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Virginia SARROPOULOU,
Kortessa DIMASSI-THERIOU, Ioannis THERIOS¹

MINERAL STRENGTH, SUCROSE LEVEL AND MANNITOL CONCENTRATION EFFECTS ON CHERRY ROOTSTOCKS MICROPROPAGATION

SUMMARY

The aim of the present study was to investigate the effects of two strength media in inorganics (full and half), two sucrose levels (15 and 30 g/l) and four mannitol concentrations (0, 5, 10, 20 g/l) in combination with 1 mg/l indole-3-butyric acid (IBA) on the morphogenic and biochemical responses in CAB-6P and Gisela 6 cherry rootstocks. In CAB-6P, root number (7.4) and rooting percentage (83.33%) were maximum in half MS medium supplemented with 5 g/l mannitol and 15 g/l sucrose. Root length was greatest (40.6 mm) with 10 g/l mannitol and 30 g/l sucrose in full MS medium. In Gisela 6, in full MS medium, 20 g/l mannitol + 15 g/l sucrose exhibited the maximum root number (6.88), while 10 g/l mannitol + 30 g/l sucrose gave the greatest root length (50.3 mm). Rooting percentage was highest (92.31%) in half MS + 15 g/l sucrose (mannitol-free) and in 10 g/l mannitol + 15 g/l sucrose (full MS) combination treatments. In CAB-6P, mannitol led to depleted chlorophyll, carotenoid and porphyrin levels in half MS medium for both sucrose levels. Mannitol resulted in elevated leaf and root carbohydrate as well as proline levels irrespective sucrose level and medium strength. In Gisela 6, mannitol + 15 g/l sucrose decreased carotenoid content (full MS) and increased leaf proline content (half MS). In roots, 10 g/l mannitol raised proline (full MS) and carbohydrate content (half MS) in both sucrose levels. Leaf carbohydrate content was higher in half MS medium supplemented with 30 g/l sucrose. In both rootstocks, higher chlorophyll levels were recorded in half MS medium supplemented with 15 g/l sucrose compared to the full MS one or with 30 g/l sucrose. In full MS medium, increase of sucrose concentration led to depleted proline levels in Gisela 6 leaves and CAB-6P roots indicating activation of osmoregulation and osmotic adjustment mechanisms located in leaves for Gisela 6 and in roots for CAB-6P. An efficient root regeneration protocol and biochemical status evaluation of micropropagated cherry rootstocks shoot tips under the combined influence of different strength media, sucrose and mannitol concentrations was established.

Keywords: carbohydrates, *in vitro* rooting, mannitol, mineral salt composition, photosynthetic pigments, proline.

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

INTRODUCTION

Growth and root initiation are high energy requiring processes that can only occur at the expense of available metabolic substrates, which are mainly carbohydrates (Calamar and De Klerk 2002). The establishment of effective shoot proliferation and root development *in vitro* is essential for subsequent success during acclimatization to autotrophic conditions (Premkumar et al. 2003). The addition of a carbon source in any nutrient medium is essential for *in vitro* growth and development of many species, because photosynthesis is insufficient, due to the growth taking place in conditions unsuitable for photosynthesis or without photosynthesis (in darkness) (Pierik, 1997).

In general, sucrose is the carbohydrate of choice as carbon source for *in vitro* plant culture, probably because it is the most common carbohydrate in the phloem sap of many plants (Fuentes et al., 2000) and because is cheap, readily available, relatively stable to autoclaving, and readily assimilated by plants (Fowler, 2000). It has also been well documented that certain plant tissues may contain and/or utilize different carbohydrates at the same time. It is then not surprising that carbon sources other than sucrose might be effective in promoting *in vitro* tissue specific growth responses in a given species (Swedlund and Locy 1993).

There are a number of species however that can grow on carbohydrates different than sucrose such as sorbitol, glycerol or mannitol (Vu et al. 1993).

In olive, mannitol is a major product of photosynthesis that reaches high concentrations in leaves that are second only to those of glucose and is translocated in the phloem (Flora and Madore 1993). Leva et al. (1995) have reported that mannitol improved the *in vitro* propagation of agamic olive explants, collected from mature trees growing in the field.

According to Ibrahim (1999), the concentration of inorganic salts plays an important role in root induction as it was shown that the reduction of MS salts strength to $\frac{3}{4}$ of the original concentration stimulated root formation in date palm tissue culture. The mineral concentration of the culture medium affects rooting characteristics, and some researchers have proposed its reduction to half normal strength for rooting improvement (Dimassi-Theriou and Economou 1993).

Therefore, the objectives of this study was to evaluate the effects of mannitol concentration, medium composition in minerals and sucrose level under *in vitro* conditions on the rooting process, vegetative growth, callusing traits as well as on biochemical parameters such as photosynthetic pigments, carbohydrate metabolism and endogenous free proline accumulation in two cherry rootstocks, namely CAB-6P and Gisela 6.

MATERIAL AND METHODS

Plant material and culture conditions

In this experiment, the effects of two different strength media in inorganics; full and half, two different sucrose levels; 15 and 30 g/l and four

different mannitol concentrations; 0, 5, 10 and 20 g/l in combination with 1 mg/l IBA on vegetative growth, rooting and callusing of CAB-6P (*Prunus cerasus*) and Gisela 6 (*Prunus canescens* x *Prunus cerasus*) cherry rootstocks were studied. Shoot tips, 1.5 to 2.5 cm long were obtained from previous in vitro subcultures and placed onto a MS (Murashige and Skoog 1962) culture medium. The pH value of the culture medium was adjusted to 5.8 and 6 g/l agar was added prior to autoclaving at 121 °C for 20 min. The experiment was comprised of 16 treatments and each treatment included 13 replications for CAB-6P and 22 replications for Gisela 6 with one microcutting placed in each 25x100 mm glass test tube with flat base that contained 10 ml of the nutrient culture medium. Afterwards, the cultures were maintained at 21-23 °C under cool white fluorescent light (Phillips, 90 µmol/m²/s) with a 16-hour photoperiod. After six weeks of culture, records and measurements were taken for root number per rooted explant, root length (mm), root fresh weight (g), rooting percentage (%), shoot length and fresh weight of the initial explant (shoot tip without roots), callus fresh weight, callus induction frequency (%), total leaf chlorophyll (a+b), carotenoid and porphyrin content, total carbohydrate and endogenous proline content in both leaves and roots.

Chlorophyll determination

For chlorophyll measurement, 0.1 g of frozen leaf material was taken and placed in glass test tubes of 25 ml volume. Fifteen ml of 96% ethanol was added in each tube, which was covered with aluminum foil to reduce ethanol evaporation. The tubes were incubated in a water bath of 79.8 °C, until complete sample discoloration and chlorophyll extraction. After chlorophyll extraction, the samples (tubes) were allowed to cool at room temperature and the level of 96% ethanol was completed to be 15 ml volume. The absorbance of chlorophylls a and b was measured at 665 and 649 nm, respectively, in a visible spectrophotometer. Chlorophyll concentration was determined according to Wintermans and De Mots (1965) from the following equations:

$$\text{Chl (a + b)} = (6.10 \times A_{665} + 20.04 \times A_{649}) \times 15/1000/\text{FW (mg/g FW)}$$

$$\text{Chl (a + b)} = (6.10 \times A_{665} + 20.04 \times A_{649}) \times 15/1000/\text{DW (mg/g DW)}$$

Proline determination

Leaf or root frozen tissue (0.1g), was chopped into small pieces and placed in glass test tubes of 25 ml. In each tube, 10 ml of 80% (v/v) ethanol was added and placed in a water bath of 60 °C for 30 min (Khan et al. 2000). The tubes were covered with aluminum foil to reduce evaporation. The extracts were filtered and 80% (v/v) ethanol was added until the total volume (ethanol extract) to be 15 ml. After extraction, the aluminum foil was removed and the tubes were allowed to cool at room temperature. In each tube, 4 ml of toluene was added and mixed well with a vortex. Two layers were visible in each tube. The supernatant (toluene layer) was removed with a pasteur pipette and was placed in a glass

cuvette. The optical density of the extract was measured at 518nm. The extract was filtered with Whatman No. 1 filter paper and free proline was measured (Troll and Lindsley 1955) with acid ninhydrin solution. Proline concentrations were calculated from a standard curve by using L-proline (Sigma Chemical Company) at 0-0.2 mM concentrations.

Carbohydrate determination

Carbohydrate determination of plant tissue was conducted by using the anthrone method (Plummer, 1987). For reagent preparation, 1g of anthrone was diluted to 500 ml concentrated sulfuric acid (96%). The extract (plant ethanolic extract) for carbohydrate determination was the same as that used for proline, with the only difference that it was diluted 10 times with 80% (v/v) of ethanol. In each test tube, 2 ml of anthrone reagent were placed and maintained in an ice bath. Subsequently, the diluted extract (10% of the initial) was added dropwise in contact with the test tube walls in order to avoid blackening of the samples. After shaking the tubes with a vortex, the samples were incubated in a water bath of 95 oC for 15 min. Afterwards, the tubes were placed in a cold water bath for cooling and optical density was measured at 625nm. Carbohydrate concentrations were calculated from a standard curve by using 0-0.2 mM sucrose concentrations.

Carotenoid and porphyrin content measurement

Carotenoid and porphyrin concentrations were determined as described Lichenthaler (1987) and Porra et al. (1989) and modified by Yang et al. (1998). Five milligrams of samples were homogenized with 5 ml of 80% acetone in a cooled mortar. Extract was centrifuged for 5 min at 1,500 g, and the supernatant was stored. The pellet was re-extracted with acetone and centrifuged again. This process was continued until the supernatant was colourless, and then the supernatant was pooled.

1. Absorbance was measured at 663.6, 646.6 and 440.5 nm, the major absorption peaks of chlorophyll a and b and carotenoids, respectively. Carotenoids were calculated using the following equation: $(4.69 \times A_{440.5} - 1.96 \times A_{663.6} - 4.74 \times A_{646.6}) \times \text{volume of supernatant (ml)} \times \text{dilution factor} / \text{sample weight (g)}$.

2. Absorbance was measured at 663.6, 646.6, 440.5, 575, 590 and 628 nm, the absorption peaks of chlorophyll a, chlorophyll b, carotenoids, protoporphyrin, magnesium-protoporphyrin and protochlorophyllide, respectively. Porphyrin content was summed (A+B+C) by the following three equations:

$A = [(12.25 \times A_{663.6} - 2.55 \times A_{646.6}) \times \text{volume of supernatant (ml)} \times \text{diluted factor} / \text{sample weight (g)}] / 892 \times 1000$

$B = [(20.31 \times A_{646.6} - 4.91 \times A_{663.6}) \times \text{volume of supernatant (ml)} \times \text{diluted factor} / \text{sample weight (g)}] / 906 \times 1000$

$C = [(196.25 \times A575 - 46.6 \times A590 - 58.68 \times A628) + (61.81 \times A590 - 23.77 \times A575 - 3.55 \times A628) + (42.59 \times A628 - 34.32 \times A575 - 7.25 \times A590)] \times \text{volume of supernatant (ml)} \times \text{dilution factor} / \text{sample weight (g)}.$

Statistical analysis

The experimental layout was completely randomized and the experiment was repeated twice. Thus, the reported data are the means of the two experiments. The means were subjected to analysis of variance (ANOVA) and compared using the Duncan multiple-range test ($P < 0.05$).

The experiment was a 2x2x4 factorial with two different strength media in inorganics (full and half), two different sucrose levels (15 and 30 g/l) and four mannitol concentrations (0, 5, 10, 20 g/l) for each of the two studied cherry rootstocks (CAB-6P and Gisela 6). The main effect of factors (medium strength in inorganics, sucrose level and mannitol concentration) and their interactions for each cherry rootstock were determined by the General Linear Model (3-way ANOVA).

RESULTS AND DISCUSSION

Effect of medium strength in inorganics, sucrose level and mannitol concentration on rooting, vegetative growth and callus induction characteristics.

In CAB-6P, best rooting results in terms of root number per rooted explant (7.4), root fresh weight (0.073 g) and rooting percentage (83.33%) were obtained with 5 g/l mannitol and 15 g/l sucrose in half strength medium (Figure 1n). On the other hand, root length reached its maximum value (40.6 mm) with 10 g/l mannitol and 30 g/l sucrose in full strength medium (Table 1, Figure 1c). Regarding the vegetative growth of the explants, 5 g/l mannitol and 30 g/l sucrose in full strength medium gave the maximum shoot length (22.31 mm) (Figure 1b) whereas better results for shoot fresh weight were recorded in the absence of mannitol (Figure 1a). Callus fresh weight was maximum (0.170 g) when 5 g/l mannitol was incorporated along with 30 g/l sucrose into the full strength medium in inorganics. Callus induction frequency reached its maximum value (100%) by adding 10 g/l mannitol to the medium containing 30 g/l sucrose irrespective of media composition in mineral salts (Figures 1c, 1k). The same maximum callus induction frequency (100%) was achieved in half MS medium with 15 g/l sucrose in the absence of mannitol (Table 2, Figure 1m).

In Gisela 6, 20 g/l mannitol + 15 g/l sucrose in full MS medium exhibited the greatest root number per rooted explant (6.88) (Figure 2h). Root length and root fresh weight were maximum (50.3 mm and 0.134 g) with 10 or 20 g/l mannitol + 30 g/l sucrose in full MS medium, respectively (Figures 2c, 2d). Rooting percentage was highest (92.31%) with 10 g/l mannitol + 15 g/l sucrose in full MS medium (Figure 2g) as well as in the absence of mannitol with 15 g/l sucrose in half MS medium (Table 1, Figure 2m).



Figure 1 Effect of MS medium strength in inorganics, sucrose level and mannitol concentration combined with 1 mg/l IBA on in vitro rooting of CAB-6P explants: (a) Full MS + 30 g/l sucrose, (b) Full MS + 30 g/l sucrose + 5 g/l mannitol, (c) Full MS + 30 g/l sucrose + 10 g/l mannitol, (d) Full MS + 30 g/l sucrose + 20 g/l mannitol, (e) Full MS + 15 g/l sucrose, (f) Full MS + 15 g/l sucrose + 5 g/l mannitol, (g) Full MS + 15 g/l sucrose + 10 g/l mannitol, (h) Full MS + 15 g/l sucrose + 20 g/l mannitol, (i) half MS + 30 g/l sucrose (j) half MS + 30 g/l sucrose + 5 g/l mannitol, (k) half MS + 30 g/l sucrose + 10 g/l mannitol, (l) half MS + 30 g/l sucrose + 20 g/l mannitol, (m) half MS + 15 g/l sucrose, (n) half MS + 15 g/l sucrose + 5 g/l mannitol, (o) half MS + 15 g/l sucrose + 10 g/L mannitol, (p) half MS + 15 g/l sucrose + 20 g/l mannitol.

Table 1. Effect of medium strength in inorganics, sucrose level, mannitol concentration and their interactions in a medium containing 1 mg/l IBA on CAB-6P and Gisela 6 cherry rootstocks rooting characteristics.

Strength MS In inorganics	Sucrose (g/l)	Mannitol (g/l)	Root number /rooted explant	Root length (mm)	Root fresh weight (g)	Rooting percentage (%)
Rootstock: CAB-6P						
full	30	0	2.79 abc	29.93 cd	0.050 abcd	70.00 h
	30	5	2.43 ab	31.63 d	0.038 ab	53.85 c
	30	10	1.71 a	40.60 e	0.032 a	38.46 a
	30	20	3.17 abc	26.46 bcd	0.037 ab	53.85 c
	15	0	4.13 bcd	31.67 d	0.068 de	80.00 j
	15	5	4.43 cd	22.00 bc	0.042 abc	53.85 c
	15	10	5.13 de	25.34 bcd	0.055 bcde	66.67 f
	15	20	2.71 abc	32.61 d	0.061 cde	53.85 c
½	30	0	3.30 abc	19.35 ab	0.036 ab	76.92 i
	30	5	3.17 abc	22.08 bc	0.038 ab	46.15 b
	30	10	2.50 ab	11.33 a	0.030 a	61.54 e
	30	20	3.33 abc	18.58 ab	0.037 ab	46.15 b
	15	0	6.44 ef	19.38 ab	0.052abcde	69.23 g
	15	5	7.40 f	20.86 b	0.073 e	83.33 k
	15	10	4.40 cd	18.35 ab	0.051 abcd	55.56 d
	15	20	3.00 abc	20.84 b	0.035 ab	38.46 a
3-wayANOVA						
Strength MS (A)			0.000*	0.363 ns	0.000***	0.000***
Sucrose level (B)			0.018*	0.000***	0.089 ns	0.000***
Mannitol concentration (C)			0.001**	0.147 ns	0.003**	0.000***
(A)*(B)			0.833 ns	0.044*	0.535 ns	0.000***
(A)*(C)			0.041*	0.158 ns	0.421 ns	0.000***
(B)*(C)			0.044*	0.295 ns	0.371 ns	0.000***
(A)*(B)*(C)			0.014*	0.003**	0.139 ns	0.000***
Rootstock: Gisela 6						
full	30	0	3.87 b	36.29 e	0.052 ab	68.18 g
	30	5	3.75 ab	36.81 e	0.066 abc	30.77 a
	30	10	4.33 bc	50.30 f	0.088 cd	42.86 d
	30	20	5.43 cd	34.13 de	0.134 f	58.33 f
	15	0	5.29 cd	28.27 bcd	0.045 a	77.27 h
	15	5	5.80 de	26.10 abc	0.119 ef	76.92 h
	15	10	3.75 ab	29.63 cd	0.081 bcd	92.31 i
	15	20	6.88 e	23.70 abc	0.100 de	66.67 g
½	30	0	3.40 ab	21.71 a	0.049 a	38.46 bc
	30	5	3.11 ab	23.24 ab	0.049 a	69.23 g
	30	10	2.40 a	23.40 abc	0.041 a	41.67 cd
	30	20	3.00 ab	32.83 de	0.083 cd	53.85 e
	15	0	5.42 cd	24.01 abc	0.096 cde	92.31 i
	15	5	5.80 de	22.60 ab	0.100 de	35.71 b
	15	10	3.64 ab	26.44 abc	0.067 abc	64.71 g
	15	20	5.33 cd	20.51 a	0.087 cd	69.23 g
3-wayANOVA						
Strength MS (A)			0.000***	0.000***	0.001**	0.001**
Sucrose level (B)			0.000***	0.000***	0.004**	0.001**
Mannitol concentration (C)			0.000***	0.000***	0.000***	0.001**
(A)*(B)			0.028*	0.000***	0.002**	0.001**
(A)*(C)			0.009**	0.014*	0.000***	0.001**
(B)*(C)			0.016*	0.000***	0.000***	0.001**
(A)*(B)*(C)			0.744 ns	0.000***	0.215 ns	0.001**

Means denoted by the same letter in each column and for each rootstock separately are not significantly different according to Duncan's multiple range test at $P \leq 0.05$. ns – non significant effect ($P \geq 0.05$). Significant effects at $P \leq 0.01$ (**) or 0.001 (***) according to 3-way ANOVA ($n = 13$ for CAB-6P and $n = 22$ for Gisela 6).

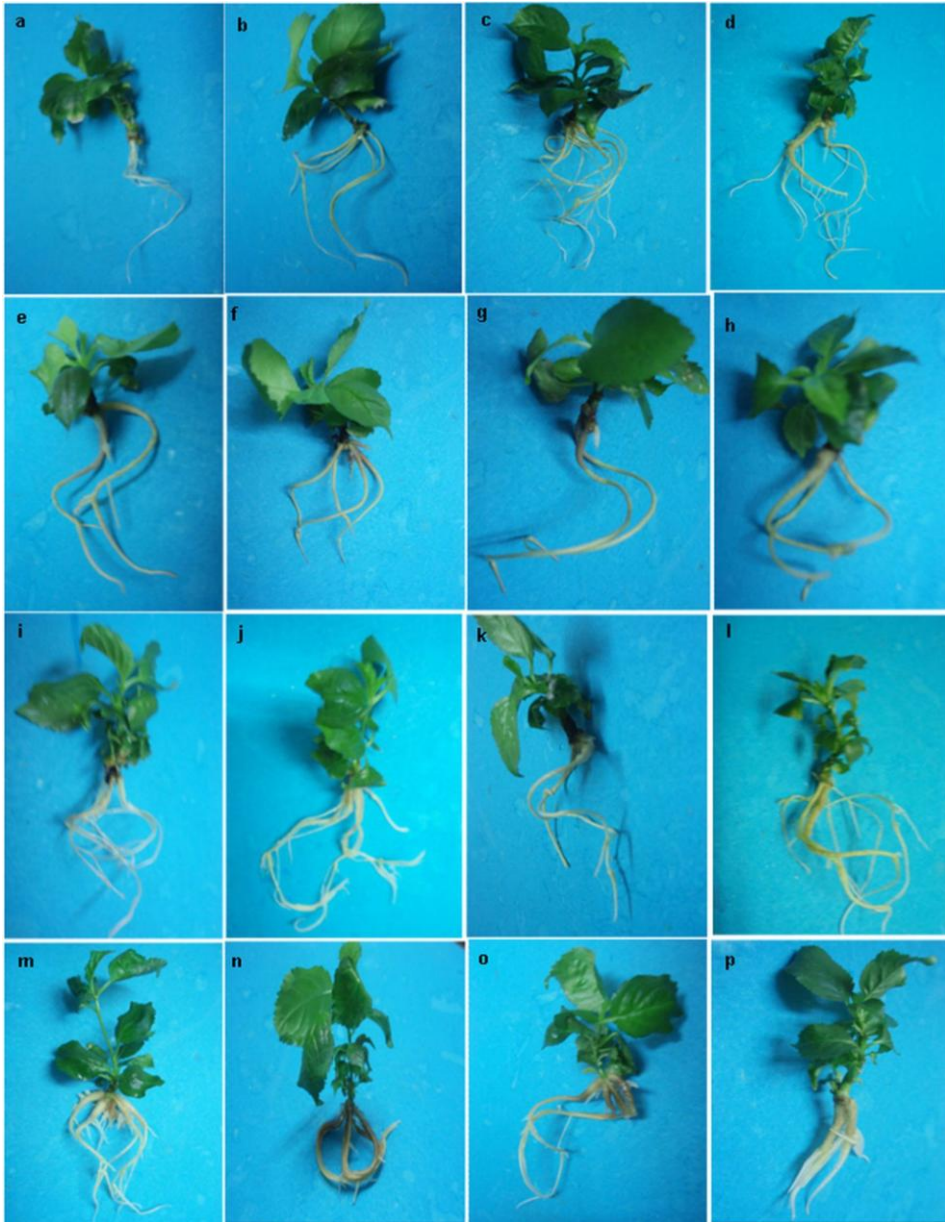


Figure 2 Effect of MS medium strength in inorganics, sucrose level and mannitol concentration combined with 1 mg/l IBA on in vitro rooting of Gisela 6 explants: : (a) Full MS + 30 g/l sucrose, (b) Full MS + 30 g/l sucrose + 5 g/l mannitol, (c) Full MS + 30 g/l sucrose + 10 g/l mannitol, (d) Full MS + 30 g/l sucrose + 20 g/l mannitol, (e) Full MS + 15 g/l sucrose, (f) Full MS + 15 g/l sucrose + 5 g/l mannitol, (g) Full MS + 15 g/l sucrose + 10 g/l mannitol, (h) Full MS + 15 g/l sucrose + 20 g/l mannitol, (i) half MS + 30 g/l sucrose (j) half MS + 30 g/l sucrose + 5 g/l mannitol, (k) half MS + 30 g/l sucrose + 10 g/l mannitol, (l) half MS + 30 g/l sucrose + 20 g/l mannitol, (m) half MS + 15 g/l sucrose, (n) half MS + 15 g/l sucrose + 5 g/l mannitol, (o) half MS + 15 g/l sucrose + 10 g/L mannitol, (p) half MS + 15 g/l sucrose + 20 g/l mannitol.

Full strength medium in inorganics with 30 g/l sucrose in the absence of mannitol (24.09 mm) (Figure 2a), full strength medium with 15 g/l sucrose + 20 g/l mannitol (23.33 mm) (Figure 2h) and half strength medium with 15 g/l sucrose + 5 g/l mannitol (23.21 mm) (Figure 2p) gave better results concerning shoot length.

In the absence of mannitol, half MS medium with 15 g/l sucrose resulted in the maximum shoot (0.190 g) and callus fresh weight (0.120 g). Callus induction frequency was maximum (59.09%) in full MS medium supplemented with 30 g/l sucrose without mannitol (Table 2).

Effect of medium strength in inorganics, sucrose level and mannitol concentration on biochemical parameters

In CAB-6P, half MS medium + 15 g/l sucrose (mannitol-free) and half MS medium + 30 g/l sucrose + 5 g/l mannitol exhibited the highest chlorophyll content.

Carotenoid and porphyrin content were greatest with 30 g/l sucrose in half MS medium in devoid of mannitol (Table 3).

Adding 10 g/l mannitol to the half MS medium with 30 g/l sucrose led to elevated leaf carbohydrate levels. Proline content in leaves was maximum by applying 10 g/l mannitol to full MS medium, irrespective sucrose level. In roots, 5 or 10 g/l mannitol combined with 15 g/l sucrose in full MS medium gave higher carbohydrate and proline content, respectively (Table 4).

In Gisela 6, chlorophyll content was higher by incorporating 10 g/l mannitol + 30 g/l sucrose or 15 g/l sucrose without mannitol into the half MS medium. Treatment of explants in full MS medium supplemented with 15 g/l sucrose resulted in the highest carotenoid content. On the other hand, medium strength in inorganics, sucrose level and mannitol concentration did not influence porphyrin content significantly (Table 3). Mannitol applied at 10 g/l in half MS medium containing 30 g/l sucrose led to elevated levels of carbohydrates in both leaves and roots. Leaf proline levels were higher by incorporating 5 or 10 g/l mannitol into the 15 g/l sucrose containing full MS medium. In the 10 g/l mannitol+15 g/l sucrose+ full MS medium treatment, free root proline was remarkably increased (Table 4).

In both rootstocks, the 2-fold increase in sucrose level from 15 to 30 g/l in both full and half MS media and in all mannitol concentrations resulted in higher root number. Similar results were obtained by Schneider (2005). In specific, half MS + 15 g/l sucrose + 5 g/l mannitol was the optimum treatment for root number and rooting percentage of CAB-6P microcuttings whereas the full MS + 15 g/l sucrose + 20 g/l mannitol treatment gave the highest root number in Gisela 6 explants. In the peach rootstock GF 677, 30 g/l sucrose gave better results regarding rooting percentage and root number, while lower (15 g/l) and higher (45 and 60 g/l) concentrations had an inhibitory effect (Ahmad *et al.* 2007). Manzanera and Pardos (1990) found that rooting percentage and root number were enhanced with increasing sugar concentration. Therefore, the tendency to form roots under the highest sugar concentration may indicate an increase in water demands of these tissues.

Table 2. Effect of medium strength in inorganics, sucrose level, mannitol concentration and their interactions in a medium containing 1 mg/l IBA on CAB-6P and Gisela 6 cherry rootstocks on vegetative growth and callus induction characteristics.

Strength MS in inorganics	Sucrose (g/l)	Mannitol (g/l)	Shoot length (mm)	Shoot fresh weight (g)	Callus fresh weight (g)	Callus induction frequency (%)
Rootstock: CAB-6P						
full	30	0	16.50 abc	0.124 f	0.070 abc	90.00 h
	30	5	22.31 d	0.060 ab	0.085 bc	84.62 g
	30	10	18.85 cd	0.056 a	0.089 bc	100 j
	30	20	15.38 abc	0.071 ab	0.070 abc	61.54 d
	15	0	15.50 abc	0.111 def	0.048 a	45.00 b
	15	5	18.46 bcd	0.090 bcde	0.106 cd	84.62 g
	15	10	17.50 abc	0.070 ab	0.073 abc	83.33 f
½	15	20	15.00 abc	0.084 abcd	0.068 ab	23.08 a
	30	0	17.31 abc	0.079 abc	0.125 de	92.31 i
	30	5	18.08 bc	0.071 ab	0.170 f	92.31 i
	30	10	17.69 abc	0.066 ab	0.142 ef	100 j
	30	20	15.38 abc	0.070 ab	0.087 bc	83.33 f
	15	0	15.77 abc	0.081 abc	0.079 abc	100 j
	15	5	17.08 abc	0.116 ef	0.091 bc	58.33 c
	15	10	13.33 a	0.066 ab	0.090 bc	44.44 b
	15	20	14.23 ab	0.070 ab	0.084 bc	76.92 e
3-wayANOVA						
Strength MS (A)			0.017*	0.001**	0.000***	0.000***
Sucrose level (B)			0.030*	0.635 ns	0.000***	0.000***
Mannitol concentration (C)			0.031*	0.000***	0.029*	0.000***
(A)*(B)			0.932 ns	0.273 ns	0.003**	0.000***
(A)*(C)			0.286 ns	0.073 ns	0.334 ns	0.000***
(B)*(C)			0.012*	0.001**	0.071 ns	0.000***
(A)*(B)*(C)			0.848 ns	0.644 ns	0.046*	0.000***
Rootstock: Gisela 6						
full	30	0	24.09 f	0.181 cd	0.046 cd	59.09 h
	30	5	19.62 bcde	0.171 bcd	0.093 j	7.69 b
	30	10	19.64 bcde	0.181 cd	0.077 h	7.14 b
	30	20	20.00 cde	0.169 bcd	0.080 h	8.33 b
	15	0	22.27 ef	0.149 abc	0.039 b	36.36 e
	15	5	23.08 f	0.181 cd	0.048 d	7.69 b
	15	10	16.54 ab	0.140 ab	0.055 e	7.69 b
½	15	20	23.33 f	0.147 abc	0.040 bc	16.67 c
	30	0	18.08 abc	0.179 cd	0.044 bcd	23.08 d
	30	5	18.46 abcd	0.173 bcd	0.086 i	46.15 g
	30	10	20.00 cde	0.171 bcd	0.070 g	25.00 d
	30	20	15.77 a	0.148 abc	0.061 f	15.38 c
	15	0	21.54 def	0.190 d	0.120 k	7.69 b
	15	5	23.21 f	0.152 abc	0.067 g	35.71 e
	15	10	19.12 bcd	0.129 a	0.041 bc	41.18 f
	15	20	18.85 bcd	0.119 a	0.000 a	0 a
3-wayANOVA						
Strength MS (A)			0.002**	0.000***	0.000***	0.000***
Sucrose level (B)			0.001**	0.189 ns	0.178 ns	0.000***
Mannitol concentration (C)			0.000***	0.001**	0.000***	0.000***
(A)*(B)			0.028*	0.934 ns	0.000***	0.063 ns
(A)*(C)			0.000***	0.099 ns	0.000***	0.000***
(B)*(C)			0.000***	0.036*	0.000***	0.000***
(A)*(B)*(C)			0.229 ns	0.117 ns	0.000***	0.000***

Means denoted by the same letter in each column and for each rootstock separately are not significantly different according to Duncan's multiple range test at $P \leq 0.05$. ns – non significant effect ($P \geq 0.05$). Significant effects at $P \leq 0.001$ (***) according to 3-way ANOVA ($n = 13$ for CAB-6P and $n = 22$ for Gisela 6).

Table 3 Effect of medium strength in inorganics, sucrose level, mannitol concentration and their interactions in a medium containing 1 mg/l IBA on CAB-6P and Gisela 6 cherry rootstocks total leaf chlorophyll, carotenoid and porphyrin content.

Strength MS in inorganics	Sucrose (g/l)	Mannitol (g/l)	Chl(a+b) (mg/g F.W.)	Carotenoids (mg/g F.W.)	Porphyrins (mg/g F.W.)
Rootstock: CAB-6P					
full	30	0	18.969 defg	0.358 abcd	5.990 cdef
	30	5	14.739 abcde	0.460 de	5.794 bcde
	30	10	11.934 abcd	0.339 abc	5.242 abc
	30	20	19.294 defg	0.389 abcd	6.726 fg
	15	0	15.068 bcde	0.411 bcde	5.451 abc
	15	5	19.563 defg	0.285 a	6.402 def
	15	10	9.716 ab	0.336 abc	5.004 a
	15	20	21.550 efg	0.386 abcd	6.893 g
½	30	0	17.755 cde	0.640 f	8.410 h
	30	5	22.879 fg	0.323 ab	6.217 defg
	30	10	15.177 bcde	0.517 e	6.753 g
	30	20	7.267 a	0.377 abcd	5.832 bcde
	15	0	25.725 g	0.451 cde	6.813 g
	15	5	13.733 abcd	0.364 abcd	5.662 abcd
	15	10	17.627 cdef	0.413 bcde	6.481 efg
	15	20	10.918 abc	0.343 abc	5.178 ab
3-wayANOVA					
Strength MS (A)			0.599 ns	0.000***	0.003**
Sucrose level (B)			0.000***	0.074 ns	0.000***
Mannitol concentration (C)			0.000***	0.000***	0.000***
(A)*(B)			0.590 ns	0.223 ns	0.505 ns
(A)*(C)			0.306 ns	0.045*	0.001**
(B)*(C)			0.083 ns	0.327 ns	0.042*
(A)*(B)*(C)			0.648 ns	0.069 ns	0.147 ns
Rootstock: Gisela 6					
full	30	0	18.245 bc	0.355 abc	7.057 a
	30	5	17.053 abc	0.374 abc	6.756 a
	30	10	12.301 a	0.298 abc	5.270 a
	30	20	13.941 ab	0.316 abc	5.897 a
	15	0	16.844 abc	0.451 c	7.159 a
	15	5	11.391 a	0.418 bc	6.937 a
	15	10	14.863 abc	0.277 ab	5.356 a
	15	20	12.830 ab	0.266 ab	5.316 a
½	30	0	17.202 abc	0.370 abc	6.917 a
	30	5	12.352 a	0.234 a	4.839 a
	30	10	20.169 c	0.346 abc	6.861 a
	30	20	16.706 abc	0.419 bc	6.558 a
	15	0	20.464 c	0.413 bc	7.231 a
	15	5	13.182 ab	0.333 abc	5.622 a
	15	10	14.849 abc	0.423 bc	6.800 a
	15	20	18.618 bc	0.356 abc	6.458 a
3-wayANOVA					
Strength MS (A)			0.000***	0.003**	0.004**
Sucrose level (B)			0.897 ns	0.379 ns	0.743 ns
Mannitol concentration (C)			0.236 ns	0.495 ns	0.490 ns
(A)*(B)			0.410 ns	0.184 ns	0.146 ns
(A)*(C)			0.730 ns	0.439 ns	0.679 ns
(B)*(C)			0.052 ns	0.120 ns	0.727 ns
(A)*(B)*(C)			0.805 ns	0.758 ns	0.880 ns

Means denoted by the same letter in each column and for each rootstock separately are not significantly different according to Duncan's multiple range test at $P \leq 0.05$. ns – non significant effect ($P \geq 0.05$) according to 3-way ANOVA (n = 13 for CAB-6P and n = 22 for Gisela 6).

Table 4 Effect of medium strength in inorganics, sucrose level, mannitol concentration and their interactions in a medium containing 1 mg/l IBA on CAB-6P and Gisela 6 cherry rootstocks total carbohydrate and proline content in leaves and roots.

Strength MS in inorganics	Sucrose (g/l)	Mannitol (g/l)	Leaf carbohydrates ($\mu\text{mol/g F.W.}$)	Leaf proline ($\mu\text{mol/g F.W.}$)	Root carbohydrates ($\mu\text{mol/g F.W.}$)	Root proline ($\mu\text{mol/g F.W.}$)
Rootstock: CAB-6P						
full	30	0	51.207 a	2.850 a	42.030 a	0.936 a
	30	5	76.033 cd	3.016 a	83.304 d	1.225 ab
	30	10	103.520 fg	22.800 f	79.113 c	2.249 d
	30	20	68.866 bc	6.282 bcd	65.434 b	1.909 cd
	15	0	55.611 ab	4.553 b	47.542 a	1.475 bc
	15	5	92.701 defg	5.472 bc	183.827 f	1.969 cd
	15	10	99.003 efg	22.139 f	93.114 e	5.956 f
$\frac{1}{2}$	15	20	76.250 cd	10.489 de	67.038 b	1.644 bc
	30	0	71.266 bcd	4.383 ab	43.148 a	1.134 ab
	30	5	97.454 efg	6.549 bcd	67.303 b	1.876 cd
	30	10	108.787 g	6.744 bcd	67.837 b	1.309 b
	30	20	75.748 cd	16.099 e	70.117 bc	1.501 c
	15	0	64.735 abc	3.223 a	40.828 a	0.945 a
	15	5	86.437 def	12.207 de	60.902 b	2.778 e
	15	10	78.900 de	11.732 de	57.592 b	1.350 b
	15	20	73.423 cd	9.898 cde	70.913 c	3.066 e
3-wayANOVA						
Strength MS (A)			0.000***	0.001**	0.002**	0.000***
Sucrose level (B)			0.000***	0.000***	0.001**	0.000***
Mannitol concentration (C)			0.797 ns	0.000***	0.280 ns	0.000***
(A)*(B)			0.056 ns	0.157 ns	0.261 ns	0.482 ns
(A)*(C)			0.000***	0.002**	0.084 ns	0.000***
(B)*(C)			0.829 ns	0.000***	0.261 ns	0.000***
(A)*(B)*(C)			0.317 ns	0.010**	0.129 ns	0.000***
Rootstock: Gisela 6						
full	30	0	25.578 ab	2.801 ab	38.320 abcd	1.385 a
	30	5	28.264 abc	2.619 ab	66.995 fg	2.173 abc
	30	10	25.941 ab	7.865 ef	50.800 bcdef	4.255 d
	30	20	23.942 ab	4.729 cde	34.019 abc	1.669 abc
	15	0	38.220 cde	6.288 de	39.108 abcde	1.476 ab
	15	5	29.819 abcd	10.255 f	57.248 ef	2.375 c
	15	10	35.332 bcde	10.904 f	52.477 cdef	5.924 e
$\frac{1}{2}$	15	20	25.840 ab	3.716 ab	33.026 ab	1.886 abc
	30	0	39.895 de	3.547 ab	39.673 abcde	1.392 a
	30	5	22.508 a	5.651 cde	39.950 abcde	2.331 bc
	30	10	45.146 e	3.333 ab	76.061 g	1.729 abc
	30	20	28.284 abc	3.099 ab	53.789 def	1.924 abc
	15	0	34.039 abcd	2.398 a	40.902 abcde	1.665 abc
	15	5	32.121 abcd	5.761 cde	26.769 a	1.483 ab
	15	10	30.687 abcd	2.729 ab	59.968 fg	1.950 abc
	15	20	30.546 abcd	3.742 cd	41.592 abcde	1.995 abc
3-wayANOVA						
Strength MS (A)			0.001**	0.000***	0.216 ns	0.000***
Sucrose level (B)			0.391 ns	0.033*	0.000***	0.000***
Mannitol concentration (C)			0.003**	0.003**	0.010**	0.000***
(A)*(B)			0.196 ns	0.003**	0.369 ns	0.000***
(A)*(C)			0.012*	0.046*	0.781 ns	0.000***
(B)*(C)			0.493 ns	0.044*	0.037*	0.000***
(A)*(B)*(C)			0.581 ns	0.015*	0.283 ns	0.000***

It is possible that at high sugar concentration, rooting reduces the ability of buds to multiply by diverting most nutrients to root formation rather than to bud formation to overcome the expected water stress under these higher concentrations.

In the absence of mannitol from the culture medium, higher rooting percentages were recorded for CAB-6P explants with 30 g/l sucrose in half MS or with 15 g/l sucrose in full MS medium. Khan *et al.* (1999) found that the increase in sucrose concentration (from 10 to 30 g/l) was positively correlated with the rooting percentage and root number in *Syzygium alternifolium*, however, sucrose concentrations higher than 40 g/l inhibited overall rooting response. On the other hand, in Gisela 6, the rhizogenetic capacity of the explants was greater with 30 g/l sucrose in full MS or 15 g/l sucrose in half MS medium. In *Eucalyptus globules* and *Eucalyptus saligna*, increase of sucrose level from 30 to 60 g/l did not influence root number and rooting percentage considerably (da Rocha Corrêa *et al.* 2005).

Root elongation of both CAB-6P and Gisela 6 microcuttings was diminished when the strength of the medium in inorganics was reduced by half. The promotory effect of mineral concentration of the culture medium on rooting can be attributed to the participation of inorganic ions in processes regulating hormonal balance (Amzallag *et al.*, 1992). The favourable effect of a diluted mineral solution on rooting can be explained by the reduction of nitrogen concentration (Driver and Suttle 1987). Dimassi-Theriou (1995) reported that reducing mineral concentration of the MS medium to half the normal value increased rooting percentage and stimulated root elongation of the GF 677 rootstock *in vitro*. Ruzic *et al.* (1984) have proposed the use of MS medium at half of normal strength for rooting improvement of the GF 677 rootstock shoots. Moncousin (1988) suggested that the dilution of salt concentration to half prepare the plants in the tubes for better adaptation to the acclimatisation medium, while shoot growth and leaf size were increased. Reducing mineral concentration of MS medium to half the normal value increased the rooting percentage, root number and root elongation of PR 204/84 (*Prunus persica* x *P. amygdalus*) (Fotopoulos and Sotiropoulos 2005). Root length of Gisela 6 explants was increased along with an increase in sucrose level (15 to 30 g/l) in full MS medium. Our findings are in line with those obtained in *Hypericum perforatum* (Cui *et al.* 2010), in *E. globules* and *E. saligna* (da Rocha Corrêa *et al.* 2005) and *S. alternifolium* (Khan *et al.* 1999). Similarly, El-Karzaz *et al.* (1997) found that in mulberry (*Morus alba* L.) plants root formation on *in vitro* shoots was most extensive on MS medium supplemented with 30 g/l sucrose.

In Gisela 6 explants grown on half MS medium, the increase in sucrose level from 15 to 30 g/l led to a significant decrease in root fresh weight. In *Hyoscyamus niger* L., 15 or 30 g/l sucrose produced good root biomass while higher concentrations had a negative effect (Hong *et al.* 2010). Sucrose generally exerts osmotic pressure and influences productivity of plants as a carbon source. In CAB-6P, on the other hand, non substantial fluctuations were observed in root fresh weight due to alterations in the MS medium regarding its sucrose and

inorganics levels. Our results in Gisela 6 are in tune with Kevers *et al.* (1999), who reported 30 g/l sucrose to be optimum for root biomass production in *Panax ginseng* and *P. quinquefolium*.

In CAB-6P explants, the higher sucrose level (30 g/l) and medium strength (full MS) promoted better shoot length and shoot fresh weight whereas in Gisela 6, 15 g/l sucrose + half MS gave better results. In micropropagated potato plantlets (Mohamed and Alsadon 2010) and in date palm (*Phoenix dactylifera* L.) cv. Khanezi (Al-Khateeb, 2001) increasing sucrose concentrations significantly increased plantlet height and shoot fresh weight. In CAB-6P, the supplementation of the full MS medium containing 30 g/l sucrose with 5 g/l mannitol positively influenced shoot length. On the other hand, Fortes and Scherwinski-Pereira (2001) in potato, Moges *et al.* (2003) in chrysanthemum and Shibli *et al.* (1999) in bitter almond found that mannitol caused a reduction in shoot growth compared to sucrose. In olive (*Olea europaea* L.), however, mannitol gave higher shoot length than sucrose (García *et al.* 2002).

In CAB-6P, callus fresh weight was greatest in the half MS medium supplemented with 30 g/l sucrose and 5 g/l mannitol. Mannitol increased callus fresh weight when added in the full MS medium with 15 g/l sucrose. In *P. dactylifera* leaf explants cultured *in vitro*, callus fresh weight was increased with sucrose concentration up to 0.1 M but then declined (Asemota *et al.* 2007). In Gisela 6, half MS supplemented with 15 g/l sucrose (mannitol-free) exhibited greater callus fresh weight. Similarly, in strawberry when sucrose concentration was raised from 30 to 90 g/l callus fresh weight was decreased (Gerdakaneh *et al.* 2010). According to Gerdakaneh *et al.* (2009), low sucrose concentration is much more effective than high levels on callus fresh weight as the rise of sucrose concentration decreases callus fresh weight and this capacity could be attributed to an osmotic effect. In CAB-6P, the increase in sucrose concentration from 15 to 30 g/l led to lower callus induction frequencies. Similar findings were reported for Gisela 6 where callus induction frequency was greatest in the simultaneous effect half MS + 30 g/l sucrose + 5 g/l mannitol. In the apple rootstock Jork 9, sucrose promoted callus formation (Pawlicki and Welander 1995). In CAB-6P, mannitol led to depleted chlorophyll, carotenoid and porphyrin levels in half MS medium regardless sucrose content, except for the half MS + 30 g/l sucrose + 5 g/l mannitol combination treatment where an increase in total chlorophyll content was observed. In accordance, mannitol decreased chlorophyll and carotenoid content of sugarcane vitroplants (Cha-Um and Kirdmanee 2008). In Gisela 6, mannitol decreased carotenoid content when explants treated with 15 g/l sucrose (full MS). A possible explanation for this decrease according to Vitova *et al.* (2002) is that mannitol creates an osmotic stress which strongly inhibits the plant cell, tissue and organ growth mainly by impairing the gain of photoassimilates e.g. by inducing stomata closure or lowering the activity of photosynthetic enzymes. In Gisela 6, on the other hand, fifty percent decrease in both macro- and microelements concentration of MS medium supplemented with 15 g/l sucrose raised chlorophyll content. Sucrose linearly increases the level of reducing sugars, starch, and total chlorophyll in citrus plantlets (Hazarika *et al.*

2000). In CAB-6P, higher chlorophyll levels were recorded in half MS medium supplemented with 15 g/l sucrose or 30 g/l sucrose + 5 g/l mannitol compared to the full MS one. Different results were reported by Mohamed and Alsadon (2010) and Serret *et al.* (2001) who observed a positive correlation between chlorophyll content and sucrose concentration. The higher chlorophyll content observed in the rooting phase in plants growing at lower sucrose concentrations such as in Gisela 6 cherry rootstock of our study may indicate that such plants could present a higher photosynthetic capacity, facilitating the acclimatization.

In CAB-6P, mannitol resulted in elevated carbohydrate and proline levels in both leaves and roots regardless sucrose level (15 or 30 g/l) and concentration of the MS medium in inorganics (full or half). Accordingly, mannitol considerably augmented endogenous proline of micropropagated sugarcane (Cha-Um and Kirdmanee 2008). Vitova *et al.* (2002) states that under high mannitol concentration, osmotic stress causes restriction of mannitol utilization and lowering availability of energy and carbon source. In Gisela 6, mannitol increased leaf proline content of leaves grown in half MS medium with 15 g/l sucrose, whereas in roots mannitol at 10 g/l raised both proline (full MS) and carbohydrate content (half MS) irrespective sucrose level. According to Ahmad *et al.* (2007) sugars are perceived by cells as chemical signals *in vitro* with very high concentration acting as stressing agents.

Proline accumulation in stressed plants is one of the vital compatible solutes to function in cellular osmotic adjustment and scavenge detoxify oxidants (Seki *et al.* 2007) and its degradation can provide carbon, nitrogen and energy source after stress (Hare *et al.* 1999). On the contrary, the increase in sucrose level from 15 to 30 g/l led to depleted proline levels in Gisela 6 leaves and CAB-6P roots (full MS) indicating mechanism of osmoregulation and osmotic adjustment located only in leaves for Gisela 6 and only in roots for CAB-6P. The carbohydrate content of Gisela 6 leaves was higher in half MS medium supplemented with 30 g/l sucrose. Sucrose may facilitate growth and development due to its impact on the adjustment of cell osmolarity as reported by Khuri and Moorby (1995). In CAB-6P roots, the increase in sucrose level from 15 to 30 g/l resulted in depleted proline levels. Our findings are in disagreement with those presented in micropropagated potato plantlets (*Solanum tuberosum* L. cv Norland) (Badr *et al.* 2011) and in strawberry (Gerdakaneh *et al.* 2010) where the amount of free proline was increased as a response to the increase in sucrose concentration. According to Badr *et al.* (2011), the presence of sucrose in the culture medium causes an osmotic stress which leads to the build up of compatible solutes, such as proline, sugars and sugar alcohols in plantlets allowing them to absorb water under these conditions.

CONCLUSIONS

It seems that mannitol concentration, sucrose level and medium strength in inorganics are involved in photosynthetic apparatus, influencing leaf chlorophyll, carotenoid or porphyrin content and participating in carbohydrate biosynthesis and metabolism as well as in proline accumulation in both leaves and roots. It is

obvious that each cherry rootstock has its own specific requirements in macro- and micronutrients, in sucrose as the main carbon and energy source and in mannitol as a supplement, all in combination for optimum rooting and shoot growth performance under in vitro conditions.

Therefore, the different responses between the two cherry rootstocks to rooting, vegetative and callusing traits as well as to various biochemical parameters are genotype-dependent.

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THE IMPACT OF ROADSIDE GREEN SPACES ON ECOLOGICAL CONDITIONS IN THE URBAN ENVIRONMENT

SUMMARY

Automobile traffic, which is considered one of the key permanent sources of different types of urban pollution, particularly contributes to environmental problems linked to cities. Negative environmental impacts produced by city traffic can be significantly reduced through the establishment of roadside green spaces along city roads. This paper examines the impact of roadside green spaces grouped according to their urban biotope type, on ecological factors with the highest impact on human comfort, including temperature, humidity, city noise intensity and wind speed. The results and conclusions of this paper provide guidelines that can be applied in urban environmental planning and the design of open urban spaces.

Keywords: ecological impact, urban biotopes, roadside green spaces, urban forestry.

INTRODUCTION

The expansion of urban landscapes accompanied by uncontrolled and unplanned urbanization has led to a series of environmental problems faced by cities of today. Automobile traffic, as one of the key sources of different types of permanent pollution in the urban environment particularly contributes to the environmental problems of cities. Large areas covered by asphalt which are occupied by city roads cause overheating, increasing the heat island effect in cities, while reducing humidity in their immediate vicinity. Due to high vehicle frequency, the amounts of pollutants and noise intensity are increased, while changes in terrain configuration caused by road routing can, among other things, cause an increase in wind speed (Taha, 1997).

