod 3 along posterior margin with 3 strong setae (fig. 4J).

Uropod 1: peduncle with dorsoexternal row of spines and dorsointernal row of setae (except distal spine) (fig. 8D), ventrodiscal tubercle absent. Outer ramus slightly shorter than peduncle, provided with 3 bunches of strong lateral spines mixed with single or paired long plumose setae, and 2-3 simple setae, at tip appears a bunch of 5 short spines (fig. 8D). Inner ramus as long as outer ramus, provided with 4 lateral and 5 distal short spines, 4 groups of 1-4 marginal plumose setae and 3 short simple setae (fig. 8D).

Uropod 2: peduncle provided with lateral and distal strong spines; inner ramus is slightly longer than peduncle, provided with 2 lateral spines accompanied by 2 long plumose setae (fig. 8D), with distal bunch of 5 spines and one plumose seta. Outer ramus slightly shorter than inner ramus, bearing 2 bunches of lateral spines and 2 plumose setae, at tip with bunch of 5 spines (fig. 8D).

Uropod 3 slender: peduncle much longer than broad (ratio: 56:26); inner ramus very short, scale-like, provided with one distal spine and short seta, and with one long plumose seta (fig. 4K). Outer ramus 2-articulated: first article along inner (mesial) margin with 6 groups of spines shorter or longer than diameter of the article itself, accompanied by short simple and long plumose seta

each; along outer margin of article 1 are attached 6 groups of unequally long spines accompanied sometimes with single simple seta. Second article is much shorter than first one (ratio: 38:150), along both margins with 2-3 bunches of simple setae, at tip appear 2 short simple setae.

Telson slightly longer than broad (ratio: 85:71), slightly gaping, incised almost  $\frac{3}{4}$  of telson length; each lobe with 3 distal spines and one small spine at mesial margin (fig. 6G); a pair of longer plumose setae is attached rather above half of telson-length.

Coxal gills ovoid, rather elongated on pereopods 3 and 4 (fig. 6C, E), and shorter on gnathopod 2 and pereopods 5 and 6 (figs. 5C, 7A, C).

**HOLOTYPE:** Male 8.2 mm. Holotype [3 slides: S-7385A/6, 7385A/7, 7385A/8] is deposited in KARAMAN's Collection in Podgorica, Montenegro.

**DERIVATIO NOMINIS.** The name "*pararhodi*" is made according to the rather similarity with *Niphargus rhodi*.

### REMARKS AND AFFINITIES.

The studied male 8.2 mm of *N. pararhodi* differs from all other known *Niphargus* species from Greece by presence of plumose setae on rami of uropods 1 and 2. This character was observed in several *Niphargus* species belonging to the subgenus *Orniphargus* S. Karaman 1950 [typus subgeneris: *Niphargus orcinus* Joseph, 1869] (Karaman, S. 1950a, 1950b) from Bosnia and Herzegovina and Montenegro: *N. vjetrenicensis* S. Karaman 1932, *N. podgoricensis* S. Karaman 1934, *N. hercegovinensis* S. Karaman 1950a, *N. kusceri* S. Karaman 1950a, *N. bilecanus* S. Kar. 1953, *N. trullipes* Sket 1958. Plumose setae on uropods 1 and 2 appear always in males and females of these species (G. Karaman, 1984). *Niphargus pararhodi* differs distinctly from all these species by combination of various morphological characters.

The members of subgenus *Orniphargus* in Macedonia (*N. macedonicus* S. Karaman 1929, *N. pellagonicus* S. Karaman 1943) and Greece (*N. lindbergi* S. Karaman 1956 and *N. lourensis* Fišer, Trontelj & Sket 2006) have rami of uropods 1 and 2 without plumose setae.

In general, the significant character of the subgenus *Orniphargus* is the absence or remarkable sexual dimorphic differences (between males and females), short distal article of uropod 3 outer ramus and short spiniferous uropod 3 and spiniferous peduncle of uropod 1. Absence of significant morphological differences between males and females is present also within the members of the subgenus *Jovaniphargus* S. Karaman 1960 (typus subgeneris: *Niphargus jovanovici* S. Karaman 1931) and *Protoniphargopsis* Sket 1957 (typus subgeneris: *Niphargus kochianus* Bate 1859), although these subgenera are created based on external morphological taxonomic characters.

*N. pararhodi* differs from *N. lindbergi* also by different number of ventroposterior spines on urosomite 1, by lower number of retinacula on pleopods 1-3, lower number of setae on inner plate of maxilla 1, etc.

*N. lourensis* differs from *N. pararhodi* also by higher number of setae on maxilla 1 inner plate, by different armature of telson, by presence of dorsointernal row of spines on uropod 1-peduncle, etc. But, despite some similarities, *N. pararhodi* probably don't belong to *Orniphargus* group.

*N. pararhodi* is also similar to *Niphargus rhodi* S. Karaman 1950 [loc. typ.: source Nimpha on Propheta Mt. (Eliasberg?) Mt., Rhodos Island, Greece], mentioned later in numerous localities of Rhodos island, including Goudouras region (G. Karaman 2017b) by various characters (uropod 3, pereopods, etc.).

The adult females and juv. males of *N. rhodi* of Rhodos island (G. Karaman, 2017b) differ distinctly from *N. pararhodi* by absence of plumose setae on uropods 1 and 2, by higher number of distal setae on maxilla 1 palpus, by subequal plates of maxilla 2, by angular epimeral plates, by different shape of gnathopods 1-2 bearing higher number of transverse rows of setae on propodus, gnathopod 2 propodus with 2 L-spines sitting laterally from S-spine.

It remains theoretical possibility that *N. pararhodi* should be the unknown male of *N. rhodi*, but we have not enough data to consider *N. pararhodi* as conspecific with *N. rhodi*. Further research on new material from Rhodos Island will put more light on the taxonomic status of both species.

### ACKNOWLEDGEMENTS.

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