The establishment of roadside green areas is one of the major strategies aimed at the reduction of negative environmental effects caused by urban traffic. Greenery along city roads can help reduce the negative effects of warming through its positive impacts on ecological microclimate conditions by creating

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the effect of cooling, while increasing overall urban comfort (Potchter et al., 2006, Zoulia et al., 2009; Georgi et al., 2010).

The use of green spaces and other landscape elements has primary importance for protection against urban noise. Soft litter of fallen leaves reduces the intensity of low-frequency sounds by absorbing their energy (Aylor, 1972), while leaves and trees help in the reduction of city noise intensity by bouncing high-frequency sound waves (Aylor and Marks, 1976). Trees can reduce pollutants from the air by absorbing particles from the atmosphere (Nowak et al., 2006). On the other hand, the presence of green spaces and greenery massifs affects air movement, causing the effect of wind force reduction, not only at the system itself but also at a certain distance (Rosenberg, 1974).

Surely, green spaces have an impact on the ecological conditions in urban areas. It is of great importance for the planning and management of urban green areas to have information on the size, dendrological structure and biophysical characteristic of green spaces that provide the best results in the modification of unfavourable environmental factors. In addition, in the early nineties of last century, due to the increasing urge to solve environmental problems of urban areas, the need was recognized to put planning and management of urban landscapes in the ecological context.

In other words, the need arose to observe the city as an ecosystem composed of its basic topographical units in ecology – biotopes. A biotope is often defined as an ecological landscape unit, characterized by particular conditions and specific populations of biota (Qui et al., 2010). The ecological division of urban landscapes into basic ecological units - biotopes greatly contributed to a clearer and more applicable methodology of research into the impacts of green spaces on environmental factors modification in the urban environment.

The registration of urban biotope types, their spatial distribution in the urban landscape, familiarity with site conditions and the evaluation of their ecological impacts constitute an important and indispensable basis for urban area planning. This paper presents a preliminary research of the impacts of roadside green spaces along the main city routes in the Belgrade area grouped according to their urban biotope type on environmental factors with the highest impact on human comfort in cities, including temperature and humidity, city noise intensity and wind speed. The study of environmental impacts of green spaces categorized according to their urban biotope type has a special added significance in the overall planning and management of urban green areas.

MATERIAL AND METHODS

The research of ecological functions of green spaces along city roads is based on the monitoring and measurement of the following environmental factors: temperature, humidity, city noise intensity and wind speed in isolated green spaces along the main city routes in the Belgrade area.

A total of 15 main routes were recorded (Zrenjaninski road, Višnjička Street, Jurija Gagarina Street, Partizanski road, King Alexander Boulevard, Rakovički road and Patrjarha Dimitrija, Boulevard JNA, the Ibar highway, Nikola Tesla Boulevard, Mihajlo Pupin Boulevard, Tošin bunar, Pančevački road, Savski road, highway E-75, Batajnički road and Cara Dušana Street) in the Belgrade city area (borders of the Master Plan of Belgrade, 2021). The research was conducted on a total of 38 representative green spaces distinguished along these city routes (Figure 1).

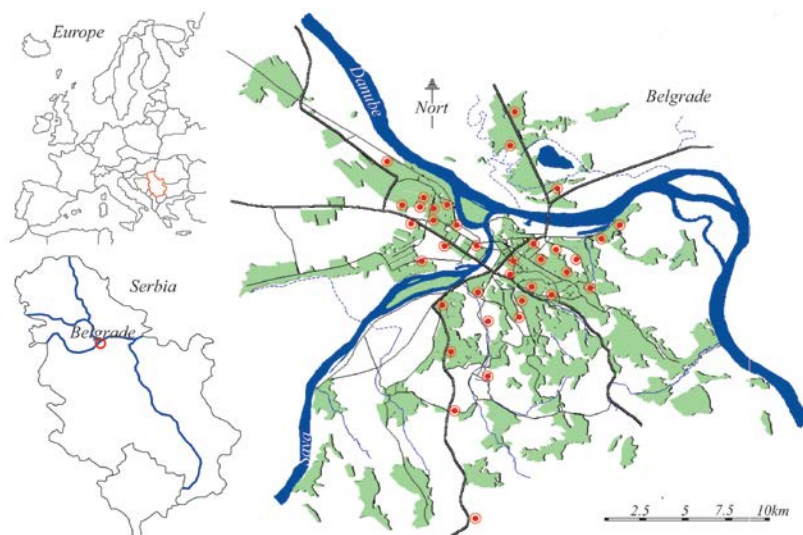


Figure 1. A schematic representation of the positions of selected green spaces along the main city routes in Belgrade (Source: A Modified map of residential and industrial areas from the Ecological Atlas of Belgrade)

The representative green spaces were selected on the basis of the following criteria: (1) *Urban biotope type* - The information basis for determining urban biotope types were results of the project "Belgrade Green Regulation" (2007), i.e. its Phase III "Mapping and Evaluation of Belgrade Biotopes". For the purpose of mapping or photo interpretation, a biotope is a clearly bordered area with a relatively unique structure of vegetation and use. The map of Belgrade biotopes was produced at the third hierarchical level of biotope typology, i.e. at the subtype level on the basis of a key for biotope mapping using the photo-interpretation method. According to the established typology of Belgrade biotopes, the investigated main routes belong to the biotope of traffic structures without green spaces along roads. Therefore, for the purpose of this study, it was necessary to determine the biotope types of green spaces along the main city routes in Belgrade. According to the Map of Belgrade, these green spaces belong to three urban biotope types: urban fallows, green structures and thickets, groves and forests, which are considered to be a protective belt. The biotope type of green structures includes more or less arranged green areas with a certain level of

maintenance. Urban fallows include various sites commonly overgrown with ruderal flora and vegetation. These spaces are subject to human impacts and unproductive. Thickets, groves and forests include all surface and line structures of trees or shrubs outside closed forests, as well as any residual forests on areas smaller than 0.5 ha which do not belong to a particular type of forest or forest plantation.

(2) *Accessibility and availability in the field*; The selection of green spaces for investigation included areas where the necessary measurements of environmental factors could be carried out over a long period of time (public and private property; accessible in the field). The research of ecological functions of the selected green areas was carried out during two research years. In each of the 38 representative green spaces measurements were performed during spring, summer and autumn of every research year in 3 series of 2 consecutive measurements, while control measurements were conducted in a series of 3 consecutive measurements. The total number of measurements was 5472, i.e. 5700 with control measurements. The measurements of air temperature, humidity and city noise intensity were performed with a digital weather station DT-8820 - CEM, UK (operating measurement range: for air temperature from -20 ° C to 750 ° C with a 0.1 ° C resolution; for humidity from 25% to 95% RH with a 0.1% RH resolution; for city noise intensity from 35 to 130dB with a 0.1dB resolution). Wind speed was measured using an AM 4220 - LUTRON, Taiwan digital anemometer with a wind vane (operating range from 0.9 to 35m/s with a 0.1m/s resolution). Measurement readings were performed at two locations, i.e. in front of the green space (side toward the road) and behind that green space. All measurements were conducted on weekdays, in the morning, and at the operational height of 130cm at each measurement point. In the aim of determination of the impact of green spaces on the environmental factors air temperature and humidity, a series of control measurements were carried out at identical distances in the direction of measurement points, both in the immediate vicinity and in open spaces without established plantings. When measuring temperature and humidity, the instruments were placed under a shield, whereas a microphone shield was used to neutralize the impact of wind on city noise intensity. The impact of green areas, i.e. the mean difference in air temperature and humidity was obtained as the difference between the mean values measured in front of the investigated green spaces and behind them and the mean values of differences in control measurements. When the environmental factors of city noise intensity and wind speed are concerned, the impact of green areas is represented as the mean value of differences in these factors measured in front of the investigated green spaces and behind them.

The research was conducted in the aim of: 1) determination of the level of impact of roadside green spaces classified according to their urban biotope type on environmental conditions in their immediate urban environment and 2) determination of possible differences between these impacts depending on the urban biotope type to which these roadside green spaces belong.

Programs IBM SPSS Statistics 21 and Microsoft Excel 2010 were used for data analysis and graphical presentation of research results. Parametric statistics was applied. Mean differences in the impact of green spaces were tested for different biotope types using one-factor analysis of variance (ANOVA). The Levene test was used for variance homogeneity testing, whereas the Tukey HSD test was used to obtain a display of statistically significant differences in the values of environmental factors, due to the best balance of type I and II errors.

RESULTS AND DISCUSSION

The representative green spaces which are the subject of this research (38 in total) were classified into three categories according to the Map of Belgrade biotopes ("Belgrade Green Regulation", Belgrade, 2007): urban fallows, green structures and thickets, groves and forests. Two roadside green spaces were classified into the urban fallows group, 26 of them in the group of green structures and 10 roadside green areas in the group thickets, groves and forests.

Table 1. The statistical parameters of mean differences in the investigated environmental factors for the analyzed urban biotope types

Environmental factor	Urban biotope type	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Min.	Max.
					Lower Bound	Upper Bound		
Air temperature (°C)	Urban fallows	0,8194	0,42347	0,07058	0,6762	0,9627	0,10	1,70
	Green structures	1,2064	0,62586	0,02046	1,1663	1,2466	0,10	3,40
	Thickets, groves and forests	1,0994	0,63421	0,03343	1,0337	1,1652	0,10	4,70
	Total	1,1670	0,62766	0,01720	1,1333	1,2008	0,10	4,70
Air humidity (%)	Urban fallows	1,0500	0,50681	0,08447	0,8785	1,2215	0,20	2,40
	Green structures	1,9268	0,97226	0,03178	1,8644	1,9892	0,10	6,20
	Thickets, groves and forests	1,9450	0,94552	0,04983	1,8470	2,0430	0,30	5,20
	Total	1,9080	0,96564	0,02646	1,8561	1,9599	0,10	6,20
City noise intensity (dB)	Urban fallows	10,9333	1,24636	0,04106	7,5349	9,7317	6,00	15,40
	Green structures	15,6411	4,58869	0,14999	15,3468	15,9355	4,00	29,90
	Thickets, groves and forests	15,9806	5,20219	0,27418	15,4414	16,5198	4,20	28,80
	Total	15,8137	4,75516	0,13029	15,5581	16,0693	4,00	29,90
Wind speed (m/s)	Urban fallows	0,6708	0,58098	0,06500	0,4428	0,5127	0,10	1,10
	Green structures	0,8470	0,72933	0,02384	0,8002	0,8938	0,10	6,20
	Thickets, groves and forests	0,8608	0,73477	0,03873	0,7847	0,9370	0,10	4,90
	Total	0,8597	0,74021	0,02028	0,8199	0,8995	0,10	6,20

The highest mean difference in air temperature (Table 1) between the measurement points was recorded for green structures ($1.21 \pm 0.63^\circ\text{C}$), a slightly lower one for spaces belonging to thickets, groves and forests ($1.10 \pm 0.63^\circ\text{C}$), and the lowest one in the urban fallows group ($0.82 \pm 0.42^\circ\text{C}$).

Green spaces belonging to the biotope type thickets, groves and forests revealed the highest mean difference between measurement points for humidity ($1.95 \pm 0.95\%$), which was close to the value obtained for green structures

($1.93 \pm 0.97\%$), whereas the lowest value was recorded for urban fallows ($1.05 \pm 0.51\%$). Wind speed reduction was under the highest impact of green areas belonging to thickets, groves and forests with a mean difference of $0.86 \pm 0.73\text{m/s}$. A lower mean difference was recorded for green spaces belonging to green structures ($0.84 \pm 0.73\text{m/s}$), and the lowest one for the urban fallows group ($0.67 \pm 0.58\text{m/s}$). The highest mean difference in city noise intensity was recorded for green spaces belonging to thickets, groves and forests ($15.98 \pm 5.20\text{dB}$), slightly lower one for green structures ($15.64 \pm 4.59\text{dB}$), while the lowest one was found in the urban fallows group ($10.93 \pm 1.24\text{dB}$).

A comparative analysis revealed that green spaces belonging to the urban biotope type thickets, groves and forests has the highest impact on humidity modification, noise intensity and wind speed, which was expected due to the significant presence of trees and shrubs. The samples from two green spaces belonging to the urban biotope type of urban fallows characterized by spontaneous ruderal vegetation or absence of woody plants proved to be the least efficient in the modification of investigated environmental factors. The results obtained are consistent with the study Wilmers (1988), which also found the highest impact of green structures on the reduction of air temperature in cities.

During two research years, mean air temperature reduction for the examined green spaces was $1.21 \pm 0.63^\circ\text{C}$. When these results are compared to literature data, we can observe a partial proportional correlation. Depending on the author, air temperature reduction ranged from 2 to 8°C (Taha *et al.*, 1991) and from 2 to 3°C (Bunuševac, 1962), or 1.3°C in 50% of measurements and 2.9°C in 15% of measurements performed in researches conducted by Amdrade and Vieira (2007). The discrepancies can be explained by different areas of studied green spaces. The listed authors investigated air temperature modification in urban parks and green spaces larger than 3ha, while 76.3% of green spaces in this study have an area below 1ha.

Statistical significance in air temperature modification between different urban biotope types was confirmed at the level of 0.01 by the results of one-factor analysis of variance (Table 2). In addition, the Tukey HSD test revealed statistically significant differences in air temperature among all urban biotope types (Table 3). Statistical significance at the 0.01 level was found for urban fallows and green structures (Sig.=0.000; $p < 0.01$), whereas statistical significance at the 0.05 level (Sig.=0.028; $r < 0.05$) was recorded for urban fallows and thickets, groves and forests, as well as for green structures and thickets, groves and forests (Sig.=0.016; $p < 0.05$). The results of one-factor analysis of variance for humidity confirmed statistically significant differences between different urban biotope types at the 0.01 level (values of statistics were 49.276 (Welchtest) and 23.093 (Brown-Forsythetest), with a 0.000 significance (in both tests)). The Tukey HSD test revealed statistically significant differences in humidity modification between urban fallows and green structures (Sig.=0.000; $p < 0.01$) and urban fallows and thickets, groves and forests (Sig.=0.000; $r < 0.01$) at the 0.01 level, while no statistically significant differences were found between

green structures and thickets, groves and forests (Sig.=0.949; $r>0.05$). These results suggest that thickets, groves and forests and green structures modify humidity more than urban fallows. Similar results were obtained by Sonne and Viera (2000), in a research of the impacts of bioclimatic urban forests. These authors found the highest cooling impact of green massifs in the city especially in terms of humidity, compared to urban green spaces with a notable absence of woody vegetation or those green spaces where woody plants occur only sporadically.

Table 2. One-factor analysis of variance of differences in the mean values of investigated environmental factors between the analyzed urban biotope types

Test of Homogeneity of Variances						
		Levene Statistic	df1	df2	Sig.	
Air temperaure		4,476	2	1329	0,012	
Humidity		6,306	2	1329	0,002	
City noise intensity		8,301	2	1329	0,000	
Wind speed		2,305	2	1329	0,100	
ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Air temperaure	Between Groups	7,445	2	3,723	9,571	0,000
	Within Groups	516,918	1329	0,389		
	Total	524,363	1331			
Humidity	Between Groups	27,326	2	13,663	14,960	0,000
	Within Groups	1213,778	1329	0,913		
	Total	1241,104	1331			
City noise intensity	Between Groups	324,108	2	162,054	7,234	0,001
	Within Groups	29771,810	1329	22,402		
	Total	30095,919	1331			
Wind speed	Between Groups	3,793	2	1,897	3,475	0,031
	Within Groups	725,472	1329	0,546		
	Total	729,265	1331			
Robust Tests of Equality of Means						
		Statistic ^a	df1	df2	Sig.	
Air temperaure	Welch	15,730	2	98,695	0,000	
	Brown-Forsythe	12,739	2	303,822	0,000	
Humidity	Welch	49,276	2	105,676	0,000	
	Brown-Forsythe	23,093	2	458,429	0,000	
City noise intensity	Welch	14,186	2	97,841	0,000	
	Brown-Forsythe	8,938	2	318,207	0,000	
a. Asymptotically F distributed.						

The results of one-factor analysis of variance for wind speed (f value is 3.475 with a 0.031 significance) revealed statistically significant differences in wind speed reduction between the green spaces belonging to different urban biotope types. The tukey hsd test confirmed statistically significant differences between urban fallows and green structures (sig.=0.023; $p<0.05$) and urban fallows and thickets, groves and forests (sig.=0.038; $p<0.05$) at a significance level of 0.05. However, no statistically significant differences (sig.=0.951; $r>0.05$) were found between the urban biotope types of green structures and thickets, groves and forests. Thickets, groves and forests and green structures reduce wind speed more than city fallows, primarily due to higher presence of woody plants.

Table 3. Statistical parameters of the Tukey test – differences between the impacts of urban biotopes on the investigated environmental factors.

Dependent variable	(I) Biotope type	(J) Biotope type	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Air temperature	Urban fallows	Green structures	-0,38697*	0,10592	0,001	-0,6355	-0,1384
		Thickets, groves and forests	-0,28000*	0,10902	0,028	-0,5358	-0,0242
	Green structures	Urban fallows	0,38697*	0,10592	0,001	0,1384	0,6355
		Thickets, groves and forests	0,10697*	0,03868	0,016	0,0162	0,1977
	Thickets, groves and forests	Urban fallows	0,28000*	0,10902	0,028	0,0242	0,5358
		Green structures	-0,10697*	0,03868	0,016	-0,1977	-0,0162
Air humidity	Urban fallows	Green structures	-0,87682*	0,16231	0,000	-1,2577	-0,4960
		Thickets, groves and forests	-0,89500*	0,16705	0,000	-1,2870	-0,5030
	Green structures	Urban fallows	0,87682*	0,16231	0,000	0,4960	1,2577
		Thickets, groves and forests	-0,01818	0,05927	0,949	-0,1572	0,1209
	Thickets, groves and forests	Urban fallows	0,89500*	0,16705	0,000	0,5030	1,2870
		Green structures	0,01818	0,05927	0,949	-0,1209	0,1572
City noise intensity	Urban fallows	Green structures	2,99220*	0,80387	0,001	1,1061	4,8783
		Thickets, groves and forests	2,65278*	0,82734	0,004	0,7116	4,5940
	Green structures	Urban fallows	-2,99220*	0,80387	0,001	-4,8783	-1,1061
		Thickets, groves and forests	-0,33942	0,29353	0,480	-1,0281	0,3493
	Thickets, groves and forests	Urban fallows	-2,65278*	0,82734	0,004	-4,5940	-0,7116
		Green structures	0,33942	0,29353	0,480	-0,3493	1,0281
Wind speed	Urban fallows	Green structures	0,33077*	0,12548	0,023	0,0363	0,6252
		Thickets, groves and forests	0,31694*	0,12915	0,038	0,0139	0,6200
	Green structures	Urban fallows	-0,33077*	0,12548	0,023	-0,6252	-0,0363
		Thickets, groves and forests	-0,01382	0,04582	0,951	-0,1213	0,0937
	Thickets, groves and forests	Urban fallows	-0,31694*	0,12915	0,038	-0,6200	-0,0139
		Green structures	0,01382	0,04582	0,951	-0,0937	0,1213

*. The mean difference is significant at the 0.05 level.

The results of one-factor analysis of variance for the environmental factor noise intensity revealed the values of statistics of 97.841 for the welch test and 318.207 for the brown-forsythe test with a 0.000 significance in both. That leads to a conclusion that the differences in noise intensity reduction between different urban biotope types are statistically significant at the 0.01 level. The tukey hsd test revealed statistically significant differences in noise intensity between urban fallows and green structures (sig.=0.01; $p < 0.05$) and urban fallows and thickets, groves and forests (sig.=0.04; $p < 0.05$), while no statistically significant differences were found between green structures and thickets, groves and forests (sig.=0.480; $r > 0.05$).

Thickets, groves and forests are green spaces which contain grown trees and usually a well-developed second storey of trees and shrubs, and therefore

have an impact on city noise intensity. In addition, Reethof (1973) argues that effective protection from city noise can be achieved with spontaneous natural vegetation or groves, especially if they contain trees. In addition, this author believes that groves containing grown trees along with the shrub storey tend to be particularly effective in the reduction of city noise intensity. The research of the environmental impacts of green spaces grouped according to their urban biotope type into green structures, urban fallows and thickets, groves and forests has revealed a particularly pronounced modification of the environmental factor air temperature. Chen and Wong (2006) also reported that the presence of urban vegetation, or the prevailing absence of greenery, has the highest impact on the modification of air temperature in the immediate urban environment, particularly in large city areas.

On the basis of the analysis conducted, it can be stated that urban fallows modify the investigated environmental factors less than green structures or thickets, groves and forests.

CONCLUSIONS

The study which explored the impact of 38 roadside green spaces grouped into three urban biotope types (green structures, urban fallows and thickets, groves and forests) along 15 main city routes in the Belgrade area on four environmental factors (temperature and humidity, noise intensity and wind speed) has revealed the following findings:

Roadside green spaces belonging to the biotope type thickets, groves and forests have the highest impact on the reduction and mitigation of the investigated environmental factors compared to green areas belonging to the urban biotope types of urban fallows and green structures. Urban fallows as a biotope type proved to be the least effective in the reduction of investigated environmental factors in the urban environment.

Compared to green areas belonging to the urban biotope types of urban fallows and thickets, groves and forests, green structures have the highest impact on the reduction of the environmental factor air temperature. Statistical significance at the level of 0.01 was found for the difference in air temperature reduction between urban fallows and thickets, groves and forests, whereas the statistical significance between green structures and thickets, groves and forests is at the 0.05 level. Differences in the reduction of the environmental factor humidity were statistically significant between urban fallows and green structures and urban fallows and thickets, groves and forests at the 0.01 level. Statistically significant differences for the environmental factor wind speed were found between urban fallows and green structures and urban fallows and thickets, groves and forests at the 0.05 level of significance.

When city noise intensity is concerned, statistically significant differences were found between urban fallows and green structures at the 0.01 level of significance and urban fallows and thickets, groves and forests at the 0.05 level of significance. However, statistically significant differences were not found between green structures and thickets, groves and forests in the reduction of the environmental factors of air humidity, wind speed and city noise intensity.

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GROWTH OF VIRGIN FORESTS IN THE SOUTHERN CARPATHIANS

SUMMARY

In the beginning, all forests were represented by natural virgin forest stands. These kinds of ecosystems stand for a model representation for the managed forests. The research area is situated in Western Romanian Carpathians. To understand the functioning baseline principles of the virgin forests the radial growth of beech in relation to breast height diameter (DBH) was studied. The experimental growth distribution was determined using Beta, Gamma and Weibull theoretical frequencies functions. For the dendrochronological series, 36 samples were used. The average time span is 361 years with a mean growth of 0,999 and standard deviation (SD) of 0,482. The pointer years of this dendrochronological series were determined and the radial growths were correlated with climatic data such as temperatures and precipitations. Once the trees' volumes determined, it has been concluded that the virgin beech stands have high eco-productive characteristics ($6,33 \text{ m}^3 \cdot \text{year}^{-1} \cdot \text{ha}^{-1}$), despite the age and climatic influences.

Keywords: virgin forest, primeveral ecosystems' growth, old-growth forest dendrochronology pattern, virgin forest autoregulation, *Fagus sylvatica* virgin forest

INTRODUCTION

Initially, all the forest ecosystems were represented by natural virgin forest stands featuring uneven age structure and obvious ecological, silvicultural and economic advantages (Giurgiu, 1988). Also, the virgin forests represent a model for their durability and high efficient eco-productivity that people strive to achieve (Bândiu, 2013). Therefore, the analysis of this types of forests is important for developing in-depth knowledge. Regarding the forest ecosystems evolution and their natural dynamics the virgin forests are considered inestimable sources of information (Motta et al., 2010) that can be used as scientific basis for improving the forest management economy (Giurgiu, 2013). During the time, various researches highlighted the structural and functional characteristics of the virgin forests. Although such studies failed to meet the requirements and to reveal their natural potential level, they are still a sound groundwork for

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furthering the research scope of primary ecosystems. Preliminary research has highlighted the virgin forests' characteristics in relation to the managed forests, and has referred, inter alia, to the stand structure relative to age as well as to the correlation between DBH and DBH growth (Giurgiu, 1974). Recent researches have focused on statistical based knowledge of a natural forest's spatial structure, concluding that these ecosystems are characterized by high amplitude of DBH (Popa, 2013).

For the reconstruction of the climate spanning the last centuries, as well as the analysis of the forest ecosystems' dynamics, researches on dendrochronological series for fir, spruce (Popa, 2013) and beech (Roibu, 2013) had been carried out. The present research study is a continuation within the afore-mentioned topic.

The most important researches related to the structure, dynamics, cause of death and regeneration progress, for the studied area, had been carried out by Tomescu and Turcu from 2004 to 2006, and others from 2009 on. The purpose of these researches was to determine the trees' age by tree sampling.

In Romania the broadleaf forest covers 69,3 % of the afforested land, of which beech comprises 30,7%, oaks - 18,2% and other broadleaf species -20,4 %. The present investigations subject to this paper have a special importance and they aim, primarily, to develop in-depth knowledge on the peculiarities of the tree growth in the primary forests. The objectives of the present research are twofold: evolution and analysis of a virgin beech stand from the growth point of view and elaboration of a dendrochronological series for beech. The aims of the dendrochronological series are as follows: radial growth and DBH tree variability; stand structure relative to tree radial growth; dendrochronological aspects; correlation between the tree radial growth and the environmental conditions; volume growth.

MATERIAL AND METHODS

The permanent research plot (Semenic P20) is situated at an altitude of 1352 m in the "Izvoarele Nerei" Natural Reserve which is located in the "Semenic-Cheile Carasului" National Park, which is a part of Western Romanian Carpathians (Figure 1).

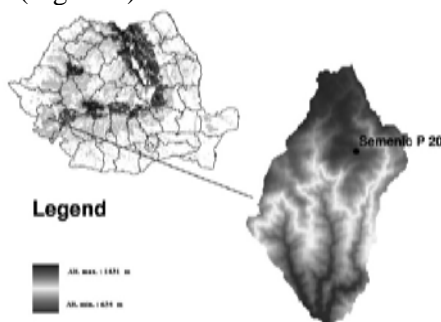


Figure 1. Semenik P20 research plot localization in the "Semenic-Cheile Carasului" National Park.

The choice of this research plot is based both on the consideration of the identification and delineation criteria of virgin forest, adopted by Romanian Government (Minister Order no. 3397/2012), and on the fact that the beech forests from the Banat Mountains are the largest and most compact century-old forests from Europe (Toader, 2004). In addition, the economical quality of this beech forest is acknowledged internationally. From the climatic point of view, the “Semenic - Cheile Carasului” National Park is located in the continental temperate region, with strong Mediterranean influences. Winters are long and cold, with a snow cover range between 60 and 100 cm, and a duration between 80 and 120 days (Tomescu, 2013), resulting in a short vegetation season (3 months). There is a tree growth variability from year to year depending on the vegetation conditions (Popa, 2004) on the one hand, and on the competition relationships between trees, on the other hand. The growth is materialized via the annual tree ring which holds important information about the time evolution of the tree growth.

For the radial growth determination, all 372 trees were sampled by means of the Pressler driller, 1,3 m above the ground, by following the cardinal points (N, E, S, V), in order to reduce the influence of the transversal form section and the radial growth, respectively. For highlighting the annual tree ring pattern, the increment cores were mounted on special wood supports and were grounded with different abrasive belts. Samples were processed by using Coorecorder 7.4, a software meant to register data from scanned pictures in order to measure tree-ring widths by registering boundary coordinates. The quality of crossdating and measurement accuracy of the tree-ring series has been assessed by the computer programme COFECHA. The ASTRAN application was used for the growth indexing of dendrochronological data series.

The volume growth is determined by means of one-inventory method using radial increment cores extracted from the living trees (Giurgiu, 1979, Leahu, 1994, Badea, 2008). This method allows to determine the volume growth by diameter classes based on the radial tree growth adjusted to the radial tree samples and was calculated as follows:

$$p_{iv} = p_{ig} + p_{ihf} - 0,01 p_{ig} p_{ihf} \quad (\text{Giurgiu, 1979}).$$

Where : p_{iv} - volume growth in volume percent by diameter classes; p_{ig} - basal area growth percent by diameter classes; p_{ihf} – reduced average height growth percent.

The basal area growth percent by diameter classes was determined as follows :

$$p_{ig} = \frac{400 i_r}{d} \left(1 - \frac{i_r}{d}\right) \quad (\text{Giurgiu, 1979}).$$

Where: i_r is average radial growth; d - diameter class.

Average height growth percent is determined using the relationship:

$$p_{ig} = n \lambda \quad (\text{Giurgiu, 1979}).$$

Where: n - number of years spanning the period on which the radial growth sampled had been averaged; λ - annual percent of tree reduced height growth.

The annual percent of tree reduced height growth is found in the production tables depending on the species, index class and in relation to the stand age (Giurgiu, 2004). In the next stage the volume growth is determined as follows:

$$i_r = 0,01 v p_{iv} \quad (\text{Giurgiu, 1979}).$$

Where: v is the volume of the diameter category.

The volume is established with the following regression equation:

$$\log v = a_0 + a_1 \log d + a_2 \log^2 d + a_3 \log h + a_4 \log^2 h \quad (\text{Giurgiu, 2004}).$$

RESULTS AND DISCUSSION

Tree radial and diameter growths variability:

Based on the information obtained by measuring the radial tree ring samples (increment cores) the chart showing the variability of the radial tree ring samples in relation to their diameters had been recorded (Figure 2). One may observe that the spread field generated by the experimental values are grouped along a second degree polynomial curve, very specific to uneven- age stand and by default, to virgin stands (Giurgiu, 1979).

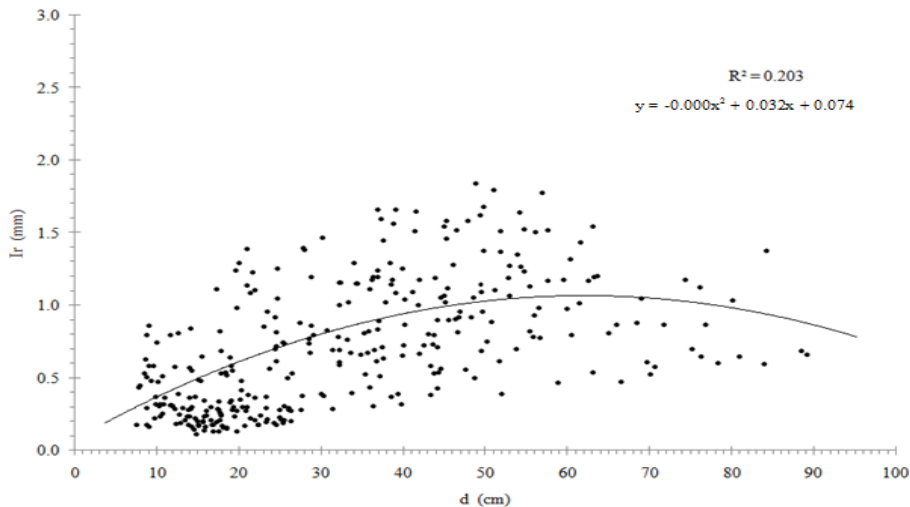


Figure 2. Variation of the radial tree growth average in relation to tree diameter, time period recorded 2003-2013.

An important characteristic pertaining to the virgin forest stands is the variation of the radial tree growth average in relation to the tree diameter, that is to say that the correlation coefficient has lower values ($r < 0.4$) in case of the virgin forest stands as compared to the managed stands (Giurgiu, 1979).

In the case study the correlation coefficient is 0.40. Due to this power of self-regulation, in case of these types of ecosystems, the radial tree growth decreases for bigger trees, the correlation field being recorded as a second order parabola shaped curve.

Distribution of values of the radial growth variation coefficient in relation to the tree diameter:

The radial growth variation coefficient in relation to diameter for the Semenik P20 virgin forest stand has a descending trend line (Figure 3), high values for the lower diameter classes being recorded, and gradually declining to the superior diameter classes.

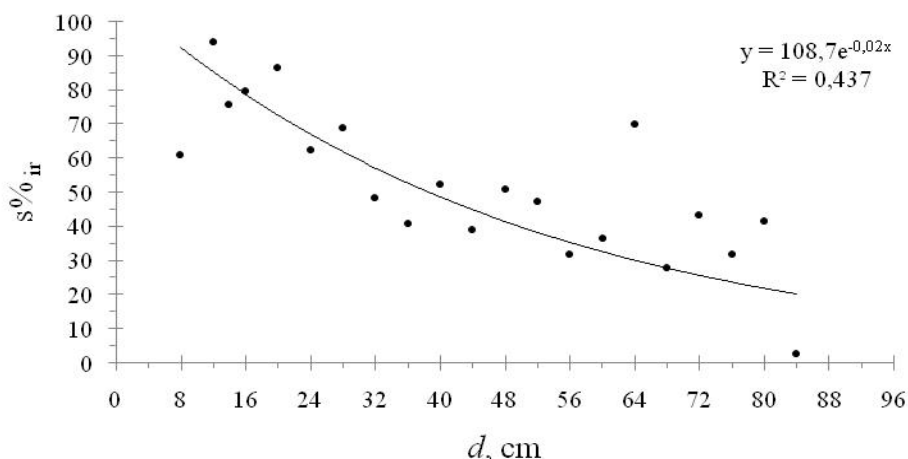


Figure 3. Distribution of values of radial growth variation coefficient in relation to the tree diameter.

The radial tree growth, as well as the diameter variation are influenced by the stand structure in relation to their age, vegetation conditions, trees health and the presence of gaps in the stand caused by windfall, snowfall or tree fall due to tree physiological death, issue specific to the virgin stands.

Virgin beech stands structure from the Semenik P20 in relation to their radial growth:

Similar to the tree diameter distribution, in the virgin forest stand the Semenik P20, the tree distributions relative to their radial growth is of exponential descending type (Figure 4), with “J” shaped allure, where trees from the lower diameter classes record the highest radial growths.

The experimental radial growth distributions were adjusted using the theoretical frequency functions: Beta, Gamma 3P and Weibull 3P.

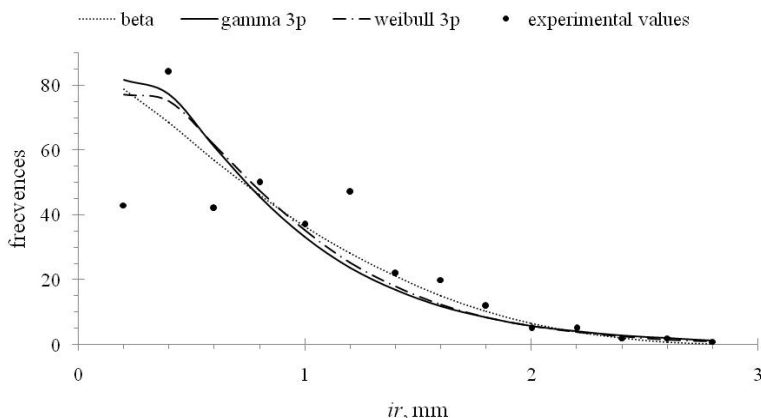


Figure 4. Tree distribution in relationship with their radial growth.

The goodness of fit was tested using χ^2 , anderson – darling (ad) și kolmogorov – smirnov (ks) tests (table 1).

Table1. Experimental values of specific goodness-of-fitness.

Distribution	Kolmogorov-Smirnov test (K-S)		Anderson-Darling Test (A-D)		χ^2 Criterion (χ^2)	
	experimental values	theoretical values	experimental values	theoretical values	experimental values	theoretical values
Beta	0,062	0,07	1,198	2,501	18,614	15,507
Gamma 3p	0,063	0,07	2,646	2,501	29,22	15,507
Weibull 3p	0,072	0,07	2,403	2,501	26,079	15,507

As the results of the komogorov – smirnov test show (table 1) one may observe that the theoretical distributions laws beta and gamma 3p are adjusting the semenic p20 virgin stand while the anderson – darlig test shows no significant differences between the experimental and theoretical values of the distributions beta and weibull 3p. In addition, the χ^2 criterion showed that no studied theoretical laws adjust the stand. These differences, between the experimental and the theoretical values, are both explained by the development stages of the stand and by the environmental factors (competition relationships) of the stand as well as natural factors (windfall, snowfall, drought, insects attack), showing once again that the primeval ecosystems have a higher structural complexity (pach and podlaski, 2015).

Dendrochronological aspects in the semenic p20 stand:

For the dendrochronological series 36 increment cores extracted with the pressler driller were used, this method being less destructive as compared to the tree cutting and sample analysis method. The graph representation of the dendrochronological series (figure 5) shows the stand evolution in time revealing the main life events as stages with high and low radial growth, influenced by

other factors such as competition relationships and multi-annual variation of the environmental conditions.

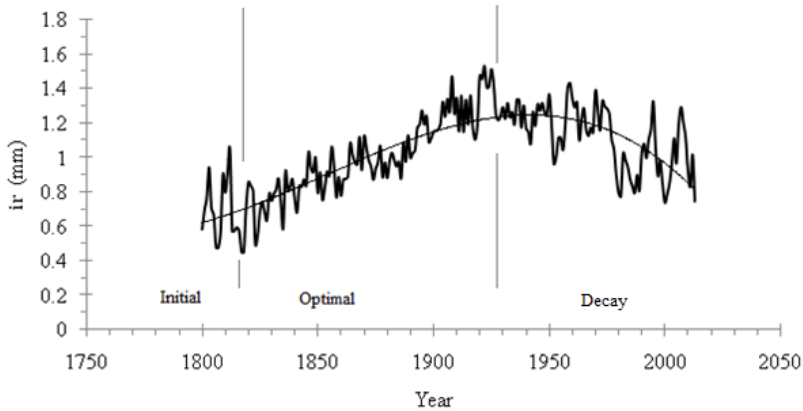


Figure 5. Standardization of polynomial model growth series.

The allure of the average radial growth curve is specific to the virgin forest stands, featuring a unimodal character, with an ascending growth until 1970 (initial and optimal development stages), followed by a decreasing trend of growth induced by the decay development stage, issue characteristic to the virgin forest stands (trotsiuk et al., 2012). Also, this decay can be explained by an increase in sulphur pollution in the 80s (castagneri et al., 2014). The average span is 361 years, time period covered is 1651-2013, mean growth is 0,999, with a standard deviation of 0,482 and mean sensitivity of 0,390 (table 2).

Table 2. Statistical parameters of the dendrocronological series (Semenic beech stand).

Parameters	STD	RES
Average length (years)	361	361
Period covered	1651-2013	1800-2013
Number of radial samples	36	36
Average radial growth	0,999	1,001
Standard deviation	0,482	0,404
Average sensitivity	0,390	0,454
First-degree autocorrelation	0,465	-0,004
Average R bar	0,068	0,034

First-degree autocorrelation is 0,465 and mean correlation coefficient is 0,068. Positive characteristic years are: 1811, 2006, 2012. Negative characteristic years are: 1805, 1836, 2010 (year characterized by drought). Further the growth is correlated with the climatic data such as temperatures and precipitations (figure 6), using cru ts3.22 climatic database for the time period 1901-2013 (ceda, 2014).

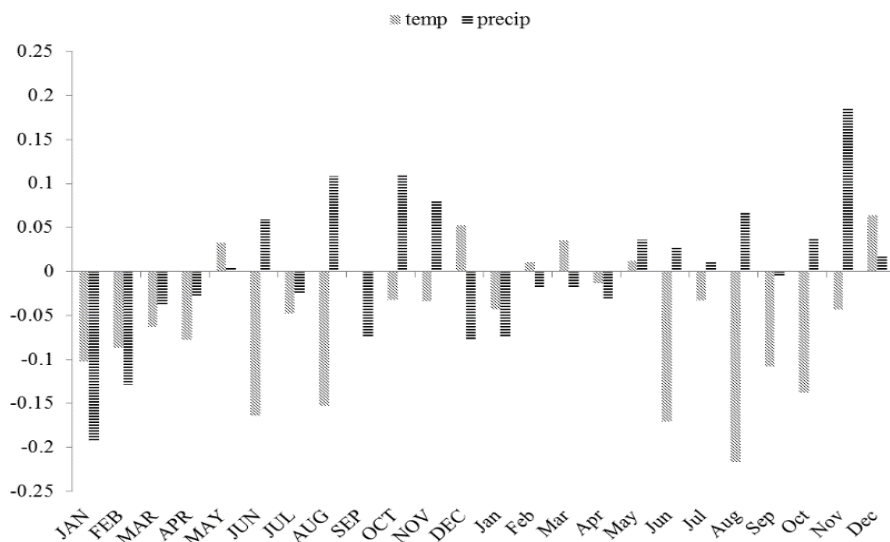


Fig. 6. Correlation between the radial tree growth and the temperatures, precipitations.

This correlation spanned 112 years and highlights the fact that the radial growth is more influenced by precipitations than by the temperature. Hence, it is demonstrated the high stability of virgin forests. Same results were found in a study conducted in Lom forest reserve in Bosnia Hertegovina (Castagneri et al., 2014).

Volume growth: To determine the volume growth of the stand, the first step was to determine the volume for each tree (v) and the growth volume percent (piv). Once the volume growth for each tree was determined, then by summing the values, the volume growth for the entire forest stand (Iv) results for the time period 2003 – 2013 ($63,32 \text{ m}^3 \cdot \text{year}^{-1} \cdot \text{ha}^{-1}$). The average of the annual growth is $6,33 \text{ m}^3 \cdot \text{year}^{-1} \cdot \text{ha}^{-1}$.

In comparison with the managed beech stands from the selection forests, where the volume growth is between $4,2$ and $6,7 \text{ m}^3 \cdot \text{year}^{-1} \cdot \text{ha}^{-1}$ (Guiman, 2007), the Semenik P20 virgin stand has the characteristics of a stand with high volume growth (even for the virgin forests). Despite the influences of the environmental and climatic factors (the stand is situated at 1532 m altitude, above the beech altitudinal limit, where the vegetation season is short and strong winds occur) as well as the age influence (there had been found trees aged over 200 years) on the volume growth, the Semenik P20 stand proves high eco-productive characteristics of virgin stands.

Thanks to their high adaptive and survival capacity in varied and heavy (even extreme) conditions, the virgin forests, which are complex ecosystems, are net superior to the managed forests, proving time and again the high stability they offer to the ecosystem to which they belong, regardless of the culture system.

CONCLUSIONS

Virgin forests represent a model for sustainability and a high efficient eco-productivity.

The graphical representation of tree radial and diameter growth variability displays the shape of a second order parabola, specific to uneven- age stand and by default, to virgin stands.

For the dendrochronological series 36 samples have been used and the graph representation shows the forest stand evolution in time. The length of this series has 361 years, first dated year being 1631. The average radial growth is 0.999 and standard deviation is 0.482. Also the first degree autocorrelation is 0.465 and average R bar is 0.068.

Climatic data as temperature and precipitations, for the time period 1901 – 2013, in correlation with radial growth show that the stand is not strongly influenced by precipitations and temperatures (with small exceptions generated by precipitations). This fact demonstrates the high stability of virgin forests;

The volume growth of Semenici P20 stand, for the period 2003 – 2013, is 63,32 m³ha⁻¹ and the annual growth is 6,33 m³ year⁻¹ ha⁻¹. In comparison with the managed beech stands from the selection forests, where the volume growth is between 4,2 and 6,7 m³ year⁻¹ ha⁻¹ the Semenici P20 virgin forest stand has the characteristics of a stand with high volume growth (even for the virgin forests);

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EMPIRICAL ANALYSIS OF INCOME CHANGES IMPACT ON FOOD CONSUMPTION EXPENDITURE

SUMMARY

This paper analyzes the impact of income and its changes on the structure of consumption by certain groups of products and in different areas in Montenegro, in the period from 2005 to 2013. The analysis of the available data on income and of its use for certain products shows how changes in income affect the structure of consumption of the population of Montenegro in the analyzed period. The results showed that the structure of food consumption population of Montenegro improved partially. Currently, the food consumption of urban residents, the share of basic food, such as meat and fruit decreases, while the percentage of nutrient foods, such as vegetables, milk, cheese and eggs is increasing. Predictably, food consumption structure of urban residents still has room for improvement.

Keywords: Empirical analysis, Engel's coefficient, consumption, per capita, income, urban residents.

INTRODUCTION

The interest in consumption patterns of households in developed and developing countries is not new and has been studied by a huge number of researchers. Some researchers differed not only in the period, scope, source and methodology but also in the finding and suggestion they forwarded to address the issue. Numerous studies used either the time series or cross section data reported in the Household Income and Expenditure Surveys (HIES). But, others were based on the area observations and memory based interviews from the residents. Irrespective of the methodology and source of data, the major question addressed by those studies was to estimate and test the validity of the relationship between income and expenditure on different commodities.

Wu at al. (1995) concluded that the results from their study provides rationale and basis for additional research into Chinese food consumption, especially processed food. Burney and Khan, (1991) while comparing the urban-rural consumption structures in Pakistan found that expenditure elasticity for different commodity groups vary with income and, in general, exhibit a cyclical pattern in terms of quantitative as well as qualitative changes in the households' consumption basket. Food consumption is a dynamic process and is greatly

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influenced by size and composition of household, number of earning hands, prices of food items, educational level, geographical, cultural and climatic conditions in the region, etc. So, economic change in the lower-income and transitional economies of the world appears to coincide with increasing, rapid social change (Guo, Xuguang, et al., 2000). Low-income countries spend a greater portion of their budget on food and are more responsive to income and food price changes than middle and high-income countries (Regmi, Anita, et al., 2001). For example, people in USA, Canada, and Netherlands spent 10.4, 13.7 and 14.4 percent of their income on food, respectively; however, in less developed countries like Sudan, India, Philippines, more than 50 percent of a household budget is spent on food commodities (Begum, Safia et al., 2010). Mmakola et al, (1997) conducted a study on food consumption patterns in South Africa and found that quality and price were both important considerations for consumer food purchases particularly for rural consumers. They also concluded that higher incomes people consumed more meat and could afford more fruit and ready-made foods. Jovanović et al, (1997, 1998, 2001, 2016) conducted that rural citizens were allocated the large part of income for food and beverages and urban citizens least.

Tozanli (1996) reported that low income population has a traditional Mediterranean consumption pattern, whereas wealthy dwellers were inclined to western behavior which had a negative effect on food industries in Turkey.

Since the 2006 Montenegro gained independence and continued with the reforms and open policy, the pace of economic growth has accelerated, the level of per capita income has improved and the average disposable income of urban residents has increased from 2,303 EUR to 4,806 EUR per capita during 2005-2013 year.

Income is the dominant variable in consumer behavior and income growth necessarily brings changes of consumption structure and consumption level. Therefore, studies on the relationship between income and food consumption structure have important guidance-giving significance for food industry policies making and speeding up the shift of the economic development model of our country. The food consumption structure in the study is such that it points to the proportion of the food expenditure such as bread, meat, milk, vegetables and fruit in the general food costs. Researches on the food consumption above show that income is an important factor in influencing food consumption structure of urban residents and most scholars focus researches on a region (country or provinces and cities, etc), lacking the comparison of areas. Numerous studies of the impact of income on food consumption were carried out in China on a significant number of samples (Dezhang, W. and W. Jialiang, 2010; Enhu, W. and L. Lutang, 2007., Jiang, L., 2010., Xinhua, G. and X. Ruihao, 2009., Zhen, Z. and L. Shuquan, 2011).

Therefore, the goal of the paper was to present the evolution characteristics of income and food consumption structure and compare the influence degree of

income on the food consumption structure in different regions- national, urban, rural and capital.

MATERIAL AND METHODS

The data were provided by the MONSTAT for Montenegro's income on private food consumption expenditure and food personal consumption by product groups in the period 2005-2013 (MONSTAT, 2014). For distribution of residents' disposable income we used data from Poverty & Equity Databank. World Bank, Development Research Group. Data are based on primary household survey data obtained from government statistical agencies and World Bank country departments. The methodology used in order to process the data was represented mainly by the index, share and comparison methods. The following indicators were used to characterize the influence of income on food consumption structure of urban residents: per capita income, standard deviation, variation, regression and Engel's coefficient.

RESULTS AND DISCUSSION

Analysis of the tendencies of income structure in Montenegro.

With the constant increase of per capita disposable income in Montenegro, per capita consumption expenditure level has gone up notably, as well as per capita food consumption level, composition of consumption and consumption concept have changed greatly, especially in the following aspects.

Food consumption level rose and Engel's coefficient declined:

Since 2005, the improvement of Montenegro's resident's income level has contributed to the growth of consumption level, especially food consumption level. In 2013, the per capita food spending is 4,366 EUR, or it is 2.11 times more than the expense 2,064 EUR in 2005. In 2013, the Engel's coefficient of Montenegro's households in Montenegro is 32.7, thus changing from well-off level to rich, as specified in Table 1².

Table 1: Per capita income, expenditure and Engel's coefficient unit: €%

Year	Per capita disposable income	Per capita consumption Expenditure	Per capita food expenditure	Engel's coefficient
2005	2,303	2,064	665	32.2
2006	3,056	2,701	930	34.4
2007	4,235	3,847	914	23.7
2008	5,037	4,562	1,213	26.5
2009	4,529	4,049	1,362	33.6
2010	4,658	4,118	1,392	33.8
2011	4,792	4,302	1,458	33.8
2012	4,738	4,241	1,405	33.1
2013	4,865	4,366	1,431	32.7

Source: MONSTAT, own calculation

² According to the United Nations Food and Agriculture Organization (FAO) proposed standards, Engel's coefficient more than 59% is poverty, 50-59% for food and clothing, 40-50% for a well-off, 30-40% for rich and less than 30 percent for the most affluent.

However, because income growth rate is faster than food spending, Engel's coefficient - food consumption expense accounts for the proportion of the total amount of personal consumption expenditure is still falling.

Food consumption expenditure structure upgrade: In longitudinal perspective, food consumption expenditure structure has shown a diversified trend and food spending has changed. On average, in eleven food expenditures, the proportion in the top five are: bread and cereals, meat, milk, cheese and eggs, vegetables and fruits. In lateral perspective, all kinds of food in the proportion of total expenditures have changed over time. In the reporting period, the most increased consumption is that of wine (40%), bread and cereals (14%) and oil and fats (9%), while it was reduced in meat (11%) and fruit (6%). The proportion of bread and cereals and wine rose from 15.17 and 2.16 in 2005 to 17.04 and 2.98 in 2013 respectively, which indicates that residents generally have not paid more attention to the balance of nutrition with increasing awareness of food safety and they have reduced the consumption of meat and fruit. The proportion of bread and cereals, fat and oils, meat, milk, cheese and eggs are basic stability in food consumption structure of urban residents, which shows that the influence of income on food consumption basic structure is unchanged and is still under the influence of the traditional way (Table 2).

Table 2: The changes of food personal consumption per capita by product groups in 2005-2013, EUR

Year	Bread and cereals	Meat	Fish	Milk, cheese and eggs	Oils and fats	Fruit	Vegetable	Other food prod	Wine	Beer
2005	105	192	20	156	22	46	68	31	15	22
2006	96	184	21	150	21	43	68	33	14	17
2007	105	214	25	161	23	44	82	33	16	24
2008	126	230	28	197	33	55	94	36	17	26
2009	107	214	22	181	22	47	87	34	16	24
2010	114	227	21	182	22	47	84	36	17	25
2011	120	203	22	164	26	44	81	35	18	24
2012	123	194	22	178	25	42	75	35	30	23
2013	120	171	21	158	24	43	73	33	21	23
2013/05	114	89	105	102	109	94	107	106	140	104

Source: MONSTAT, own calculation

The distribution of residents' disposable income.

Following the quinque section method, the residents are divided into five equal groups, as follows: the lowest income households (20%), lower-income households (20%), middle-income households (20%), higher income households (20%), and the highest income households (20%). We then calculate the income proportion for each group (Table 3). Thus we can analyse the distribution of residents' income.

Table 3: The major food consumption structure of urban residents in different income groups unit: €

Classifications	Average	Income share held by highest 20%	Income share held by the fourth 20%	Income share held by the third 20%	Income share held by the second 20%	Income share held by lowest 20%
Bread and cereals	112.88	43.5071	25.6076	19.5121	7.1892	9.5302
Meat	203.22	78.3267	46.1019	35.1280	26.5637	17.1575
Milk, cheese and eggs	169.66	65.3918	38.4885	29.3269	22.1769	14.3241
Fruit	45.66	17.5986	10.3582	7.8926	5.9684	3.8550
Vegetable	79.11	30.4912	17.9466	13.6747	10.3408	6.6791

Source: poverty & equity databank. World bank, development research group. Data are based on primary household survey data obtained from government statistical agencies and world bank country departments. Own calculation

The food consumption demands are far apart in different income groups of urban residents in montenegro: the income gap between urban residents will lead to the change of food consumption expenditure. From table 3 we can see that the food consumption structure in different income groups of urban residents is different. The 20% lowest income households are spending most on meat and milk, cheese and eggs, with the expenditure of 17.15 eur and 14.32 eur per capita, respectively. However, the 20% highest income households are spending most on meat and milk, cheese and eggs, with expenditure of 78.32 eur and 65.39 eur per capita, respectively. For example, in 2013, the milk, cheese and eggs expenditure of the supreme income households is 158 eur per capita; it is 11.03 times more than the lowest income households spending of 14.324 eur and bread and cereals is the 12.5 times of the lowest income households. To sum up, with the development of economy and urbanization, the “food revolution” of urban residents has quietly spread and engel’s coefficient has declined. The bread and cereals and vegetable consumption gradually rose, reversely, the consumption of meat and fruit gradually fell and milk, cheese and eggs and fish were basically unchanged. The food consumption of urban residents has moved from staple food towards nutritious and healthy food structure.

Table 4: the influence of income on the per capita on food consumption structure

	Montenegro	Urban area	Rural area	Podgorica
Bread and cereals	0.66276	0.45152	0.10291	0.30766
Meat	0.20702	0.49989	0.23659	0.47861
Milk, cheese and eggs	0.64671	0.77220	0.01370	0.79125
Fruit	0.25424	0.26183	0.00392	0.25661
Vegetable	0.61380	0.82599	0.03548	0.74295
Wine	0.19410	0.08201	0.07019	0.05412

Source: Own calculation

Empirical analysis on the influence of income on food consumption structure of montenegrin residents.

In order to further analyze the influence of income on food consumption structure and explain the changes of food consumption structure of residents in montenegro from time and space dimensions, the study will analyze the food consumption structure of different regions in 2005-2013. The data cover per capita disposable income and all kinds of food expenditure of all residents, urban residents, rural residents and residents in the capital of montenegro. In the analyzed model, per capita disposable income is the independent variable and the dependent variables are per capita total food spending, bread and cereals, meat, milk, cheese and eggs, fruit, vegetables and wine. Using excel software, the study makes regression analysis of the available data and all the regression results concerning meat, milk, cheese and eggs, fruit are significant at 1% significance level, indicating that income has very significant influence on food consumption structure. Table 4 shows the influence of income on the per capita food consumption structure of urban residents, rural residents and capital residents.

The minimum influence coefficient of three foods are meat, fruit and wine in 2005-13 year. The phenomenon above shows that the overall food consumption is declining with improvement of per capita income level of urban residents and people's living standard is improving, food consumption tend to convenient and nutrient food.

The influence coefficient of wine (urban area), milk, cheese and eggs and fruit (rural area) are minimal the coefficients being 0.19410, 0.01370 and 0.00392 respectively, meaning that urban and rural residents have stable expenditure for these three foods. In addition, the food consumption structure is different in the urban and rural area and podgorica. In urban area, the income demand elasticity coefficients of eleven foods rank first three are vegetable, milk, cheese and eggs and meat. In rural area, the food with higher influence degree of income on food consumption structure are meat, bread and cereals and vegetable. In podgorica, the main foods ranked as the first three are vegetable, milk, cheese and eggs and meat.

in 2005-2013, bread and cereals, milk, cheese and eggs and vegetable are still the main food for residents in montenegro, people consumption habits are basically stable, but the income demand elasticity has changed greatly in eastern, central and western regions.

Through comparison of food consumption structure in time and space dimensions, we can see that dining out plays an important role in food expenditure of urban residents and its influence coefficient has been growing, while the income demand elasticity of other food has reduced under the price system influence. Bread and cereals and vegetable have replaced meat and vegetables and become the main food spending in the montenegro. This phenomenon indicates that the influence of regional income disparity on food consumption structure in different regions is great. With the improvement of income level, the food consumption structure has changed for healthy, nutrient

food and residents have paid more attention to balanced staple and non-staple food, while food consumption of the midwest is changing from “eating good” to “eating nutritious”.

CONCLUSIONS

With the improvement of income level, the trend of Engel’s coefficient has continued to fall, which indicates that urban residents living standard has improved and income has significant influence on food consumption structure. Therefore, raising the income level of residents and enhancing the purchasing power of safe food are the effective measures to stimulate the economic development of Montenegro.

The structure of food consumption population of Montenegro was partially improved. Currently, the food consumption of urban residents, the share of basic food, such as meat and fruit decreases, while the percentage of nutrient foods, such as vegetables, milk, cheese and eggs is increasing. Predictably, food consumption structure of urban residents still has room for improvement. Therefore, the change of regime development of traditional food industry and industrial structure adjustment of the direction of development of the food industry is needed.

There is still certain difference in structure in different income groups and different regions in Montenegro. Therefore, the government should speed up the economic development in the rural area, improve the people’s income level and reduce the gap of food consumption structure between different regions and different income groups so as to improve the food quality.

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**ASSESSMENT AND PREDICTION OF VIABILITY AND METABOLIC
ACTIVITY OF *TILIA PLATYPHYLLOS* IN ARID STEPPE
CLIMATE OF UKRAINE**

ABSTRACT

Urban phytocenoses preserving under the warming climate becomes especially urgent problem in the arid areas, including the steppe zone of Ukraine. Expected elimination of the susceptible woody plants defines necessity of species composition enrichment by introduction. In order to estimate *Tilia platyphyllos* adaptive capacity, leaves growth and metabolic features were determined for both shaded and lighted trees grew at the plots polluted with transport exhausts in Dnipro city. Reducing leaf surface area by 29 – 60% compared to conventional control (the Botanical Garden) was associated with level of pollution and lighting as well. Leaf weight (per cm²) exceeded the control (4 – 25%) at the most contaminated plots, but diminished with increasing distance from the pollution source. Compared to control, stomata density increased in all leaves, especially at the most polluted and shaded plots (50% above control).

Total chlorophyll content was below the control value (maximum 16%) at almost all polluted plots, while Chl a/Chl b ratio exceeded the control. Redox state of glutathione reached the maximum in leaves at the Botanical Garden, decreasing notably at contaminated plots, whereas the total accumulation of glutathione was enhanced.

Activity of glutathione-S-transferase was the highest in the most lighted leaf even on contaminated plot, while was inhibited (11-32% below control) by pollutants in shaded leaves. Results showed variability of morphometric characteristics and metabolic properties of large-leaved linden, depending on local environmental conditions. Phenotypic plasticity of urban *T. platyphyllos* trees is quite capable play a key role in adaptation to climate change allowing survival of the species.

Keywords: large-leaved linden, climate change, adaptability, morphometric and metabolic traits

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

INTRODUCTION

In recent decades, many studies have been focused on the identification of climate change trends and prediction of the consequences of their effects on ecosystems. With regard to Europe, the basic climate shifts can be expressed as higher mean summer temperatures and prolonged summer drought periods (Scherrer et al., 2011), and lower summer precipitation as well (Frei et al., 2006). It was suggested that changes in rainfall, increasing temperatures and drought risks may affect plant species distribution and community composition (Fraser et al., 2008). More dramatic prognosis focuses on complication of woody plants survival (Talbi et al., 2015), and even on extinction of European forests populations with low ecological plasticity (Bussotti et al., 2015).

In steppe zone of Ukraine, the urgent need to preserve and expand planted woody communities is dictated by extremely small natural forests area. The role of urban trees can not be overemphasized in the industrial cities given the numerous environmental, social, economic, aesthetic, and health benefits (Gillner et al., 2015). However, tree community composition is limited in the phytocenoses of Dnipro city because of inappropriate climate and anthropogenic load. Herein planted wood communities were created involving both the indigenous and successfully introduced species. In particular, genus *Tilia* is currently represented by *T. cordata* Mill, which is an autochthonous species in arboreal flora of Steppe zone, and by introduced species *T. amurensis*, *T. platyphyllos* Scop. (large-leaved linden), *T. tomentosa* Moench, and *T. x europea* L. as well..

All species of *Tilia* genus are remarkable ornamental trees with a dense crown and broad leaf surface; linden trees are able to provide the aesthetic aspect and a comfortable microclimate in places of recreation of people. Moreover, *T. platyphyllos* is able to accumulate some heavy metals (Marković et al., 2013) and mercury (Kowalski et al., 2016). *T. platyphyllos* is widely planted throughout the temperate areas as an ornamental tree in parks and city streets. Large-leaved linden was characterized as a species with the moderate drought sensitivity (Scherrer et al., 2011) and rate of net photosynthesis (Gillner et al., 2015) in the urban conditions of Central Europe

The expected consequences of climate change can be particularly severe for tree species in arid regions (Talbi et al., 2015). In this context, the predictive assessment of viability of woody species in the face of increasing aridity is justified. According to Bussotti et al. (2015), useful tree genotypes should have features of adaptation to drought first of all, which can be reflected in variation at the morphological, physiological and phenological level. It was established, that plants have the thermal sensors to program metabolic level and provide acclimation to short-term fluctuations or adaptation to gradual temperature change (Bahuguna, Jagadish, 2015). Therefore, the study of intraspecific morphological and metabolic variations influenced by local adverse environmental conditions can indicate the possible ways of adapting of tree species. The objective of our study was to reveal variability of morphological and metabolic features of *T. platyphyllos* in order to assess the species adaptation

capacity to environment adverse impacts and to predict the probability of survival in the urban plant communities when climate changes.

MATERIAL AND METHODS

Study area

The study was conducted in urban phytocenoses of Dnipro city (steppe zone of Ukraine). Steppe climate is continental with sharp fluctuations in temperature, unstable moisture, and seasonal drought periods, which accompanied by high temperature and dry winds. The annual amount of evaporation exceeds precipitation by 2–3 times, because an average annual rainfall is 472 mm, while it could fall to 250 mm in dry years. In addition, the successful development of urban trees is complicated due to city environment pollution with the motor exhausts and industrial emissions.

Study sites were located in four planted woody plant communities, in which *T. platyphyllos* Scop. (large-leaved linden) was introduced 50 – 55 years ago (Figure 1).

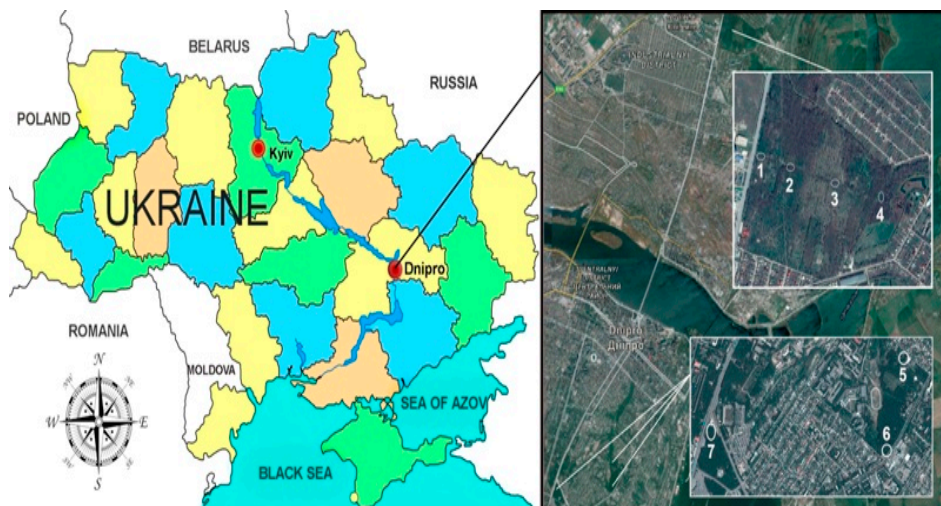


Figure 1: Study areas of the urban phytocenoses in city of Dnipro, Ukraine

Plot 1, plot 2, plot 3, and plot 4 were located at the territory of “Peoples’ Friendship Park” (48°32'04.22" – 48°31'56.13"N, 35°05'15.91" – 35°06'00.45"E), which lies on the north-eastern outskirts of Dnipro city, and occupies an area of 93.7 ha. Being created in the 60s of last century, the park was practically free of any human care in the last 20 years. Now, arboreal community is represented by 61 tree and shrub species with the dominance of *Quercus robur* L., *Acer platanoides* L., *Robinia pseudoacacia* L., *Ulmus minor* Mill., *Fraxinus excelsior* L., *Fraxinus pennsylvanica* Marsh., *Tilia platyphyllos* Scop., *Populus alba* L., *Gleditzia triacanthos* L., *Larix sibirica* Ledeb., *Acer pseudoplatanus* L., *Phellodendron amurense* Rupr. Herein, *T. platyphyllos* trees grew in the second

tier, hence were shaded. In addition, undergrowth (5 – 7 years old) was found around the adult large-leaved linden trees. One side of the park is adjacent to the highway with non-stop intercity heavy traffic of passenger and a truck transport. Four study sites were chosen at a distance of 20 m, 270 m, 660 m and 965 m from the highway (plot 1, plot 2, plot 3, and plot 4 respectively).

Plot 5. Botanical Garden of Dnipropetrovsk National University (48°26'14.09"N, 35°02'35.11"E) was considered as conventional control because of low pollution level. Herein, *T. platyphyllos* trees were planted as a mono-species community, and exposed to sunshine abundance.

Plot 6. It was a land along the roadside of Gagarin Avenue (48°25'49.75"N, 35°02'27.19"E), where *T. platyphyllos* solitary trees were subjected to chronic influence of the passenger cars exhausts and sunshine.

Plot 7 located at the square "20th anniversary of Victory Day" (48°25'53.92"N, 35°01'00.22"E). It has an area of about 2.0 ha, surrounded on all sides by trucks highways. Woody plant community is represented by *A. platanoides*, *R. pseudoacacia*, *F. excelsior*, *U. minor*, *T. platyphyllos*, *Betula pendula* Roth, *A. negundo*, *G. triacanthos*. Here, large-leaved linden trees grew in the second tier in the shade, and were exposed to chronic strong pollutant action.

The plots 1, 2 and 7 are under two factors impact: a) shading; b) strong pollution. The local environmental conditions for the rest plots are following: plot 3- a) shading; b) moderate pollution; plot 4 – a) shading; b) slight pollution; plot 5 (control) – a) light; b) low pollution; plot 6 – a) light; b) strong pollution.

Data collection

Data on the composition of the woody communities at the study plots were obtained during the period May-June 2016. The leaves of *T. platyphyllos* were collected into a clear dry weather in the mean of July 2016 from 5–7 same-age trees in each studied plot simultaneously. Leaves intended for morphometric measurements and counting of the stomata were placed in plastic bags, while the second part of the leaves was frozen immediately for biochemical analysis.

Data analysis

The measurement of stomata density was performed in accordance with Grant and Vatnick (2004) using a light microscope Carl Zeiss Jena, and the results were expressed as stomata number per mm² of leaf surface.

Chlorophyll content (Chl a, Chl b, and a total chlorophyll value) was determined according to Wintermans and De Mots method (1965) in the ethanol extracts of tree leaves. Results were expressed in mcg of chlorophyll per g fresh weight (mcg/g FW).

Glutathione-S-transferase activity was assessed with 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate according to method of Habig et al. (1974). The assay mixture, contained 0.1 M Tris buffer, pH 8.0, 100 µl of GSH, and 200 µl of sample, was incubated during 10 min at 30° C. The change of the optical density was detected at 340 nm during four minutes after addition of 100

μl CDNB, and the enzyme activity was expressed in nanoM CDNB/ sec \cdot g FW (nanokatal/g WW).

The reduced glutathione (GSH) content determination was based on spectrophotometric registration of reaction with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB, Ellman's reagent) in accordance with method of Anderson (1985) in modification. No-protein extracts were obtained by homogenization of 200 mg fresh leaves with 2.5 ml of 5% sulfosalicylic acid followed by centrifugation at 10,000 g for 10 min. Optical density of assay mixture (110 μl of 0.1 M K-phosphate buffer contained EDTA, pH 7.8, and 300 μl of sample) was detected at 412 nm 3 minutes after addition of 0.013 M Ellman's reagent. GSH content was calculated by using calibration graph, and expressed in nanoMol GSH/g FW. Total glutathione content was determined through the same procedure after reduction of oxidized glutathione in the non-protein extracts using zinc dust (20 mg/ml of sample) as described by Woodward and Fry (1932). Oxidized glutathione (GSSG) content was determined from the difference between total glutathione and GSH followed by halving, and result was expressed in nMol GSSG /g FW.

Catalase (CAT) activity evaluations according to Goth (1991) as well as guaiacol-peroxidase (GPOD) activity determination in accordance with Ranieri et al. (2001) were conducted as has been described before (Lykholat et al., 2016). All determinations of morphometric indexes and stomata number, as well as metabolites content and enzymes activity required three replicates. Data represent mean values and standard deviations (\pm SD). Significance of differences was estimated using Student's t-test ($P < 0.05$).

RESULTS AND DISCUSSION

Variability of morphometric indexes, stomata density, and chlorophyll content was revealed in leaves of *T. platyphyllos* depending on the local environmental conditions, as shown in Table 1.

Table 1. Effect of local environmental conditions on growth and chlorophyll content of *T. platyphyllos* leaves (Mean \pm SD)

Plot	Parameter, units				
	Leaf surface area, cm^2	Leaf FW/surface area, mg/cm^2	Stomata density, units per mm^2	Total chlorophyll, mg/g FW	Ratio Chl a/Chl b
1	40.9 \pm 11.0	1.16 \pm 0.09	452 \pm 18	3.25 \pm 0.14	4.00
2	41.5 \pm 10.8	0.84 \pm 0.21	442 \pm 9	3.87 \pm 0.14	3.10
3	44.3 \pm 12.9	0.83 \pm 0.09	393 \pm 12	3.82 \pm 0.12	3.80
4	55.7 \pm 14.5	0.68 \pm 0.12	337 \pm 14	3.47 \pm 0.14	4.20
5	100.9 \pm 21.1	0.92 \pm 0.23	302 \pm 14	3.87 \pm 0.13	3.65
6	72.1 \pm 13.7	0.96 \pm 0.31	440 \pm 9	3.85 \pm 0.15	3.68
7	69.5 \pm 12.1	0.81 \pm 0.11	395 \pm 11	3.45 \pm 0.13	4.21

Reaching its maximum at the conditional control, leaf surface area of *T. platyphyllos* was decreased both at most contaminated plot 1, plot 6 and plot 7 (60%, 29% and 31% below control, $P < 0.05$), and away from pollution in shaded leaves (45% and 56%, at plot 3 and plot 4 respectively, $P < 0.05$). Similar differences of leaf area were revealed by Gillner et al. (2015) in sun adapted and shaded leaves of different urban trees. In contrast, stomata density, being a minimal in control linden leaves, increased markedly (in range 12%–50%, $P < 0.05$) in both lighted and shaded leaves under pollutant influence. Results agree with Carins et al. (2013) data that leaf size plasticity can provide an efficient acclimation of stomatal conductance to contrasting evaporative conditions of sun and shade. In addition, the predominant stomatal density in relatively more polluted and lighted leaves are in accordance with opinion of Fraser et al. (2008) that highest stomatal density will be at high combined stress. In our study, rather high correlation ($r = -0.66$) between leaf surface area and stomata density pointed to coordinated physiological features change in linden leaves, depending on increase in light and pollution. This assumption is in one line with data about stomatal regulation as an efficient short term dynamic adaptation of *T. platyphyllos* to water stress (Breda et al., 2006). Control linden leaf thickness was exceeded due to strong pollution influence both in shaded and lighted leaves (25% and 11%, respectively at plot 1 and plot 6, $P < 0.05$). Results resonate with Sperlich et al. (2015) data about severe drought-induced increasing leaf mass per area. They can also be attributed to lignification processes under stressful conditions (Ranieri et al., 2001). High total chlorophyll content was found not only in control leaves of *T. platyphyllos*, but in shaded and lighted leaves at contaminated plots. The lowest chlorophyll levels (16% and 11% below control, $P < 0.05$) were found in shaded leaves on the most polluted plot 1 and plot 7, respectively. At the same time, Chl a/Chl b ratio exceeded the control in all linden leaves, excluding leaves at plot 2. The results obtained indicate intraspecific differences in the rate of photosynthesis in leaves of large-leaved linden, caused by local environmental conditions. The variability of the photosynthetic process allows its adaptation to water stress (Aranda et al., 2015), as well as seasonal acclimation to drought in sunlit and shaded leaves (Sperlich et al., 2015). Total glutathione content was higher in leaves of *T. platyphyllos* at the most polluted plot 1, plot 6, and plot 7 (respectively 7%, 15%, and 7% above control, $P < 0.05$) as shown in Figure 2 (a). However, pool of reduced glutathione was the highest in control leaves, falling in all other cases (4–8%). Control GSH/GSSG ratio (equal to 2.4) was also reduced at all contaminated plots, reaching a minimum (1.3) at plot 6. Varying the glutathione redox status in mature linden leaves means adaptation to local environmental conditions and reflects the adaptive capacity in general. GST activity was declined in shaded leaves at the polluted plots (in a range 11 – 32% below the control level), but not in lighted polluted leaves (Fig.2, b).

Glutathione-S-transferases are large family of enzymes, which catalyze the reaction of GSH conjugation with various xenobiotics providing their

detoxification (Edwards et al., 2000). Decrease in defense enzyme activity in *T. platyphyllos* leaves could be related to its inhibition due to excessive pollutant influence, just in the shaded leaves. Multilevel protection system provides that plants are able also to use non-enzymatic pathway detoxification of pollutants, including through direct conjugation with reduced glutathione (Noctor et al., 2002).

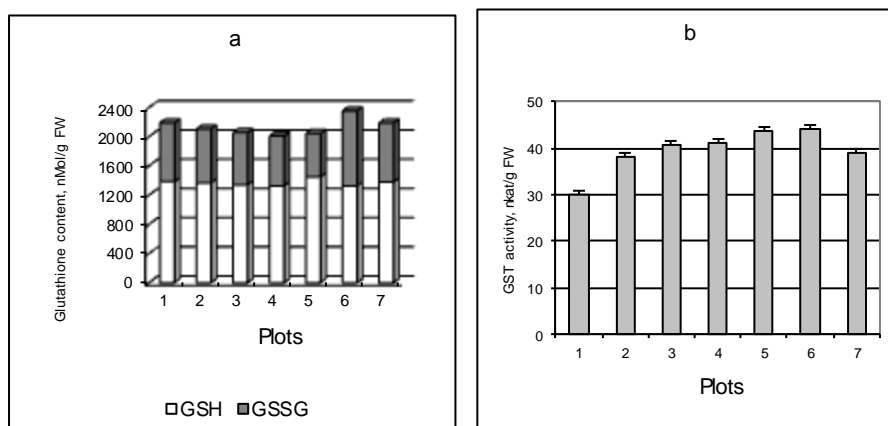


Figure 2: Effect of growth local conditions on *T. platyphyllos* leaves glutathione (GSH and GSSG, nanoMol/g FW) content (a) and activity (nano Mol/sec ·g FW) of glutathione-S-transferase (b).

It is quite possible that enhanced glutathione accumulation could play a compensatory role in toxicants neutralization in case of inhibiting the enzymatic pathway in shaded polluted linden leaves.

At the same time, glutathione-dependent system functioning in sunlit polluted leaves of *T. platyphyllos* requires more detailed study. In our study, activities of catalase and guaiacol-peroxidase were the highest in the lighted linden leaves at polluted plot 6 (Table 2).

Table 2. Effect of local environmental conditions on catalase and guaiacol-peroxidase activity of *T. platyphyllos* leaves (Mean \pm SD)

Plot	Factors	Enzyme, units	
		CAT, cMH ₂ O ₂ /sec·g FW	GPOD, mM/sec·g FW
1	Shading; strong pollution	9.55 \pm 0.9	40.21 \pm 2.6
2	Shading; strong pollution	7.94 \pm 0.7	33.07 \pm 1.4
3	Shading; moderate pollution	7.52 \pm 0.8	36.86 \pm 3.8
4	Shading; slight pollution	6.48 \pm 0.6	29.14 \pm 1.3
5	Light; low pollution	19.65 \pm 1.8	38.76 \pm 2.3
6	Light; strong pollution	20.78 \pm 1.7	53.33 \pm 2.8
7	Shading; strong pollution	4.85 \pm 0.5	10.64 \pm 1.1

Activity of CAT in shaded leaves of *T. platyphyllos* was significantly lowered relative to conditional control (in a range 51 – 67%) at all plots, especially at plot 7. The results obtained are consistent with previously reported (Lykholat et al., 2016) catalase activation in leaves of oak and ash for countering photorespiration due to temperature and light increasing. However, low level of catalase activity in the shaded linden leaves can not be unambiguously regarded as the evidence of insufficient active hydrogen peroxide eliminating. Difficulties in assessing the role of catalase appear because of complexity and redundancy of plant antioxidant system (Mhamdi et al., 2010). Anyway, intraspecific variation in catalase activity is undoubtedly an important element in *T. platyphyllos* adaptation capacity. Activity of guaiacol-peroxidase exceeded the control level in both shaded and sunlit linden leaves at polluted plot 1 (4% above control, unreliable difference) and plot 6 (38%, $P < 0.05$), while declined at all other plots. Results reflect the multiplicity peroxidase role in physiological processes since has been shown (Ranieri et al., 2001; Lee et al., 2007) that stress-induced peroxidase activation may be more related to enzyme involving in processes of lignifications than in protecting against oxidative stress. Taking into account the increase in linden leaf thickness founded at plot 1 and plot 6, we can assume that the increase in lignification is an adaptive response to pollution regardless of light level.

CONCLUSION

In the present study, large range of intraspecific variability of *T. platyphyllos* leaves morphological and metabolic traits induced by local environmental conditions was observed. Dissimilar effects of pollutant action have been identified in the sunlit and shaded linden leaves. Pollution-induced decrease in leaf area was more marked in the shaded leaves, whereas leaf thickness and stomata density were more declined in the lighted leaves at contaminated plots. Sunlit polluted leaves contain a greater total chlorophyll amount; whereas shaded leaves accumulated more Chl a. Lighted polluted leaves have the highest total glutathione pool together with lowest redox state of glutathione. Pollution-induced activation of glutathione-S-transferase, guaiacol-peroxidase and catalase was higher in the sunlit linden leaves. Comparative analysis of sunlit and shaded leaves features away from the pollution allowed predicting responses of *T. platyphyllos* to increase light intensity and temperature. Presumed adaptive changes may include increase in leaf area and leaf thickness. However decrease in stomata density, enhancing total chlorophyll content together with Chl b increasing; growth of glutathione redox state. Despite a slight increase in the total glutathione pool, moderate activation of peroxidase followed by increasing lignification; multiple increase in catalase activity. It can reduce the negative effect of photo respiration. Study results permit to conclude that the forecast for the survival of *T. platyphyllos* trees under climate change in steppe zone is more favorable than negative

ACKNOWLEDGEMENTS

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CALCULATION OF SOIL LOSS FROM THE S7-3 CATCHMENT OF THE SHIRINDAREH WATERSHED, IRAN USING THE RIVER BASIN MODEL

SUMMARY

Soil erosion is one of the major environmental problems in Iran. Factors such as soil erodibility, density of the river network of the river basin and its asymmetry, slope length and steepness, agricultural practices and the other physical-geographical characteristics, were surveyed and soil loss rate were calculated using an empirical River Basin Model (RBM). The objective of this research was to introduce a new method based on Erosion Potential Method (EPM) for the estimation of soil erosion on the catchment scale. Calculated peak discharge from the river basin was $35 \text{ m}^3 \text{ s}^{-1}$ for the incidence of 100 years and the net soil loss was $3182 \text{ m}^3 \text{ yr}^{-1}$, specific $164 \text{ m}^3 \text{ km}^2 \text{ yr}^{-1}$. Supplementary research is needed to address model limitations regarding the further development in relation to the GIS adaptations.

Key words: Soil erosion, River Basin Model, Sediment yield, Shirindareh watershed.

INTRODUCTION

Soil degradation caused by erosion, as one of the most important environmental problems in the world (Stoffel and Huggel, 2012) and sediment transport is not only the cause of an imbalance of natural rivers and streams, but also the cause of change in the river channel and sediment accumulation behind dams reducing their storage volumes (Sadeghi *et al.*, 2014).

Soil loss is a serious ecological concern in various environments worldwide (Kisic *et al.*, 2016; Ballesteros-Cánovas *et al.*, 2015; Ristic *et al.*, 2001, Curovic *et al.*, 1999). Sediments are responsible for transporting a significant fraction of nutrients and contaminants. Large suspended sediment fluxes in river catchments, which result from soil loss due to water erosion, constitute a major environmental issue (Louvat *et al.*, 2008), heavily affecting

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

sustainable land management in various environments (Ballesteros - Cánovas *et al.*, 2015; Stoffel *et al.*, 2013).

Study of soil erosion and sediment yield is one of the basic necessities to achieve integrated land management and soil and water conservation (Khaledi Darvishan *et al.*, 2014).

Direct measurements of erosion in the catchment are valid for a number of year's measurements of solid transport in the closing-section (Behzadfar *et al.*, 2014a and Behzadfar *et al.*, 2014b). The water and sediment sampling in given intervals need a lot of time and is costly (Khaledi Darvishan *et al.*, 2010) and the assessment of sediment yield using soil erosion models have been used more and more (Spalevic *et al.*, 2013a, 2013b, 2013c).

The modelling of the erosion process has progressed rapidly and a variety of models have been developed to predict both runoff and soil loss. We used the computer-graphic "River Basin" model (Spalevic, 2011; Spalevic *et al.*, 2000; Spalevic, 1999) for prediction of soil erosion intensity from the watershed area.

The objectives of this research were to quantify the sediment yield in the studied S7-3 Watershed of the Shirindareh River Basin testing the possibility of application of the River Basin model in the conditions of the Caspian Sea Watersheds.

MATERIAL AND METHODS

We calculated soil loss from the S7-3 Catchment (19 km²), of the Shirindareh Watershed, a tributary of the river Atrak (Caspian Sea Watershed), located in the north eastern parts of the mountainous area of Iran (Figure 1).



Figure 1. Study area of the S7-3 Catchment of the Shirindareh River Basin

Morphometric methods were used to determine the slope, the specific lengths, the exposition and form of the slopes, the depth of the erosion base and the density of erosion rills. Google Earth and Google Maps were used for further studying of the morphology of the features.

We used available data on Soils and of Geology of North Khorasan province, based on the research of the National Geological Survey Organization (Bolourchi, 1987).

Climatological data were received from the meteorological stations located in North Khorasan province of Iran. We analysed torrential rains, annual air temperatures, and average annual precipitations.

Directly observing of large-scale hydrological processes is difficult. Modelling has become a key research tool at the basin scale studies (Fu *et al.*, 2011).

The “River Basin²” physically-based model (Spalevic *et al.*, 2000) as a computer-graphic catchment-scale hydrological model, with the Erosion Potential Method – EPM (Gavrilovic, 1972) rooted in the procedure of this model, was used for soil loss calculation from the studied watershed.

According to the method sediment yield is calculated using the following calculation:

$$W_{yr} = T \cdot H_{yr} \cdot \pi \cdot \sqrt{Z^3} \cdot F$$

where W_{yr} is the annual erosion in m^3yr^{-1} ; T, the temperature coefficient; H_{yr} , the average yearly precipitation in mm; Z, the erosion coefficient.

The erosion coefficient, Z, was calculated as follows:

$$Z = Y \cdot X \cdot (\phi + \sqrt{I})$$

where, Y is Soil erodibility coefficient; X is Soil protection coefficient; ϕ is Erosion development coefficient (tables for Y, X and ϕ coefficients available at Gavrilovic, 1972). F is the watershed area in km^2 .

The actual sediment yield was calculated as follows:

$$G_{yr} = W_{yr} \cdot R_u$$

where, G_{yr} is the sediment yield in m^3yr^{-1} ; W_{yr} , the total annual erosion in m^3yr^{-1} ; R_u is sediment delivery ratio.

The actual sediment yield was calculated as follows:

$$R_u = \frac{(\sqrt{O \cdot D})}{0.2 \cdot (L + 10)}$$

where, O is perimeter of the watershed in km; D is the average difference of elevation of the watershed in km; L is length of the catchment in km.

RESULTS AND DISCUSSION

The climate of the studied area is continental, with the absolute maximum temperature of 34.6°C and the negative of -24.4°C , respectively. Average annual air temperature, t_0 , is 11.8°C and the Temperature coefficient of the region, T, is calculated on 1.13; The amount of torrential rain, hb, on 33.53 mm. The average annual precipitation, H_{yr} , is 303.2 mm (Source: Data from the North Khorasan Meteorological stations of Iran).

The coefficient of the river basin planning is calculated on 0.7. The coefficient of the vegetation cover is calculated on 0.8.

(A)symmetry coefficient indicates that there is a possibility for large flood waves to appear in the river basin. The value of G coefficient of 1.85 indicates

² Link to the “River Basin” exe file: www.agricultforest.ac.me/Spalevic/River

there is high density of the hydrographic network. The value of 23.51% indicates that in the river basin prevail steep slopes.

According to the erosion type, it is mixed erosion. Surface erosion is the most pronounced on the steep slopes without vegetation cover. In the studied river basin some problems of overgrazing and livestock traces are recorded also.

Calculation of Sediment yield of the S7-3 Watershed of the Shirindareh River Basin of Iran is presented at the "River Basin" Report 1.

Report 1. The "River Basin" report for the S7-3 Watershed

Inputs: River basin area, **F**, 19.33 km²; The length of the watershed, **O**, 24.76, km; Natural length of the main watercourse, **Lv**, 12 km; The shortest distance between the fountainhead and mouth, **Lm**, 10.96 km; The total length of the main watercourse with tributaries of I and II class, **ΣL**, 35.67 km; The area of the bigger river basin part, **Fv**, 10.58 km²; The area of the smaller river basin part, **Fm**, 8.75 km²; Altitude of the first contour line, **h0**, 1200 m; The lowest river basin elevation, **Hmin**, 1119 m; The highest river basin elevation, **Hmax**, 1758 m; A part of the river basin consisted of a very permeable products from rocks (limestone, sand, gravel), **fp**, 0.08; A part of the river basin area consisted of medium permeable rocks (slates, marls, brownstone), **fpp**, 0.25; A part of the river basin consisted of poor water permeability rocks (heavy clay, compact eruptive), **fo**, 0.67; A part of the river basin under forests, **fs**, 0; A part of the river basin under grass, meadows, pastures and orchards, **ft**, 1; A part of the river basin under bare land, plough-land and ground without grass vegetation, **fg**, 0; The volume of the torrent rain, **hb**, 33.53 mm; Incidence, **Up**, 100 years; Average annual air temperature, **t0**, 11.8 °C; Average annual precipitation, **Hyr**, 303.2 mm; Types of soil products and related types, **Y**, 1.1; River basin planning, coefficient of the river basin planning, **Xa**, 0.7; Numeral equivalents of visible and clearly exposed erosion process, **φ**, 0.64.

Results: Coefficient of the river basin form, **A**, 0.4; Coefficient of the watershed development, **m**, 0.77; Average river basin width, **B**, 2.41, km; (A)symmetry of the river basin, **a**, 0.19; Density of the river network of the basin, **G**, 1.85; Coefficient of the river basin tortuousness, **K**, 1.09; Average river basin altitude, **Hsr**, 1316.28 m; Average elevation difference of the river basin, **D**, 197.28 m; Average river basin decline, **I_{sr}**, 23.51%; Coefficient of the region's permeability, **S₁**, 0.88; Coefficient of the vegetation cover, **S₂**, 0.8; Maximal outflow from the river basin, **Q_{max}**, 34 m³s⁻¹; Production of erosion material in the river basin, **W_{yr}**, 15842 m³ yr⁻¹; Coefficient of the deposit retention, **Ru**, 0.201; Real soil losses, **G_{yr}**, 3182, m³ yr⁻¹; Real soil losses per km², 164 m³ yr⁻¹ km⁻².

This approach is also in use: Bosnia and Herzegovina, Brazil, Bulgaria, Croatia, Czech Republic, Italy, Macedonia, Montenegro, Morocco, Saudi Arabia, Serbia, South Africa and Slovenia (Al-Turki et al., 2015; Gazdic et al., 2015; Spalevic et al., 2015a, 2015b, 2015c, 2015d, 2015e, 2015f, 2015g, 2015h, 2015i, 2015k; Vujacic & Spalevic, 2016; Kostadinov et al., 2014; Spalevic et al., 2014a, 2014b, 2014c). The provided methodology have been successfully used in Iran in the regions of Chamgardalan, Kasilan, Kermanshah, Razavi Khorasan (Spalevic et al., 2016; Draganic et al., 2015a; Draganic et al., 2015b; Behzadfar et al., 2015; Barovic & Spalevic, 2015; Sadeghi, 2005) and other regions.

CONCLUSIONS

Based on the calculation of sediment yield it can be revealed that:

- Production of erosion material in the river basin, W_{yr} , was $15842 \text{ m}^3 \text{ yr}^{-1}$;
- Calculated soil losses are $3182 \text{ m}^3 \text{ yr}^{-1}$ and specific soil losses were $164.66 \text{ m}^3 \text{ yr}^{-1} \text{ km}^{-2}$;
- The peak discharge was calculated on $34 \text{ m}^3 \text{ s}^{-1}$ (incidence 100 yr).

This study confirmed the findings of Barovic *et al.* (2015); Behzadfar *et al.*, 2015, Zia Abadi & Ahmadi (2011); as well as Amiri (2010) in possibility of implementing the “River Basin Model” for the other river basins similar to the the Shirindareh Watershed of Iran, when hydrological stations are missing. The model is a good tool for rapid assessment of erosion risk to support decision-making and policy development.

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*Halil BOLU*¹

**SOUTHEASTERN ANATOLIA REGION INSECT FAUNA I
(COLEOPTERA II: CURCULIONOIDEA, TENEBRIONOIDEA)
OF TURKEY**

SUMMARY

The aim of this study was to determine Insect fauna Southeastern Anatolia Region. Surveys on insect species in various ecologies have been conducted in the provinces (Adıyaman, Batman, Gaziantep, Diyarbakır, Mardin, Siirt, Şanlıurfa, Şırnak) of Southeastern Anatolia region between the years 1948-2013. Almost 2600 species and subspecies among 180 families belonging to 13 insect orders are defined owing to these studies. Coleoptera species formed about 20% of the collected insects. Coleoptera included 32 families were recorded. During this study totally 248 species were found in 9 families and in 2 superfamilies of Coleoptera. Those superfamilies are Curculionoidea and Tenebrionoidea. The distribution of determined insect types according to the provinces, plant hosting and feeding type is also done. Information about their identification, host plants, and distribution in Southeastern Anatolia Region was presented as detailed.

Keywords: Insect Fauna, Coleoptera, Curculionoidea, Tenebrionoidea, Turkey.

INTRODUCTION

Insects (Insecta) are the most numerous group of animals in the world, with over one million species that have been described (Price, 1997). Insects are difficult to study because they represent the most species-rich, yet one of the least known, of all taxa of living organisms, a problem that is compounded by a dearth of skilled entomologists. Although the number of described insect species is uncertain due to synonyms and the lack of a global list, most authorities recognize 900 000–1 000 000 named morpho-species, representing 56% of all species known on Earth (Groombridge, 1992; Anonymous, 2003). Sensible estimates of the number of insects yet to be discovered range from another 1 million to 30 million species (Erwin, 1982; 1991), although most predict around 2–8 million more species (May, 1990; Gaston, 1991; Stork, 1997; Ødegaard, 2000).

Turkey in fact seems to be like a small continent in terms of biological diversity. Despite the Anatolia is not a continent alone, it contains all properties of a continent that should have an ecosystem and habitat. Each of seven geographical regions in Turkey has a distinguishable climate, flora and fauna.

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

The aim of this study is to make contribution to some faunistical records of Coleoptera of the Southeastern Anatolia Region of Turkey.

MATERIAL AND METHODS

Southeastern Anatolia Region (Adıyaman, Batman, Gaziantep, Diyarbakır, Mardin, Siirt, Şanlıurfa, Şırnak) entomology studies on insect species in different ecological provinces were made between the years 1948-2013 (Fig. 1).

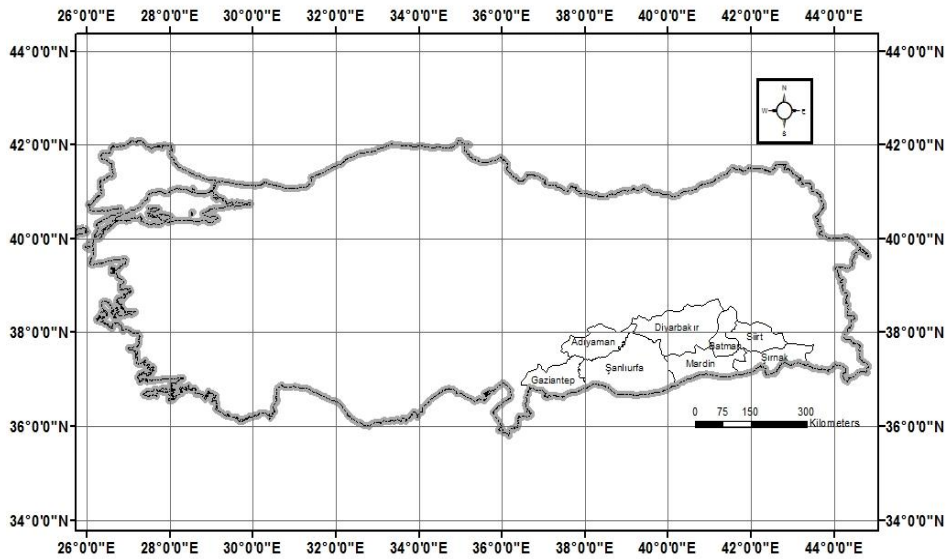


Figure 1. Sampling localities in Southeastern Anatolia Region of Turkey

In this study, the inventory has reached the major advantage of the waterways:

- Currently in Turkey, published or unpublished entomology journals related to scanning,
- Giving more weight to faunistic studies, and in the meantime, the insect fauna of our country foreign scientific journals that publishes articles about scanning,
- Faculty of Agriculture, Faculty of Science and Regional Plant Protection.

RESULTS AND DISCUSSION

Surveys on insect species in various ecologies have been conducted in the provinces (Adıyaman, Batman, Gaziantep, Diyarbakır, Mardin, Siirt, Şanlıurfa, Şırnak) of Southeastern Anatolia region between the years 1948-2013. Almost 2600 species and subspecies almost 180 families belonging to 13 insect orders are defined owing to these studies. During this study totally 248 species were found in 9 families and in 2 superfamilies of Coleoptera. Those superfamilies are Curculionoidea and Tenebrionoidea. The number and percentages of the species of the families are given on table 1.

Table 1. Number of species of coleoptera on southeastern anatolia region

Superfamily	Family	Number Species	%
Curculionoidea	Curculionidae	171	68,95
	Brentidae	20	8,06
	Rhynchitidae	10	4,03
	Scolytidae	6	2,42
	Attelabidae	2	0,81
Tenebrionoidea	Meloidae	26	10,48
	Tenebrionidae	10	4,03
	Anthicidae	2	0,81
	Mycetophagidae	1	0,40
Total	9	248	100,00

The distribution of insect species in the Southeastern Anatolia Region as a result of studies carried out in different provinces ecology, information on the host status and feeding has been given below.

Order Coleoptera (beetles)
Superfamily Curculionoidea
Family Rhynchitidae

Coenorrhinus aequatus (Linnaeus, 1767) Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Prunus avium* L.

Epihynchites smyrnensis (Desbrocher des Loges, 1869) Distribution: Adıyaman, Diyarbakır, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L.

Involvulus hungaricus (Herbst, 1784) Distribution: Adıyaman, Mardin Host plant: Weeds

Rhynchites auratus (Scopoli, 1763) Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Prunus avium* L.

Rhynchites aequatus Linnaeus, 1767 Distribution: Mardin Host plant: *Prunus avium* L.

Rhynchites hajastanicus Ter-Minassian, 1965 Distribution: Adıyaman Host plant: Weeds

Rhynchites purpureipennis Voss, 1973 Distribution: Gaziantep Host plant: Weeds

Rhynchites smyrnensis Desbrochers, 1869 Distribution: Adıyaman, Diyarbakır, Mardin, Siirt, Şanlıurfa, Host plant: *Amygdalus communis* L.

Rhynchaenus subfarinus (Klima, 1935) Distribution: Gaziantep Host plant: Weeds

Tatianaerhynchites aequatus (Linnaeus, 1767) Distribution: Adıyaman, Diyarbakır, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L.

Family Brentidae

Alocentron curvirostre (Gyllenhal, 1833) Distribution: Diyarbakır Host plant: *Amygdalus communis* L.

Aspidapion radiolus (Marsham, 1802) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Catapion pubescens (Kirby, 1811) Distribution: Diyarbakır Host plant: *Amygdalus communis* L.

Catapion burdigalense (Wencker, 1859) Distribution: Mardin Host plant: *Amygdalus communis* L.

Ceratapion basicorne (Illiger, 1807) Distribution: Diyarbakır Host plant: *Amygdalus communis* L.

Ceratapion beckeri (Desbrochers, 1875) Distribution: Mardin Host plant: *Amygdalus communis* L.

Ceratapion carduorum (Kirby, 1808) Distribution: Diyarbakır Host plant: *Amygdalus communis* L.

Ceratapion fremuthi (Wanat, 1995) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Ceratapion gibbifrons (Hustache, 1932) Distribution: Diyarbakır Host plant: *Amygdalus communis* L.

Eutrichapion sp. pr. punctigerum (Paykull, 1792) Distribution: Diyarbakır, Host plant: *Amygdalus communis* L.

Malvapion malvae (Fabricius, 1775) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Oxystoma ochropus (Germar, 1818) Distribution: Mardin Host plant: *Amygdalus communis* L.

Protapion trifolii (Linnaeus, 1768) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Protapion truquii (Reiche et De Sauley, 1858) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Protapion varipes (Germar, 1817) Distribution: Mardin Host plant: *Amygdalus communis* L.

Rhopalapion longirostre (Olivier, 1807) Distribution: Diyarbakır Host plant: *Amygdalus communis* L.

Squamapion atomarium (Kirby 1808) Distribution: Mardin Host plant: *Amygdalus communis* L.

Squamapion elongatum (Germar, 1817) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Squamapion phocopus (Eppelsheim, 1888) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Squamapion vicinum (Kirby, 1808) Distribution: Mardin Host plant: *Amygdalus communis* L.

Family Attelabidae

Coenorrhinus aequatus (Linnaeus, 1767) Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak, Host plant: *Amygdalus communis* L., *Prunus avium* L.

Lasiorrhynchites praeustus (Voss, 1933) Distribution: Gaziantep Host plant: Weeds

Family Curculionidae

Alocentron curvirostre (Gyllenhal, 1833) Distribution: Southeastern Anatolia Region Host plant: Weeds

Anthonomus amygdali Hustache, 1930 Distribution: Adıyaman, Diyarbakır, Gaziantep, Mardin, Şanlıurfa Host plant: *Amygdalus communis* L.

Anthonomus baudueri Desbrochers, 1875 Distribution: Diyarbakır, Gaziantep Host plant: *Amygdalus communis* L., *Prunus persica* (L.)

Anthonomus bituberculatus Thomson, 1868 Distribution: Diyarbakır, Mardin, Host plant: *Amygdalus communis* L.

Anthonomus brunnipennis Curtis, 1840 Distribution: Diyarbakır, Mardin, Host plant: *Amygdalus communis* L.

Anthonomus rubripes Gyllenhal, 1835 Distribution: Adıyaman, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L.

Anthonomus variabilis (Hoffman, 1963) Distribution: Diyarbakır, Mardin, Host plant: *Amygdalus communis* L.

Apion aeneum Fabricius, 1775 Distribution: Diyarbakır Host plant: Winter quarters

Apion aestimatum Faust, 1890 Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L., *Avena sativa* L., *Medicago sativa* L., *Onobrychis sativa* Lam., *Vicia sativa* L.

Apion arrogans (Wencker, 1858) Distribution: Adıyaman, Diyarbakır, Mardin, Şanlıurfa Host plant: *Zea mays* L., Winter quarters

Apion apricans Herbst, 1797 Distribution: Diyarbakır Host plant: Winter quarters

Apion assimile Kirby, 1808 Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L., *Avena sativa* L., Winter quarters

Apion beckeri Desbrochers, 1875 Distribution: Adıyaman, Diyarbakır Host plant: *Medicago sativa* L., *Onobrychis sativa* Lam., *Vicia sativa* L.

Apion brenskei Desbrochers, 1895 Distribution: Adıyaman Host plant: *Medicago sativa* L., *Onobrychis sativa* Lam., *Vicia sativa* L.

Apion carduorum Kirby, 1808 Distribution: Diyarbakır Host plant: Winter quarters

Apion dissimile Germar, 1817 Distribution: Diyarbakır Host plant: Winter quarters

Apion jordanicum (Voss, 1964) Distribution: Adıyaman, Host plant: *Medicago sativa* L., *Onobrychis sativa* Lam., *Vicia sativa* L.

Apion longirostre Olivier, 1807 Distribution: Gaziantep Host plant: Weeds

Apion loti Kirby, 1808 Distribution: Gaziantep Host plant: Weeds

Apion marchicum Herbst, 1797 Distribution: Gaziantep Host plant: Weeds

Apion neorelictum (Bajtenov & Lodos, 1977) Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Amygdalus communis* L., *Medicago sativa* L.

Apion (Kalcaption) pallipes Kirby, 1808 Distribution: Diyarbakır, Mardin, Host plant: *Amygdalus communis* L.

Apion persicum (Desbrochers, 1893) Distribution: Adıyaman Host plant: *Medicago sativa* L., *Onobrychis sativa* Lam., *Vicia sativa* L.

Apion scalptum Mulsant & Rey, 1858 Distribution: Adıyaman Host plant: *Medicago sativa* L., *Onobrychis sativa* Lam., *Vicia sativa* L.

Apion seniculus Kirby, 1808 Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Amygdalus communis* L., *Avena sativa* L.

Apion urticarium Herbst, 1784 Distribution: Gaziantep Host plant: Weeds

Apion tenue Kirby, 1808 Distribution: Gaziantep Host plant: Weeds

Apion transversum Bajt. et Lodos (1977) Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Amygdalus communis* L., *Avena sativa* L., *Medicago sativa* L., *Onobrychis sativa* Lam., *Vicia sativa* L.

Apion (Protapion) trifolii (Linnaeus, 1768) Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Amygdalus communis* L., *Avena sativa* L., *Medicago sativa* L., *Onobrychis sativa* Lam., *Vicia sativa* L.

Apion (Protapion) truguii Reiche, 1857 Distribution: Gaziantep Host plant: Weeds

Aspidapion radiolus (Marshall, 1802) Distribution: Southeastern Anatolia Region Host plant: Weeds

Bangasternus planifrons (Brulle, 1882) Distribution: Gaziantep Host plant: Weeds

Bangasternus orientalis (Capiomont, 1873) Distribution: Diyarbakır, Gaziantep, Mardin Host plant: Weeds

Calosirus ovulum (Schultze, 1897) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Corimalia pallida (Olivier, 1807) Distribution: Gaziantep Host plant: *Tamarix* sp.

Catapion burdigalense (Wencker, 1859) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Catapion pubescens (Kirby 1811) Distribution: Southeastern Anatolia Region Host plant: Weeds

Ceratapion basicorne (Illiger, 1807) Distribution: Southeastern Anatolia Region Host plant: Weeds

Ceratapion beckeri (Desbrochers, 1875) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Ceratapion carduorum (Kirby, 1808) Distribution: Southeastern Anatolia Region Host plant: Weeds

Ceratapion fremuthi (Wanat, 1995) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Ceratapion gibbifrons (Hustache, 1932) Distribution: Southeastern Anatolia Region Host plant: Weeds

Ceutorhynchus peyerimhoffi Hustache, 1916 Distribution: Şanlıurfa Host plant: Weeds

Ceutorhynchus abbreviatus Redtenbacher, 1849 Distribution: Gaziantep Host plant: Weeds

Ceutorhynchus trimaculatus (Fabricius, 1775) Distribution: Diyarbakır Host plant: Winter quarters

Ceutorhynchus angustus Dieckmann & Smreczynski, 1972 Distribution: Diyarbakır Host plant: Winter quarters

Ceuthorrhynchus caucasicus Kirsch, 1877 Distribution: Diyarbakır Host plant: Winter quarters

Ceuthorrhynchus deplanatus Schultze, 1901 Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L., Winter quarters

Ceuthorrhynchus sp. nr. *suturalis* Fabricius, 1775 Distribution: Diyarbakır Host plant: Winter quarters

Ceutorhynchus trimaculatus (Fabricius, 1775) Distribution: Southeastern Anatolia Region Host plant: Weeds

Ceutorhynchus carinatus Gyllenhal, 1837 Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Ceutorhynchus contractus (Marsham, 1802) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Ceutorhynchus erysemi Olivier, 1790 Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L., *Prunus avium* L.

Ceutorhynchus sinapicola Dieckmann, 1975 Distribution: Diyarbakır Host plant: *Amygdalus communis* L.

Ceutorhynchus sophiae (Gyllenhal, 1837) Distribution: Diyarbakır Host plant: *Amygdalus communis* L.

Ceutorhynchus subpilosus C.N.F. Brisout de Barneville, 1869 Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Ceutorhynchus sulcicollis (Paykull 1800) Distribution: Diyarbakır, Gaziantep Host plant: Winter quarters

Cionus merkli Stierlin, 1882 Distribution: Gaziantep Host plant: Weeds

Cionus olivieri Rosenschold, 1838 Distribution: Gaziantep Host plant: Weeds

Chlorophorus varius (Müller, 1766) Distribution: Southeastern Anatolia Region Host plant: Weeds

Coeliodes rubricus Gyllenhal, 1837 Distribution: Adıyaman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Pistacia vera* L.

Coenorrhinus aequatus Voss, 1933 Distribution: Adıyaman, Diyarbakır, Mardin, Siirt, Şanlıurfa, Host plant: *Amygdalus communis* L.

Coniatus splendidulus Fabricius, 1781 Distribution: Gaziantep Host plant: *Tamarix* sp.

Conioeleonus nigrosuturatus (Goeze, 1777) Distribution: Gaziantep Host plant: Weeds

Curculio nucum Linnaeus, 1758 Distribution: Mardin Host plant: *Prunus avium* L.

Epirhynchites smyrnensis (Desbrochers des Loges, 1869) Distribution: Diyarbakır, Mardin, Host plant: *Amygdalus communis* L.

Eptacus arachnoides (Stierlin, 1861) Distribution: Diyarbakır Host plant: Winter quarters

Eusomus mniszechi Hochhuth, 1851 Distribution: Gaziantep Host plant: Weeds

Eusomus ovulum Germar, 1824 Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Amygdalus communis* L., *Avena sativa* L., *Medicago sativa* L., *Onobrychis sativa* Lam., *Vicia sativa* L.

Eutrichapion sp. pr. *punctigerum* (Paykull, 1792) Distribution: Southeastern Anatolia Region Host plant: Weeds

Gymnetron asellus Schoenherr, 1838 Distribution: Gaziantep Host plant: Weeds

Gymnetron germari Faust, 1889 Distribution: Gaziantep Host plant: Weeds

Gymnetron labile Herbst, 1795 Distribution: Gaziantep Host plant: Weeds

Gymnetron tetrum (Fabricius, 1792) Distribution: Gaziantep Host plant: Weeds

Gymnetron vittipenne Marseul, 1876 Distribution: Gaziantep Host plant: Weeds

Holotrichapion pisi (Fabricius, 1801) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Hypera farinosa (Boheman, 1842) Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Amygdalus communis* L., *Avena sativa* L., *Medicago sativa* L., *Prunus avium* L., *Onobrychis sativa* Lam., *Vicia sativa* L.

Hypera meles (Fabricius, 1793) Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Amygdalus communis* L., *Avena sativa* L.

Hypera nigrirostris (Fabricius, 1775) Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Amygdalus communis* L., *Avena sativa* L., Winter quarters

Hypera jucundus Capiomont, 1868 Distribution: Diyarbakır Host plant: Winter quarters

Hypera postica (Gyllenhal, 1813) Distribution: Diyarbakır, Gaziantep Host plant: *Amygdalus communis* L., Winter quarters

Hypera trilineata Marsham, 1802 Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Amygdalus communis* L., *Avena sativa* L.

Hypera variabilis (Herbst, 1795) Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Amygdalus communis* L., *Avena sativa* L., *Medicago sativa* L., *Onobrychis sativa* Lam., *Prunus avium* L., *Vicia sativa* L.

Hypera zoilus Scopoli, 1763 Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Amygdalus communis* L., *Avena sativa* L., *Medicago sativa* L., *Onobrychis sativa* Lam., *Vicia sativa* L.

Larinus brenskei Faust, 1900 Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Larinus curtus Hochhuth, 1851 Distribution: Gaziantep Host plant: Weeds

Larinus flavescens Germar, 1824 Distribution: Gaziantep Host plant: Weeds

Larinus jaceae (Fabricius, 1775) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Larinus latus (Herbst, 1784) Distribution: Diyarbakır Host plant: *Gundelia tournefortii* L.

Larinus lucidirostris Penecke, 1936 Distribution: Gaziantep Host plant: *Onopordum* sp.

Larinus minutus Gyllenhal, 1836 Distribution: Gaziantep Host plant: *Cirsium* sp., *Onopordum* sp., *Vitis vinifera* L.

Larinus sp.pr.nanus Lucas, 1846 Distribution: Southeastern Anatolia Region Host plant: Weeds

Larinus onopordi (Fabricius, 1787) Distribution: Gaziantep Host plant: Weeds

Larinus orientalis Capiomont, 1874 Distribution: Gaziantep Host plant: *Cirsium* sp., *Onopordum* sp. Weeds

Larinus rudicollis Petri, 1907 Distribution: Gaziantep Host plant: *Cirsium* sp., *Onopordum* sp., Weeds

Larinus sibiricus Gyllenhal, 1835 Distribution: Adıyaman Host plant: Weeds

Larinus syriacus Gyllenhal, 1835 Distribution: Gaziantep Host plant: Weeds

Larinus turbinatus Gyllenhal, 1835 Distribution: Diyarbakır Host plant: *Cirsium arvense* L.

Lepidotychius winkleri Franz, 1940 Distribution: Southeastern Anatolia Region Host plant: *Amygdalus communis* L.

Limobius borealis (Paykull, 1792) Distribution: Gaziantep Host plant: Weeds

Lixus albomarginatus Boheman, 1843 Distribution: Mardin Host plant: *Amygdalus communis* L.

Lixus ascanii (Linnaeus, 1767) Distribution: Diyarbakır Host plant: Winter quarters, Weeds

Lixus cardui Olivier, 1807 Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L., *Onopordum* sp. *Prunus avium* L.

Lixus circumcinctus Boheman, 1835 Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Lixus convexicollis Petri, 1904 Distribution: Mardin Host plant: *Amygdalus communis* L., Weeds

Lixus elegantulus Boheman, 1843 Distribution: Diyarbakır, Gaziantep, Mardin Host plant: *Amygdalus communis* L., *Rhus* sp.

Lixus junci Boheman, 1836 Distribution: Southeastern Anatolia Region Host plant: Weeds

Lixus iridis Olivier, 1807 Distribution: Gaziantep Host plant: Weeds

Lixus obesus Petri, 1904 Distribution: Southeastern Anatolia Region Host plant: Weeds

Lixus scolopax Boheman, 1835 Distribution: Gaziantep Host plant: Weeds

Lixus speciosus Miller, 1861 Distribution: Gaziantep Host plant: *Prunus communis* L.

Lixus subtilis (Boheman, 1836) Distribution: Southeastern Anatolia Region Host plant: Weeds

Malvaption malvae (Fabricius, 1775) Distribution: Southeastern Anatolia Region Host plant: Weeds

Mecaspis alternans (Herbst, 1795) Distribution: Diyarbakır Host plant: Winter quarters

Melanobaris dalmatina (H. Brisout, 1870) Distribution: Southeastern Anatolia Region Host plant: Weeds

Microlarinus lypriformis (Wollaston, 1861) Distribution: Gaziantep Host plant: Weeds

Microtrogus cuprifer (Panzer, 1799) Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Amygdalus communis* L., *Avena sativa* L.

Mylocherus damascenus Miller, 1861 Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Amygdalus communis* L., *Avena sativa* L.

Otiorhynchus peregrinus Stierlin, 1861 Distribution: Adıyaman, Diyarbakır, Mardin, Siirt, Şanlıurfa, Host plant: *Avena sativa* L., *Vitis vinifera* L., *Zea mays* L.

Oxystoma ochropus (Germar, 1818) Distribution: Southeastern Anatolia Region Host plant: Weeds

Pachytychius hordei (Brullé, 1832) Distribution: Adıyaman, Diyarbakır, Mardin, Şanlıurfa, Host plant: *Amygdalus communis* L., *Cucurbita* spp., *Lens culinaris* Medic, *Pistacia vera* L., *Punica granatum* L., *Triticum* sp., Winter quarters

Phyllobius fulvago Gyllenhal, 1834 Distribution: Gaziantep, Mardin Host plant: *Pinus* sp.

Polydrusus gracilicornis Kiesenwetter, 1864 Distribution: Gaziantep Host plant: *Quercus* sp.

Polydrusus ponticus Faust, 1888 Distribution: Adıyaman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Host plant: *Amygdalus communis* L., *Pistacia vera* L.

Polydrusus roseiceps Pesarini, 1974 Distribution: Adıyaman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Host plant: *Amygdalus communis* L., *Pistacia vera* L.

Protapion fulvipes (Geoffroy, 1785) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Protapion trifolii (Linnaeus, 1768) Distribution: Southeastern Anatolia Region Host plant: Weeds

Protapion varipes (Germar, 1817) Distribution: Southeastern Anatolia Region Host plant: Weeds

Protapion truqui (Reiche et De Sauley, 1858) Distribution: Mardin Host plant: Weeds

Pseudocoeliodes rubricus (Gyllenhal, 1837) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Rhinosoythropus serripes eibesensis (Reitter) Distribution: Gaziantep Host plant: *Quercus* sp.

Rhinusa sp. Distribution: Diyarbakır Host plant: *Amygdalus communis* L.

Rhopalapion longirostre (Olivier, 1807) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Stenocarus cardui (Herbst, 1784) Distribution: Diyarbakır Host plant: Winter quarters

Sibinia arenariae Stephens, 1831 Distribution: Gaziantep Host plant: Weeds

Sibinia bipunctata Kirsch, 1870 Distribution: Mardin Host plant: *Amygdalus communis* L.

Sibinia femoralis Germar, 1824 Distribution: Diyarbakır Host plant: Winter quarters

Sibinia reitteri Desbrochers, 1895 Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Sibinia subelliptica (Desbrochers, 1873) Distribution: Gaziantep Host plant: Umbelliferous plants

Sibinia subirrorata Faust, 1885 Distribution: Mardin Host plant: *Amygdalus communis* L.

Sibinia syriaca Faust, 1890 Distribution: Gaziantep Host plant: *Onobrychis sativa* (Sainfoin)

Sibinia phalerata (Gyllenhal, 1836) Distribution: Mardin Host plant: *Amygdalus communis* L.

Smicronyx sp Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Sitona bicolor sub sp. concavirostris Fähræus, 1840 Distribution: Diyarbakır Host plant: Winter quarters

Sitona callosus Gyllenhal, 1834 Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L., Winter quarters

Sitona crinitus (Herbst, 1795) Distribution: Adıyaman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Host plant: *Avena sativa* L., *Lens culinaris* L., *Medicago sativa* L., *Onobrychis sativa* Lam., *Pistacia vera* L., *Vicia sativa* L.

Sitona humeralis Stephens, 1831 Distribution: Adıyaman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L., *Pistacia vera* L., *Medicago sativa* L., *Onobrychis sativa* Lam., *Vicia sativa* L.

Sitona lineata (Linnaeus, 1758) Distribution: Gaziantep Host plant: Weeds

Sitona lineellus (Bonsdorff, 1785) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Sitona lividipes Fahæus, 1840 Distribution: Diyarbakır Host plant: Winter quarters

Sitona longulus Gyllenhal, 1834 Distribution: Adıyaman, Diyarbakır, Mardin Host plant: *Amygdalus communis* L., *Medicago sativa* L., *Onobrychis sativa* Lam., *Vicia sativa* L.,

Sitona macularius (Marshall, 1802) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Sitona puncticollis Stephens, 1831 Distribution: Adıyaman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa Host plant: *Pistacia vera* L., Winter quarters

Sitophilus granarius (Linnaeus, 1758) Distribution: Adıyaman, Diyarbakır, Mardin, Siirt, Şanlıurfa Host plant: *Cucurbita* spp., Storage pests, the pests of flour, *Triticum* spp.

Sitophilus oryzae (Linnaeus, 1763) Distribution: Adıyaman Host plant: Storage pests

Squamapion atomarium (Kirby, 1808) Distribution: Southeastern Anatolia Region Host plant: Weeds

Squamapion elongatum (Germar, 1817) Distribution: Southeastern Anatolia Region Host plant: Weeds

Squamapion phocopus (Eppelsheim, 1888) Distribution: Southeastern Anatolia Region Host plant: Weeds

Squamapion vivinum (Kirby, 1808) Distribution: Southeastern Anatolia Region Host plant: Weeds

Stenocarus cardui (Herbst, 1784) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Strophomorphus damaisoni Desbrochers, 1875 Distribution: Gaziantep Host plant: Weeds

Strophomorphus porcellus (Schoenherr, 1832) Distribution: Gaziantep Host plant: Weeds

Strophomorphus sublaevigatus Desbrochers, 1875 Distribution: Gaziantep Host plant: Weeds

Tanymecus dilaticollis Gyllenhal, 1834 Distribution: Adıyaman Host plant: *Medicago sativa* L., *Onobrychis sativa* Lam., *Vicia sativa* L.

Tychius aureolus Kiesenwetter, 1852 Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Amygdalus communis* L., *Avena sativa* L., *Medicago sativa* L., *Onobrychis sativa* Lam., *Vicia sativa* L.

Tychius bicolor (C. Brisout, 1862) Distribution: Adıyaman, Diyarbakır, Mardin Host plant: *Amygdalus communis* L., *Medicago sativa* L., *Onobrychis sativa* Lam., *Vicia sativa* L.

Tychius callidus Caldara, 1990 Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Tychius consputus Kiesenwetter, 1864 Distribution: Southeastern Anatolia Region Host plant: Weeds

Tychius meliloti Stephens, 1831 Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Amygdalus communis* L., *Avena sativa* L., *Medicago sativa* L., *Onobrychis sativa* Lam., *Vicia sativa* L.

Tychius picirostris (Fabricius, 1787) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Tychius tibialis Boheman, 1843 Distribution: Mardin Host plant: *Amygdalus communis* L.

Tychius quinquepunctatus (Linnaeus, 1758) Distribution: Adıyaman, Batman, Diyarbakır, Gaziantep, Mardin, Siirt, Şanlıurfa, Şırnak Host plant: *Amygdalus communis* L., *Avena sativa* L., *Medicago sativa* L., *Onobrychis sativa* Lam., *Vicia sativa* L.

Xenotychius strigicollis Reitter, 1897 Distribution: Gaziantep Host plant: *Lens esculenta* Moench

Family Scolytidae

Chaetoptelius vestitus (Mulsant & Rey 1860) Distribution: Adıyaman, Batman, Diyarbakır, Siirt, Şanlıurfa Host plant: *Pistacia vera* L.

Carphoborus perisi Chapuis, 1873 Distribution: Adıyaman, Batman, Diyarbakır, Mardin, Siirt, Şanlıurfa Host plant: *Pistacia vera* L.

Hylesinus vestitus Mulsant & Rey, 1861 Distribution: Adıyaman, Gaziantep, Şanlıurfa Host plant: *Pistacia vera* L.

Phloeotribus scarabaeoides (Bernard, 1788) Distribution: Adıyaman, Gaziantep, Mardin, Şanlıurfa Host plant: *Olea europaea* L.

Scolytus rugulosus (Müller, 1818) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L., *Prunus avium* L.

Scolytus amygdali Guérin-Ménéville, 1847 Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Superfamily Tenebrionoidea

Family Anthicidae

Notoxus appendicinus Heberdey, 1936 Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Anthelephila caeruleipennis (La Ferte-Senectere, 1847) Distribution: Diyarbakır, Mardin Host plant: *Amygdalus communis* L.

Family Meloidae

Alosimus armeniacus (Faldermann, 1837) Distribution: Diyarbakır, Siirt Host plant: Weeds

Alosimus castaneus Escherich, 1896 Distribution: Diyarbakır, Mardin, Siirt Host plant: Weeds

Alosimus chalybaeus (Tauscher, 1812) Distribution: Şanlıurfa Host plant: Weeds

Alosimus decolor (Abeille de Perrin, 1880) Distribution: Mardin Host plant: Weeds

Alsimum luteus (Waltl, 1838) Distribution: Adıyaman Diyarbakır, Siirt, Şanlıurfa Host plant: Weeds

Alosimus syriacus (Linnaeus 1758) Distribution: Şanlıurfa Host plant: Weeds

Apalus necydalaeus (Pallas, 1782) Distribution: Diyarbakır Host plant: Weeds

Cerocoma dahli Kraatz, 1863 Distribution: Adıyaman, Diyarbakır, Şanlıurfa Host plant: Weeds

Cerocoma malatyensis Kaszab, 1951 Distribution: Siirt Host plant: Weeds

Cerocoma scovitzii intermedia Mañan, 1944 Distribution: Gaziantep Host plant: Weeds

Cerocoma syriaca Abeille de Perrin, 1880 Distribution: Diyarbakır, Siirt Host plant: Weeds

Lydus gibbiger Escherich, 1896 Distribution: Adıyaman, Şırnak Host plant: Weeds

Micromerus bitlisensis Kaszab, 1958 Distribution: Diyarbakır Host plant: Weeds

Micromerus dersinensis Kaszab, 1968 Distribution: Diyarbakır Host plant: Weeds

Mylabris apicenigra Sumacov, 1924 Distribution: Adıyaman Host plant: Weeds

Mylabris ciliciensis (Escherich, 1899) Distribution: Diyarbakır Host plant: Weeds

Mylabris fabricii Sumakov, 1924 Distribution: Mardin Host plant: Weeds

Mylabris fusca (Olivier, 1811) Distribution: Diyarbakır, Mardin, Siirt Host plant: Weeds

Mylabris humerosa (Escherich, 1899) Distribution: Mardin Host plant: Weeds

Mylabris quadripunctata (Linnaeus, 1767) Distribution: Mardin, Şanlıurfa Host plant: Weeds

Mylabris zebraea (Marseul, 1870) Distribution: Adıyaman, Gaziantep, Mardin Host plant: Weeds

Meloe cicatricosus Leach, 1815 Distribution: Diyarbakır Host plant: Weeds

Meloe deflexus Reitter, 1889 Distribution: Adıyaman Host plant: Weeds

Meloe mediterraneus Müller, 1925 Distribution: Diyarbakır Host plant: Weeds

Stenodera caucasica (Pallas, 1781) Distribution: Diyarbakır Host plant: Weeds

Stenodera puncticollis (Chevrolat, 1829) Distribution: Adıyaman Host plant: Weeds.

Family Tenebrionidae

Blaps tibialis Reiche, 1857 Distribution: Diyarbakır Host plant: Weeds

Dailognatha quadricollis (Brullé, 1832) Eschschaltz Distribution: Adıyaman, Gaziantep Host plant: Weeds

Dendarus (Pandarinus) tenellus Mulsant & Rey, 1854 Distribution: Southeastern Anatolia Region Host plant: Weeds

Gonocephalum costatum (Brullé, 1832) Distribution: Southeastern Anatolia Region Host plant: Weeds

Gonocephalum (s. str.) granulatum pusillum (Fabricius, 1791) Distribution: Weeds Host plant: Weeds

Palorus subdepressus (Wollaston, 1864) Distribution: Adıyaman Host plant: Storage pests

Pedinus (s. str.) strabonis Seidlitz, 1893 Distribution: Diyarbakır Host plant: Weeds

Tribolium confusum Jacquelin du Val, 1868 Distribution: Adıyaman, Diyarbakır, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L., *Cucurbita* spp., Storage pests

Tribolium castaneum (Herbst, 1797) Distribution: Adıyaman, Diyarbakır, Mardin, Siirt, Şanlıurfa, Host plant: *Amygdalus communis* L., *Cucurbita* spp.

Opatroides punctatus Brullé, 1832 Distribution: Adıyaman, Diyarbakır, Mardin, Siirt Host plant: *Nicotiana tabacum* L.

Family Mycetophagidae

Typhae stercorea (Linnaeus, 1758) Distribution: Adıyaman, Diyarbakır, Mardin, Siirt, Şanlıurfa Host plant: *Amygdalus communis* L., *Cucurbita* spp.

The aim of this study is to make contribution to some faunistical records of Coleoptera of the Southeastern Anatolia Region of Turkey. This region has different climatic conditions and contains different habitats. Coleoptera contains both important phytophagous and also zoophagous insects.

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THE INFLUENCE OF TIME AND FRUITLET INTERSPACE THINNING ON YIELD AND FRUIT QUALITY OF PEACH AND NECTARINE GROWN IN MONTENEGRO

SUMMARY

The study results over a three year period (2007-2009) on three cultivars of peach and nectarine (Early Crest, May Crest, Springbelle, Adriana, Rita Star and Caldesi 2000) has been presented in the paper. The study includes: time of flowering, pollen germination percentage, the percentage of fruit set, interspaces and time of fruit thinning and their influence on fruit weight and total yield of the examined cultivars. The study found a high percentage of fruit set especially in cv. May Crest (86.60%) and the lowest was in cv. Springbelle (80.51%). Interspace thinning of 5.0 cm gave the highest average of fruit weight (119.0 g) in cv. Caldesi 2000 and the lowest (54.3 g) in cv. Early Crest. At a distance of 10 cm interspace thinning the highest fruit weight was again in cv. Caldesi 2000 (141.0) and the lowest in cv. Early Crest (59.3 g). Our research shows that the most favorable period for thinning of peaches in our agroecological conditions is at the end of April, when the initial fruit weight is 3-5 g.

Keywords: peach, cultivar, thinning, fruit quality, yield

INTRODUCTION

Peach - *Prunus persica* L. Batsch., a plant of the *Rosaceae* family, originating from China, although it could be concluded otherwise according to its Latin name (*Persian plum*). It began to grow in China 4000 years ago. From its homeland it was first transferred to Iran (ex Persia) by which it was named "persica". Then it was transferred in Europe at the beginning of a new era. Peach is one of the most important fruit species in the world, which besides Europe, it is represented in North and South America, Australia, Africa and Asia. It is grown in the subtropical zone and in areas with moderate continental climate.

The observances of the production of the fruits from temperate areas, with the exception of tropical and subtropical fruits, then peach ranks in the third place behind apples and pears. Currently (according to FAO data) Europe is the largest producer of peaches in the world (48.6%), North America (22.9%), Asia (17.1%), South America (6.9%), and Africa (3.3%). Among the European

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countries the largest producers are Italy, then Greece, Spain and France. In the world Italy and the United States are considered as the biggest producers and together they achieve over 40% of total world production (Bulatović-Danilović, 2007).

In Montenegro the peach arrived through Minor Asia, Greece and Italy, in the second half of the 19th century. Organized cultivation of cultivated peach cultivars in Montenegro started in the middle of the last century, mainly by private holdings. The first cultivars were imported from Italy, with European and American origin. In the initial stages cultivars with white and later with yellow mesocarp were imported, and they were 'frestone' cultivars with high quality.

Peach is present in the surroundings of Podgorica and the Adriatic coastal area, mainly early and medium early cultivars. In Montenegro, the peach had a turbulent and dynamic development as only a few other fruit species which clearly indicates its importance and the future perspective. Of the total peach production, 60% applies to consumption of fresh peach, 25% of nectarine and 15% of peach for processing. In the structure of fruit growing in Montenegro, peach orchards are in fifth place and in the structure of stone fruits, immediately after the plums. It should be noted that it is in the group of fruits that are grown on intensive and semi-intensive plantations in the country (Prenkić *et al.* 2010).

Peach contains 87% of water, 12% carbohydrates, very low fat (0.1%) and proteins (0.5%). Cellulose fibres are about (2.3%), but mainly in the cortex. Among the vitamins peach contains mostly beta-carotene, vitamin C, group B, PP and provitamin A (antioxidant).

The peach also contains minerals such as potassium, phosphorus, magnesium, sodium, sulfur and calcium. Peach is among the rare fruits that contain selenium, which helps in reducing free radicals. Peaches not only have a refreshing effect on the human organism, but also curative (diuretic, lowers blood pressure, purifies the body, encourages and facilitates digestion, etc.) (Jovanović, 1987).

Peach trees reach full productivity in their fifth and sixth year after planting. In good ecological conditions it bears abundantly and regularly (30 - 40 t ha), which is more than enough that a yield covers the investment already in the second harvesting year. Peach has a quite short period of exploitation (15-16 years) (Medin, 1998).

Peaches and nectarines in a full crop form several thousands of flower buds which 20 - 50% of them give a fruit. For a high and quality yield it is enough 15% of them is enough. Such abundant yield decreases the quality of the fruit. Branches are more susceptible to breakage and growth of fruit shoot holders in the coming year is compromised, which may lead to alternate bearing (Ognjanov, 2005).

The aim of this study was to determine the time and thinning interspace for some peach and nectarine cultivars, in order to get the greatest amount of first class fruits, and thus more stable and quality yield that will achieve a significantly higher price at the market.

MATERIAL AND METHODS

Investigations were carried out in productive – experimental plantation DD "Plantaze" at Čemovsko field in the period 2007 - 2009. Plantation of newly introduced peach cultivars was set up in the spring of 1993 and nectarine plantation in spring of 1998. The planting space was 4.5 x 2.0 m (1.111 trees ha), where each tree has 9 m² area. Planting direction among the experimental plots is north - south. Growing form is the slender spindle (Fusseto), and in cultivars that were set up in 1998 are modified palmettes. All varieties are grafted at the vineyard peach.

The study comprised the following cultivars of peaches and nectarines: Early Crest, May Crest, Springbelle, Adriana, Rita Star and Caldes 2000. Each cultivar was presented 5 trees per variety. Beginning of flowering was recorded when 10% of flowers was opened. Full flowering was noted when 90% of flowers were opened and the end was when 90% of petals had fallen (Slović, 1972).

Pollen germination was tested in a nutrient medium with 1% of agar, supplemented with 15% sucrose at 25°C, according to Warner and Chang (1981). After three hours pollen grains were counted under the microscope, and the percentage of germination was determined by mathematical proportion.

Fruit set was determined by counting the open flowers on five trees per cultivars of each cultivar studied. Then fruit set was counted before their natural falling off the tree.

Fruit thinning was done according to time and on three occasions: in peach on 22 April, 29 April and 6 May and in nectarine on 29 April, 6 May and 13 May and the most favorable moment for carrying out thinning in peach and nectarine plantation was determined. Also it was determined thinning of fruit, depending on the distance (at 5 and 10 cm) and its impact on the number of fruits per branch and per tree, fruit weight and yield of tested peach and nectarine cultivars, expressed in kg/tree and t/ha. They were mathematically calculated by multiplying the number of fruits on the tree with their average weight and number of trees per ha. All cultivars were grouped according to time of their maturity. Statistical analysis of the observed characteristics (should be specify which, like fruit mass etc.) performed by analysis of variances a two-factorial trials. The comparison of means was tested by LSD for the level of 95 and 99%. Fruit mass of 50 fruit were measured in three trees per cultivars replicates.

Fruit maturity in peach and nectarine were recorded according to Gvozdenović (1990), based on visual changes of basic and additional color skin, easiness of separation of fruit from a branch. The organoleptic evaluation and determination of dry matter content was done by refractometer and calculation of its index.

Agro ecological conditions

Podgorica is located at 42 m above sea level and 42° 26' N latitude, and 19°15' E longitude. It is characterized by a Mediterranean climate, modified by

the influence of peripheral high mountains; opposed to the narrow coastal area, which are hotter with drier summers and wetted winters.

Temperature is an important factor affecting the growth of peach, its yield and the quality of fruits. Annual course of air temperature in this region has elements belonging to temperate latitudes, because the typical minimum and maximum points are located in the central summer (July-August) and winter months (January-February). The mean annual air temperature for a period of 10 years (1990-2000) was 17.6°C and 18.2°C during the study, which is 0.6°C higher than the ten-year average. Mean temperature in the vegetation period of investigation (01st March – 30th November) was 20.7°C (Table 1).

Table 1. Mean monthly, annual and vegetation air temperatures in Podgorica, °C.

	I	II	III	IV	V	VI	VII	VIII	VIII	IX	X	XI	XII	Average
2007	8.0	9.8	12.2	17.6	21.2	26.1	30.1	28.2	20.3	16.2	9.2	5.4	17.0	18.4
2008	6.6	7.7	11.1	15.1	21.0	25.0	27.3	28.7	21.4	16.8	12.0	7.6	16.7	18.1
2009	6.3	6.3	9.9	17.0	22.0	23.5	27.5	28.6	23.0	15.1	10.7	9.1	16.6	18.0
Average 2000-2009	5.8	7.1	10.7	15.3	20.9	25.0	27.8	27.3	21.3	16.7	11.0	7.1	16.3	18.2

Besides temperature conditions precipitation is certainly the most important for intensive and cost-effective cultivation of peach. Podgorica region, in regard to precipitation, is characterized by specific features of the Mediterranean climate. Maximum rainfall is in the last two months of the year (November-December), and minimum in July and August.

The average amount of precipitation during the vegetative period of study was only 142.2 mm/m², while the amount of annual rainfall was extremely high (1357.6 to 2036.3 mm/m²), but not so useful because of the soil is skeleton and has a little water capacity. Due to all the water from rainfall sinks and washes the nutrients into the deeper layers, while the capillary rise of water is very small.

Unfortunately, distribution of the precipitation is unfavorable, so their deficit appears in the period of June-August, which negatively affects the process of growth and fruiting of peach, especially in mid-growing season (Table 2).

The soil where the research was conducted is formed on conglomerate of fluvio - glacial sediments transferred from the mountain massif from the east of "Ćemovsko field." the sediments are mostly composed of lime or dolomite rocks, gravel and sand. The main characteristic of this soil is that it is sandy and relatively shallow. Granulometric composition is characterized by a high content of skeleton which is 80% throughout the soil profile with small soil particles less than 20%.

Table 2. Mean monthly, annual and vegetation sum of precipitation in the Podgorica, mm m².

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Average
2007	95.6	228.5	194.8	32.2	72.6	40.4	0.2	43.9	119.7	139.8	273.7	116.2	1357.6
2008	209.1	63.1	326.4	98.4	40.8	142.4	28.9	14.8	75.6	150.8	221.7	353.9	1725.9
2009	343.6	134.6	202.0	33.9	31.2	234.9	17.6	12.9	62.6	260.3	331.0	371.7	2036.3
Average 2000-2009	187.2	151.5	177.6	111.1	62.3	82.1	31.2	57.9	176.4	156.2	255.4	248.8	1697.6

RESULTS AND DISCUSSION

Flowering time and duration of studied cultivars were quite uniform in the years of research (Table 3.). The shortest interval of flowering energy was recorded in the cultivar Adriana (11 days) and the longest in the cultivar Rita Star (16 days). The earliest beginning of flowering was in cultivar Rita Star (09.03.), and the latest in cultivars Early Crest and Springbelle (29.03.).

Pollen germination of studied cultivars (Table 4.) was in average 77.52%. Cultivar Adriana had the highest pollen germination (79.00%) and cultivar May Crest the lowest (75.20%).

Fruit set was in average (83.07%), (Table 4.). The greatest number of fruit set was in variety May Crest (86.60%) and the lowest in cultivar Springbelle (80.51%). The favourable climatic conditions (Table 1 and 2) prevent the risk of late spring frosts which caused a high percentage of fruit set in the years of research. Lučić et al. (1996) state that for a normal peach yields it is necessary to set 10-20% of comparison or fruitless. Ripening of tested cultivars (Table 4.) shows that the earliest harvest date arrived in the cv. Early Crest (28.05.), and the latest in Caldesi 2000 (22.06.).

Table 3. The time of flowering of summer peaches cv., 2007-2009.

Cultivar	Beginning of flowering	Full flowering	End of flowering	Flowering duration
Early Crest	16.03	22.03	29.03	12
May Crest	12.03.	19.03.	26.03.	14
Springbelle	16.03.	22.03.	29.03.	13
Adriana	17.03	23.03.	28.03.	11
Rita Star	09.03.	17.03.	25.03.	16
Caldesi 2000	16.03	22.03.	28.03.	12
X				13

According to the time of ripening all tested cultivars belong to early to mid-early cultivars (Bellini, 1984). Time of ripening in peaches varied slightly in the three-year period of investigation (Table 2.). Namely, the favourable environmental conditions at the time of flowering until fruit maturation influenced to relatively uniform maturation of the tested peach and nectarine cultivars.

Table 4. Pollen germination, fruit set up and fruit ripening, %, 2007-2009.

Cultivar	Pollen germination	Fruit set up	Time of ripening	
			X	(0)
Early Crest	78.40	84.36	28.05.	-16
May Crest	75.20	86.60	06.06.	-6
Springbelle	77.66	80.51	12.06.	0
Adriana	79.00	81.33	15.06.	+3
Rita Star	76.33	82.30	19.06.	+7
Caldesi 2000	78.50	83.33	22.06.	+10
X	77.52	83.07		

Influence of fruit thinning on initial fruit weight: Thinning of peach fruits was performed three times: 22. 04. (I, Table 5), 29. 04.(II), and 06. 05.(III), and in nectarines 29. 04.(I), 06. 05.(II), and 13. 05(III). During the first peach thinning, fruit weight was 1.2 g in Early Crest to 1.1 g in May Crest and Springbelle (Table 5.). In second thinning of fruit weight has increased by 2.5 times, so the cultivar Early Crest had fruit weight of 3.7 g, May Crest 3.2 g and Springbelle 3.0 g. In third thinning, peach fruits weight reached 10.6 g in Early Crest, 9.3 g in May Crest and 8.6 g in Springbelle. Statistical analysis of comparison of means shows no significant differences in initial fruit weight within the varieties and the time of fruit thinning.

Table 5. Influence of fruit thinning on initial fruit weight, g, 2007-2009

Cultivar	Early Crest	May Crest	Springbelle	Adriana	Rita Star	Caldesi2000
Date of thinning						
I	1.2	1.1	1.1	1.2	1.4	1.1
II	3.7	3.2	3.0	3.2	4.4	2.4
III	10.6	9.3	8.6	11.6	12.6	8.5
LSD 005	2.45	3.23	2.07	2.55	3.58	3.3
LSD 001	3.51	5.18	2.8	3.28	5.25	5.18

In the first thinning in nectarine fruit mass was 1.2 g in Adriana. 1.4 g in Rita Star and 1.1 g in Caldesi 2000. In the second thinning fruit weight was 3.2 g in Adriana. 4.4 g in Rita Star and 2.4 g in Caldesi 2000. In the third thinning fruit weight was 11.6 g in Adriana. 12.6 g in Rita Star and 8.5 g in Caldesi 2000.

During thinning in nectarines average fruit weight was similar as in peach except in the third thinning when the fruits were slightly larger due to favorable weather conditions. Namely temperatures were from 20 to 25°C in that period which influenced intensive fruit growth. Also the soil moisture in this period was very favourable due to heavy winter and spring rainfall. and soil was enriched with nitrogen fertilizer in March.

Influence of interspace thinning on fruit weight and yield: The average number of fruits that were not been thinned in three-year period ranged from 305.4 in cultivar Adriana to 560.3 in Springbelle (Table 6.).

After the thinning was done at 5.0 cm. the most numerous fruits were recorded in Springbelle (175.5). and the least in Rita Star (151.8). The greatest number of fruits in thinning made at 10 cm was in cv. Springbelle (121.5). and the smallest in Rita Star (105.1).

These interspaces thinning affected the fruit weight of tested cultivars and their yield. In thinning at 5.0 cm interspace. maximum average fruit weight had a Caldesi 2000 (119.0 g) and lowest Early Crest (54.3 g). At a thinning distance of 10 cm the highest fruit weight was also in Caldesi 2000 (141.0 g) and the lowest in cultivar Early Crest (59.3 g). Biometric data processing did not show significant differences in fruit mass within cultivars or fruit thinning space of 5 and 10 cm. The average yield of tested peach and nectarine cultivars at a distance of 5.0 cm was significantly higher and ranged from 9.65 t/ha in cultivar Early Crest to 18.3 t/ha in cultivar Caldesi 2000. At a thinning distance of 10.0 cm the highest average yield was recorded in Caldesi 2000 (15.0 t/ha) and the lowest again in Early Crest cultivar (7.28 t/ha).

The influence of favourable agricultural conditions in the investigated region has contributed to the fact that tested cultivars of peaches and nectarines are classified into groups of early and medium flowering cultivars (Bellini *et al.* 1984). Agro-ecological conditions in the locality Čemovsko field. primarily slightly lower temperature and relatively high precipitation in the years of research have influenced later flowering of tested peach and nectarine cultivars. According to Ognjanov (1991) it is necessary that the temperature from 1st of March should be 8°C or higher in a 14-15 day period in order to begin flowering phenophase which totally agrees with our studies. Observing the time of flowering and ripening time of tested peach and nectarine cultivars. can be concluded that there was no correlation between them. as it is the case with other fruit species (Baldini and Scaramuzzi. 2005). Flowering duration of studied cultivars is 3-5 days longer than in studies done by Bulatović and Mratinić (1996). They carried out their research in the areas with lower annual temperature. while the mean temperature in Podgorica is 18.2 °C.

Table 6. The influence of thinning interspace on fruit weight and yield. g. kg/ tree. t/ha. 2007-2009.

Thinning	Variety	Early Crest	May Crest	Spring belle	Adriana	Rita Star	Caldesi 2000	LSD 005
								LSD 001
The average number of fruit branches per tree		61.3	58.7	67.5	63.7	58.4	60.8	2.62 3.90
Before thinning	The average number of fruits per fruit branch	8.2	7.6	8.3	4.8	5.4	6.1	4.26
								7.28
Before thinning	The average number of fruits per tree	502.7	446.1	560.3	305.8	315.4	370.9	2.62 3.9
After thinning	The average number of fruits per tree at 5.0 cm	159.4	152.6	175.5	165.6	151.8	158.1	2.74
								3.95
After thinning	The average number of fruits per tree at 10 cm	110.3	105.7	121.5	114.7	105.1	109.4	2.42
								3.70
After thinning on 5.0 cm	Average fruit weight (g)	54.3	107.0	75.7	93.3	105.0	119.0	2.81
								3.95
After thinning on 10 cm	Average fruit weight (g)	59.3	122.7	85.0	105.0	119.7	141.0	4.02
								7.68
After thinning at 5.0 cm	The average yield per tree (kg)	8.67	16.3	13.3	15.5	15.9	18.8	2.42
								3.75
After thinning at 10 cm	The average yield per tree (kg)	6.54	13.1	10.3	12.0	12.6	15.4	2.25
								3.38
After thinning at 5.0 cm	The average yield per ha	9.65	17.3	14.6	16.6	17.6	18.3	2.4
								3.55
After thinning at 10 cm	The average yield per ha	7.28	11.9	11.3	13.0	13.8	15.0	2.07
								2.9

All tested peach and nectarine cultivars had high pollen germination (over 70.00%). which fully agrees with literature data of Bulatović (1992). According to Ognjanov (1991) great importance to germination of pollen has the same environmental conditions that influenced on the flowering time but the microsporogenesis of peach starts in most of our areas in the second half of February or at the beginning of March and lasts 2-3 days.

In this study a high percentage of fruit set (83.07%) was recorded so the thinning was necessary. which completely corresponds with the data presented by Nicotra *et. al.* (1994). It is important to note that the natural fruit drop represents a preliminary thinning. and thus an intentional thinning should be considered. in order to ensure an optimum yield of peach and nectarine.

It could be concluded that the moment of peach ripening in Mediterranean conditions differed in regard to different localities of peach growing in our country and in the world. Our tested peach cultivars arrived earlier about 15 days to harvest in comparison to studies done by Bassi and Intrieri (1983) near Bologna. Kupci (2000) noted that the differences in time of ripening of early ripening cultivars are greater between continental and Mediterranean areas than in late varieties of middle and late ripening time.

It could be concluded that the peach fruit growth was almost identical with the growth of nectarine fruit although peach thinning was done a week earlier. Therefore if we compare early varieties of peaches and nectarines, peaches are still ripening earlier and are holders of the first income. It is very important to note that the most favourable period for peach thinning is the second thinning which is done at the end of April in our agroecological conditions due to the danger of natural fruit falling. Therefore, deliberate thinning must be taken into account, so the optimal yield can be ensured. This agrees with the allegations of Cross - Raynaud (1985), which referred to three periods of fruit falling: falling of small fruits that have not been fertilized, the second falling in June when embryos abort at certain points and the third falling after the pit hardening.

According to IBPGR all tested peach cultivars were classified in three groups of productivity: low, medium and high. The yield of peaches depends on hereditary characteristics, as well as of agro-ecological conditions in a given locality. Since the examined peach and nectarine cultivars were in a declining productivity, their yield was lower than in the research of Della Strada (1992). For each year there was no linear increase observed in yield of all cultivars, but the yield decreased slightly, depending on research year. However, some of the test cultivars such as Caldesi 2000, Rita Star, May Crest and Adriana have shown relatively high yields in years of research. Although, environmental conditions were favorable and can also be considered that other biological-physiological factors, such as moderate vigour and optimum load of mixed fruit bearing branches after thinning at 5.0 cm, have positive impact on this phenomenon (Odalović, 2002). The thinning at 10 cm significantly influenced to lower yield of tested peach and nectarine cultivars, that were at the end of their ontogenetic cycle.

CONCLUSIONS

Based on the results obtained in the three-year period (2007-2009), it can be concluded the following:

Beginning of flowering of studied cultivars largely depends on agroecological conditions in the locality. The earliest flowering beginning was in cultivar Rita Star (09.03.), and the latest end of flowering was in cultivar May Crest (26.03.). Research results showed that in terms of flowering time tested cultivars are in the range of medium early to medium flowering cultivars.

Tested peaches and nectarines have a high percentage of pollen germination that is over 70%. The highest pollen germination was in cv. Adriana (79.00%) and the lowest in cultivar May Crest (75.20%).

In our research there was a high percentage of set up fruits (83.07%), so their thinning was necessary. The greatest number of fruit set was in cultivar May Crest (86.60%) and the lowest in cultivar Springbelle (80.51%).

It is important to note that the most favorable period for peach thinning is the second one, which in our agroecological conditions was in late April when the initial fruit weight ranged from 3-5 g. The reason for this is in fact that earlier thinning increases the danger of natural fruit falling off and therefore the deliberate spacing must be taken into account in order to ensure the optimum yield.

Thinning spaces of 5.0 and 10 cm influenced to fruit weight of tested cultivars and their yield. At the thinning of 5.0 cm. the highest average fruit weight was in cv. Caldesi 2000 (119.0 g) and the lowest in Early Crest (54.3 g). At a thinning of 10 cm the highest fruit weight was also in Caldesi 2000 (141.0 g) and the lowest in Early Crest (59.3 g). The average yield of tested peach and nectarine cultivars at a distance of 5.0 cm was significantly higher and ranged from 9.65 t ha in cultivar Early Crest to 18.3 t ha in cultivar Caldesi 2000. The thinning distance of 10 cm resulted in the highest average yield in cultivar Caldesi 2000 (15.0 t ha) and the lowest also in cultivar Early Crest (7.28 t ha).

Some of the studied cultivars such as Caldesi 2000, Rita Star, May Crest and Adriana have shown relatively high yields in years of research at thinning distance of 5.0 cm even though they were at the end of their ontogenetic cycle and their exploitation period can significantly be extended. On the other hand, thinning space of 10 cm significantly influenced on lower yield in the tested cultivars of peach and nectarine.

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Shourav DUTTA¹

**NODULATION STATUS AND NITROGEN FIXING POTENTIAL
OF AKASHMONI (*Acacia auriculiformis*) SEEDLINGS
IN TROPICAL MIXED PLANTATIONS**

SUMMARY

A study was carried out to assess biological nitrogen fixation and observe nodulation status of *Acacia auriculiformis* (Akashmoni) at mixed plantations in the Seed Research Laboratory and nursery of the Institute of Forestry and Environmental Sciences, Chittagong University (IFESCU), Bangladesh. The plantations consist of one pure planting plot (100% A) and eleven mixed planting plots (1A:1S, 1A:1G, 1A: 1G: 1S, 1A:2S, 1A:2G, 1A:3S, 1A:3G, 2A:1S, 2A:1G, 3A:1S and 3A:1G) of the three common plantation tree species of Bangladesh namely *Acacia auriculiformis* (A), *Swietenia macrophylla* (S) and *Gmelina arborea* (G). In Seed Research Nursery, seedlings of three species were raised in a randomized blocks with three replicates of twelve treatment plots. Nodule number, color, size, shape, form, structure, nodule fresh weight, oven-dry weight and total nitrogen accumulation in the soil of *A. auriculiformis* seedling were recorded. At the age of 10 month, highest nodulation (143 number) of *A. auriculiformis* were recorded in the mixed plot (1A:2S) in comparison with pure 100%A plot. Fresh and oven dry weight of roots of the *A. auriculiformis* seedlings were found significantly ($p < 0.05$) highest in 1A:2S mixed plot. The findings of this study suggest that in comparisons with pure plot, *A. auriculiformis* fix better nitrogen in mixed plantations.

Keywords: *Acacia auriculiformis*, Bangladesh, Mixed Plantation, Nitrogen fixation, Nodule, Symbiosis.

INTRODUCTION

One of the major management objectives of tropical forests of Bangladesh was to replace the heterogeneous natural forests by the mixed plantations of valuable timber species (Dutta *et al.* 2014). The management of mixtures of two dominants is more difficult; they can usually only be managed on specific sites and often result in the suppression of one of the species (FAO 1992, Hossain 2008). Nitrogen fixing trees in tropical environments appear to offer both high growth rate and soil enrichment (Binkley and Giardiana 1997). Nitrogen fixing trees may increase the supply of available nitrogen in the soil, benefiting both N-fixing and non-N fixing trees (Binkley *et al.* 2000). The success of mixed species plantations depends on species attributes and site factors (Forrester *et al.* 2005)

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that affect the balance of positive and negative interactions between the species (Boyden *et al.* 2005). Nitrogen fixation by NFS is supposed to improve soil N availability (Bouillet *et al.* 2008, Khanna 1997, Voigtlaender *et al.* 2012), alleviating N limitation and facilitating the growth of the target species in N₂ limited soils (Forrester *et al.* 2007). In addition, nitrogen fixing species may enhance phosphorus (P) availability through the rhizosphere acidification due to nitrogen fixation (Hinsinger *et al.* 2011), increasing the amount of P that cycles in mixed-species plantations compared to monocultures (Forrester *et al.* 2005). For increasing the effectiveness of the biological nitrogen fixation, studies are needed to carry out on the nodulation and nitrogen fixation in the existing legume flora in different parts of the world (Mahmood and Iqbal 1994). In Bangladesh, information on comparative seedling growth and nodulation of legumes are very scanty (Aryal *et al.* 1999). There is a wide range of nitrogen fixing plants that have been used in forestry with an objective of raising soil nitrogen levels and subsequently improving the growth of the non-nitrogen fixing forest species (Turvey and Smethurst 1983). But still research on nitrogen-fixing species in general has lagged behind that on food, feed, and forage crops.

Estimates of N₂-fixation for tropical evergreen forests are extremely rare and highly variable (Cleveland *et al.* 1999). Over 600 tree species have been reported to fix nitrogen (MacDicken, 1994). In Bangladesh, a total of 98 genera and 332 sub-generic taxa of nitrogen-fixing species, both wild and cultivated have been recorded under the families Caesalpiniaceae (20, 60), Mimosaceae (14, 39) and Fabaceae (64, 233) (Khan *et al.* 1996). *Acacia auriculiformis* and *Albizia* spp. are two most common nitrogen fixing species commonly used in road side and forest plantation, agroforestry, community forestry and homestead plantation programs in Bangladesh. Various exotic nitrogen fixing plants with native nitrogen fixing trees are planted all around the country. A common expectation is that an N₂-fixing species will enhance nitrogen availability to the other and accelerate nitrogen cycling, so that total growth will be greater. Recently N₂-fixing tree species such as *Acacia auriculiformis*, *Albizia* spp., *Leucaena leucocephala* etc are planted with non-N₂ fixing tree in the homegarden of Bangladesh (Alam *et al.* 2005). *Acacia auriculiformis*, *Swietenia macrophylla* and *Gmelina arborea* are the three major plantation tree species proved successful in trials and in large scale plantation programs of Bangladesh (Hossain and Hoque 2013). The present study was aimed to investigate the optimum mixing and benefits of nitrogen fixation of N₂-fixing *A. auriculiformis* with non-nitrogen fixing species (*S. macrophylla* and *G. arborea*). The present attempt was also made with a view to studying the nodulation potential and nitrogen fixing ability of *A. auriculiformis* used in the mixed plantations of Bangladesh.

MATERIAL AND METHODS

Experiment site

The study was conducted in the nursery seed bed of Seed Research Laboratory of the Institute of Forestry and Environmental Sciences, University of Chittagong

(IFESCU) campus, Chittagong, Bangladesh. The experimental design was carried out over a period of ten months from February to November, 2015 in the IFESCU nursery. The experimental site (nursery) lies approximately at the intersection of 91°50' east longitude and 22°30' north latitude (Khan *et al.* 2004). The nursery site enjoys a tropical monsoon climate characterized by hot, humid summer and cool, dry winter (Mahmud *et al.* 2005). The average monthly mean temperature varied 29.75°C maximum and between 21.14°C minimum (Ahmed, 1990). The annual rainfall in the nursery is 2500–3000 mm which mostly takes place between June and September (Gafur *et al.* 1979). Relative humidity was generally the lowest (64%) in February and highest (95%) in June–September (Mahmood *et al.* 2005).

Experimental plot design

Seeds of *Acacia auriculiformis* (Akashmoni), *Swietenia macrophylla* (Mahagony) and *Gmelina arborea* (Gamar) were collected from the Bangladesh Forest Research Institute (BFRI), Chittagong, Bangladesh in the month of February, 2015 and seedlings were raised in the nursery bed of Institute of Forestry and Environmental Sciences, University of Chittagong (IFESCU) with standard nursery techniques (Fig. 1). The soil samples collected from the barren hills of the Chittagong University Campus was sieved well (< 3mm) and then fill up the seed bed. The hills consist of moderate to strongly acidic soils (Osman *et al.* 1992) and an average soil pH 5.5 (Badrudin *et al.* 1989). The soil used in the nursery was moderately coarse to fine textured (Mahmood *et al.* 2005). Seedlings were raised in a randomized blocks with three replicates of twelve treatment plots. One pure planting plot of *A. auriculiformis* along with ten mixed planting plots of two species (either *Acacia* × *Swietenia* or *Acacia* × *Gmelina*) was established. One mixed planting plots of three species [*Acacia* (A) × *Swietenia* (S) × *Gmelina* (G)] were established. Pure planting plot was P₁ (100% A) and eleven mixed planting plots were –

- a) M₁ (1A:1S), M₂ (3A:1S), M₃ (1A:3S), M₄ (2A:1S), M₅ (1A:2S);
- b) N₁ (1A:1G), N₂ (3A:1G), N₃ (1A:3G), N₄ (2A:1G), N₅ (1A:2G);
- c) M₆ (1A: 1G: 1S).

Each plot was 200 cm × 60 cm in size with 30 seedlings at a spacing of 20 cm × 20 cm (seedling to seedling distance: 20 cm). Within each mixed plot of 1A:1S or 1A:1G, seedlings of *Acacia* were planted with *Swietenia* or *Gmelina* alternatively. In 2A:1S or 2A:1G plot, two seedlings of *Acacia* followed by one seedlings of either *Swietenia* or *Gmelina* sequentially. Similarly, in 1A:2S or 1A:2G plots one seedling of *Acacia* followed by two seedlings of *Swietenia* or *Gmelina* sequentially. Again, within each mixed plot of 3A:1S or 3A:1G, three seedlings of *Acacia* followed by one seedlings of *Swietenia* or *Gmelina* sequentially. Similarly, in 1A:3S or 1A:3G plot, one seedling of *Acacia* followed by either three seedlings of *Swietenia* or *Gmelina* consequently.



Fig. 1. All the experimental plots in the Seed Research Nursery (a: Two months old seedlings, b: Five months old seedlings).

Harvesting and data collection in the seed research laboratory and nursery

Five randomly selected seedlings of each species were harvested carefully from each mix plot and ten seedlings from pure plantation plot at 10 months after germination. The harvested seedlings were washed in tap water and then in distilled water to clean the root regions off all soil particles. Fresh weight of the root of *A. auriculiformis* was measured after removal of all water from the root portion of the washed seedlings. Dry weight of roots was recorded after oven dried at 70°C for 72 hours.

Evaluation of nodulation

For *A. auriculiformis* nodule numbers per seedling were recorded after measuring the root weight. Then nodules were separated from individual plants and further washed carefully to remove all the soil particles (Solaiman, 1999). The root nodules were counted and their fresh weight was recorded. Nodule status, shape, color and structure were recorded. Nodule numbers in roots were recorded and nodule score was evaluated using the following table (Table 1):

Table 1. Classification criteria used to evaluate the nodulation in the roots (Corbin 1977)

Soil level	Nodule number in roots	Nodule score
0 – 5 cm	0	0
>5 cm	0	
0 – 5 cm	<5	1
>5 cm	0	
0 – 5 cm	5 - 10	2
>5 cm	0	
0 – 5 cm	>10	3
>5 cm	0	
0 – 5 cm	>10	4
>5 cm	<5	
0 – 5 cm	>10	5
>5 cm	>10	

Effectiveness of nodules was represented as follows (Peoples, 1989):

Nodule Score	Representation
4 - 5	Excellent nodulation; excellent potential for N ₂ -fixation
3 - 4	Good nodulation; good potential for fixation
2 - 3	Fair nodulation; N ₂ fixation may not be sufficient to supply the Nitrogen demand of the crop/plant.
0 - 2	Poor nodulation, little or no N ₂ -fixation.

Collection of soil samples and determination of nitrogen

In the nursery, soil samples were collected from each plot at different positions from a depth of 30–35 cm near each seedling. Soil samples were then air dried, ground in agate mortar and passed through a 2 mm mesh sieve. The soil samples were analyzed in the chemistry laboratory of institute of forestry and environmental sciences, Chittagong University (Ifescu), Bangladesh to determine total nitrogen. The total nitrogen content was determined by the Kjeldahl method (Bremner, 1965).

Statistical analysis

Analysis of variance (ANOVA) and tests for means ($p < 0.05$) were run using the means of each variable from each of the three replicate plots. The statistical evaluations of the data obtained as a result of research was carried out though variance analysis according to randomized blocks experimental design. In order to determine the difference between the averages, the Duncan's multiple range test (DMRT) was utilized. All the data collected were analyzed statistically by using spss (Aryal et al. 1999, khan et al. 2004).

RESULTS AND DISCUSSION

Nodulation status of *A. auriculiformis* seedling

Nodules of *A. auriculiformis* were elongated to ovate with/ without branching and pink/ brown in color. *A. auriculiformis* nodules were found both in primary and secondary roots of the seedlings (Fig. 2). Nodule number differed significantly among pure and mixed plots (Table 2).

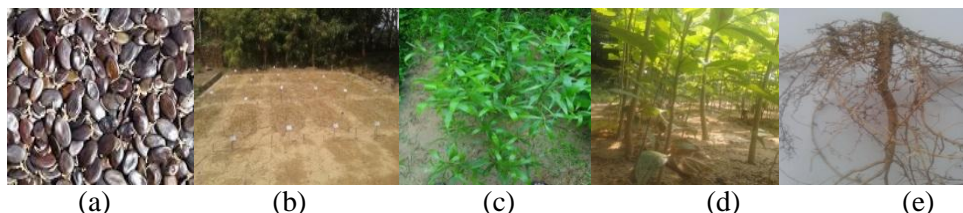


Fig. 2. Biological nitrogen fixation of *A. auriculiformis* through nodulation (a: Seeds of *A. auriculiformis*, b: Germination of seeds in seed bed, c: Three months old seedlings under nursery condition, d: Species interactions among *A. auriculiformis*, *S. macrophylla* and *G. arborea*, e: Nodule formation of *A. auriculiformis* seedlings both in primary and secondary roots).

Table 2. Nodule number, nodulation status, color, shape, branching status and distribution of nodules of *A. auriculiformis* in pure and mixed planting plots at 10 months after germination.

Treatments	Nodule number	Nodule condition	Color	Shape	Branch/Cluster	Distribution in roots
P ₁	28.34 ^{abc*}	Sparse	Pink	ovate to obovate	Branch and Cluster	Primary and Secondary
M ₁	29.35 ^{abc}	Sparse	Brown to dark brown	elongate to elongate	Branch	Secondary
M ₂	21.67 ^{abc}	Sparse	Pink	elongate to elongate	Branch	Primary
M ₃	38.67 ^{bc}	Moderate	Brown	elongate to ovate	Cluster	Primary
M ₄	32.34 ^{abc}	Moderate	Brown to dark brown	elongate to ovate	Branch and Cluster	Secondary
M ₅	58.67 ^c	Abundant	Brown	elongate to elongate	Cluster	Primary and Secondary
M ₆	18.35 ^{ab}	Sparse	Pink	ovate to obovate	Branch and Cluster	Secondary
N ₁	14.3 ^{ab}	Sparse	Pink	elongate to elongate	Branch	Secondary
N ₂	18.36 ^{ab}	Sparse	Brown	elongate to ovate	Cluster	Secondary
N ₃	20.67 ^{ab}	Sparse	Dark Brown	ovate to obovate	Cluster	Secondary
N ₄	12.32 ^{ab}	Sparse	Pink	elongate to ovate	Branch and Cluster	Primary
N ₅	6.68 ^a	Sparse	Pink	elongate to elongate	Branch	Secondary

(*) Means followed by the same letter(s) in the same column are not significantly different at $p < 0.05$ (DMRT).

Nodules of *A. auriculiformis* in pure and mixed plots were found pink or brown in color. (Fig. 3). Nodule size varied from 2.6×3.3 mm in N5 to 5.2×6.3 mm in M5. *A. auriculiformis* possessed nodules both in single and aggregate forms. Variations in nodule size, form and structure were observed among the treatment plots (Table 3).

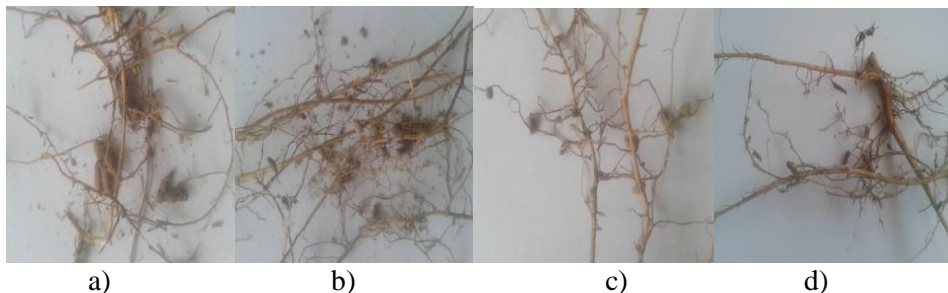


Fig. 3. Nodulation in *A. auriculiformis* seedlings (a: Nodules in Cluster, b: Ovate to obovate nodules, c: Elongate to ovate nodules, d: Nodules in lateral roots).

Table 3. Nodule size and Structure of ten months old seedlings of *A. auriculiformis* grown under nursery condition

Treatments	Nodule size(mm)	Form	Nodule structure
P ₁	3.1 × 3.6	S*	Advance indeterminate
M ₁	2.8 × 3.4	A	Primitive indeterminate
M ₂	3.2 × 3.7	S & A	Advance indeterminate
M ₃	3.5 × 4.2	S	Advance determinate
M ₄	3.7 × 4.4	A	Primitive indeterminate to Advance determinate
M ₅	5.2 × 6.3	S & A	Advance determinate
M ₆	2.7 × 3.9	A	Advance determinate
N ₁	3.7 × 4.5	S	Advance indeterminate
N ₂	4.2 × 4.9	S & A	Primitive indeterminate to Advance determinate
N ₃	3.8 × 4.6	A	Advance indeterminate
N ₄	3.6 × 3.8	S	Primitive indeterminate to Advance determinate
N ₅	2.6 × 3.3	A	Primitive indeterminate

*S: single and A: aggregated.

Maximum number (143) of nodule was recorded in M5 followed by 38 in M3 and 32 in M4. Nodule number was found minimum (6) in N5. *A. auriculiformis* seedlings showed excellent and good nodulation status in both pure and mixed plots. *A. auriculiformis* fixed more Nitrogen through nodule formation in their root systems with *S. macrophylla* than with *G. arborea*. *A. auriculiformis* seedlings showed excellent, good or fair nodulation status in mixed plots except N5 plot. Nodulation status found fair in N2 and N4 plots and poor in N5 plot (Fig. 4).

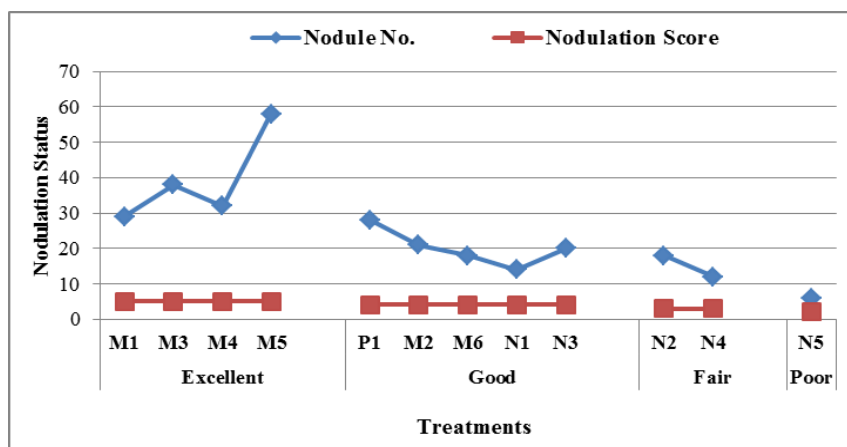


Fig.4. Treatment-wise nodulation status and nodulation score of *A. auriculiformis* seedlings.

At the time of *A. auriculiformis* seedling harvest, the highest root fresh weight (27.26 g) was recorded in M5 followed by 20.06 g in P1 and 19.07g in M3. Compared with pure plot, the fresh weight of root was significantly higher in mixed plots. The result of variance analysis for root dry weight and nodule fresh

weight *A. auriculiformis* of revealed that the effect of planting pattern was significant ($p < 0.05$). Mean comparison using Duncan multiple range test (DMRT) showed that the highest root dry weight obtained in mixed plot M5. Compared with pure plot (10.06 g), the dry masses of root were also significantly higher in M5 (19.28g) followed by M2 (10.82 g). The result of analysis of variance revealed that planting patterns had significant effect on the nodule fresh weight of *A. auriculiformis* (Table 4).

Table 4. Comparative root fresh weight (g), nodule fresh weight (g), root dry weight (g) and total nitrogen (%) of ten months old *A. auriculiformis* seedlings.

Treatments	Root Fresh Wt. (g)	Root Dry Wt.(g)	Nodule Fresh Wt.(g)	Total N (%)
P ₁	20.06 ^{cd*}	10.06 ^{cde}	1.19	0.29 ^{ab}
M ₁	14.51 ^{abc}	7.64 ^{abcd}	1.54	0.36 ^{ab}
M ₂	18.53 ^{bcd}	10.82 ^{de}	1.98	0.93 ^c
M ₃	19.07 ^{bcd}	9.34 ^{bcde}	1.76	0.47 ^{ab}
M ₄	14.11 ^{abc}	7.70 ^{abcd}	0.98	0.40 ^{ab}
M ₅	27.26 ^d	19.28 ^e	2.01	0.22 ^{ab}
M ₆	10.71 ^{abc}	7.03 ^{abcd}	1.50	0.38 ^{ab}
N ₁	6.33 ^a	4.2 ^a	1.29	0.32 ^{ab}
N ₂	9.66 ^{ab}	5.28 ^{abc}	0.86	0.56 ^b
N ₃	7.74 ^a	4.83 ^{ab}	1.47	0.34 ^{ab}
N ₄	8.42 ^a	5.25 ^{abc}	1.67	0.31 ^{ab}
N ₅	6.49 ^a	4.62 ^{ab}	1.04	0.11 ^a

(*) Means followed by the same letter(s) in the same column are not significantly different at $p < 0.05$ (DMRT).

Highest values of total Nitrogen (0.93%) was measured in mixed M2 plot. Compared with pure plots, the total nitrogen were significantly higher in mixed plots M2 followed by N2 (0.56%) and M3 (0.47%). Among all the plots the sequence of total nitrogen (%) were M2 > N2 > M3 > M4 > M6 > M1 > N3 > N1 > N4 > P1 > M5 > N5 (Fig. 5).

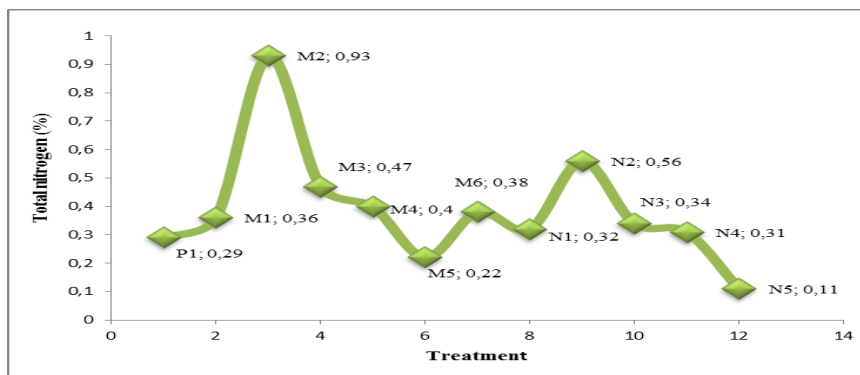


Fig. 5. Percentage of total nitrogen in the soil of twelve experimental plots

The present study reveals a total of 143 nodules per tree for *A. auriculiformis* in the mixture with *S. macrophylla* were comparatively more than Aryal *et al.* (1999) and Khan *et al.* (2004). Aryal *et al.* (1999) recorded 55 nodules per tree in a 14 months old plantation and Khan *et al.* (2004) recorded 61 nodules per tree in a 3 months old plantation for *A. auriculiformis* respectively. Compared with the findings of several other researchers, the findings of the present study were similar to that of Aryal *et al.* (1999) and Khan *et al.* (2004). The present findings also revealed that *A. auriculiformis* showed excellent nodulation behavior in the mixture with *S. macrophylla* than with *G. arborea*. The present investigation also recorded nodule fresh weight (2.01 g) for *A. auriculiformis* which was greater than Aryal *et al.* (1999). Aryal *et al.* (1999) recorded 1.34 g nodule fresh weight for *A. auriculiformis*.

A. auriculiformis (Akashmoni) is extensively planted in plantations all over the country for its short rotation, wider adaptability and faster growth. *S. macrophylla* (Mahagony) and *G. arborea* (Gamar) are two non-nitrogen fixing species extensively planted in forests, marginal lands, institutes, roadsides, railway sites, field borders and homesteads of Bangladesh. From the present investigation, it is evident that the mixed stands possess better effects than the pure stands in improving soil nitrogen.

In this study, the nodulation status of *A. auriculiformis* seedlings was measured at ten months after germination. In this respect, given the values presented in Table 2, it has been determined according to variance analysis applied that there were statistically significant differences among nodulation of this species in various treatments ($p < 0.05$). According to the results of Duncan Multiple Range test implemented, it has been found that the nodulation behavior of *A. auriculiformis* in pure plots were lower than in mixed plots. The present study also indicates the increment of nitrogen in the soil through plantation of nitrogen fixing tree species.

Though monoculture of exotic species such as *A. auriculiformis* has some effect on native biodiversity of Bangladesh, mixed plantation of *A. auriculiformis* with valuable indigenous tree species particularly *G. arborea* may show marvelous results. *A. auriculiformis* may also be planted in degraded hilly areas because it was reported to thrive well on moderately acid, poorly fertile soil and also improves pH, physical and chemical properties of soil. The present investigation indicates three most common plantation tree species (*A. auriculiformis*, *G. arborea* and *S. macrophylla*) of Bangladesh which were similar to Das and Sarkar (2014) conducted in the Bhawal Sal forest, in the central region of Bangladesh. Das and Sarkar (2014) observed significant positive relationship between species growth, diversity and productivity in four mixed species plantations and the subject species were *A. auriculiformis*, *G. arborea*, *S. macrophylla* and *A. mangium*.

From the present investigation it was also found that nodule size of *A. auriculiformis* seedlings varied from 2.6×3.3 mm in N_5 plot to 5.2×6.3 mm in M_5 plot which was greater than Khan *et al.* (2004). Khan *et al.* (2004) recorded

3.2 × 6.1 mm nodules in three months old *A. auriculiformis* seedlings under nursery condition.

Nodule number and nodule mass or nodule weight per unit dry weight of the whole plant or root system are often used in trial comparisons; however, similar information can be obtained by visually scoring nodulation on a 0-5 basis taking into account nodule number, size, pigmentation and distribution. Nodule score is judged by the number of effective nodules in the crown-root zone (regarded as the region 5 cm below the first lateral roots) and elsewhere on the root system (Corbin, 1977). The present study indicates an excellent nodulation status of *A. auriculiformis* in plantation forests by increasing nodule number below (5 cm) the first lateral roots.

The present study reveals that mixed species plantations of *A. auriculiformis* with other non-nitrogen fixing species (*G. arborea* and *S. macrophylla*) have the potential to improve nodulation, nitrogen fixation and stand productivity over that of *Acacia* monoculture through catalytic effects on soils by *Acacia*, which is similar to that of Forrester *et al.* (2007). A study was conducted by Forrester *et al.* (2007) reported that nitrogen fixation and productivity were higher in mixed stands of *Acacia mearnsii* with non-nitrogen fixing *Eucalyptus globulus* than in *A. mearnsii* monocultures.

Effectiveness of nodules can generally be gauged by the degree of pink or red coloration of N₂-fixing bacteroid tissue inside each nodule (Corbin 1977). As a general rule, white or green nodules are inactive and would not be considered when classifying active nodulation (Corbin 1977, Peoples 1989). The present study revealed that nodules of *A. auriculiformis* in pure and mixed plots were pink or brown in color. So the result indicates all the nodules in the root system were active.

CONCLUSIONS

The results of present investigation showed biological nitrogen fixation and nodulation status of *Acacia auriculiformis* in mixed plantations in comparison to pure plantations. During the present study, fixation of biological nitrogen in the soil through the root system of *A. auriculiformis* seedlings was considered as the only way of nitrogen increment in the soil, rejecting all other factors such as rainfall, storm, thunder-bolt, fertilizer treatment etc. The present findings would be so much helpful to carry out further investigation on the growth and development of different nitrogen fixing species to both acidic and alkaline soils before recommending them in mixed plantation programs. These results are based on 10-month old seedlings. So, further researches are necessary to assess the exact performance of *A. auriculiformis* in fixation of biological nitrogen in the soil.

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THE EFFECT OF HERBICIDES ON THE CHEMICAL CONTENT OF WHEAT GRAIN

SUMMARY

The importance of herbicides for weed control in wheat crop is extensively studied, but there is limited research about their impact on the chemical content of wheat grain. With the aim to analyse the effect of five commonly used herbicides on the chemical content of winter wheat crop (cv. Euclide) grown on Vertisol soil, field trial was carried out during 2012-13 in the central part of Kosovo. The five applied post-emergence herbicides were: Sekator WG (a.i. iodosulfuron-methyl-sodium + amidosulfuron + mefenpyr-diethyl-protectant), Lintur 70 WG (a.i. triasulfuron + dicamba), Granstar 75 WG (a.i. tribenuron-methyl), Mustang (a.i. florasulam + 2,4-D 2-EH) and a combination of Sekator WG (a.i. iodosulfuron-methyl-sodium + amidosulfuron + mefenpyr-diethyl-protectant) + Furore super EW (a.i. fenoxaprop-P-ethyl). There were seven treatments: five herbicide treatments, mechanical control (hand check) and an untreated control. The field trial was set in a randomized block design with four replications and elementary plots of 9 m². The chemical content of wheat grain (crude proteins, crude fibre, fat, starch, ash and moisture) was analysed using NIRS technique. Milled samples of 20 g were placed in special boxes and sealed with a lid of cardboard and placed in the NIRS's apparatus for the analyses. Three replications for each treatment were used for the statistical analysis. There was significant effect of herbicides for crude proteins ($F_{2,21} = 5.10$, $p < 0.05$), fat content ($F_{2,21} = 5.36$, $p < 0.05$), crude fibre ($F_{2,21} = 13.0$, $p < 0.05$) and ash ($F_{2,21} = 2.84$, $p < 0.05$). However, no significant effect of the herbicides on starch content was observed. Based on our results we suggest to additionally consider the effects of herbicides on the chemical content of wheat grain in the process of choosing appropriate herbicides for weed control.

Keywords: food quality, crude proteins, crude fibre, fat, starch.

INTRODUCTION

The winter wheat is one of the most important crops in Kosovo and is produced on about 102.000 ha with an average yield of 4.0 t/ha (KAS, 2014), but this depends and from wheat cultivars (Fetahu et al. 2013). Pests, plant diseases

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

and weeds cause high crop loss. Yield losses due to weeds have been reported to range from 5% up to 26% (Oerke, 2006; Dangwal et al. 2010). Thus, in wheat production, it is necessary to undertake control of weeds, and herbicide use is common to reduce weed infestation and thus crop loss.

The importance of herbicides for wheat control in wheat crop is extensively studied, but there is very limited research about the effects of herbicides on the chemical content of wheat grain. However, the dietary value of wheat is enormously important as wheat takes an important place among the crop species being widely grown as staple food sources. Many researchers investigated the chemical content of wheat grain (Demaj et al. 2009; Hala and Ibrahim 2011; Šramková et al. 2009, Kastrati, 1997; Kryeziu, 2008) or the content of protein in wheat grain (Bramble et al. 2002; Horvatic et al. 1981), other cereals and maize (Gellrich et al. 2003; Kastrati, 1997; Kryeziu, 2008; Mehmeti et al. 2010). However, to our knowledge, the effects of herbicides on the chemical content of wheat grain were only studied by Demaj et al. (2009) and Hala and Ibrahim (2011). Given this background, the aim of our study is to analyse the effect of applied herbicides on the chemical content of wheat grain.

MATERIAL AND METHODS

Field trial was carried out during 2012-13 on Vertisol soil in the central part of Kosovo to analyse the effect of five commonly used herbicides on the chemical content of winter wheat grain (crude proteins, crude fibre, fat, starch, ash and moisture). The chemical content was analysed using NIRS technique (Near Infrared Reflectance Spectroscopy; FOSS 6500) and the software WIN ISI III at department in Biotechnology in Zootechny. There were seven treatments: five herbicide treatments were applied at the end of tillering, mechanical weed control (hand check) and an untreated control (Tab.1). The field trial was set in a randomized block design with four replications and elementary plots of 9 m². The winter wheat crop (Euclide cultivar) was sown in beginning of November, by using 300 kg/ha seeds in good tilled soil, apparently treated with fertilizer NPK 15:15:15 in doses of 200 kg/ha.

During the vegetation of wheat crop in spring the supplementary fertilization with ammonium nitrate in doses 200 kg/ha was used. Field trial was treated using the Farmate special knapsack sprayer of the capacity of 20 l was used, and amount of water used was 400 l/ha.

For the chemical analysis around 2 kg sample of wheat grain were taken, which were milled into the grinder. Milled samples of 20 g were placed in special boxes and sealed with a lid of cardboard and placed in the NIRS's apparatus for the analyses. Three replications for each treatment were used for the statistical analysis.

Statistical Analysis

The data were analyzed following an Analysis of Variance (ANOVA) technique, and the mean values of treatments were calculated and significant

differences were determined by the Fisher's least significant differences (LSD) test, using the statistical computer programme VVStat (Vukadinović, 1994).

Table 1. Basic information on the applied herbicides

Treatments	Product	Active ingredient	Rate l, kg/ha	Time of application
1	Sekator OD	iodosulfuron-methyl Na+ amidosulfuron mefenpyr-diethyl-protectant	0.150 l/ha	Post-emergence
2	Lintur 70 WG	triasulfuron + dicamba	0.180 kg/ha	Post-emergence
3	Granstar 75 WG	tribenuron-methyl	0.015 kg/ha	Post-emergence
4	Mustang SE	florasulam + 2,4- D-2-EHE	0.600 l/ha	Post-emergence
5	Sekator OD + Furore super EW	iodosulfuron-methyl Na+ amidosulfuron mefenpyr-diethyl-protectant fenoxaprop-P-ethyl	0.150 l/ha 0.800 l/ha	Post-emergence
6	mechanical control (hand check)			once during vegetation
7	Control			

Agroecological conditions

In general, the climate of Kosovo is characterised by warm summers and cold winters, and the air temperature may range from -20°C to 35°C . However, in the western part of Kosovo, the average air temperature (11.0°C) and the annual rainfall are higher (about 780 mm), and the frost-free period (up to 225 days) is longer than in the eastern part (9.8°C ; 635 mm; 170 to 220 days, respectively). Data presented in (Tab. 2) were obtained from the meteorological station of Prishtina. The amount and distribution of rainfalls as well and temperature define the main characteristic of the climate and directly affect the germination and growth of wheat.

Table 2. Mean air Temperature ($^{\circ}\text{C}$) and Rainfall (mm) in Prishtina 2012/13 and between 1951-1980 ('average year') based on (Zajmi, 1996)

Year	Months											
	IX	X	XI	XII	I	II	III	IV	V	VI	VII	Ave.
Temperature												
2013/14	20.0	13.8	8.6	-0.6	1.6	3.9	6.6	12.8	16.7	19.0	21.6	11.8
Long term aver.	15.8	10.5	5.8	0.7	-1.2	1.3	4.8	9.8	14.4	18.0	19.7	9.1
Rainfall												
2013/14	13.7	60.4	29.6	65.9	38.7	5.8	70.3	40.4	122	55.3	32.6	48.6
Long term aver.	47.5	56.1	64.2	53.5	36.7	37	35.3	51.4	75.3	56.9	48.6	51.1

RESULTS AND DISCUSSION

According to achieved results, the chemical content of the harvested wheat grain differed between the herbicide treatments (Tab. 3). There were significant effects of the herbicides on crude proteins ($F_{2,21} = 5.10$, $p < 0.05$) and on the fat content ($F_{2,21} = 5.36$, $p < 0.05$). Also, significant differences were observed for crude fibre ($F_{2,21} = 13.0$, $p < 0.05$), ash ($F_{2,21} = 2.84$, $p < 0.05$) and moisture ($F_{2,21} = 5.67$, $p < 0.05$). In contrast, no significant effect of the herbicides on the starch content was found.

In the plots treated with triasulfuron + dicamba, the content of crude proteins in wheat grain was (15.25%) higher than in all other treatments (Tab. 3). The lowest content of crude proteins in wheat grain was measured in plots with mechanical control (13.72%). However, compared to the other treatments and beside the plots treated with the herbicide tribenuron-methyl significant differences were also found between the plots treated with the herbicide triasulfuron+dicamba (crude protein content 15.25%).

The results for the content of crude proteins are in accordance with Demaj *et al.* (2009), wherein the average content of proteins was 14.83%, in comparison with our results (14.14%). But, the results do not match with Krasniqi (2011), wherein the average content of proteins was lower (12.72%). However, according to Kastrati (1997), the crude protein content in winter wheat grain may range between 9 to 17%.

Moreover, the results achieved by Kryeziu (2008) for the winter wheat, using two different methods for analyses of crude proteins, showed that the crude protein contents are almost similar using the Kjeldah method (11.63%) or the NIRS apparatus (11.66%).

Table 3. The chemical content of winter wheat grain (% of dry matter)

Treatments	CP	Fat	CF	Ash	Moisture
iodosulfuron-methyl-natrijum + amidosulfuron mefenpir-diethyl +(protectant)	13.87 ^b	2.18 ^a	1.12 ^b	1.74 ^a	10.59 ^b
triasulfuron + dikamba	15.25 ^a	2.04 ^c	0.74 ^a	1.84 ^b	10.74 ^a
tribenuron-methyl	4.68 ^{ac}	2.13 ^b	1.10 ^b	1.85 ^b	10.64 ^{ac}
florasulam + 2,4- D-2-EHE	13.94 ^b	2.14 ^b	1.10 ^b	1.82 ^b	10.66 ^a
iodosulfuron-methyl-natrijum + amidosulfuron +mefenpir-diethyl +(protectant) + fenoxsapro-P-ethyl	13.74 ^b	2.21 ^a	1.26 ^c	1.79 ^{ab}	10.49 ^b
mechanical control (hand check)	13.72 ^b	2.12 ^b	1.23 ^c	1.81 ^b	10.66 ^a
control	14.33 ^{bc}	2.10 ^b	1.13 ^c	1.84 ^b	10.72 ^a

Legend: CP-Crude Proteins; CF-Crude Fibber; LSD-Least Significant Difference; Means within columns that do not share a letter are significantly different.

Compared to all other treatments, the wheat grain of the plots treated with the herbicide triasulfuron + dicamba had the lowest content of fat (2.04%; Tab. 3). Additionally, compared to the other treatments and beside the plots treated with the herbicide iodosulfuron-methyl-sodium + amidosulfuron mefenpyr-diethyl significant differences were also found between the plots treated with the herbicide combination iodosulfuron-methyl-sodium, amidosulfuron + mefenpyr-diethyl + fenoxaprop-P-ethyl (fat content 2.21%).

Again our results are in accordance with Kastrati (1997), who found a range of fat contents in winter wheat grain from 0.85 to 4.0%. However, the mean total fat content of the winter wheat grain of our study (2.13%) differs from results of Demaj et al. (2009), where the average content of total fat was (1.67%).

The content of crude fibre was higher in the plots treated with a combination of the herbicides iodosulfuron-methyl-sodium, amidosulfuron + mefenpyr-diethyl + fenoxaprop-P-ethyl (1.26%), while the lowest content of crude fibre (0.74%) was found in the plots treated with triasulfuron + dicamba.

In general, the differences in the chemical content of wheat grain between the studies cited above could be due to different cultivars tested in these studies (e.g. we analysed the wheat cultivar 'Euclide', while Demaj et al. (2009) used the variety 'Novosadska rana 1'), since cultivar-specific protein contents are well known for wheat (cf. e.g. Yahata et al. 2005).

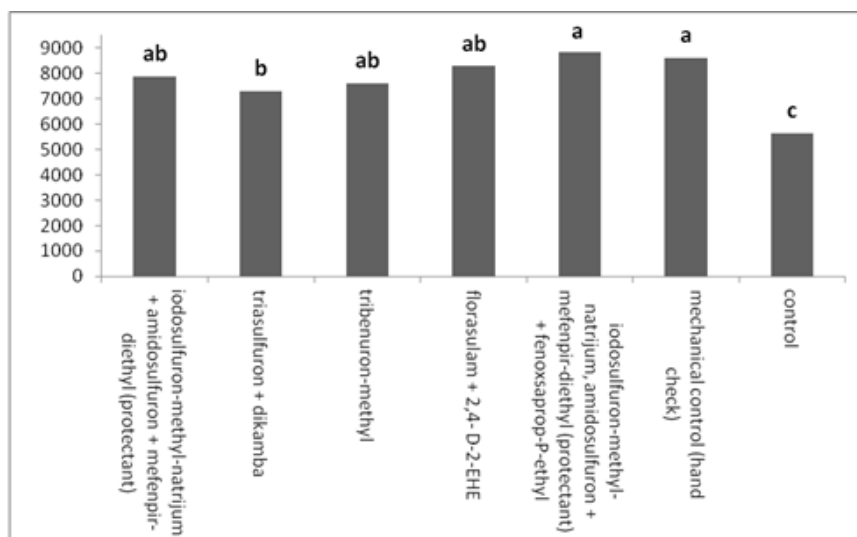


Figure. 1. Yield (kg/ha) of winter wheat depending on treatments.

LSD0.05 = 822; Means that do not share a letter are significantly different.

Moreover, climatic conditions may also affect the quality of grains and grain yields (cf. Cesevičienė et al. 2009; Tarakonavas and Ruzgas 2007; Mehmeti and Demaj 2010; Lalić et al. 2007), and may influence the amount of proteins of wheat grain. In this context, precipitation and temperature are particularly important (Benzian and Lane 1986). Finally, also soil type and

structure, water amount and nutritional mineral substances may impact the chemical content of wheat grains (Kirkman et al. 1982; Moss et al. 1981; Smika and Greb 1973).

In our study, the used herbicides also affected the yield of wheat (Fig. 1). These results are in accordance with Mehmeti and Demaj (2010), Knežević et al. (2014), Abbas et al. (2009), and Kika (2012). Furthermore, it is important that besides choosing appropriate herbicides to control weeds and affect the yield of wheat in future herbicides should be selected in base of their impact on the chemical content of wheat grain.

CONCLUSIONS

The conclusions of the study are:

- The applied herbicides affected the chemical content of the wheat grain.
- The highest content of crude proteins was found in plots treated with the herbicide triasulfuron + dicamba (15.25%), and the lowest in the plot with mechanical control (hand check) with (13.72%).
- The highest grain yield has found in the plots treated with the herbicide combination iodosulfuron-methyl-sodium + amidosulfuron mefenpyr-diethyl-protectant + fenoxaprop-p-ethyl (8.825 kg/ha).
- These results are based on a one-year field trial. Thus, additional research considering more than one year and more than one parcel are needed to come to more accurate conclusions. However, given the results of our study, the future process of choosing appropriate herbicides for weed control may additionally consider the effects of herbicides on the chemical content of wheat grain and thus on food quality.

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ASPECTS OF URBAN AND RURAL LAND CONSOLIDATION AND ACCURACY PROBLEMS OF GIS DATABASE FOR THE NEEDS OF SPATIAL PLANNING DOCUMENTS

SUMMARY

Spatial development of urban and rural areas is a priority task in an adequate urban land management of all modern societies. Although provided by the Law on Agricultural Land, unfortunately such measures are not implemented in Montenegro. One of the main problems is in unresolved legal property relations as well as in the out of date existing cadastral records. A cadastral record is a variable category that should be adjusted to the current situation through the maintaining process. Practice has shown that the rights holders do not report timely a large number of changes of the registered status in the cadastre to the administrative authority.

A particular problem is disagreement of surfaces in graphic and alphanumeric GIS database, but all that together affects big problems when adopting the cadastral maps as conditionally accurate on the development and implementation of detailed urban plans. Problem persists in operational cadastre on the entire territory of Montenegro, and its genesis and way of resolving it are specifically analysed in examples in the Municipality of Bar.

Keywords: land consolidation, cadastral maps, detailed urban plan, property title, GIS, database, DKP Bar.

INTRODUCTION

Operational cadastre represents a complete documentation of public and private rights and restrictions for users and land owners, which is based on state surveying (Kaufmann and Steudler, 1998). Its statement is geodetic and cadastral information system with a database in which the land registration and cadastral mapping are aligned. Also, human beings and activities of a society in the geographic space are at the same time both a problem and systematic solution for sustainable development.

Some of the challenges faced today have a critical geographic dimension- especially when it comes to natural disasters, climate changes or the process of urbanization. The catastrophic earthquake that took place on 15th of April 1979 and the consequences left on the area Montenegrin coast, especially in the Municipality of Bar, imposed the need to use the survey data with greater

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accuracy and more homogenously. The characteristic found in Bar is that it had a major disagreement of the points of geodetic networks between each other. To understand the etiology and exploratory nature of this problem, it is necessary to explain theoretical and practical base of the city trigonometric networks.

Territory of Montenegro with 1382623 hectares is divided into 23 political and 796 cadastral municipalities, and according to the cadastral record data, there are about 1 300 000 cadastral plots. The major problem shown in practice is quality of the existing documentation which is to be used in new surveying. Namely, in the most cadastral municipalities for which the surveying is to be done, there has been into force the listed cadastre which is used mainly for fiscal purposes and only for calculation of cadastral income. Value and quality of property record established in such way are limited on multiple grounds, especially in rural areas.

Agriculture is an activity that has high economic potential for the Municipality of Bar- arable land includes about 5.551 hectare. From the point of geo-ecological conditions in the existing spatial planning documentation (GUP Bar 6.118.58 ha) two parts are clearly separated: narrow coastal area (indented seashore with a significant number of larger and smaller bays and capes) and mountain ranges of Rumija, Lisinja and Sutorman. The spatial statement of geo-ecological resources within the analysed documents is resumed to: optimal climate of sea and lake (Skadar Lake) shore, its distinctive morphology (46 km) with unique beaches, extreme wealth of biodiversity, appealing environment of old and new urban settlements. The existing documentation recognizes area of Bar as one of the three areas of intensive agriculture: the total area of olive trees complex is 477.7 ha (nearly 110 000 trees), which are located in the areas around the fast traffic road; the total area of other agricultural land (around 100 000 citrus trees, orchards 2217 ha, vineyards 245 ha, field crops, etc.) is 826.81ha. Particular problem is that these areas are mostly within the mixed space intended for housing and work (the area below the existing railway plants and the main road up to Gorak, part of Polja, Čeluga, Tomba, Zaljevo as well as Pečurica and Kunje).

For many years Montenegro is facing the issue of constructing objects of various types, surface and purpose, which are in contrary to the positive legislation of Montenegro which regulates this field. There is an established term "informal" construction for such phenomena. It is a construction without a building permit or without the required legal and technical documentation, which in the long term permanently changes and destroys the space and directly reduces both current and future development opportunities. Illegal construction has recently taken hold in huge proportions, thus creating big problems in terms of public utility, transportation, hygiene and environment, organization and legal property relations. In the future, in these areas it is necessary to resolve all problems in technical, economic and legally rational and feasible way.

Number of constructions built contrary to regulations is measured by tens of thousands. The field of spatial development and construction in Montenegro

legal system is regulated by the Law on Spatial Planning and Construction ("Official Gazette of Montenegro", no. 51/08, 40/10, 34/11, 40/11 and 47/11), which does not provide specific procedure when it comes to development of planning documents that are used for integration of constructions built without building permits - since the issue has been resolved systematically. In the process of developing the planning documents, full attention is unquestionably dedicated to constructions built without a building permit. Vienna Declaration on Informal Settlements in South Eastern Europe, which Montenegro signed in September 2004, envisages that signatory countries aim at the complete regional resolution of informal settlements by the year 2015 and signatories shall undertake in-situ regularization and upgrading to the maximum extent.

The old Law on Construction Land ("Official Gazette of Montenegro" no. 28/80 and 12/86) did not recognize the ownership in the construction zone. In fact, natural and legal entities could have had the right to manage, use or permanently use and dispose the land in the social ownership and now it is a state property as long as the state is the owner. It was only when the new Law on Construction Land has been adopted ("Official Gazette of RM" no. 55/00) and the Law on Ownership and Legal Relations ("Official Gazette of RM", no. 19/09) with the Article 419 that this right is transferred into ownership right. However, this change of the right is not made *ex officio*, but at the request of the party. Attempts to abuse this right are often. Namely, many natural persons in the second half of the last century, when the recordings for real estate cadastre were done, registered the state property for their use and more recently tried to transfer it into their ownership. Many took advantage of that interim period and sold land for which they had only the right to use it and only the adoption of the Law on Ownership and Legal Relations led to determination of these abuses.

MATERIAL AND METHODS

In this paper we define these problems and we used the results within the case study which has been done on the example of cadastral records of Bar. The study in spatial terms covers also parts of the rural area of municipality. The analysis includes its geo-ecological problems, particularly those related to agricultural land.

With the analysis we have covered both the planning documentation and targeted field observations along with geodetic and cadastral information system with a database, which confirmed the existence of so-called the primary problems in this area: along with those related to conversion and endangered land, there is also its visible fragmentation especially due to the construction of family houses and cottages.

The next big problem is disputable cadastral records and inaccuracies in the existing GIS database, which cumulatively creates problems in development and implementation of urban plans and compromises their application in practice.

With modern GIS tools all problems become visible when using imprecise old cadastral maps and databases: with digitizing and georeferencing plans of old

graphic surveying, the problem is that such substrates are often useless in overlapping with ODK or DOF, along with the out of date cadastre and disagreement of cultures in the cadastre with the ones on the ground. Many cadastral plots are converted and registered under a different culture. It is often the case that the same plot has one data or culture in cadastral record and different one in the land registry.

That is the reason why we offered concrete proposals, which are based on previous results and/or experiences from ex YU region, and we provided original solutions with a highlighted explorative character. Also, possibilities of implementing consolidation projects have been considered.

Urban and rural land consolidation with possible application in Montenegro

Land is limited and most valuable resource on disposal to the mankind. With this in mind, it is clear that land management has a very important role in development of mankind and that the existence of an efficient system of land management is necessary condition for development of each country. Although the land management always has had high importance, it seems that such importance has never been greater than in modern times because of the rapid urbanization that is taking place in all countries of the world, though not at the same time (Šoškić, 2016).

Land consolidation (in Dutch: ruilverkaveling; in French: remembrement; in German: Flurbereinigung) means "a comprehensive procedure of deploying existing rural areas with fragmented agricultural or forest landholdings or their parts" (Vitikainen, 2004). It can be defined as the agrarian-technical operation which represents socio-economic and political measure, and which aims to group and encompass land properties in order to provide optimum conditions for profitable production (Lukic, 1977). Along with grouping and creating plots with proper shape, land consolidation increases the surface of productive land, reduces processing costs, creates conditions for expansion of commodity production, creates favourable conditions for association of farmers, carries out rationalization and construction of road and drainage network and provides basis for urbanization of settlements.

First land consolidations are known in Italy as early as in XIV century, then Keptan in south eastern Bavaria in the mid of XVI century. The first land consolidation in the former Yugoslavia when a settlement was planned took place (subdivision) in Tornjoš, a small settlement during consolidation of Municipality of Senta (1963-1966).

Taking into account the way of using and population of the area where it is done, the rough distinction could be made between rural and urban land consolidation.

Rural land consolidation is used as a synonym for integrated rural development, although it is common for this expression to represent an outdated type of agricultural structural development. Integrated rural development is generally referred to the development of rural areas. "Between the land

consolidation and spatial planning as a measure for spatial development, there must be a connection as required by the legislation. The question is what are such connections, whether the spatial plans provide a solid basis for development of land consolidation project and to which extent the items of spatial plans can be achieved through the process of land consolidation? Problems arise when a certain part of space is not "covered" with spatial or urban plan and then within such space the territory is used and developed spontaneously. If a territory is not covered by such plans, then on the margins of the construction zone of the planned settlement the population can spontaneously settle without the threat that their residential and other buildings could be later demolished "(Trifkovic, 2001). In such cases, the geodetic experts leading the land consolidation are given the difficult task to practically legalize the existing situation within the spatial development of construction zone.

"The most effective way of land consolidation of rural development is a comprehensive land consolidation, but in some cases other approaches such as simplified land consolidation, voluntary group land consolidation and individual initiative for land consolidation can bring a benefit" (Čvorić, 2011).

The land consolidation project provides also a project of planning and reconstructing the rural settlement and the land for the common needs of the settlement is allocated, by adopting a common coefficient of reduction for all participants in land consolidation, in a way which takes from each owner a land in proportion to his/her possession.

FAO (Land Tenure Studies 6, 2003) recommendations for improvement of rural areas are "good practices" for countries in transition. One of important European positions is to reduce the gaps between rural and urban areas through improvement of situation in the villages. The range of objectives of land consolidation in rural development is very broad, starting from agricultural development to reconstruction of villages and protection and improvement of environment. Land consolidation can be used as a very efficient instrument and a starting point of rural development so the landowners have more opportunities for improvement of their situation.

Urban land consolidation as an instrument for development of construction land has been used in Western Europe for more than a century. In recent decades urban land consolidation is used in many developed countries as well as in developing countries worldwide. It relies on the spatial or urban plan of the observed area and usually is an instrument for their implementation. It develops land in modern way that all participants have interest in. A land achieves significantly higher market value through its conversion from agricultural land into construction land. In such process a new cadastre record and land register are established, on the basis of exposing the data on surveying and ownership to the public.

Larsson (Larsson, 1997) lists a number of ways for implementing measures of urban planning. The first one is to adopt a new urban plan and to wait its realization through the usual procedures, over time. This requires a lot of

time, but also problems arise in coordinating its implementation. Another way is to purchase the entire land of the territory plan by a single owner, whether it to be one private or public entity or a state institution. This process is very expensive and also requires a longer period of time. The third way is urban land consolidation, which is being increasingly applied in recent times. It is an organized method that has a formal organization. In this process, the structure of land boundaries is to be changed but the land owners are not changed. Urban land consolidation in a number of countries in the world is increasingly used as an instrument for implementation of measures for urban development. The process of urban development can be described in three main phases which include planning, land development and construction of infrastructure. Urban land consolidation is classified as a stage of land management although, in certain cases, it can be a part of the planning phase which includes development of spacial and urban plans.

In Montenegro, the land consolidation has been carried out on the basis of the Law on Agricultural Land ("Official Gazette of Montenegro", No.15/92, 59/92 and 27/94). Work's program on developing agricultural territory through land consolidation refers to works that are envisaged by the Strategy for Development of Food Production and Rural Areas.

Montenegro does not have a lot of large agricultural areas at the same complex. The plots are small and fragmented and in such conditions, the production costs are high which increases a sales price. Production in difficult natural conditions on very fragmented property does not enable stronger modernization, which causes low competitiveness. It is even worse that there is no legislation which would prevent further property fragmentation. There is a big problem in conversion of purpose of agricultural land, by which this non-renewable resource is permanently lost. Also, one of the main problems for adoption of such measures is in unresolved legal property relations as well as the out of date and inaccurate existing cadastral records. There is a characteristic example in the attempt of implementing such project in Mrčevo Polje and Grbalj behind the beach Jaz, where after trying to determine the factual situation the project was stopped. We are witnesses that these unresolved relations still persist on this attractive territory.

The total area of agricultural land in 2012, according to MONSTAT data was 515,717 hectares, which represents 37.34 percent of total territory of Montenegro, out of which 190,000 hectares are cultivated. Statistical data focus our attention in two levels: first, it points out that if we look at the total agricultural land, Montenegro has valuable and rich resource - 0.82 ha/per capita. However, if we reduce it to the category of arable land, this value is significantly lower - 0.30 ha / per capita. If we follow the methodology of EU countries, which in the category of arable land does not include meadows, then this geoecological potential at the level of Montenegro is very low -0.009 ha / per capita and thus very sensitive and endangered. This level focuses our attention to physical, chemical and biological degradation of agricultural land, including pollution. A

particular problem is conversion and "consumption" of land for urbanization, roads, industrial and other facilities. This resource in municipalities of Montenegrin coast is particularly threatened and its protection, optimal and sustainable management involves primarily the existence of valid and practically operational GIS database records on immovable property.

In the period from 1998 to 2002, the works on agricultural land consolidation were carried out only on the territory of Municipalities of Ulcinj on the area of 100 ha and Municipality of Herceg Novi (Sutorinsko Polje and Bračkovina) on the area of about 120 ha. This program included a plan on developing an agricultural land consolidation on the area of about 240 ha in Municipalities of Budva and Kotor (Mrčevo).

So far in Montenegro urban land consolidation has not been applied for construction land development, at least not in a form and a manner in which all of its potentials would be used. The urban land consolidation is the easiest way of providing necessary land for public space, infrastructure facilities and other public purposes, without which any development of settlement is unimaginable. Providing a land for common needs is not possible without reducing the surface of property of participants in urban land consolidation. On the other hand, this is a way which the land not suitable for construction converts into the land suitable for construction and its market value increases significantly which compensates to participants the loss of a certain part of the property.

Among many effects it is important to resolve and manage all legal property relations among the participants of land consolidation. It is indisputable fact that actual situation on the ground in a large percentage is not in line with the status that is registered in the land cadastre records and land registry. There are many effects that would be achieved with the land consolidation projects but unfortunately the budget or any announced investments do not mention them. Perhaps a model of public-private partnership would be a good way in which the private sector and the state could find common ground. However there are many problems which are obstacles to starting these processes as well as in adopting the very urban plans and hereinafter only some of them will be described.

Updated cadastral maps and use of the existing GIS database

Operability and importance of new IC technologies in geodetic and cadastral records of urban units, is the fact that changes the way and dynamics of its use. GIS with a database is the "fuel" that accelerates these changes, especially in developing digital maps and their database, in dynamics of inserting the changes and in the way of maintaining a modern cadastre (Longley et al., 2005).

On the other hand, flows of data manipulation in cadastral record impose the need for distributing spatial information to a wide range of users. User requirements relate to the review and research of data in the form of maps, to the search for list of entity attributes according to certain criteria, to the control of displaying layers and finally, to generating appropriate reports and/or basis.

When it comes to advanced users of these technologies, it is primarily about creating physical and logical data model for spatial information with integration of available data, and creating the appropriate topology and data analysis. At the state and local level advanced software systems are mainly used for managing spatial data through ESRI ArcGIS architecture (ArcExplorer, ArcMap, ArcCatalog, ArcPad), where the data can be manipulated from a local computer or as clients with a full functionality with the request to use GIS data from remote server over the Internet. For clients with full functionality, most of the operations related to manipulation of spatial data take place on the client's side, where the role of server is to make available the shared information. ESRI ArcIMS is used for distribution of maps, data and meta-data via the Internet. The system is designed for easy creation of maps, for development of web pages with interactive maps and for administration of WEB-GIS sites. The architecture of ArcIMS refers to the presentation, the level of business logic and data level.

The database of agricultural land is based on: legal frames and Development Strategy for Food Production and Rural Areas, GIS-oriented information system which is expressed through the alphanumeric and graphic cadastral data and DOF, and the data related to records, mode of use and management. It can be modularly upgraded; it allows control of the collected data and assignment of attributes based on the spatial relations between geometric objects. Its ultimate goal is to know the status i.e. sustainable management of each cadastral plot.

Cadastral municipality is a territorial unit that as a rule covers an area of inhabited settlement, which has the statutory name and which represents the basic unit where surveying is performed and real estate cadastre is made. Administration body establishes borders of cadastral municipality, by placing geodetic marks and describing borders in the borders record book (National Assembly of the Republic of Montenegro (2007).

RESULTS AND DISCUSSION

In the Municipality of Bar there have been a number of cases in which during the establishment of the land cadastre in 1974, the registration of the right to use was carried out without any legal basis, and in the archives of the cadastre of the period there are only lists that cannot be taken as proof of ownership. Therefore, when establishing the Real Estate Cadastre the previous entry should have been taken as a record of the property at that time and there should have been an obligatory request for the legal basis for acquisition through exposure of data to public and establishment of actual rights on real estate.

Previous realisations of urban plans consisted of designing and determining the boundaries of construction plot in which, in most cases, the existing parcelling did not match the new projected boundaries or the rules important for construction and public needs. Thus during the establishment, the new building plots usually consist of parts of the existing cadastral plots that belong to different owners, which in practice very often presents insurmountable

obstacles for resolving ownership and legal relations. The end result is that the construction plot is to belong to one entity, which would later get the right to build on it. The conflict between designed situation and ownership situation is supported with the emergence of incompliance between the boundaries of using the land in the field and the actual legal status of plots boundaries in the real estate cadastre.

The surfaces of cadastre plots are determined in different ways: from the original measure, from the measures taken graphically from cadastral map, from the coordinates of detailed points of cadastre plots from cadastre maps or by planimetry – an instrument for mechanical calculation of surface.

In practice, we meet a number of examples in which the difference between the surface of plot registered in the property title and the surface that is obtained from the coordinates of detailed points of cadastral map is bigger than the allowed value that is obtained by the formula (Rulebook on content and method for state survey of immovable properties, draft version, 2015):

$$\Delta = 0.0007 * \sqrt{P} * M \quad (1)$$

where M is the denominator of the plan scale and P is the plot surface.

Example 1: *The difference between the plot surface in property title and cadastral map*

Cadastral plot no. 4386/1 KO Novi Bar from the property title number 1173, which is located within the plan that was developed in the scale of 1: 1000. The surface registered in the property title is 310 m². When the surface area is calculated from the coordinates of the turning points, than the following is obtained P = 202 m². That plot has approximately 35% less surface on the map and at the same time it is the surface that could be marked on the ground.

The difference in the surface value is in this case 108m², which means that the potential buyer could be mistaken when purchasing a plot, i.e. the person could pay the sum as for 310m², although in fact 108m² do not exist, having in mind that the legal transactions are based on data from the cadastral documentation in accordance with the principle of trust in the real estate cadastre. According to this principle, a conscientious right obtainer can rely on credibility of what is written, which means truthfulness and completeness. Usually the parties are familiar with these "problems in Cadastre" and in a purchase contract (as it must be linked to data from property title) the surface from the numerics is stated but in fact the real price comes down to what the plot contains on the field.

The consequence of this problem is also in spatial planning where for the needs of calculating urbanistic parameters (urbanistic potentials) there are used and exclusively taken as the reference value those surfaces registered in the cadastral records.

Proposal for solution: a solution to this problem has a legal and geodetic aspect. From the geodetic aspect it is possible to survey the site and determine whether the boundaries on the field correspond to the boundaries in the cadastral map and in that case it would be possible to carry out a correction of surface in property title. However, from a legal point of view changes in the surface require

a legal basis. If, for example is taken the case where the purchase contract is a legal basis of registered owner, that owner could with the previous owner conclude an annex to the contract which would state the exact surface from the plan but the limitation is in the rule that the seller cannot transfer more rights to the buyer than those rights possessed by the very seller. Usually, in practice the predecessor after selling a real estate is not interested in the problems of followers, so these annexes are difficult to obtain. In addition, the surface which is registered in the property title represents the basis for calculating property tax, property transfer tax, etc. One solution is for the Real Estate Administration to adopt an act that would implement a surface from the map and the field through the property title. There should be defined an "Elaborate on Compliance of Surfaces" which are calculated in the previous period and surface areas from the coordinates without changing geometry of plots. Such elaborate would have multiple usages in the process of corrections in the real estate cadastre, in issuing urbanistic and technical conditions and building permits, etc.

In addition to errors in the procedure of establishing a real estate cadastre and cadastral maps at certain locations there are wrong positions of boundaries of cadastral plots due to non-compliance with regulation in the process of maintaining the real estate cadastre when primarily the parcelling of cadastral plots was done on the basis of measurements of fronts (Djurovic, 2011).

The cadastral map is developed on the basis of aero-photogrammetric surveying where the owners were required to lawfully mark the boundaries of their plots.

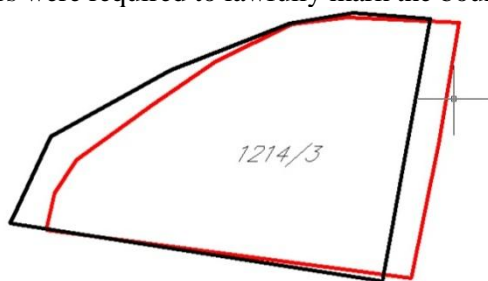


Figure 1. Example of problem in reconstruction of boundaries

Example 2: Cadastral plot no. 1214/3 KO Mišići, the Municipality of Bar. Black colour shows a cadastral plot and red colour shows the position of plot obtained on the basis of surveying the existing boundaries on the spot. Obviously this is a translational displacement. When in such case the boundaries of the parcel on the ground should be marked based on the coordinates from cadastral map, the absurd situation arises in which the spot on the ground is marked with 2-3m of distance compared to the existing border based on which the boundary is established in cadastre.

Proposal for solution: to determine the age of the boundary i.e. whether the boundary was established before the aero-photogrammetric surveying. If so, and during the presentation of the surveying data to public and during the registration

of the right no complaints from the neighbours were submitted, a border that is on the ground should be adopted through the procedure of planning the borders.

Example 3: *A concrete example of disagreement of boundaries of cadastre plot in relation to their actual position on the ground*

At the same site there is an example in which the position of the road is completely misrepresented on the cadastral map so that when its position is marked on the ground it "goes" over the existing facilities and the road really exists on the ground but it is in fact located behind the facility on its northeast side.

After the surveying, there were decrypted on photo plans and marked the details and the boundaries of plots and the cultures and facilities were drawn. In this procedure, which consists of several phases the errors occur which results in the mapping of the details in wrong position. On the other side, the obvious flaw of boundaries in the real estate cadastre should be able to get re-transferred at any time to the field through professional cadastral surveying with reasonable accuracy which means that the borders can be reproduced on the site (field). This return transfer of established boundaries is carried out within the framework of marking the boundaries of the plots.

Figure 2 shows the position of cadastral plot no. 825/171 KO Šušanj where the blue line is the actual position on the basis of surveying with polar method on the ground and the black line is position on the cadastral map. On the north-east side of the plot there is a road which according to the cadastral map "goes" over the recorded existing facilities.

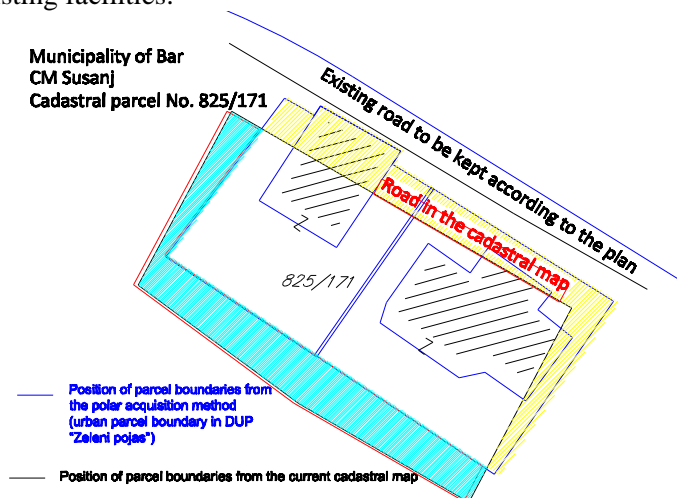


Figure 2. Overview of boundaries of cadastral plot no. 825/171 KO Šušanj.

Proposal for solution: Based on the position of the existing borders of immovable property on the field and the position of boundaries of cadastral plot no. 825/141 on the cadastral map there can be concluded that the property owner keeps on the ground a yellow hatched area within the property and that the owner is not registered as the holder of the property but according to the cadastral data it

represents a fraction of non-categorized road (although in fact the road is located above the presented position) while the area which is hatched in green is beyond the fence of the owner's property although in the cadastral register the owner is registered as holder of the right on ownership on that part of the plot.

Accepting the cadastral boundaries as conditionally accurate and by planning the road on the position shown on the cadastral map we would come into a situation that the road paves the way over the existing facilities and such facilities could not be registered in the cadastre. Given that these discrepancies of boundaries of immovable property are not the result of shift in boundaries and occupation on the field, but are result of incorrect mapping on the cadastral map, there would be a great harm to owners of real estate that is not caused by their fault but by the work of state bodies. For these reasons there is a necessity to check GIS database and for accurate and precise determination of the current situation before development of planning documents in order to avoid problems in implementation of the plan.

Based on the given examples we can conclude that the position of boundaries of cadastral plots which are shown on the cadastral map cannot be accepted as a priori true but there is a need to provide for development of detailed urban plans a quality and updated geodetic basis but in practice, usually due to the lack of funds we stick to alternative solutions and use the existing cadastral and topographic maps as well as the data gathered by authorized geodetic organizations in the process of maintaining the real estate cadastre.

CONCLUSIONS

Agricultural land consolidation increases the income from agriculture and simultaneously creates material and social conditions for improvement of socio-economic conditions in rural areas, as well as for overcoming negative interpersonal relationships arising from disputes over land. Although most of the rural territory of Montenegro with its geomorphological characteristics is not the most favourable for implementation of these projects in typical form, for this neglected part there should be found one of already applied models from developed countries.

Increasing urbanization, if not accompanied by appropriate mechanisms for development of urban land, can cause a number of problems. One of the biggest problems is the provision of land for public purposes (streets, green surfaces, parks, kindergartens, schools, health institution, etc.). Another problem is provision of appropriate structures of plots (shape, size, position) in accordance with their intended purpose. From the above mentioned, it can be concluded that it is necessary to develop urban land management system that will effectively resolve all of these problems (Šoškić, 2016).

Out of major issues and projects that are waiting in the near future, the inevitable one is the problem of illegally constructed buildings which according to some estimates are in the amount over 100 000 in Montenegro and are mainly concentrated in small and medium-sized settlements around urban areas. It is an

unofficial data arising from the fact that significant number of informal constructions is not registered in the real estate cadastre, i.e. there are much more of them than shown by the official data.

The greatest challenges faced today have a critical geographic dimension- especially when it comes to natural disasters, climate change or uncontrolled urbanization process. Disadvantages of cadastral records and inaccuracies in the existing GIS databases, which are described in this paper as causes to the problems in development and implementation of urban plans, can be divided into three categories: the out of date cadastral data, which is reflected in great number of changes that have occurred among real estates on the field and which are not recorded at the cadastral map and cadastral records; inaccuracy in boundaries positions of cadastral plots and in topographic details as well as difference of surfaces registered in property title and surfaces that are obtained by calculations from the cadastral map.

Operability and the importance of new IC technologies in geodetic and cadastral urban complexes is the fact that changes the way and dynamics of its use. GIS with a database is the "fuel" that accelerates these changes, especially in terms of dynamic in inserting changes and maintaining a modern cadastre. Most of the plots in Montenegro have a problem of discrepancy between data in graphic database and in alphanumeric one. In this paper due to the problems in uniformity of geodetic networks in Bar we analysed examples and offered solutions for the territory of that Municipality.

Through the procedure of parcelling the cadastral plots according to planning document there can be resolved the problem of deviations of boundaries in cadastral plots compared to the position of the existing borders on the ground provided that the drafter of the plan before making the parcelling plan is equipped with up to date and quality cadastre - topographic maps. Otherwise, accepting the cadastral defined boundaries position of real estate and topographic details eliminates the possibility of harmonizing the current situation on the field with the situation in the cadastral records.

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SELECTION FOR TRAITS ASSOCIATED WITH DROUGHT TOLERANCE IN DURUM WHEAT

SUMMARY

This study was conducted to evaluate the 22 durum wheat genotypes based on morphological, phenological and physiological traits, to determine trait relations with yield in different levels of drought stress; and to assess their potential use in breeding for drought tolerance in durum wheat. The genotypes were evaluated in three cropping seasons (2008-11) under rainfed conditions, which the variation in the annual rainfall was provided a range of drought scenarios in durum wheat trials. The measured traits included grain yield (GY), plant height (PH), peduncle length (PL), flag-leaf length (FL), spike length (SL), days to heading (DH), days to maturity (DM), harvest index (HI), 1000-kernel weight (TKW), number of seeds per spike (NSPS), relative water content (RWC), relative water loss (RWL) and chlorophyll content (SPAD reading). Based on the results drought tolerant genotypes were characterized for higher TKW, HI, SPAD, PH and PL/PH and lower DH, RWL, DM and SL. Stepwise regression analysis indicated that PH, RWC, RWL and short grain filling period could be instrumental in predicting the drought tolerance of durum wheat genotypes. In conclusion, relative estimates of genotypes response to drought couldn't be obtained in a single growing season in Mediterranean conditions i.e., Kermanshah region in west of Iran, and the effect of drought depends on the severity, frequency and duration of stress.

Keywords: durum wheat, drought stress, regression analysis, trait selection.

INTRODUCTION

Durum wheat (*Triticum turgidum* L. var. durum Desf.) is grown in 10% of the world's wheat area. It occupies about 11 million ha in the Mediterranean basin. Rainfall and temperatures in Mediterranean dryland areas show large and unpredictable fluctuations within and across cropping seasons (Mohammadi et al. 2011). Drought stress is the most important reason to yield loss in this area. Drought tolerance is the ability of a plant to survive periods with insufficient

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

uptake of water. The effects of drought stress vary depending on the frequency, duration, and intensity of stress and growth stages affected (Munoz-Peraet al. 2006). The improvement of crop productivity under drought conditions requires genotypes with good agronomic traits, drought tolerance and yield stability. Selection for grain yield under drought stress conditions is difficult due to its low heritability resulting from variations in the intensity of the stress throughout the field (Blum, 1988, Ludlow and Muchow 1990). Nevertheless, the probability of increasing yield where there is significant stress and genotype x environment (G×E) interaction is high (Blum, 1989).

As a major crop, wheat has gained special attention in respect to morphological and physiological characters and traits affecting drought tolerance. Agronomic traits such as grain yield and yield components have also served as criterion for evaluating drought tolerance (Dencic et al. 2000). Rong_Hua et al. (2006) concluded that chlorophyll content (SPAD reading) could be considered as a reliable indicator in screening barley genotypes for drought tolerance. Water deficient was found to reduce the relative water content (RWC) in plant leaves. The high RWC and low relative water loss (RWL) have been suggested as important indicators of water status (El-Tayeb 2006; Gunes et al. 2008). The number of kernels per spike is the most affected yield component and this has been proposed as an important selection criterion for drought tolerance (Shpiler and Blum 1986, 1991).

The objectives of this study were to evaluate the 22 durum wheat genotypes (breeding line, old and new varieties) based on morphological, phonological and physiological traits in different levels of drought stress to determine the traitrelations with yield in durum wheat; and to estimate heritability and gain from selection for different traits and assess their potential use in breeding for drought tolerance in durum wheat.

MATERIAL AND METHODS

Twenty two durum wheat genotypes including 20 breeding lines along with one improved new cultivar (cv. Saji) and one durum wheat old variety (Zardak) were evaluated in three cropping seasons (2008-11) under rainfed conditions. Each trial was conducted in a randomized complete block design with three replications. The trials were carried out at Sararood research station (47° 16' N; 34° 19' E; 1351 masl) of Dryland Agricultural Research Institute (DARI), Iran. Plot size was 7.2 m² (6 rows, 6 m long, with 20-cm row spacing). 45 kgha-1 Nitrogen and 45 kgha-1 phosphate fertilizers applied before planting by urea and super phosphate triple. For control of weeds used 1.5 litha-1 of 2-4-D in wheat tillering. The grain yields were measured on a plot basis and converted to kg ha⁻¹ for the statistical analyses. In addition to grain yield, the other measured and recorded traits included plant height (PH), peduncle length (PL), flag-leaf length (FL), spike length (SL), days to heading (DH), days to maturity (DM), harvest index (HI), 1000-kernel weight (TKW), number of seeds per spike

(NSPS), relative water content (RWC), relative water loss (RWL) and chlorophyll content (SPAD reading). SPAD was recorded on three flag leaves for each genotype at anthesis, using a chlorophyll meter (SPAD 502 Plus, Spectrum Technologies, Plainfield, IL, USA). Days to heading was designated as the time 50% of the plants in a plot had at least one open flower. Days to maturity was recorded when 50% of the plants in a plot had yellow leaves. PH, PL, FL and SL were measured for each genotype at physiological maturity. HI measured from harvesting biomass of one meter length of each plot and calculated by dividing grain yield to biomass. To measure RWC, fresh leaves were taken from each genotype and each replication at anthesis stage and weighted immediately to record fresh weight (FW). Then they were placed in distilled water an overnight and then weighted again to record turgid weight (TW), and subjected to oven drying at 70°C for 48 h to record dry weight (DW). The RWC was calculated using the following equation (Turner, 1986):

$$\text{RWC} = ((\text{FW} - \text{DW})/(\text{TW} - \text{DW})) \times 100$$

To measuring relative water loss (RWL), five youngest fully expanded leaves were sampled for each of three replications under rainfed conditions at early flowering stage. The leaf samples were weighted (W1), wilted for 2 hour at 35°C, reweighed (W2), and oven-dried for 48 h at 70°C to obtain dry weight (W3). The RWL was calculated using the following formula (Yang et al. 1991):

$$\text{RWL (\%)} = [(W1 - W2)/W3]/((t2 - t1)/60)$$

Where, t1 and t2 are the measuring time for initial and wilted weight (in minutes).

Combined analysis of variance was used to partitioning of variance explained by year (Y), genotype (G) and G×Y interactions. Biplot methodology based on principle component analysis (PCA) was applied to study the relationships among studied traits and to characterize of tested genotypes.

RESULTS AND DISCUSSION

Climatic conditions were considerably different from year to year and crops experienced different level of drought stress during three cropping seasons (Fig. 1). Total rainfall was 288.3, 453.9 and 342.5 mm in three consecutive cropping seasons, respectively. The precipitation patterns were obviously different in three years (Fig. 1). Amount and distribution of precipitation and higher average temperature in winter prepared a favorable growth season for the crop in 2009-10 (Y2), but in 2008-09 (Y1) and 2010-11 (Y3) low rainfall with unsuited distribution accompany with lower temperature in winter, provided an unfavorable growth season and drought stress, especially in Y1. Results of ANOVA showed effect of year was significant on all studied traits indicating the

existence of different climatic conditions in different years (Table 1). Genotypes were significantly different in RWL, FL, PL, PH, PL/PH, NSPS, DH, DM and TKW. Genotype \times year ($G \times Y$) interaction was significant for all the traits except for DH, DM and RWC. The significant $G \times Y$ interaction for the traits suggests that the rank order of genotypes changed significantly from one year to other. So relative estimates of genotype response to drought couldn't be obtained in a single growing season in a specific location, Kermanshah, Iran, and the effect of drought depends on the severity, frequency, and duration of stress. Genotype effect wasn't significant for grain yield (GY). Heritability founded in traits showed PL, PH, SPAD and DM had the highest heritability among studied traits, and GY had the least heritability (Table 1) that shows it is a polygenic trait and is more variable in different conditions. Mean comparisons based on LSD test at 5% level of probability for each studied trait is presented in Table 2. The results indicated significant differences between genotypes for each of the traits, showing genetic variation among genotypes for the studied traits.

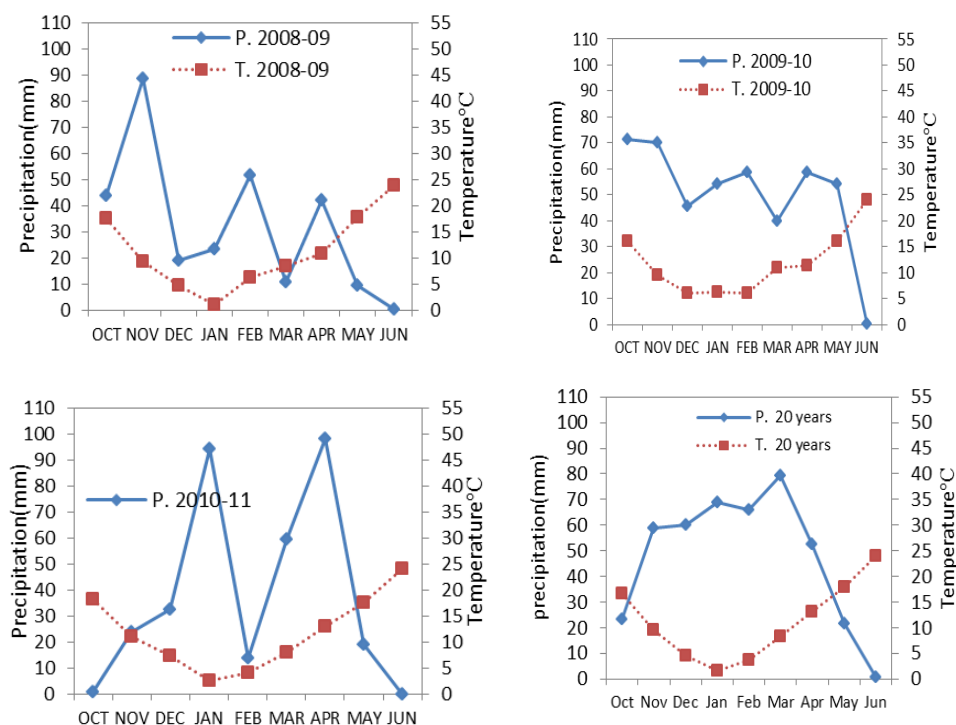


Figure 1. Ombrothermic diagram of 2008-9, 2009-10 and 2010-11 in compared with average long-term (20 years).

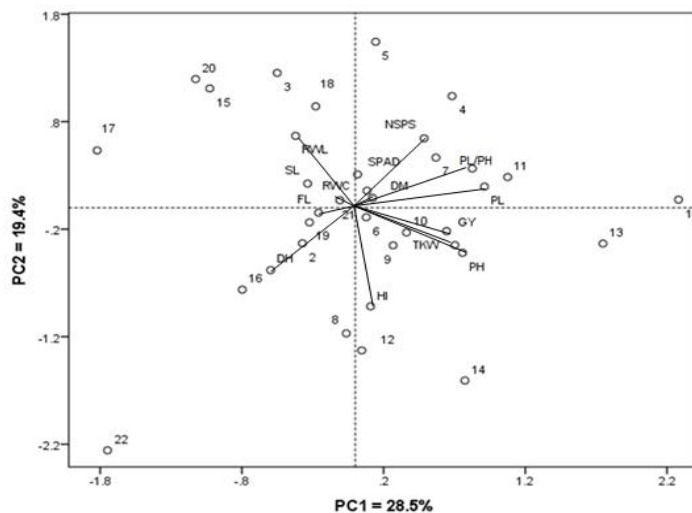
Table 1 .combined analysis of variance and heritability for developmental, productive and physiological traits of 22 durum wheat genotypes across three years

Traits	Year	Rep/Y	Genotype	G×Y	Error	Heritability
df	2	6	21	42	126	0.17
RWC	3913.7*	708.5	115.7 ^{ns}	72.6 ^{ns}	62.4	0.28
RWL	2.7**	0.1	0.0*	0.02*	0.0	0.32
SPAD	1215.3**	4.9	29.7 ^{ns}	13.0**	8.9	0.44
SL	58.4**	0.5	1.0 ^{ns}	0.2**	0.3	0.38
FL	643.0**	0.4	9.4**	3.9**	2.1n	0.41
PL	4112.4**	5.7	28.6**	14.6**	3.5	0.51
PH	19002.0**	38.4	238.0**	79.5**	23.1	0.47
PED/PH	0.3**	0.0	0.0**	0.0013**	0.0	0.34
NSPS	3011.2**	23.9	112.5**	59.9**	25.1	0.32
DH	2264.4**	2.5	12.6**	2.5 ^{ns}	5.3	0.22
DM	3190.4**	3.0	4.6**	1.7 ^{ns}	2.3	0.44
TKW	6566.2**	16.8	26.2**	9.5**	4.5	0.33
GY	417336000**	164246	736219 ^{ns}	431378**	158766	0.09
HI	0.344**	0.004	0.005 ^{ns}	0.005*	0.003	0.17

*, ** significant at 5% and 1% level of probability; ns: non-significant

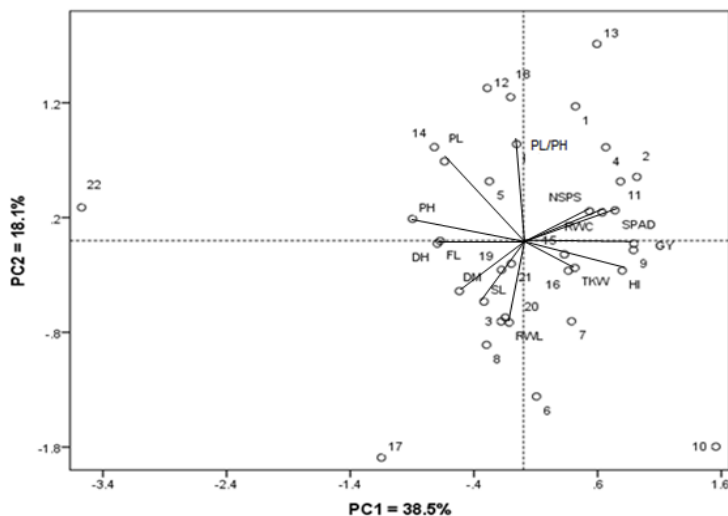
ph: plant height; pl: peduncle length; fl: flag-leaf length; sl: spike length; dh: days to heading; dm: days to maturity; grain gy: grain yield; tkw: 1000-kernel weight; nsps: number of seeds per spike; rwc: relative water content; rwl: relative water loss; spad: chlorophyll content; hi: harvest index.

A biplot based on pc analysis for evaluated traits of 22 durum genotypes was generated for each dataset year. In 2008-09 (severe drought stress, in compare to long term data, see fig. 1), pc1 accounted for 28.5% of variance which gy, tkw, ph, pl, pl/ph had high value of this component and dh and rwl had negative values of pc1. Pc2 accounted for 19.4% of variance that nsps, rwl had high values and hi, ph, tkw and dh had negative values of this component. Based on the first two pcs, a biplot was constructed for graphic analysis of data (fig. 2). The traits which located in the same direction of biplot have positive correlation together. Gy, ph, tkw, pl, pl/ph were positively correlated and the genotypes no. 1, 13 and 11 can be selected according to these traits. Rwl and dh had negative correlation with grain yield as indicated by obtuse angle between vectors, showing that the shorter dh resulting in higher grain yield. Geravandi et al. (2011) found negative correlation between gy and rwl. The traits including rwc, dm, fl and spad had the shortest length vectors, so they were not correlated with other traits information. Thus, under severe drought stress condition higher ph, tkw, pl and pl/ph and shorter dh and lower rwl may be used as useful indices for genotype selection.



*Numbers are stand for genotypes and vectors for traits. Ph: plant height; pl: peduncle length; fl: flag-leaf length; sl: spike length; dh: days to heading; dm: days to maturity; grain gy: grain yield; tkw: 1000-kernel weight; nsps: number of seeds per spike; rwc: relative water content; rwl: relative water loss; spad: chlorophyll content; hi: harvest index.

Figure 2. Genotype by trait biplot showing the interrelationship among the studied traits of 22 durum wheat genotypes in 2008-09.



*Numbers are stand for genotypes and vectors for traits. Ph: plant height; pl: peduncle length; fl: flag-leaf length; sl: spike length; dh: days to heading; dm: days to maturity; gy: grain yield; tkw: 1000-kernel weight; nsps: number of seeds per spike; rwc: relative water content; rwl: relative water loss; spad: chlorophyll content; hi: harvest index.

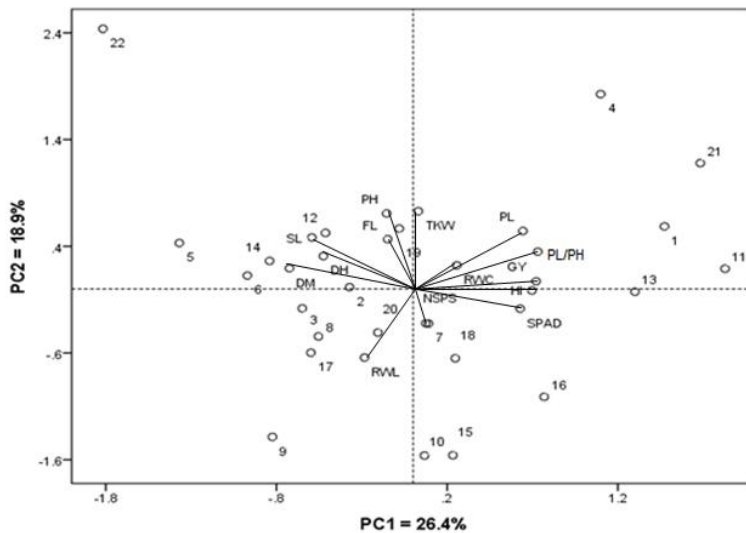
Figure 3. Genotype by trait biplot showing the interrelationship among the studied traits of 22 durum wheat genotypes in 2009-10.

Table 2. Mean comparison and descriptive statistics for the developmental, productive and physiological traits for 22 durum wheat genotypes across three years.

Entry	RWC*	RWL	SPAD	SL	FL	PL	PH	PL/PH	NSPS	DH	DM	TKW	GY	HI
1	63.1	0.37	50.3	5.74	14.5	16.7	76.8	0.211	34.0	184.0	219.8	28.7	3357.8	0.33
2	71.5	0.42	52.3	6.22	14.9	11.6	74.5	0.146	36.0	185.8	219.5	27.3	2997.6	0.33
3	74.9	0.53	51.1	6.69	15.9	11.9	71.9	0.156	31.5	184.8	220.7	24.8	2971.1	0.27
4	73.7	0.45	50.5	6.42	15.2	15.8	79.4	0.190	36.4	184.7	219.5	29.6	2953.9	0.32
5	71.5	0.51	50.8	6.39	15.0	12.9	77.2	0.159	36.8	185.8	221.3	27.5	2777.0	0.29
6	72.6	0.47	48.9	6.13	14.9	12.2	70.0	0.166	31.9	186.7	221.3	29.2	2813.9	0.30
7	67.3	0.42	52.1	6.17	13.7	12.4	76.0	0.156	32.7	184.2	221.2	28.6	3126.2	0.32
8	71.3	0.43	47.6	5.82	13.5	11.5	73.6	0.148	31.4	187.2	221.2	26.3	2989.8	0.36
9	69.8	0.42	49.9	5.90	12.5	11.7	70.4	0.154	29.8	184.5	219.8	27.5	2835.1	0.33
10	75.4	0.50	49.9	5.95	12.6	9.6	69.9	0.132	28.1	183.5	219.7	29.2	3415.4	0.35
11	76.9	0.40	50.7	5.76	15.0	14.5	73.0	0.192	34.1	184.7	219.7	26.4	3456.3	0.33
12	68.5	0.37	48.4	5.75	13.9	14.0	79.1	0.164	31.4	187.2	220.7	25.7	2900.6	0.33
13	76.1	0.41	51.1	5.70	14.5	16.4	75.1	0.210	30.3	184.5	219.5	29.0	3257.3	0.35
14	72.5	0.45	47.7	5.59	14.4	13.8	80.8	0.161	29.5	187.7	220.3	28.0	2850.7	0.35
15	73.2	0.63	48.3	5.61	14.3	13.1	73.4	0.167	38.1	185.5	219.5	24.4	2871.2	0.30
16	72.3	0.49	51.0	6.04	14.3	11.9	74.2	0.150	28.3	185.2	219.8	27.9	3018.1	0.33
17	65.5	0.49	51.2	6.71	14.5	11.6	75.3	0.139	30.0	186.8	221.0	24.4	2762.4	0.31
18	67.4	0.53	51.0	5.84	15.2	13.1	73.0	0.169	35.5	185.2	219.0	24.2	2904.4	0.30
19	71.6	0.44	49.6	5.97	13.9	13.3	74.8	0.165	29.2	186.0	220.5	28.4	2687.1	0.32
20	68.6	0.58	48.8	6.01	14.5	11.9	73.5	0.151	30.6	185.5	220.2	25.9	2935.0	0.28
21 (Saji)	71.2	0.40	52.2	5.94	12.8	13.2	79.4	0.156	30.4	185.3	220.0	28.2	3067.0	0.33
22 (Zardak)	66.3	0.43	44.7	6.65	16.9	15.4	94.2	0.140	22.7	187.3	221.0	27.2	2087.1	0.32
SE	2.8	0.05	1.2	0.15	0.7	1.3	3.0	0.012	2.6	0.5	0.4	1.0	218.9	0.02
LSD (5%)	8.1	0.13	3.4	0.44	1.9	3.6	8.5	0.034	7.4	1.5	1.3	2.9	624.8	0.07
Mean	71.0	0.5	49.9	6.0	14.4	13.1	75.7	0.2	31.8	185.5	220.2	27.2	2956.1	0.32
Max	76.9	0.63	52.3	6.71	16.9	16.7	94.2	0.211	38.1	187.7	221.3	29.6	3456.3	0.36
Min	63.1	0.37	44.7	5.59	12.5	9.6	69.9	0.132	22.7	183.5	219.0	24.2	2087.1	0.27

*rwc: relative water content; ph: plant height; pl: peduncle length; fl: flag-leaf length; sl: spike length; dh: days to heading; dm: days to maturity; gy: grain yield; tkw: 1000-kernel weight; nsps: number of seeds per spike; rwl: relative water loss; spad: chlorophyll content; hi: harvest index.

In 2009-10 (favorable condition which was near to normal year according to 20 years data, Fig. 1) PC1 accounted for 38.5% of variance which GY, SPAD, HI, TKW and RWC had high value of PC1 and PH, DH, FL and PL had negative values of PC1. PC2 accounted for 18.1% of variance that PL/PH and PL had high values and RWL and SL had negative values of PC2. The traits GY, SPAD, HI, TKW, RWC and NSPS were positively correlated together and the genotypes no. 9, 11 and 2 were found to be superior based on these traits. FL, DH, PH, DM, SL and PL were negatively associated with grain yield as indicated by acute angle between their vectors (Fig. 3). So, under favorable condition higher SPAD, HI, TKW, RWC and NSPS and lower FL, DH, PH, DM, SL and PL may be used as useful indices for genotype selection.



*Numbers are stand for genotypes and vectors for traits. ph: plant height; pl: peduncle length; fl: flag-leaf length; sl: spike length; dh: days to heading; dm: days to maturity; gy: grain yield; tkw: 1000-kernel weight; nsps: number of seeds per spike; rwc: relative water content; rwl: relative water loss; spad: chlorophyll content; hi: harvest index.

Figure 4. genotype by trait biplot showing the interrelationship among based on mean values for 22 genotypes in 2010-11.

In 2010-11 (mild severe drought stress, in compare to long term data, see Fig. 1) PC1 accounted for 26.4% of variance which GY, HI, SPAD and PL/PH had high value of PC1 and DM, SL and DH had negative values of PC1. PC2 accounted for 18.9 % of variance that TKW and PH had high values and RWL had negative value of PC2. The traits of GY, HI, PL/PH, PL and RWC showed positive correlations, and the genotypes no. 11, 13, 1 and 21 were found to be superior based on this group of traits. DM, DH, SL, RWL and FL had negative correlations with grain yield as indicated by obtuse angle between their vectors (Fig. 4).

In favorable condition, selection for higher HI, SPAD and PL/PH and lower DM, SL and DH will be enhanced yield productivity in durum wheat genotypes. In both drought years (Y1 and Y3; sever and mild severe drought stress), PL was positively associated with grain yield, whereas RWL and DH negatively correlated with grain yield indicating that positive and negative selection for these traits will be enhanced yield productivity in durum wheat under severe drought condition. In three level of stress, selection for lower DH will be positively associated with high grain yield, showing the importance of early flowering under stress conditions.

The results of stepwise regression analysis based on grain yield for each level of drought stress condition are presented in Table 3. Under severe drought

condition, grain yield was significantly influenced by RWC, RWL and PH, while under favorable condition the traits RWC, RWL, FL, PH, NSPS and HI most affected grain yield and under mild drought condition the grain yield significantly influenced by the traits of SPAD, NSPS, DH and DM. PH and RWC were important in severe drought stress (Y1) for selecting genotypes, while these traits in year with mild stress(Y3)had no effect on grain yield and in year with favorable condition (Y2) had negative effect on grain yield.

In favorable year (Y2) FL, NSPS and HI had positive effect on grain yield. In mild stress condition (Y3) short grain filling period (GFP) was important to produce higher yield because DH and DM had positive and negative effects to grain yield, respectively. SPAD and NSPS were contributed positively and negatively to grain yield productivity, respectively. However, traits including PH, RWC, RWL, DH, DM and SPAD can be considered as traits associated with drought tolerance in durum wheat genotypes.

Table 3. Stepwise regression analysis for traits associated with grain yield under different level of drought stress.

Drought stress level	variables	Regression coefficient	R ² -adjusted
Y1: Severe stress	Constant	-1557.1	49.7
	RWC	10.8	
	RWL	-604.1	
	PH	43.6	
Y2: Favorable year	Constant	14385.5	87.1
	RWC	-68.7	
	RWL	-7580.3	
	FL	113.5	
	PH	-74.1	
	NSPS	24.2	
	HI	7457.4	
Y3: Mild stress	Constant	9287.2	42.5
	SPAD	53.8	
	NSPS	-22.3	
	DH	62.7	
	DM	-95.4	

*PH: plant height; FL: flag-leaf length; DH: days to heading; DM: days to maturity; NSPS: number of seeds per spike; RWC: relative water content; RWL: relative water loss; SPAD: chlorophyll content; HI: harvest index.

Improvement of grain yield depends on genetic variability for yield and its components. There were significant differences among the genotypes for the studied traits, which can be exploited for improving of grain yield under drought prone environments.

In severe drought stress condition (Y1) higher PH, TKW, PL and PL/PH and lower DH and RWL were found to be useful indices for genotype selection. Dencic et al. (2000) stated that in wheat cultivars, the number of kernels per spike, TKW and particularly grain yield were more drought sensitive, than plant

height and number of spikelet per spike. RWC is widely used as indicator of leaf hydration status, which is controlled by the balance of water loss and capacity for water uptake (Rachmilevitch et al. 2006). Maintenance of leaf water status is important for protection of physiological and biochemical function during drought stress (Damayanthi et al. 2010). Sun et al. (2013) stated that RWC had positive correlation with net photosynthesis rate, stomatal conductance, and negatively correlated with canopy-air temperature difference. Khakwaniet al. (2012) suggested that rainfed cultivars retained higher stomatal conductance and relative water content (RWC) under water stress conditions. A very high variability for this trait had been observed by Morgan (1980) in the *Triticum* genus. In our study SPAD and HI were possessed in common in two years (Y2 and Y3) in positive association with grain yield.

DH was only trait which its low values was index for higher GY in these three different conditions, so it is the most important traits under rainfed conditions. Zhong- hu and Rajaram (1994) found that yield, kernels per spike, biomass and plant height were more drought sensitive compared with spike number and 1000 kernel weight. Gasura et al. (2014) found a strong positive correlation of grain yield with the grain-filling traits that include EGFD (effective grain filling duration), and TGFD (total grain filling duration) that shows the influence of these traits on GY formation in maize. Longer EGFD and TGFD results in the accumulation of more photo-assimilates in the grains during grain-filling (Lee and Tollenaar2007).

Longer grain-filling durations imply more dry matter accumulation, and hence high kernel weight that translates into high yield (Gasura et al. 2013). In the two droughty years (Y1 and Y3), PL was positively associated with grain yield whereas RWL and DH negatively correlated with grain yield indicating that positive and negative selection for these traits will be enhanced yield productivity in durum wheat under severe drought condition.

Based on the results, the traits of PH, RWC, RWL, DH, DM and SPAD can be considered as indicators of drought tolerance in durum wheat genotypes. In many crops, leaf chlorophyll content and its indirect assessment via SPAD readings proved to be heritable traits related to leaf physiology, yield, and quality (Ramesh et al. 2002, Le Bail et al. 2005, Songsri et al. 2008).

These were evidenced despite the putative bias from both abiotic and biotic factors, which may curb the leaf chlorophyll–SPAD correlation especially at high values (Markwell et al. 1995, Uddling et al. 2007).

CONCLUSIONS

In order to evaluate the effectiveness and reliability of phenological, physiological and grain yield traits for screening drought tolerant genotypes, many studies were conducted at controlled and field conditions. Based on the results of this research, according to PCA we concluded that the results indicated the drought tolerant durum genotypes had the higher TKW, PH, HI, SPAD and PL/PH and the lower DH, RWL, DM and SL. Regression analysis permitted us to

conclude that PH, RWC, RWL and short period between DH and DM could be instrumental in predicting the drought tolerance of durum wheat genotypes. In summary, relative estimates of cultivar response to drought couldn't be obtained in a single growing season in Mediterranean conditions i.e., Kermanshah region in west of Iran, and the effect of drought depends on the severity, frequency, and duration of stress.

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*Aleksandra Despotović*¹

INFLUENCE OF EDUCATION ON DEPOPULATION OF VILLAGE

SUMMARY

Population is a significant factor in rural development, because it recruits its workforce from the existing population, but also because of the fact that the population is a holder of needs which are the factor of consumption or production.

Demographic situation in Montenegro has been constantly deteriorating. Economic and social consequences of the demographic aging of the population are numerous: reduction of the share of economically active population, decelerating of technological progress, etc. From the aspect of rural development, negative consequences of demographic (depopulation and deagrarisation) are reduction of agricultural population. It is one of the most important changes in the socio-economic structure of the population. This led to disorder of age and gender structure of the agricultural population, in terms of increased share of elderly („senilisation”) and increased participation of women („feminization”).

Educational structure of the population is of special importance for rural development. However, if it is considered the education level of the agricultural population, it can be concluded that it is very unfavourable. One of the reasons is the process of deagrarisation, which led to a massive abandonment of agriculture and rural areas by working population. From a sociological point of view, a transfer of farmers into non-agricultural activities is not only a consequence of industrialization, but also a number of other factors.

One of them is enhanced communication with other worlds (spread of literacy, radio, television, etc.), which increased a social mobility of once poor farmers even more movable (Maksimović, 2011).

This paper analyzes the sociological factors that led to the depopulation and deagrarisation. Indicators of the educational structure (level of literacy and educational attainment) in fifty year period (1961-2011) have been analyzed. Conducted analysis shows that the process depopulation was not caused only by economic, but also by a range of non-economic factors. T

he most creative people were leaving rural areas what had a very adverse impact on agricultural production. Population and identification and management of natural resources is of great importance for future development of rural communities.

Keywords: education, population, depopulation, agriculture

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INTRODUCTION

In modern development conditions of any society, education appears as an essential condition and a basic factor whose impact has far-reaching consequences, both on overall development of the intellectual and physical capabilities and on the development of economy and society as a whole (Skara, Lj., 1972). Educational equity is a constitutive element of human capital and, therefore, one of the basic factors of intellectual equity (Koković, 2009, Petty, Guthrie, 2000). Before beginning the process of modernization of society (the second half of the twentieth century) Montenegro was an agrarian country where agriculture was the dominant branch. In the period after World War II, Montenegro had high agrarian population in relation to the Yugoslav average (Vukčević, 1963). The educational structure of the population in general and agricultural was characterized by a high proportion of illiterate people and even those without school. The lowest percentage of illiterate population in Montenegro was in Kotor (26.8%), and it was followed by Cetinje with 47.9% (Vujošević, 1990). Also, in Kotor was the largest number of residents who lived from non-agricultural activities, (Vujošević, 1990).

After World War II, Montenegro faced process of depopulation and deagrarisation. The unfavourable position of agriculture and private households, as well as favourable conditions in the city resulted in escape from the countryside and from agriculture, (Čikić, J. 1983). Economic theory of the twenties of the twentieth century tried to prove that the main cause of rural depopulation was low productivity of agricultural labour in relation to work in non-agricultural activities (Clark, 1955). Sociological theory emphasises „revolution of civilisation“, which disintegrated rural communities and seemed supportive in relation to abandonment of the village.

Transfer of knowledge „from generation to generation „was an integral part of the socialization of future farmers. Education of children in a peasant family was unnecessary luxury, because the workforce was being lost in such cases (Čikić, 2012). Despite the progress made in agro-industrial complex, education reform did not provide assistance to farmers to produce sufficient quantities of products, and to protect environment and prevent devastation of area at the same time (Petak, 1988). Gap in the level of education between people from city and countryside deepened. Continuation of unfavourable characteristics of the educational structure was influenced by the low social status of the peasantry, primarily the lack of funds needed for education. Only wealthy members of peasant society went to school but education was considered as a status symbol (Čikić, 2012). Educated groups were marginal among the rural and agricultural population. According to Petak, the rule is that all those who are educating leave the village and farms, and all those who do not see their future in the village, try to use education as a channel of deagrarisation. After the first wave of uneducated migrants from countryside who had found their job opportunities among unqualified and skilled workers, the next wave of migrants focused their education on non-agricultural activities (Čikić, 2012). The post-war

technological progress has caused decrease in demand of labour and thus had made room for its education, but mostly for non-agricultural occupations. Changes that occurred were in conflict with the principles of traditional peasant economy, according to which the impact of production on peasant holdings depends on the available number of hands, and number of household members (Mandras, 1986).

Today, education is the most important factor of social mobility of particular young people. Due to the underdevelopment of rural communities, young people see education as an opportunity „to escape from the countryside“. An increasing number of young people opt for non-agricultural occupations and remain to work in larger urban centres. Unfavourable position of agriculture and peasantry had led to fewer parents wanting their children to be engaged in agriculture (Novak, 2010).

The aim of this paper was to analyze the indicators of the educational structure in Montenegro in the fifty year period 1961-2011 and their impact on the depopulation of rural areas.

MATERIAL AND METHODS

The paper analyzes the indicators of the educational structure in Montenegro and their impact on the depopulation of rural areas. The analysis was conducted for the period 1961 - 2011, when censuses were conducted in Montenegro. The indicators were analyzed in two aspects: participation of illiterates aged 10 and over in the total population and participation of all levels of education for the population aged 15 and over. In preparing the paper, the official data of the Statistical Office of Montenegro (MONSTAT) were used, as well as scientific and professional papers that dealt with this issue. In order to display the data, the statistical tables and graphs were used. Using the relative numbers of structure, it is shown the share of illiterate people in the total population aged over 10 years, the share of the population aged 15 and over by educational attainment and gender. Dynamic statistical analysis, namely, the method of calculation of basic and chain indices were used, as well as the methods of descriptive statistics. The paper uses the methods of research at the table "desk research" and methods of comparison. The paper aims to draw attention to the causes of uneven regional development in Montenegro.

RESULTS AND DISCUSSION

Period after 1945 represents milestone in the development of socio-economic relations in Montenegro. Changes made in all aspects of social and economic development had positive effect on the development of educational structures, in both its essential aspect - participation of illiterates and participation according to educational qualifications. Literacy is the basis, initial form of acquiring further education and education level of an individual. It is a prerequisite for cultural development, which has a capital importance for communication and understanding between people (Skara, Lj., 1972). A large

number of studies have confirmed a visible impact of literacy on the positive effects of employees in manufacturing. Strumlin wrote „that literacy a worker acquires after only one year of primary education contributes to increasing of labour productivity by about 30%, while the length of service of illiterate workers increases the qualification and the effects by 12 to 16%“, (Skara, Lj. 1972). Illiteracy is inevitably accompanied by poverty, unemployment and other social vices. Table 1 shows the participation of illiterate people in the total population aged 10 and over in Montenegro in inter-censuses period that took place between 1961-2011.

Table 1. Participation of illiterate people in the total population aged 10 and over in Montenegro, 1961-2011.

	1961	1971	1981	1991	2003	2011
Total number of illiterate people aged 10 and over	352.04 9	418.17 7	477.07 7	511.91 4	537.89 8	542.64 9
Total number of illiterate people	76.368	69.805	44.633	30.443	12.617	8.149
% participation of illiterate people in total number of population aged 10 and over	21,70	16,70	9,40	5,90	2,35	1,50

Source: Calculation of the corresponding author according to the data in Montenegro, 1961-2011

Data in Table 1, show a social progress of the changes in the number of illiterate population. Specifically, total number is gradually decreasing. Percentage of illiterate population in 1961 stood at 21.7% - it was reduced to 1.5% according to the 2011 census. In the fifty years of the reporting period, the number of illiterates decreased by approximately 68,129 (decreased from 76,368 to 8,149).

Table 2. Trend of illiterate people aged 10 and over in Montenegro according to basic and chain indices, 1961-2011.

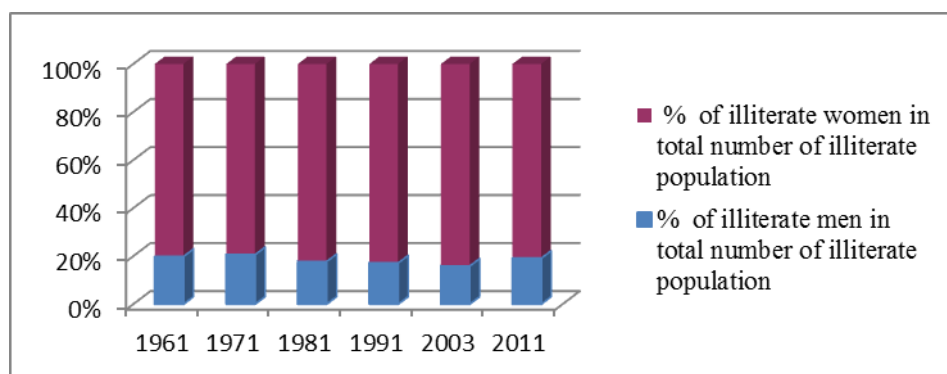
Year	Basic index 1961 =100	Chain index
1961	100	-
1971	91,40	91,40
1981	58,44	63,99
1991	39,86	68,20
2003	16,52	41,44
2011	10,67	64,58

Source: Calculation of the corresponding author according to the data in Montenegro, 1961-2011.

On the basis of the calculated indices where 1961 is taken as the base year, it is clearly shown a drop of illiteracy, which was the most obvious at the 2011 census and it was about ninety index points compared to the base year of 1961.

Chain indices show a decline in illiteracy even by individual census years. However, despite the results achieved over a period of fifty years, we should point out the fact that the official statistics of Montenegro continues to lead the data on literacy, which speaks volumes that illiteracy has not been eradicated. Contrary to our practice in a number of countries in which illiteracy and registration of this phenomenon ceased to exist long ago. Thus, for example - Austria ceased with practice of putting the question of literacy in Census questionnaire in 1934. In the forties of the twentieth century, this practice was discontinued in Japan, Denmark, Sweden, Finland, Switzerland and Canada. Immediately after the end of World War II, question on literacy was omitted in the Netherlands, Czechoslovakia, Great Britain, Norway, Luxembourg, Australia and some other countries.

Tendency of decreased participation of illiterate population is evident, but also different if it is observed by gender.



*Source: Calculation of the corresponding author according to the data in Montenegro, 1961-2011.

Figure 1. Percentage of the participation of illiterate people according to gender in total number of population aged 10 and over in Montenegro, 1961-2011.

In the fifty year period, illiteracy rate was reduced both in men and women. The share of illiterate women in the total number of illiterate population aged 10 years and over was significantly higher than the proportion of men and it was at a level of about 80%. The number of illiterate women decreased by approximately 54,593 (reduced from 61,183 to 6,590) and men by about 13,626 (reduced from 15,185 to 1,559). Analysis of illiteracy by gender shows that a social status of women was lower than the position of men. Women acquired all necessary knowledge on the basis of direct personal experience or by inheritance from her mother, grandmother, sister. Therefore, it is no wonder that women in traditional village were mostly illiterate (Isić, 2008). After World War II there was a change in the educational structure of women which was a result of the development of non-agricultural activities. Disadvantage of agriculture and peasantry led to the fact that parents had decreasingly wanted their children to be engaged in agriculture (Novak, 2010). According to the survey, which was

conducted on three generations of farmers, none of the participants would have wanted their daughter to be interested in agriculture, and only 8.4% in 1968, 6.6% wanted their son to be involved in the field of agriculture in 1978 and 10.8% in 1988 (Martić, 1988).

Powerful and rapid pace of industrialization in Montenegro influenced the distribution of population in the agricultural and non-agricultural. This process in Montenegro was very dynamic, and this indicates the fact that in 1953 the share of urban population in the total population amounted to 14.2% and 34.2% in 1971. In this period, Montenegro recorded the highest growth in urban population compared to the other republics. A greater degree of urbanization and other benefits offered by city attracted the rural population. Many people had seen education as an opportunity to move towards the city, and some were engaged in industry and other branches. A huge role in professional education of farmers had a village primary school. With reform of 1952, a 8 year primary school became a mandatory starting school. Petak (1988) points out that secondary, higher schools and universities that educated population for the field of agriculture were accounted as a form of deagrarisation and depopulation. Rule is that all those who want to educate themselves leave countryside and farms and thus seek a better future in the towns and non-agricultural activities. For these reasons, the older segments of the population remained in rural areas and those who are prevailing in the group of illiterate population (Table 4).

Table 3. Rate of illiterate population aged 10 and over in Montenegro based on age, Census 1961-2011 expressed in %.

	1961	1971	1981	1991	2003	2011
Age	21,7	16,7	9,4	5,9	2,35	1,5
10-19 years	3,0	2,7	1,2	0,8	0,66	0,8
20-34 year	10,33	3,5	1,3	0,9	0,52	0,7
35-64 years	37,23	26,2	11,1	5,4	0,98	0,7
65 and over	60,3	58,9	45,9	33,0	11,75	6,1
Unknown		-	21,7	5,7	6,3	12,9

*Source: Calculation of the corresponding author according to the data, 1961-2011.

If the illiteracy rate, is observed it is apparent that it is highest among the population aged 65 and over. In the fifty years of the reporting period, the illiteracy rate had been decreasing but it still evident in the age group of 65 and over.

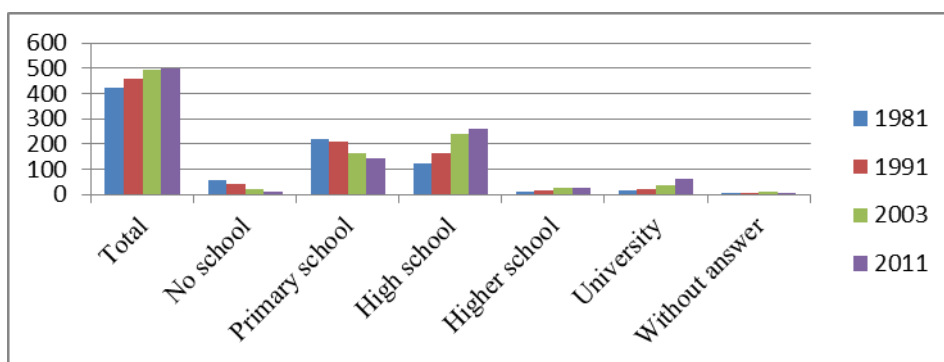
If the illiteracy rate of the population aged 10 and over in Montenegro by gender and age, 1961 to 2011 census is observed, it is apparent that there were more illiterate women than men. Illiterate female population was concentrated in the age group of 65 and over, which was partially due to the unfavourable position of women in the past (Table 4).

Table 4. Percentage of illiterate population aged 10 and over in Montenegro by gender and age, 1961-2011 Census.

	1961		1971		1981		1991		2003		2011	
Overall illiterate population	21,7		16,70		9,40		5,90		2,35		1,50	
	M	F	M	F	M	F	M	F	M	F	M	F
10-19 years	5,64	3,0	8,5	3,3	6,5	2,2	7,4	1,7	15	3,5	20,6	5,3
20-34 years	10,22	17,4	5,3	6,2	7,6	3,7	9,8	3,3	14,7	3,8	22,1	8,2
35-64	48,2	55,35	40,7	56,5	28,6	48,0	29,9	38,4	26,2	16	31,1	19,1
65 and over	36,0	24,31	45,5	34,0	55,7	48,0	51,7	55,4	41,48	74,28	25,9	67,0
unknown- ²	-	-	-	-	1,6	1,0	1,2	1,2	2,59	2,8	0,3	0,4

Source: Calculation of the corresponding author according to the data, 1961-2011

“Educational attainment“ is used besides a literacy level as an indicator of educational structure. Table 5 shows the population aged 15 and over by educational attainment in Montenegro, 1981-2011. Data indicate the growth of the population aged 15 and over in the fifty year period, the largest growth was recorded in the census period of 2011 and it amounted to 18% compared to the base year of 1981.



*Source: Calculation of the corresponding author according to the data, 1961-2011.

Figure 2. Population aged 15 and over by educational attainment in Montenegro, 1981-2011, (in thousands)

Data show that there had been a continuously growing number of people with completed secondary, higher and university education. A change in structure of the population by educational attainment or increasing the number of people with higher education affected the qualification structure and allowed a change in the structure of the population by occupation. Education is one of the major changes in the social position of the individual. Table 5 shows the trend of the

² There are no term „unkown“ in source documents for 1961 and 1971

population aged 15 and over by educational attainment in Montenegro in the period 1981-2011.

Table 5. Trend of the population aged 15 and over by educational attainment in Montenegro from 1981 to 2011, base index.

Year	Total	No school	Elementary school	High School	Higher school	University	Without answer
1981	100,00	100	100	100	100	100	100
1991	108,45	69,30	96,75	133,82	143,00	167,22	606
2003	116,26	36,09	73,77	198,71	202,33	267,33	927
2011	118,29	19,27	64,87	216,70	213,33	431,6	226

*Source: Calculation of the corresponding author according to the data, 1961-2011.

Analysis based on the base index (1981 = base) shows a declining trend of population without school and with primary education, and there was a trend of increasing population with secondary and university education. In absolute numbers the largest population finished secondary education. According to the 2011 census, population with completed secondary education increased by 116% compared to the base year of 1981.

Table 6. Population aged 15 and over by educational attainment and gender in Montenegro, 1981-2011

Year	Total	No school	Primary school	High school	Higher school	University	Without answer
1981	Total	423.763	58.764	217.660	120.105	12.268	13.846
	Male	207.233	13.803	99.367	75.494	8.111	9.870
	Female	216.530	44.961	118.293	44.611	4.159	3.976
1991	Total	459.577	40.724	210.607	160.735	17.559	23.154
	Male	225.998	9.328	94.533	92.712	10.787	14.998
	Female	233.579	31.486	116.074	68.023	6.772	8.156
2003	Total	492.684	21.210	160.571	238.671	24.822	37.017
	Male	239.322	5.024	65.323	127.621	14.186	21.155
	Female	253.362	16.186	95.248	111.050	10.636	15.862
2011	Total	501.278	11.324	141.198	260.277	26.170	59.773
	Male	244.470	2.171	56.916	139.389	15.035	29.630
	Female	256.808	9.153	84.282	120.888	11.135	30.143

*Source: Calculation of the corresponding author according to the data, 1961-2011

Highest growth was recorded at the level of university education and it reached 331 index points. Introduction of compulsory primary education in Montenegro caused a mobility of the population and their desire for further education. If these phenomena are observed in terms of depopulation, it can be said that education constituted a form of indirect transfers from rural areas. It is called „indirect“ because those who are educating themselves are leaving agriculture and train to perform some other activities. De facto, young people are getting educated and do not participate in agricultural activities (Puljiz, 2002). In this way, younger and more capable workforce leaves countryside and it results

in numerous groups of older households. City gets superiority over village and becomes a driving force of social and economic change. Data in Table 8 indicate that males are more educated.

In the category of „no schools“, prevails female population. According to the 1961 census, its share was at the level of 76.51%, and according to the 2011 census it was at the level of 80.8%. Such phenomena in education resulted that more male workforce was influenced by deagrarisation than female one. There are two causes of such phenomena: 1. rapid industrial development hired more men; 2. there is a traditional suspicion towards a woman leaving a house. Overall increase in the importance of education influenced the changes in the educational structure of rural women, particularly by providing them the opportunity to educate themselves. Unfavourable position of agriculture, led to that in the patriarchal system decided that son remained on the farm and in agriculture and female children was offered education as compensation for not inheriting family property (Korać, 1990).

Table 7. Participation of population aged 15 and over by educational attainment and gender in Montenegro, 1981-2011

Year	Total		No school	Primary school	High school	Higher school	University	Without answer
1981	Total	100	13,86	51,36	28,34	2,89	3,26	0,26
	Male	100	6,66	47,9	36,42	3,91	4,76	0,28
	Female	100	20,76	54,63	20,60	1,92	1,83	0,25
1991	Total	100	8,86	45,82	34,97	3,82	5,03	1,48
	Male	100	4,12	41,82	41,02	4,77	6,63	1,65
	Female	100	13,47	49,69	29,12	2,89	3,49	1,31
2003	Total	100	4,30	32,59	48,44	5,03	7,51	2,10
	Male	100	2,09	27,29	53,32	5,93	8,83	2,51
	Female	100	6,38	37,59	43,83	0,41	6,62	1,73
2011	Total	100	2,3	28,16	51,90	5,20	11,92	0,50
	Male	100	0,88	23,33	57,00	6,15	12,12	0,55
	Female	100	3,56	32,80	47,10	4,30	11,73	0,50

*Source: Calculation of the corresponding author according to the data, 1961-2011.

Data in Table 7 show that men are more educated. The percentage of women with no schooling (not) completed primary school ranges from 75.39% according to the census of 1981 to 36.40% according to the 2011 census. According to census (1981-2011) there is decline of participation of women (without) primary, but it is still high. There is a largest share of the population with secondary education. The percentage of participation of the population with university degrees increases and it ranges between 3.26% according to the census of 1981 to 11.92% according to the 2011 census. There is a lower participation of female population in the structure of the population with university degrees. Contemporary literature emphasizes the importance and role of women in strengthening the rural economy (Babović, Vuković, 2008, Bock, Shortall, 2006, Goverde, de Haan, Baylin, 2004).

Observed indicators of education in the fifty year period specify a tendency of reducing the share of illiterate population, as well as reduction of disparities in literacy of males and females. There is a continuous increase in the number of people with completed secondary, higher and university education. On the other hand, increased level of education has caused some social changes. It is believed that those who study (educated) must become very competent in all phases of the development cycle of the economy and society (Zjalić, 2009).

Positive educational trends have influenced the shift of population from agriculture to other activities, which led to the emptying of rural areas in Montenegro. In comparison with other republics, Montenegro experienced the fastest and greatest transformation. From the perspective of sociological theory, with a decline of illiteracy as of 1961 comes a rapid process of urbanization and reduction of share of the agricultural population in the total population of Montenegro (Table 10). Economic theory holds that the reduction of the share of the agricultural population is a consequence of economic development which increasingly requires a workforce from agriculture.

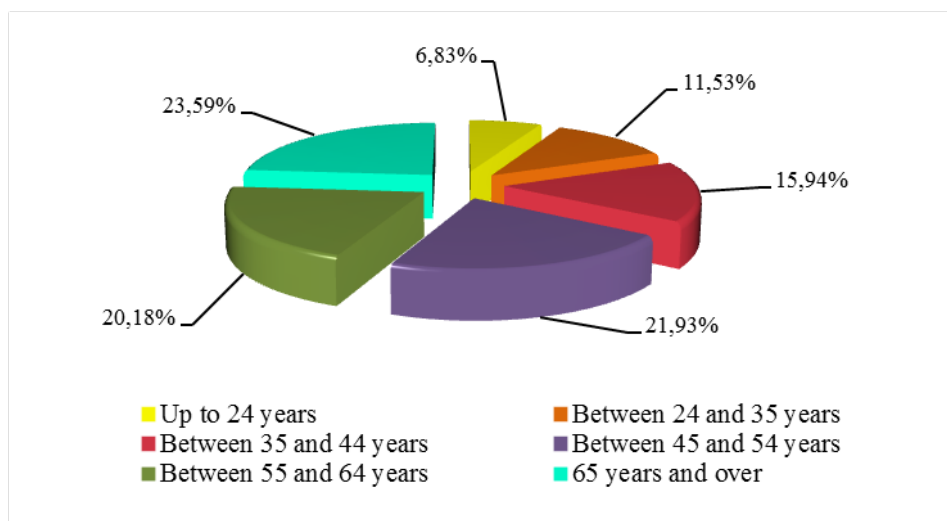
Table 8. Participation of the agricultural population in the total population of Montenegro in the period 1948-2003.

Year	1948.	1961.	1971.	1981.	1991.	2003.
Participation of the agricultural population in the total population	75,40	48,0	42,60	13,00	7,10	5,3

*Source: Ž. Andrijašević, Š. Rastoder, 2006.

Data on the trend of participation of agricultural population in the total population of Montenegro point to the significant decrease in the observed period. After 1945, as a result of the overall economic development and industrialization of the country, mass migration reduces agricultural and general population in the mountainous area, therefore in 1961 it amounted to an average of about 60% of the total population (Marković, P. 1974). School became the main channel, through which the agricultural exodus occurred. As a result of the outflow of population resulting changes in the demographic structure of rural areas and come up with specific social problems. It was significantly increased „mortality“ of production units in agriculture.

Today Montenegrin agriculture is characterized by the aging of the rural population and substantially lower average level of education. More than 44% is older than 55, 65% is older than 45. More than half (55.3%) employed in agriculture completed secondary school, and only 9.1% have completed higher school or university (Agricultural Census, 2010, Structure of agricultural holdings, 2011b.). Taking into account the age and educational structure of farmers, it would be difficult to talk about the professionalization of work on the farm. Professional activity is characterized by systematic rounded theory, social sanctions, the Code of Ethics and a specific subculture (Milošević, 1997).



*Source: Monstat, 2010

Figure 3. Age structure on family agricultural holdings by age.

Unfavourable age structure on family farms is a result of social and economic factors. New technologies are changing the character of work, status, position of employees, working conditions, new quality of a working man which affects the change in the role and importance of education (Jovanović, 2011). In order for a farmer to be successful and happy he must be professionally trained, because it is the only way to be able to achieve the quality of life and for agricultural to be accepted as a professional orientation. Education of farmers has wider significance than economic objectives and creation of commodity producers. Therefore, education on the one hand a necessary factor in the modernization of agricultural production, on the other hand - represents a certain „danger“, because young people use education as a way to „escape from the countryside.“ Therefore, it is necessary to continue work on improving demographic, social and economic policies toward the village.

CONCLUSIONS

Transfer of population from agriculture to other activities is a result of the development of social division of labour. Reducing the number of inhabitants and demographic depopulation of certain areas is one of the dominant demographic processes in Montenegro after the Second World War. Unfavourable demographic trends have influenced on the development of agriculture in Montenegro. Montenegrin agriculture is characterized by the aging of the rural population and substantially lower average level of education. More than 44% of them are older than 55, 65% are older than 45. More than half (55.3%) employed in agriculture completed secondary school, and only 9.1% completed higher

school or university. According to the sociological theory, one of the reasons of mentioned process is a transfer of farmers into non-agricultural activities. In the fifty years of the reporting period (1961-2011) education indicators indicate a reduction of illiteracy and increase in the participation of citizens with a higher level of education in the total population. In absolute numbers, the highest number of population finished secondary school. Process of education and training has led to the depopulation of rural areas, as well as to the process of deagrarisation. Overall increase in the importance of education influenced the changes in the educational structure of rural women, particularly by providing them the opportunity to be educated.

It is necessary to identify and manage available resources for a successful economic development. Three basic groups of resources that have a large impact on economic development are: population, natural resources and infrastructure. Population is a resource that has a great importance for the further development of rural areas. Education of farmers should be focused on the ability to accept new knowledge, but not to present a possibility of leaving countryside and educating for non- agricultural activities as it happened in the past.

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THE SOCIOECONOMIC AND HEALTH EFFECTS OF GREEN INFRASTRUCTURE ON THE VRACAR MUNICIPALITY, CITY OF BELGRADE

SUMMARY

Green spaces are the important components of the green infrastructure of cities that provide a wide range of services. Recent demographic reports have shown that the most people live in urban areas and that quality of living and work environment directly affects the physical and mental health of city residents. Health-promoting areas and elements within urban areas such as green spaces are recognized to support the residents' possibilities to cope with morbidity and to have a beneficial effect on general health. The Vracar municipality represents one of the three central municipalities in the territory of the Belgrade, with the low percentage of green areas and the high population density. This paper examines the relation between the socioeconomic and health characteristics of the residents with a quality of a green infrastructure of the Vracar municipality. According to the results of the survey, on one side a high number of the Vracar residents visit local parks less than 3 times a week and stay less than 30 minutes, while on the other, 69% of the residents suffer from the respiratory infections and have frequent annual visits to a doctor. The results of this study indicate that exposure to the green environment and spending free time outdoors in local parks, have a clear influence on the general health and the degree of urbanity and the higher proportion of green space affect a life quality of the Vracar residents.

Keywords: green infrastructure, socioeconomic and health characteristics, urban residents, the Vracar municipality, city of Belgrade

INTRODUCTION

Recent demographic statistics have shown that the most people live in cities, and this trend will likely continue in the future (Dye, 2008; Arnberger and Eder, 2015). Urban green spaces are essential and consciously influence on the experience and emotions of the city residents, while the factors that evaluate the quality of these areas affect the physical and mental health, as well as the living and work environment (Mitchell and Popham, 2007). The quality of urban areas is increasingly recognized to benefit to human health and wellbeing (Nilsson et

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al, 2007). The supply and maintenance of health-promoting areas and elements within urban areas such as green spaces are recognized to support residents' possibilities to cope with morbidity and to have a beneficial effect on general health (Tyrväinen *et al.*, 2014). The terms "open spaces", "urban green" and "green spaces" according to general understanding are related to the design elements intended for recreation or to the improvement of urban spaces. Public and private areas, city parks, trails, streets, squares, school yards and other surfaces planted trees, shrubs and other plants can function as elements of green infrastructure and can influence in the heat reduction, air pollution and affect the aesthetics values (Konijnendijk, 2008; Lee and Maheswaran, 2010). However, there is also another benefit of green infrastructure and it is related to the resource that contributes to the improvement of public health (Williams *et al.* 2000, Girardet 2004). Concern over the quality of urban environments, including noise levels and declining quality of green space, has grown over the past decade with increasing emphasis on assessing everyday physiological health and economic values of urban residents (Irvine *et al.*, 2009).

There is a growing interest in the relation between the environment and human health, followed by investment and efforts to preserve the natural environment by organizations and sectors of public health (Stiglitz *et al.*, 2009; Bowler *et al.*, 2010). Such initiatives can promote the importance of urban green infrastructure for the benefit of the public health and wellbeing, by pointing to many benefits of preserving nature and green areas. The physical benefits are reflected in the reduction of physical illness and positive effects on the cardiovascular system, respiratory diseases, diabetes, obesity and reduced blood pressure (Bowler *et al.*, 2010). They also observed positive effects on psychological morbidity including anger reduction and stress and feeling of depression, which is the main cause of suicide (Lee & Maheswaran, 2010). Recent studies have demonstrated a positive relationship between the amounts of green space in people's living environment and their perceived general health (Maas *et al.*, 2006; Mitchel and Popham, 2007). Variations between living environments are in relation to social and financial capabilities of individuals. The conclusions reached on the basis of tests carried out in the Netherlands on a large number of samples obtained by testing in the general population provide an interesting picture of the impact and presence of green space on the psychological experience (de Vries *et al.*, 2003; Maas *et al.*, 2009; Van den Berg *et al.*, 2010). Comparing the responses of people who live in different locations that each has a different level of the greenery, it is possible to estimate how their life in these areas reflected their wellbeing (White *et al.*, 2013).

Many studies underline the links between the environment, living conditions and public health. This paper examines the relation between green space and health, based on research and a systematic review of data collected from the field, in order to assess the socioeconomic and physiological benefits of green infrastructure to the residents of Vracar municipality, one of the three central municipalities in the territory of the Belgrade, with the lowest percentage

of green areas and highest percentage of residents suffering from respiratory and nervous diseases (Vujcic et al., 2015).

MATERIAL AND METHODS

Survey and Location

The research relies on the special survey that was conducted among the visitors at four sites in the Vracar municipality (Figure 1). On-site, face-to-face interviews were carried out between October and November 2015. A survey contained a combination of different types of questions highlighting the socioeconomic and health aspect with a recreational aspect (Arnberger and Eder, 2015). The participants in this research (n=101) were residents of the Vracar municipality with an age range between 18 and 65 years. The survey respondents were selected via systematic sampling in which every second visitor was interviewed, respecting representatives of both genders equally (Bankovic, 2003).

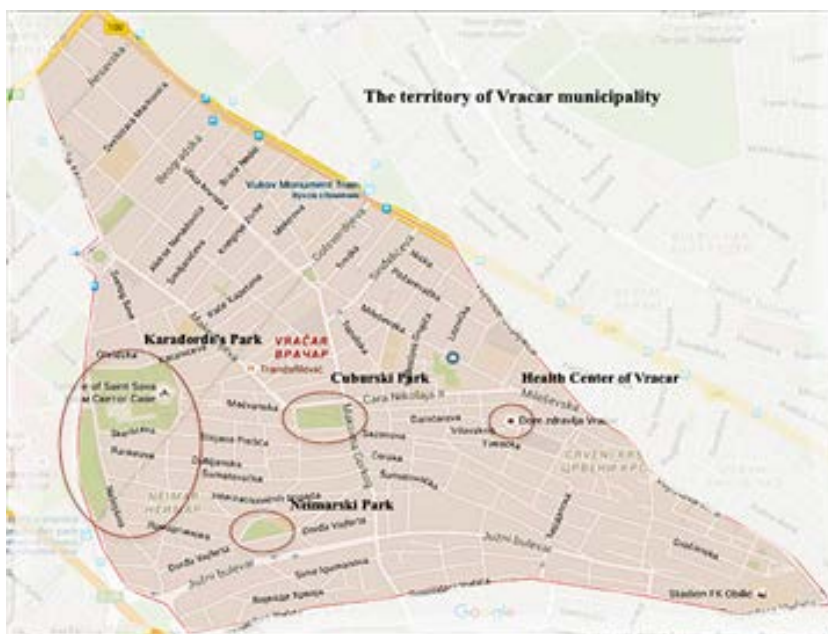


Figure. 1. The target locations for the survey at the Vracar municipality.

Locations, where the visitors were interviewed included three public parks, Karadjordje's Park, Neimarski Park, Cuburski Park and Health Center of Vracar municipality (Figure 2, 3, 4 and 5). All these parks vary in many aspects like size, function, design and location; some parks are used for daily recreation while the others are also tourist attractions. Karadjordje's Park represents a historical park and tourist attractions while Neimarski and Cuburski Parks represent smaller local parks favourite among the residents of Vracar municipality. All three green spaces are under the jurisdiction of the public utility company. The

Health Center Vracar represents a modern primary health care institution in the city of Belgrade. On one side the municipality of Vracar counts about 80 thousand residents and less than 3 km² of space and on the other, it represents the Belgrade's municipality with the highest population density. Excessive population density and urbanization at such a small area with a lack of green space, certainly have influenced a general health of the Vracar's residents (Republic of Serbia, Institute for Statistics, 2011).

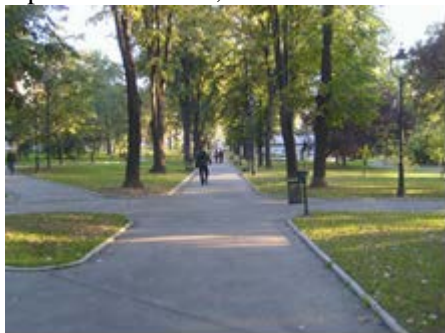


Fig. 2. Karadjordje's Park



Fig. 3. Neimarski Park



Fig. 4. Cuburski Park



Fig. 5. The Health Center of Vracar

RESULTS AND DISCUSSION

Relying on the Biophilia Hypothesis in the context of daily life situations, there is a systematic preference for natural compared to built settings and also a link between features such as biodiversity richness and human health appreciation of urban green spaces (Kaplan and Kaplan, 2011; Thompson and Aspinall, 2011). These evaluations should also be reflected in a smaller capacity of settings with lower biodiversity levels vs. settings with higher biodiversity levels, to induce community health outcomes (Scopelliti and Giuliani, 2004; Carrus *et al.*, 2013). The overview of health characteristics of the Vracar residents in the past few years (2009-2014) is presented according to the statistical report of The Health Center Vracar, based on a total number of morbidity and patients with the respiratory diseases and mental disorders (Figure 1). According to the report, in the year 2013 it was registered the highest number of the residents of Vracar municipality suffering from the acute respiratory

infections (16.2%), and in the year 2010 the highest number of the patients with the chronic respiratory infections (2.3%). Also, in the year 2012 about 11.2% of the Vracar residents suffered from the mental and behavioral disorders.

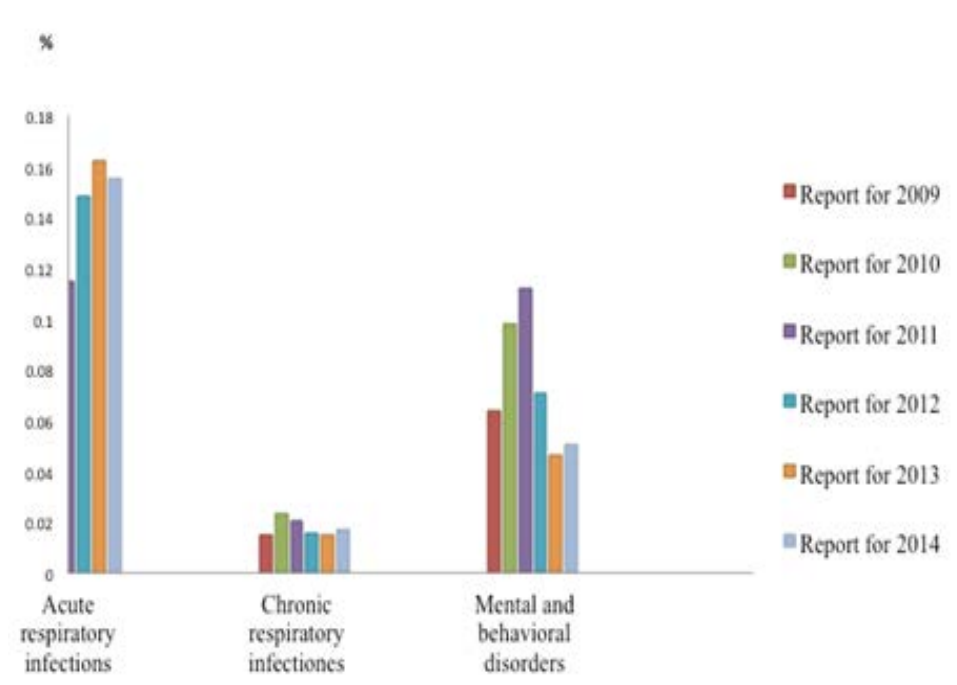


Figure 1. An overview of a total number of morbidity and patients with the respiratory diseases and mental disorders (HCV, 2009-2014)

In order to have an objective view of the situation and taking into the consideration the socioeconomic and demographic structure of the visitors on four selected survey points, a more diverse sample of the respondents was included. The main demographic and socio-economic characteristics are presented in Figure 2. The study included 101 participants (mean 40.6 years) 55 female respondents, pointing out that the larger number of the respondents had a higher level of education (58%) and single marital status (37%).

In order to measure the relation between the experiences of urban nature across the four selected locations on the Vracar municipality, with their physical health following a hypothesis that higher levels of nature exposure lead to greater health outcomes, the Vracar residents were asked about their general health condition, regarding the respiratory infections, nervous disorders and the use of medications (Kardan et al., 2015; Seresinhe et al., 2015).

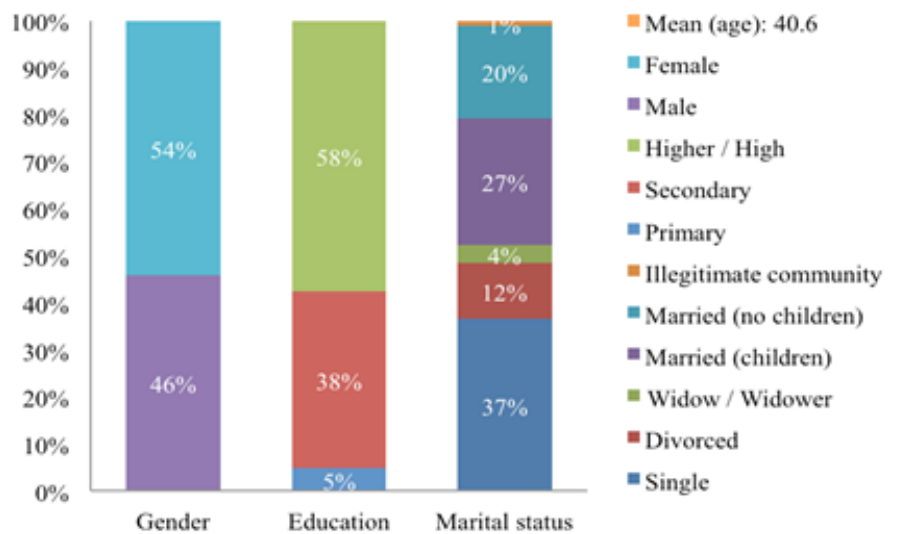


Figure 2. Demographics and the socio-economic structure of visitors

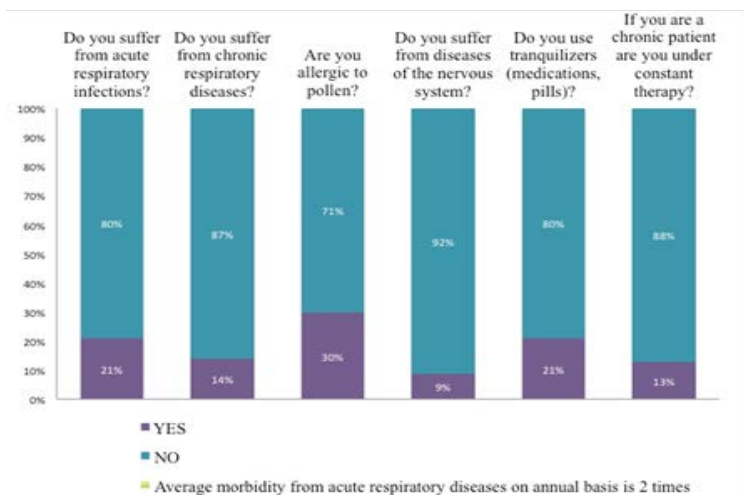


Figure 3. The health characteristics of the respondents of Vracar municipality

About 21% of the respondents reported to suffer from the acute respiratory infections and use medications, and 9% reported to suffer from the nervous disorders. Also, according to collected data about 30% of the Vracar residents reported to suffer from the allergies (Figure 3).

Evaluating the relation between the health characteristics with the usual frequency of the average duration of visits to green space across a week, the respondents were asked to estimate their everyday habits regarding spending free time outdoors in the local parks with a visit to a doctor and monthly expenses on medications (Figure 4). The average duration of green space visits was estimated based on self-reported time spent during each visit across the survey week. The chosen timeframe provided a short and recent reference period to improve accuracy (Schwarz and Oyserman, 2001). This measure of duration was necessarily linked to frequency as to achieve a duration measure the respondent must have visited a green space at least once during the survey week. Duration was selected from a time category (1–29 minutes; 30 minutes to one hour and more than one hour).

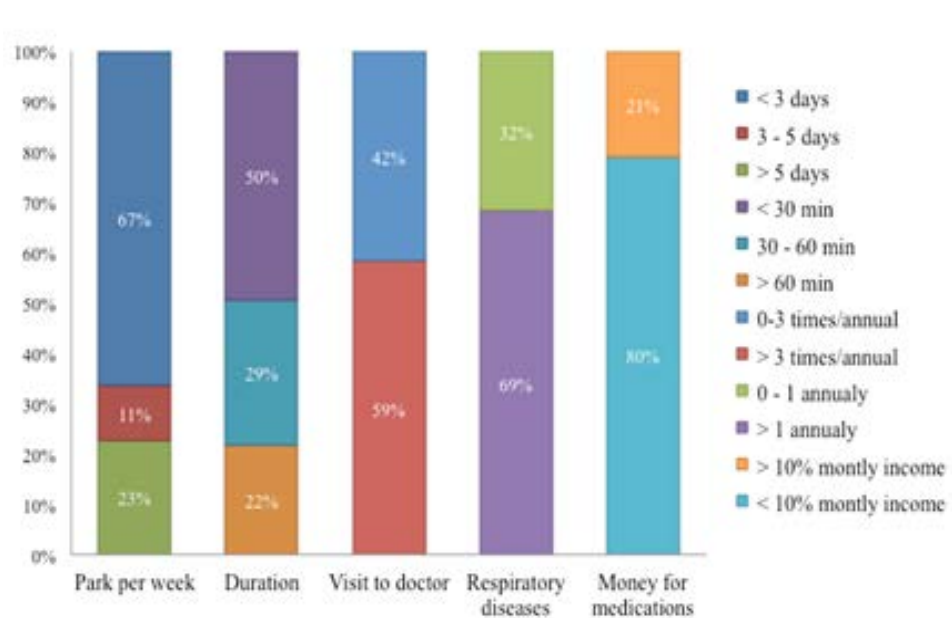


Figure 4. A relation between the health and recreational characteristics of the Vracar residents

Figure 4. has also shown that the different components of experiences of urban nature, including the frequency, duration or intensity, variously influence the health outcomes of the Vracar residents. 67% of the respondents visit the local parks less than 3 days a week and stay less than 30 minutes. It was recorded that 59% of the respondents visit a doctor more than 3 times during a one year (an average visit to a doctor is 5 times annually) and often suffer from respiratory diseases (69%). Concerning the socioeconomic aspect of nature dose effects on respondents, about 21% of the Vracar residents spent more than 10% of monthly income on medicaments and therapies. Similar studies have examined the scale of the population health benefits that could arise if the nature dose

recommendations were met and the impact of this on the public health occurred, finding that the proportion of cases of depression and high blood pressure in the city population who failed to spend an average of 30 minutes or more during a weekly green space visit. Also, studies highlighted that the efforts to unpack the nature-health relationship will be vital to combating the emerging public health challenges associated with urbanization and instrument that investment in green space provides value for money (Shanahan et al., 2016).

CONCLUSIONS

The main causes of disease and premature death in the Europe and the other urbanized parts of the world are related to the everyday habits and lifestyle with the environment in which one lives. High level of urbanization leads to air and noise pollution, which adversely affect the general health of the city residents. The presented Vracar municipality is located in the central zone of the capital of Belgrade, and as such contains a low percentage of green areas and a high population density. Reviving the relationship between the health outcomes and four selected components of nature dose, four presented survey locations, also allows for the assessment of dose-response connections, including whether there is a minimum dose where some effect of nature on health might be seen. Nature cohesion, or the distinction in the way people view their interaction with the urban nature, could both drive interactions with nature and enhance general health in its own right. This study has found that higher levels of nature cohesion could predict better socioeconomic cohesion and higher levels of positive health outcomes. The minimum dose, like 30 minutes, of everyday nature experience could provide better measurable health characteristics of the urban residents including better respiratory and nervous medical conditions. Also, the potential of green space for mental health benefits is not just to be found through physical activity. However, there is strong evidence, which demonstrates the restorative value of green space showing that more passive forms of usage, or even just access to views of green space, can have a beneficial impact on mental well-being and cognitive function. This findings support other research, which has found that people with higher nature relatedness scores, also often report better well-being, happiness, and life satisfaction and lower levels of anxiety. This study indicates that everyday exposure to the urban green spaces and spending free time outdoors in local parks has a clear influence on the general health and moderate socioeconomic aspect of the Vracar residents. Also, these facts speak volumes about how the living environment and life quality of the Vracar residents depend on the degree of urbanity, the higher proportion and quality of a green space.

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NUTRITION REGIMES OF ERODED LANDS IN THE NORTHERN STEPPE ZONE OF UKRAINE

SUMMARY

The research of quantitative and qualitative composition of nitrogen, phosphorus, potassium, and soil enzymes activity in arable soils on the slopes and watersheds was performed. Soil erosion is considered as a result of a complex interaction of natural and agricultural factors. Among the main ones are the natural terrain, erosion resistance, vegetation soil-protective role, climatic and meteorological conditions that determine the rainfall, nature of soil freezing, the size and intensity of surface runoff snowmelt and rainwater. An intensity of erosion processes depends on the slopes characteristics, their steepness, shape, length, exposure. These factors different combinations give specificity of soil profile forming in slopes, generally similar, but not identical with soils of the neighbouring northward and southward latitudes. The experiments reported here compare soils from three types of landscapes: level soils (0-1% slope) with no observable erosion (E0), mildly sloped soils (1-3%) with mild erosion (E1, 0-10 cm topsoil loss), and moderately sloped soils (5-7%) with moderate erosion (E2, up to 30 cm topsoil loss). The content of available forms nitrogen assimilation varies depending on the soil erosion degree. Urease activity decreases significantly with the depth, especially at E2 soils. The reduction of digestible phosphorus under the erosion impact was fixed two times in the slope of the southern exposure, in comparison with plain soil. The data obtained indicate the phosphatase activity decrease under the influence of flushing by 37 – 83 %. The decrease of potassium level in the slopes of the northern and the southern exposition reaches 1.5 and 2.5 times accordingly. A larger value of the ratio of nitrogen and phosphorus in the underlying eroded soils layers in the two test crops is associated with a much greater adaptive potential of peas comparative to the barley.

Keywords: soil, erosion, nutrients, uptake, biological activity.

INTRODUCTION

Ukrainian natural zones consist of three zones - forest, forest - steppe and steppe zone. Black soils take 76.8% or 11.9 mln.ha (Kravchenko, 2012). The Ukraine steppe can be divided in two subzones: northern and southern parts.

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

Unsteady agriculture in the steppe zone of Ukraine is in most cases by rather changeable weather conditions relief and diversity of soil covering. Precipitation is less than 350mm in dry years i.e. them dry steppe zone, in damp years more 550mm live in forest-steppe zone (average perennial value 470 mm). The relief makes worse the influence of the zonal climate, with redistribution climatic ecological recourses leads to draining of soil formation of soil erosion, deterioration of fertility of it (Kisic et al., 2016; Barovic et al, 2015). Agricultural zones differences are caused by horizon zones, vertical and similar ecological effect of soils of different granular composition (Kharytonov et al., 2009).

It is necessary to emphasize that black soils are prevalent in the steppe zone of Ukraine. Generally, black soils are not rich in available phosphorus. They have available K content ranging from medium to high. The reduction of fertilizer inputs leads to nutrition balance infringement and the development of degradation processes in the black soil (Shikula M.K., 2000). Erosion processes within steppe landscapes reach 40-50%. Dnepropetrovsk region territory stretching for only 200 km from north to south and 270 km from east to west is characterized by the wide variety of ecological conditions that predetermine its division into separate agricultural zones and subzones (Kharytonov et al., 2009).

There are similar agricultural resources distribution connected with vertical zoning within separate elements of landscape. The educational farm "Samarsky" of DSAEU is known due to V.A.Kovda (1989) as farm with high level of agriculture including some elements of ecological farming: crop rotations with long and short - term grass and leguminous mixtures, forest land conservation, etc. Investigation of typical for center of Ukraine steppe the landscape has been done. Slopes with steepness 1,5 degree had weak-expressed erosion soils. Slopes with steepness 1,5-7 degree had washed off upper horizon of humus more than 2 times (Kharytonov et al., 2002).

In our research we set the following goal: to study the changes of nutrition regimes of arable lands (in plain and slopes of the northern and the southern exposure).

MATERIAL AND METHODS

Field observations were carried out at the ecological field station of the "Samarsky" farm located in the Dnipropetrovsk oblast in the northern part of the steppe zone of Ukraine. Soil for controlled experiments and laboratory analysis was sampled from the same location. The farm coordinates are: 48°30' E lat. and 35°15'N long. The field station was used for many years as an area for intensive agricultural production and research. It is located far away from the city of Dnipropetrovsk (25-30km) enough to avoid industrial pollution effect (Anisimova et al., 2009).The training farm "Samarsky" – agri-landscape scheme was highlighted to demonstrate the idea of the case study. Comparison of the received information regarding the crop yield with the landscape features offers the possibility to differentiate the agricultural resource potential of the area.The research field occupies an area of 14 hectares and it is crossed by three ravines.

One of them is of 30 m depth with a slope of $> 7^\circ$, the other two have the slopes up to 3° . Studies were performed on plain (full-height normal soil), on the northern exposition slope (low eroded soil), the slope of the southern exposure (middle level of erosion). Special attention was paid to the one of the three ravines: flat terrain, slopes of the southern and the northern exposure. Thus, the experiments reported here compare soils from three types of landscapes: level soils (0-1% slope) with no observable erosion (E0), mildly sloped soils (1-3%) with mild erosion (E1, 0-10 cm topsoil loss), and moderately sloped soils (5-7%) with moderate erosion (E2, up to 30 cm topsoil loss).

Coincidentally, in the study area, the mildly sloped soils have the northern exposure, and the moderately sloped soils have the southern exposure. The soil type in the experimental area is referred to the central steppe chernozem in the FSU (former Soviet Union) system (Kharytonov, et al., 2004). Numerous soil characteristics were determined for each erosion severity and each 10 cm depth increment from bulk samples. Generally, duplicate determinations were made and averaged. The depth increments were assigned to general soil horizons, according to the Russian soil taxonomy system (Kovda, 1989). These are defined as follows (with correspondence to the Ukrainian soil taxonomy system (Kharytonov et al., 2004). Soil samples were taken every 10 cm to a depth of 100 cm.

Up to 100 soil samples were taken in topsoil of ecological field. Percent of organic N (N%) and mineral N was determined by Keldahl analysis of the bulk sample, including the organic matter. Nitrification energy was determined by the following procedure. A portion of a bulk sample for each erosion severity and depth was allocated to each of two 50 ml Erlenmeyer flasks. NO_3 concentration was determined for each flask immediately after the soil sampling, and again after 7 days incubation at 28°C with daily addition of water just enough to keep the soil moisture for the period of experiment. NO_3 concentration was determined using an ЭБ-74 ion meter (Ion-meter factory, Gomel, Belorussia) and a nitrate selective ion electrode for NO_3 (Ion Meter Factory, Tbilisi, Georgia). Using 4 equally-spaced concentrations of KNO_3 from 10^{-2} to 10^{-5} M (approximately equivalent to 0.78 to 780 mg NO_3/kg of solution), a standard curve was prepared. For greater accuracy, this curve was divided into two segments (10^{-2} to 10^{-4} M and 10^{-4} to 10^{-5} M). Humus concentration was determined by placing 0.1 g of soil in 10 ml of 0.4 N $\text{K}_2\text{Cr}_2\text{O}_7$ aqueous solution, followed by titration with salt of Moore ($\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4$). The C:N ratio was calculated from determinations of N% (as above) and C%. C% was determined as humus/1.72. Mobile P was determined by the colorimetric method of Denizhe, following offered by the Machigin extraction method. The method of Machigin is basically an extraction by a 1% solution of ammonia. Exchangeable K was evaluated by flame emission spectrophotometry after extraction by 1% $(\text{NH}_4)_2$ carbonate. Ca and Mg were determined by titration with EDTA. Urease was determined for each 10 cm up to the depth of 1 m, phosphatase was determined for 3 pooled depths: 0-20, 20-40, and 40-60.

The pea and barley experiments involved two erosion levels (E1 and E2). It was made to establish the eroded soils effective fertility. Soil samples were taken every 10 cm to a depth of 1 m on each slope. The vessel volume is 5 kg, repetition - 3. The experiments duration 90 days in greenhouse conditions under natural light and sufficient supply of water. N, P₂O₅ and K₂O uptake with pea and barley mass for each soil layer was accounted. The ratio for each nutrient between pea and barley was calculated as well. The nitrogen and phosphorus concentration in plant samples was estimated using Kjeldahl method. Total P concentrations of the applied residues were determined by sulfuric acid digestion (Thomas *et al.*, 1967). Potassium was determined with flame photometry. Statistical analysis of each experiment was performed on Excel (2000), using the analysis package add-in.

RESULTS AND DISCUSSION

The different character of interaction of factors on the slopes and plains has led to the phenomenon of the soils biological activity diversity in arable landscapes. The ratio of rate C/N changes at different depths (Fig 1).

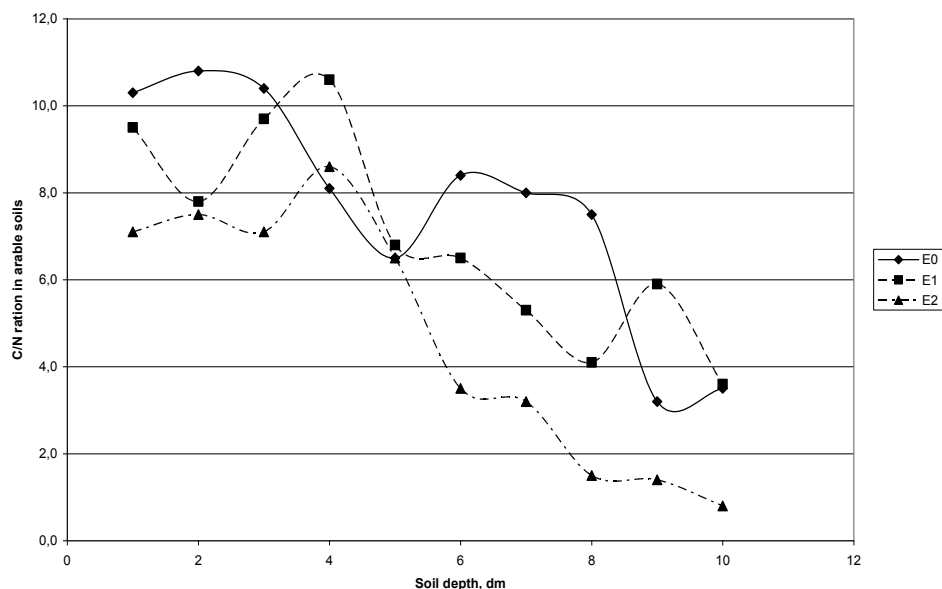


Figure 1. The C : N ratio profile distribution in arable soils

The data analysis showed that the amount of humus decreases with the depth. The nitrogen content also decreases, but more slowly. This may explain the decrease in the C : N ratio along the profile. The biological process of nitrification in the soil happens depending on several factors: soil moisture, temperature, aeration, and pH value. The nitrification process is the final step in

the organic nitrogen transformation to available form for plants. The content of nitrogen assimilation available forms varies depending on the soil erosion degree. The data on arable soils nitrification energy are shown in the figure 2.

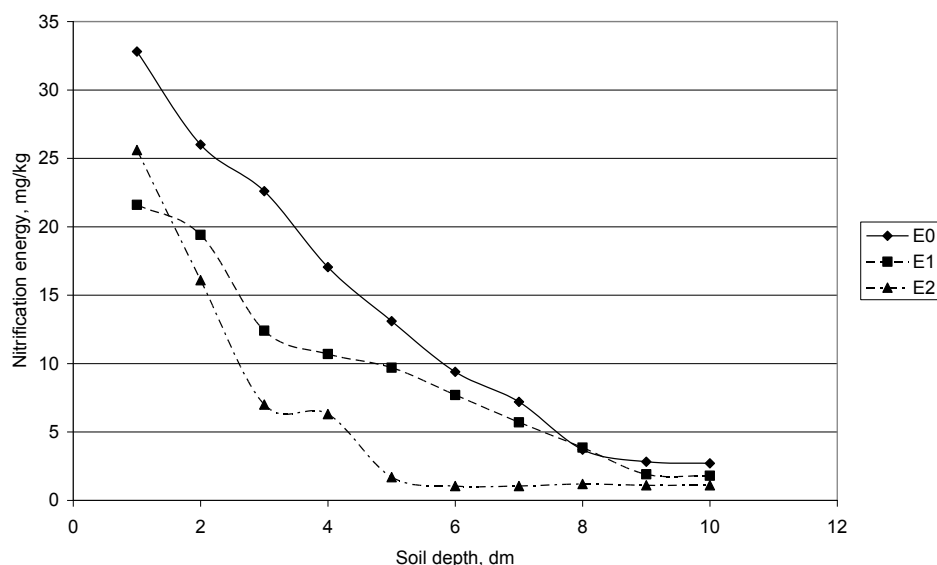


Figure 2. Nitrification energy profile distribution in arable soils

The greatest energy of nitrification (27,1 mg/ kg) was in the E0 topsoil (0-30 cm). Its value in the topsoil of slopes: 17.8 mg/ kg (E1) and 16.2 mg/ kg (E2). A common trend is the decrease of nitrate transformation rate with the depth.

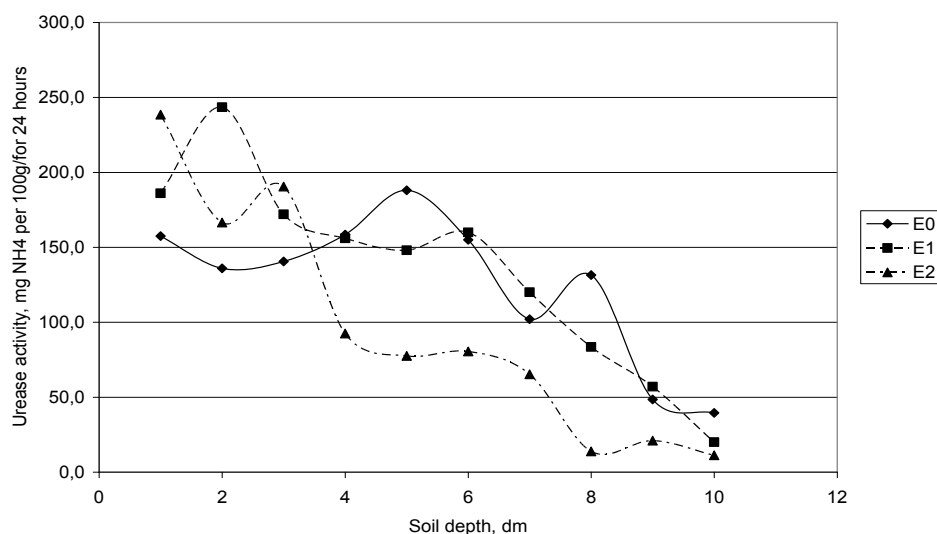


Figure 3. Urease activity in soil profiles (mg NH₄ per 100 g/for 24 hours).

As it is known such enzyme as urease has great influence on the soil nitrogen mobilization. The data portion on urease activity distribution in soil profiles are shown in the figure 3.

Data show urease activity increasing on the slopes of the northern and the southern exposure. However this pattern is observed in the only three topsoil layers: 0-10, 10-20 and 20-30 cm. Urease activity decreases significantly with the depth, especially at E2 soils. As it is known, phosphorus in the soil is accumulated in organic and mineral compounds. Mainly the phosphorus substances in soil are inaccessible compounds to plants (Kabata-Pendias, 1987). In this regard, the total phosphorus content in soil cannot give the trust information on the availability to the plants. Soil microorganisms and biochemical processes have a large impact on the mobility of phosphate in soil. The mobility of phosphorus stimulates the enzymatic system of the soil microorganisms. Biochemical processes contribute to the soluble and mobile phosphorus compounds accumulation. The lack of digestible phosphorus in eroded soils caused by the action of two factors: the washout and the amount of carbonates that reduce the mobility of phosphate (Shikula, 2000)]. A transitional horizon (Hp+Ph) is poor in the most mobile forms of phosphorus. This is due to the approximation to the surface of the rocks that have a low mobility of phosphates and significant phosphorus uptake crops yield. Both phosphate compounds mobilization and fixing in less mobile mineral and organic compounds occur in soil under the microbiological and biochemical processes influence. As a result of erosion processes in the humus horizon, rich in organic and mineral compounds, is washed away, and the surface are depleted with nutrients. The content of digestible phosphate is significantly reduced with the increasing of erosion degree. The digestible phosphate decreasing ranged from 165 mg/ kg to 104 mg/ kg. Both total and recoverable reserves of phosphorus in eroded soils were determined in our studies. The phosphorus gross reserves can be considered as indicators of soil fertility as well. As usual, the nitrogen gross reserves predominate over phosphorus reserves in ordinary black mollisols. That is why, in favorable conditions (in the absence of factors that inhibit the nitrification process) the plants are often lack of phosphorus. A number of chemical and biological processes that influence the phosphates availability occur as a result of washout in addition to the mechanical soil particles removal. The ploughing is an additional factor that leads to carbonate uptake to the day surface. At that the mobile phosphorus content decreases. The data on content of digestible phosphate profile distribution in arable soils are showed in the figure 4. The reduction of digestible phosphorus under the erosion impact was fixed two times in the slope of the southern exposure, in comparison with plain soil. The content of digestible phosphate drop to a depth of 50 cm and further the indices are stabilized at a level of 7.3 to 8.5 mg/ kg.

Along with the content the stocks of digestible phosphorus are significantly reduced. An available phosphorus reserve is reduced by 30 – 50 % in the layer of one meter thick under the erosion influence. The soluble

phosphorus accumulation in soil occurs in the process of hard forms decomposition. These transformations occur under the enzymes influence. An enzymes activity in soils depends on the amount of organic matter and activity of microflora. The phosphatase activity distribution in soil profiles was observed (Fig.5).

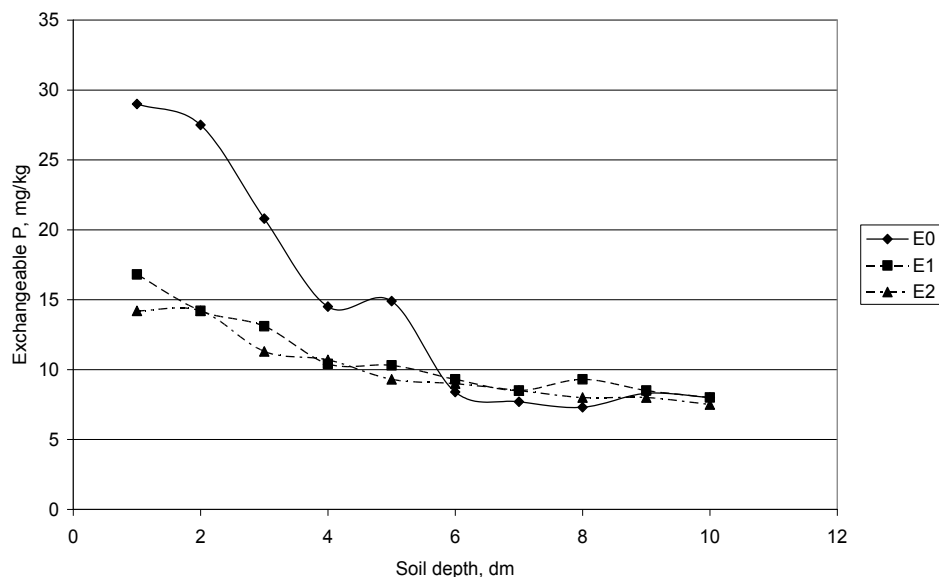


Figure 4. The digestible phosphate profile distribution in arable soils.

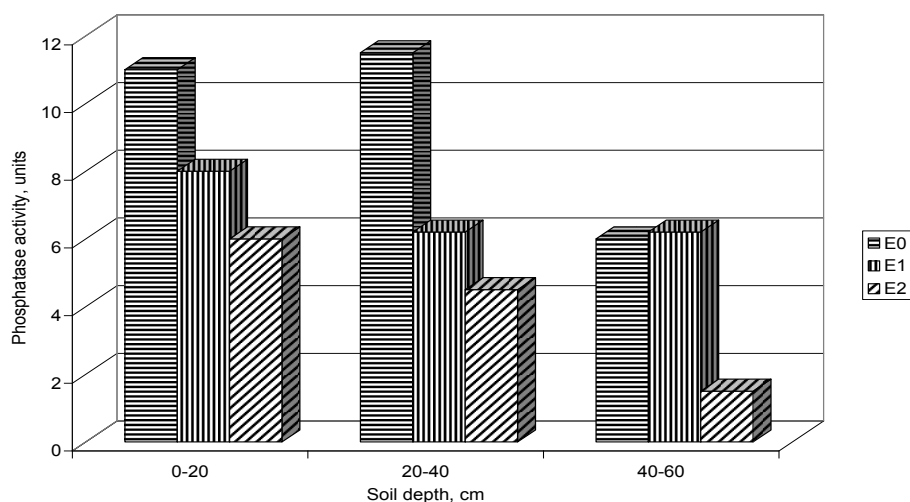


Figure 5. The phosphatase activity profile distribution in arable soils.

The data obtained indicate the phosphatase activity decrease under the influence of flushing by 37 – 83 %. The phosphatase activity reduction reaches 4 times in E2 soil profile at a depth of 40 – 60 cm in comparison with E0.

This indicates a low mobilization of soil phosphates in eroded soils, especially at the exit of the transition horizon Hp to the surface.

Thus, the amplification of surface flushing is a risk factor for significant losses of phosphorus in the surface layer of eroded soils. The data on exchangeable potassium in ordinary black mollisols are shown in the figure 6.

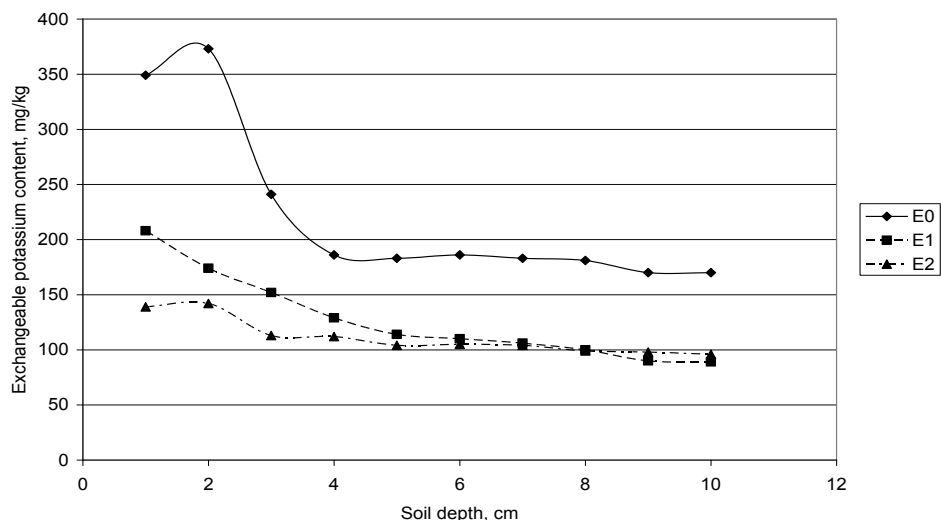


Figure 6. The exchangeable potassium profile distribution in arable soils.

The content of exchange potassium in the E0 soil is significant. The decrease of potassium level in the slopes of the northern and the southern exposition reaches 1.5 and 2.5 times accordingly.

Such a significant drop can be explained, firstly by flushing some part of the soil and leaching of potassium in aqueous solution.

The pea and barley were studied as biological indicators to estimate eroded soils fertility. A pea is largely due to nitrogen fixation removes the restrictions due to different nitrogen content in the soil genetic horizons. Barley is much more demanding of soil fertility.

Barley productivity is in direct proportion to the consumption of nutrients – nitrogen, phosphorus and potassium. The ratio for each nutrient to compare pea and barley adaptive potential for two types of eroded soils was calculated (Fig. 7 and 8).

The ratio index for pea and barley for nitrogen, P_2O_5 , K_2O along E1 profile was 2.2-4.2; 1.3-4.5 and 1.2-2.4 times. At the same time the nitrogen and P_2O_5 distribution along E2 profile was 3.1-4.2 and 2.2-4.3. The similar uptake was fixed was potassium.

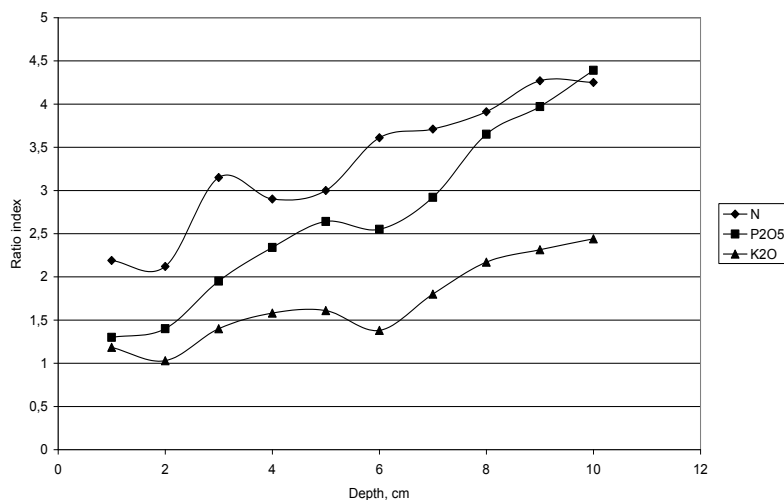


Figure 7. The pea/ barley ratio nutrition indexes profile distribution in E1 profile.

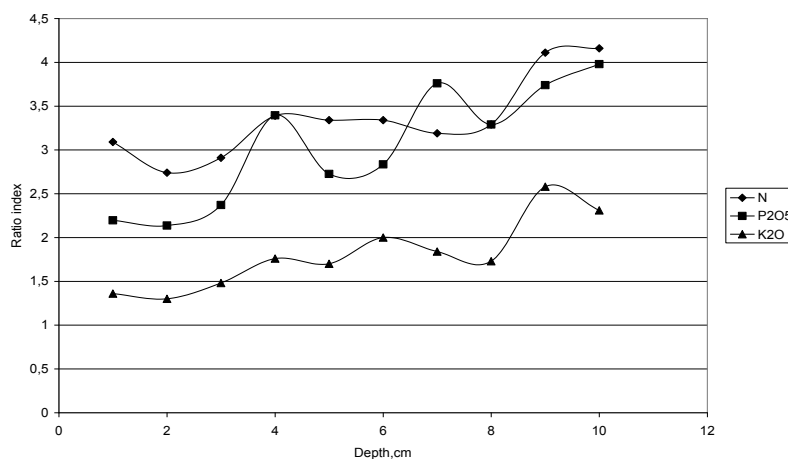


Figure 8. The pea/ barley ratio nutrition indexes profile distribution in E2 profile.

CONCLUSIONS

The research of quantitative and qualitative composition of nitrogen, phosphorus, potassium, and soil enzymes activity in arable soils on the slopes and watersheds was performed. On the basis of it the dependence between the eroded soils fertility and environmental conditions was established. The slope soils are represented by the variety of watershed types of black mollisols. Essential changes in the content of available forms of nitrogen, phosphorus, potassium, and enzyme activity were established for eroded and non eroded soils.

The content of exchangeable phosphate and potassium is significantly reduced with the increasing of erosion degree. It was fixed different character of

main nutrients uptake in two eroded soil types. The maximum consumption of peas of nitrogen, phosphorus and potassium was in the E1 soil profile in the layer 0-10 and 10-20 cm.

ACKNOWLEDGEMENTS

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THE INFLUENCE OF NITROGEN FERTILIZER ON THE TOTAL NUMBER OF MICROORGANISMS AND AMINOAUTOTROPH DYNAMICS UNDER "UGAR" AND SOWN MAIZE

SUMMARY

Soil microorganisms have a key role in creation and maintaining of soil fertility. Anthropogenic impact on the soil microorganisms is extremely powerful. The application of fertilizer represents the most significant one.

Applying fertilizers increase biological productivity of agroecosystem and microbiological activity of soil, but, at the same time, they cause certain changes inside them through the influence on microorganisms. This research examines how microbiological activity of soil is influenced by the quantity of applied N fertilizer and "ugar" or tillage and the connection between this given activity and the maize quality. The total number of microorganisms and the number of amino-autotrophs depend on a phenophase of a plant, "ugar" or tillage and the quantity of applied N fertilizer. Less quantity of applied N fertilizer affects the increase of a number of microorganisms and components of the maize income whereas higher concentration of applied N fertilizer has an inhibitory effect.

Keywords: soil, fertilizer, total number of microorganisms, number of amino-autotrophs, maize.

INTRODUCTION

Biogeochemical cycles of matter and energy flowing are basic for obtaining life on the planet, they are constant, developing themselves in the ecosystems due to microorganisms. In the agroecosystems, which cover nearly one ninth of the whole Earth's surface (Lješević, 2005), soil microorganisms have a primary role in these processes. Through the enzymes which are being free into surrounding area, these soil microorganisms take part in almost all soil biochemical reactions which are present in C, N, P, S cycles and microelements, where organic substances of soil are produced and degraded and fertilizers again become available for plants. Decomposing of organic synthesis of carbon

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and nitrogen, dissolving of phosphate, H_2S oxidation, nitrification, de nitrification and nitro fixation, transformations of microelements and heavy metals are just some of the processes which continuously happen in soil thanks to metabolic activity of microorganisms as well as to enzymes which are being released. Enzymes are very significant protein molecules which allow chemical reactions that are life important (Topisirović, 1998). Plants use released mineral salts, which are the result of the enzyme catalyzed processes. At the same time, microorganisms also compete with plants for these biogenic elements in soil which they also include in the biosynthesis of their cells' plasmas (Kuzyakov, 2002). Microorganisms are uncontested the most important factor in forming of soil fertility. Having the possibility to rule microbiological processes in soil, many perspectives for soil fertility refinement are promising. The success in the first place depends on as better evaluation of soil biogenesis as possible whereas the number and the enzyme activity are the most remarkable indicators. The number and enzyme activity stand for the current state of microbiological soil activity, so, for a better considering of biogenesis, it is preferable to observe these parameters for several times during the vegetation of the growing plants (Jarak et al. 2006). Apart from abiotic factors, microbiological soil activity is also affected by biotic factors among which anthropogenic have a leading function. In agro ecosystems, the application of fertilizers is one of the most significant anthropogenic. Mineral fertilizers increase biological productivity of agroecosystems (Marković et al. 1985, Nedić and Šuput 1977). Among the N, P and K elements which are being used as fertilizers, nitrogen is considered to have the most important part in soil fertility, its microbiological activity, growing and development of cultivating plants (Albinska et al. 2002; Popović, 2010; Glamočlija et. al. 2015). On the other hand, fertilizers which are being used cause a number of changes in the given agroecosystem, they have an impact on the physical and chemical soil features, the structure of microbiocenosis and all the former mentioned processes which happen in a soil - microorganism - plant system. Considering the key function of microorganisms, the appearing changes can influence the cycles of matter and energy flowing as well as the creation and maintaining of soil fertility (Doran and Zeiss 2000).

Examining the activities of soil microorganisms under the tillage of cultivated plants and noticing the correlation between the given activity and growth and development of a plant in conditions under the applying doses of the concentration of mineral fertilizer, it is possible to determine the optimal concentration of fertilizer by which a plant productivity is highly improved and the costs of production are decreased in great measure. The research in this area enters the essence of the ecosystem functioning, that is, the way of biogeochemical cycles of matter and energy flowing as the basis of life and it conducts us how to get back the de-arranged courses into their natural forms by using anthropogenic influence. As it is mentioned before, the aim of this research is establishing the influence of different concentration of nitrogen fertilizer on microbiological soil activity under the sown maize.

MATERIAL AND METHODS

The research of the impact of nitrogen fertilizer concentration on microbiological soil activity, type "cernozem" under the sown maize is carried out in the area of Eastern Srem (The Maize Institute "Zemun Polje"). During the research field micro tests are used, conducted by the split plots method in four goes. The surface of the main plot was 403,2 squares, the subplot was 16,8 squares (6,0 x 2,8 sqrs) and the accounting plots for a grain yield was 8,4 sqrs.

Testing covered the following systems of maize fertilizing as well as the variations on "ugar", i.e. without tillage:

1. follow up (without fertilizing);
2. $P_{90} K_{60} N_{30}$ kg/ha (basis, phon);
3. $P_{90} K_{60} N_{60}$ kg/ha;
4. $P_{90} K_{60} N_{120}$ kg/ha;
5. $P_{90} K_{60} N_{180}$ kg/ha.

During the tests the standard agro technique is applied. In the both years of the research, the prior crop was winter wheat. After the wheat harvest, the ground is plown on the depth of 10-15 cm. Before the fundamental soil cultivation, 300 kg/ha of mineral fertilizer NPK10:30:20 formulations are used.

The fundamental soil cultivation had been finished during the autumn on the depth of 25 cm. During the spring, the additional cultivation was carried out, as well as the additional nitrogen KAN fertilizer (27%N), in the concentration of 30, 90, 150 kg/ha of active matter and finally the pre- harvest preparation took place.

The seed used for sowing was maize hybrid ZPSC- 578 (FAO group of reaping 500). The harvest (gathering) was done manually in the physiological reap of the seeds. The chemical structure of the maize yield was determined on the DICKEY-JOHN NIR Analyzator machine. Soil samples were taken in the period of 2006 and 2007, before maize sowing, in the phase of a period of silking and at the end of the vegetation period on the depth of 0-30 cm, on "ugar" and under the sown maize. During the research meteorological conditions were also taken into consideration.

In the soil samples the basic chemical features were determined by the following methods:

1. reaction (pH in H_2O and KCl) of soil- by electrometric method;
2. % $CaCO_3$ - by Scheibler's method;
3. % humus - by Turin's method, Simankov's modification;
4. % total percent of nitrogen-by Kieldahl's method;
5. the percent of mineral nitrogen (NO_3 and NH_4)- by Bremner's method;
6. the percent of P_2O_5 and K_2O - by AL- method (Egner and Reihm).

Microbiological analysis has determined the total number of microorganisms and aminoautotrophs. The soil samples were taken (under the

sown maize and on "ugar") on the depth of 30 cm. The number of microorganisms is detected by the standard indirect method of sowing diluted soil samples on selective nutritious substrata, which were afterwards incubated on the temperature of 28 degrees. The number is expressed in grammas of completely dry soil.

The following features were determined in soil: - the total number of microorganisms on nutritious substratum tripton- soya agar (TSA), (10^{-5});

- the number of aminoautotrophs on the starch- ammoniac agar substratum, (10^{-4}).

The research results were analyzed by descriptive statistics method.- the number of aminoautotrophs on the starch- ammoniac agar substratum, (10^{-4}). The research results were analyzed by descriptive statistics method.

Agroecological conditions during the experiment conducting

The Eastern Srem is settled down in the area of medium- continental climate and it borders to the north, east and south with two big rivers, the Danube and the Sava. This area belongs to wide Pannonia lowland which has convenient climate and soil conditions for arable farming. The exception is an average rainfall and its unequal arrangement during years.

Meteorological conditions. Average monthly air temperatures in the research years as well as the lasting several years' average (1995-2004), during the vegetation period of maize is shown in the table 1. In the both years of the research, the average month temperatures increased from April until July after which they decreased. The average air temperatures for the maize vegetation period was lower in 2006 and it was $19,7^{\circ}\text{C}$ which is $1,1^{\circ}\text{C}$ lower than in 2007, but on the several years' level. ($19,6^{\circ}\text{C}$ April is characteristic for higher average monthly temperature in the both years regard to the lasting several years' period. Air temperature in April 2006 was higher for $1,2^{\circ}\text{C}$ and in 2007 for $1,8^{\circ}\text{C}$. In May 2006, the average monthly temperature was $17,6^{\circ}\text{C}$. According to the lasting several years' period it was lower for $1,1^{\circ}\text{C}$. In the same month of 2007, the temperature was higher for 1°C than in the lasting several years' period. Similar tendency was confirmed in June, too. In this month, the average air temperature in 2006 was $20,3^{\circ}\text{C}$ and it was lower for $1,5^{\circ}\text{C}$ than in the several years' period ($21,8^{\circ}\text{C}$). June 2007 was warmer for $1,8^{\circ}\text{C}$ than a decade period. In the both research years, July was warmer than the several years' period ($23,2^{\circ}\text{C}$). In 2006, the average air temperature was higher for $1,4^{\circ}\text{C}$ and in 2007 for $2,5^{\circ}\text{C}$.

On 24th july 2007 recordly high temperature of $44,9$ degrees was registered. In august 2006, the average monthly temperature was $21,7^{\circ}\text{C}$, which was a bit lower for $1,7^{\circ}\text{C}$ in comparison to the several years' period ($23,4^{\circ}\text{C}$). On the other hand, august 2007 was warmer for $1,0^{\circ}\text{C}$ than average and for $2,7^{\circ}\text{C}$ than the first month in the first year of the research. On the contrary, the

average monthly air temperature in september was lower in 2007 and it was 16,8 o c which was lower for 1,0 oc than the several years' period and for 2,9 oc at 2006, table 1

Table 1. Temperature and precipitation, 2006–2007, Zemun Polje, Serbia

Month	Temperature		Average long 1995-2007	Precipitation		Average long 1995-2007
	1. year	2. year		1. year	2. year	
April	14.1	14.7	12.9	97.0	4.0	65.0
May	17.6	19.7	18.7	40.0	79.0	57.0
June	20.3	23.6	21.8	137.0	108.0	79.0
July	24.6	25.7	23.2	22.0	18.0	81.0
August	21.7	24.4	23.4	123.0	72.0	57.0
September	19.7	16.8	17.8	26.0	85.0	82.0
Average	19.7	20.8	19.6	445.0	366.0	421.0

The facts about the amount of rainfall during the months of a vegetation period of maize in the research years as well as the annual average (1995-2004) are shown in table 1.

The years when this research took place distinguished between themselves according to the total amount of rainfall during the vegetation period of maize and its proportion in certain months. Bigger amounts of rainfall for the vegetation period of maize (iv-ix) were registered in 2006 (445 mm) than in 2007 (366 mm). Comparing this with the lasting several years, in the first year of the research the amount of rainfall was bigger for 24.0 mm and in the second year it was smaller for 55.0 mm and it affected growing and development as well as the maize productivity. In April 2006, the amount of rainfall was 97.0 mm which was for 32.0 mm more than in the lasting several years' average (65mm). On the contrary, April 2007 characterized itself with 4.0 mm of rainfall. The situation was reversed in May. The bigger amount of rainfall in this month was registered during 2007 and it was 79.0 mm which was more for 22.0 mm than average and for 39.0 mm more than the amount in 2006. During June, in the both years of the research, there were suitable humidity conditions for growing and development of maize. In 2006 in June 137.0 mm of rainfall was registered and in 2007 this amount was 108,0 mm. In comparison with the lasting several years' average (79.0 mm) these amounts were bigger for 58.0 mm, that is 29.0 mm. In July, in contrast to June, humidity conditions were inconvenient for growing and development of maize. In 2006 in this month, the amount of rainfall was 22.0 mm and in 2007 it was 18.0 mm. In comparison with the lasting several years' period (81.0 mm) these amounts were smaller for 59.0 mm, that is, 63.0 mm. During august in 2006 the amount of rainfall was 123.0 mm which was more for 66.0 mm than a ten-year period average in Zemun Polje (57.0 mm). In the same month in 2007 the rainfall was smaller for 51.0 mm than in the first year of the

research. The amount of rainfall in September also varied. In this month in 2006 the amount was 26.0 mm which was smaller for 56.0 mm than in the lasting several years' period (82.0 mm). On the other hand, September 2007 was distinguished with the amount of rainfall on the lasting several years' level (85 mm). On the basis of these results, it can be seen that more suitable temperature and humidity conditions for microbiologic activity of soil and maize cultivation were in 2006 in comparison with 2007.

RESULTS AND DISCUSSION

The research of the impact of nitrogen fertilizer quantity on microbiological activity of soil under the sown maize was conducted in the "cernozem" soil type. According to the pedological study of the soil in Zemun Polje, carbon cernozem is characterized by humus- accumulative (Ah) horizon of 0-50 cm of depth, dark black color and powdery clay argil texture. On the 0-30 cm depth its structure consists if small lumpy and grainy particles and going more deeply by the end of Ah horizon it is more compact and its structure is lumpy and nut shaped.







Table 3. Chemical analysis of soil (Zemun Polje)

Depth (cm)	pH		CaCO ₃ (%)	Humu s (%)	Total N (%)	Available N (ppm)		mg in 100 g soil	
	H ₂ O	KCl				NH ₄ ⁺	NO ₃ ⁻	P ₂ O ₅	K ₂ O
0-30	7.71	7.3 4	4.4	2.86	0.19	4.9	17.5	25.4	22.2

On the basis of the chemical analysis results (Table 3), which are performed in the agrochemical laboratory of the Faculty of Agriculture in Zemun, it can be said that the reaction of the soil mixture was mild alcal. Medium humus and nitrogen concentration are present in this soil and easily accessible phosphor and potassium are highly proportioned. A micro field testing station is positioned in Zemun Polje on 88 metres altitude.

The total number of microorganisms represents a general soil biogenesis and together with enzyme activity it is a reliable fertility indicator. Using the variable analysis of it is established that the total number of microorganisms was affected in great measure by all the examined factors: a phenophase of a plant (A), ugar or tillage (B) quantity of the applied fertilizer (C), and their interactions (Table 4). The number is notably expressed in a plant reaping phenophase than in a cub blooming phenophase, which can be explained by meteorological conditions, which were prevailing. The plant reaping phenophase was more liable to hydrotermic regime, i.e. more rainfall, drought absence and more equal temperatures.

Table 4. The total number of microorganisms in the soil on account of a phenophase of a plant, ugar or tillage and a quantity of nitrogen fertilizer (105 g-1) in the first and the second years of the research.

1. year					2. year				
A	C	B			A	C	B		
V	N	ugar	Under crop		V	N	Ugar	Undrer Crop	
Flowering	1.	75,7	71,0	73,35	Flowering	1.	78,0	34,9	56,45
	2.	83,2	87,3	85,25		2.	84,8	43,8	64,3
	3.	142,3	113,5	127,9		3.	120,3	56,7	88,5
	4.	128,0	94,0	111,0		4.	101,9	52,9	77,4
	5.	64,9	80,1	72,5		5.	53,3	36,8	45,05
		98,92	89,18	94,0					
Maturing	1.	153,7	119,4	136,55	Maturing	1.	359,1	330,9	345
	2.	163,0	134,5	148,75		2.	391,2	367,1	379,15
	3.	193,0	161,7	177,35		3.	501,2	410,0	455,6
	4.	140,0	100,3	120,15		4.	378,9	432,8	405,85
	5.	121,3	109,0	115,15		5.	225,7	69,9	147,8
		154,2	124,98	139,59			371,22	322,14	346,68
1. Control, 2. P ₉₀ K ₆₀ N ₃₀ kg/ha; 3. P ₉₀ K ₆₀ N ₆₀ kg/ha; 4. P ₉₀ K ₆₀ N ₁₂₀ kg/ha; 5. P ₉₀ K ₆₀ N ₁₈₀ kg/ha;									

Year	LSD	A	B	C	AB	BC
1.	0.5	7,69	5,13	12,16	10,88	17,20
	0.1	10,29	7,03	16,27	14,55	23,01
2.	0.5	2,05	1,73	3,24	2,30	3,71
	0.1	3,72	2,91	4,33	3,89	5,23

Microbe populations and their biochemical activities in the soil are subjected to the season fluctuations (Matinizadeh et al., 2008). Equal temperatures and humidity stimulate proliferation of soil microorganisms, and water availability is a crucial environmental parameter which affects the quantity and diversity of soil microflora (Anderson, 1984).

In the plant reaping phenophase the excretion of the root sugar secretes is more intensified likewise the organic acid, amino acid, vitamins, hormones and other organic matters so an easier development of microorganisms is probably connected to reachable resources of carbon and energy from plants.

The total number of microorganisms is more expressed on ugar than under the sown maize (table 4). Knowing the fact that "ugar" is soil without tillage, a higher number of microorganisms can be explained by the absence of competitive interactions with roots for nutrients. The variable analysis showed very significant differences in the total number of microorganisms within different quantity of the applying fertilizer (table 4). Apart from the control less quantity of fertilizer (n30 and n60 kg/ha) had a great effect on increasing of the

total number of microorganisms while higher quantity of fertilizer (n180 kg/ha) worked as inhibitors.

Mineral fertilizers intensified microbiological processes in soil and they affected an income increase at the beginning, but when they are used in less quantity, their effect was visible on increasing the total number of microorganisms and their systematic and physiologic groups while their higher quantities had a diverse effect (Epanchinov, 1975, Milošević, 1990, Đodević, 1998, Hajnal - Jafari, 2010).

Nevertheless, in a short period, mineralization of humus is quickened as well as of other nitrogen synthesis, nitrate is quickly accumulated, the part of biological nitrogen in plant is shortened which leads to worse conditions of soil features and decrease of culture income. Higher concentration of mineral fertilizers affects the total number of microorganisms and general soil biogenesis in negative manner (Arnus et al., 1995, Đukić and Mandić, 1997).

These testing also proved the pattern of influence of the applying fertilizers on the total number of microorganisms. Beside the total number of microorganisms, the number of some systematic and physiologic groups of microorganisms also becomes one of the general soil biogenesis indicators.







Aminoautotrophs stand for bacteria which are physiologically united by their capability to use mineral synthesis of nitrogen metabolically in metabolic purposes (Đukić i Jemcev, 2000). The variable analysis showed that the number of aminoautotrophs was in a great part influenced by the following factors: a phenophase of a plant (A), ugar or tillage (B), and the concentration of the applying fertilizer (C) as well as the interaction of factors AC, BC and ABC (Table 5).

In the first year of the research, interactions of AB factor were not statistically significant and in the second year B factor (ugar or tillage) didn't affect the number of aminoautotrophs as well (Table 5).

In the both years of the research, there were more aminoautotrophs (table 5) in a phenophase of a plant reaping than in a phenophase of a cob blooming. The growth of a number of microorganisms in the plant reaping phenophase is stimulated in the first place by the higher quantity of rainfall and dry periods' absence. In the phenophase of a plant blooming, especially during 2007, extremely high temperatures were recorded and that had an adverse effect on development of microorganisms. The quantity of nutrients, supstrat availability, soil humidity, temperature, and all other ecologic conditions of importance can have a strong mutual dependance and affect, in that way, biologic and biochemical processes in soil in a complex way (seifert et al., 2001, tabaković et al. 2016). There is no a simple answer in which way these factors have an impact on microorganism populations and their activities. Ugar or tillage in the first year

of the research had a significant influence on autotrophs' development in both plant phenophases. Although in the both years of the research the number on ugar was more explicit in the both phenophase than on tillage, the differences are statistically very important just in the phenophase of a cob blooming in 2006 (table 5). The concentration of the applied fertilizer had a notable impact on the number of autotrophs during the both years of the research. In average, the most outstanding increase of the number in comparison with the control is noticed in the test variation n120 kg/ha although the quantity n60 kg/ha also influenced in a very stimulative way the number of this group of microorganisms. The most numerous number of aminoautotrophs was established in n120 kg/ha applying variation and it is $364,3 \times 10^4 \text{ g}^{-1}$ while the lowest one was in n180 kg/ha and it was $31,4 \times 10^4 \text{ g}^{-1}$ (table 5).

Table 5. The number of the aminoautotrophs in soil depending on the phenophase of a plant, ugar or tillage and quantity of n fertilizer (104 g^{-1}) quote in year one (5a) and in year two (5b) of the research.

1. year					2. year				
A	C	B			A	C	B		
V	N	ugar	Under crop		V	N	Ugar	Undrer Crop	
Flowering	1.	204,0	113,0	158,50	Flowering	1.	101,8	118,9	110,35
	2.	185,5	193,4	189,45		2.	60,5	75,8	68,15
	3.	220,3	142,7	181,50		3.	150,7	123,4	137,05
	4.	168,0	178,1	173,05		4.	153,0	82,0	117,05
	5.	133,1	127,0	130,05		5.	89,9	31,4	60,65
		182,18	150,84	166,51			111,18	86,3	98,74
Maturing	1.	251,3	217,7	234,50	Maturing	1.	263,1	306,0	284,55
	2.	265,1	237,0	251,50		2.	314,9	320,5	317,70
	3.	298,4	279,9	289,15		3.	343,0	310,0	326,50
	4.	320,0	364,3	342,15		4.	322,1	312,0	317,05
	5.	205,2	172,7	188,95		5.	228,1	290,2	259,15
		268,0	254,32	261,16			294,24	307,74	300,99
1. Control, 2. $\text{P}_{90}\text{K}_{60}\text{N}_{30} \text{ kg/ha}$; 3. $\text{P}_{90}\text{K}_{60}\text{N}_{60} \text{ kg/ha}$; 4. $\text{P}_{90}\text{K}_{60}\text{N}_{120} \text{ kg/ha}$; 5. $\text{P}_{90}\text{K}_{60}\text{N}_{180} \text{ kg/ha}$;									

Year	LSD	A	B	C	AB	BC	ABC
1.	0.5	14,80	12,13	23,40	33,10	30,53	47,33
	0.1	19,80	16,71	31,31	44,28	41,02	61,82
2.	0.5	12,54	19,83	17,74	27,56	20,17	37,91
	0.1	16,78	26,54	23,73	36,87	25,37	49,10

Quantity of 180 kg/ha nitrogen decreased in great manner the number of aminoautotrophs in comparison with the control in the both years of the research.

Besides the number of microorganisms, it was also examined how the quantity of nitrogen affected maize productivity within the productivity parametres, maize income and contents of proteins in grains. The results of this research showed that within the average for the tested factors, the maize income was 8,65 t/ha (table 6).

Table 6. The impact of nitrogen quantity on the grain (maize) income, t/ha

Quantity of nitrogen (B)	Year (A)		Average	Index (%)
	2006	2007		
1	8.05	7.54	7.80	100.0
2	8.27	7.78	8.03	102.9
3	8.82	8.48	8.65	110.9
4	9.92	9.04	9.48	121.5
5	9.78	8.81	9.30	119.2
Average	8.97	8.33	8.65	-
Index (%)	100.0	92.9	-	-
1. Control, 2. P ₉₀ K ₆₀ N ₃₀ kg/ha; 3. P ₉₀ K ₆₀ N ₆₀ kg/ha; 4. P ₉₀ K ₆₀ N ₁₂₀ kg/ha; 5. P ₉₀ K ₆₀ N ₁₈₀ kg/ha;				

LSD	A	B	BxA	AxB
0.05	0.17	0.17	0.24	0.28
0.01	0.31	0.23	0.33	0.45

In 2006, the grain income was higher for 0.64 t/ha than in 2007. The income difference between these two years statistically was very significant.

In average for these years, when the quantity of the applied n fertilizer was 120 kg/ha, the grain income increased and then it decreased. The lowest grain income (7,80 t/ha) was measured on the control, without fertilizing and the highest one (9,48 t/ha) was on the variable with 120 kg/ha n. The differences in quantity of fertilizer and the grain income were significant and very significant (table 6).

The research results show that, in average, for the tested factors, the concentration of proteine in a grain was 8.58% (table 7). Higher concentration of proteins in a grain is established in the year with less rainfall and higher temperatures (2007) during the maize vegetation period. With the increasing quantity of nitrogen to 120 kg/ha, the concentration of proteins increased and then decreased. In the average for these years, the smallest concentration of proteins in grains (8,26%) was measured on the control and the highest concentrations (8,87%) was measured at 120 kg/h use. In both years of the research, there was a tendency for increasing proteins with graduate increasing of dosis n (table 7).

Table 7. The impact of N fertilizer quantity to concentration of proteins in grain, %

Quantity of nitrogen (B)	Year (A)		Average	Index points
	2006	2007		
1	8.02	8.49	8.26	100.0
2	8.13	8.72	8.43	102.1
3	8.34	8.75	8.55	103.5
4	8.81	8.92	8.87	107.4
5	8.69	8.88	8.79	106.4
Average	8.40	8.75	8.58	-
Index points	100.0	104.2	-	-
1. Control, 2. P ₉₀ K ₆₀ N ₃₀ kg/ha; 3. P ₉₀ K ₆₀ N ₆₀ kg/ha; 4. P ₉₀ K ₆₀ N ₁₂₀ kg/ha; 5. P ₉₀ K ₆₀ N ₁₈₀ kg/ha;				

CONCLUSIONS

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

Tested factors, a phenophase of a plant, ugar or tillage and quantity of the applied nitrogen fertilizer affected in a remarkable way the total number of microorganisms and aminoautotrophs.

The number of microorganisms is more expressive in a phenophase of a plant reaping on ugar.

Regard to the control the quantity of nitrate fertilizer N30, N60 and N120 kg/ha induced the total number of microorganisms and aminoautotrophs whereas the quantity of the applied nitrate fertilizer N180 kg/ha functioned as inhibitor on the tested parameters of soil biogenecy.

The applied quantity of nitrogen had a positive effect on nutritive values of grains and the maize income.

On "cernozem" and in the climate conditions of the Eastern Srem, the appropriate nourishment with nitrogen hybrid ZP 578 implies the use of 120kg/ha N, on a phon 90 kg/ha phosphore and 60 kg/ha potassium.

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INSECTICIDAL ACTIVITY OF *Citrus sinensis* (L.) and *Parkia biglobosa* (Jacq.) EXTRACTS AGAINST *Trogoderma granarium* Everts

SUMMARY

In this study, ethanolic extracts of *Citrus sinensis* (L.) (leaves/peels) and *Parkia biglobosa* (Jacq.) (leaves/seeds) were screened for insecticidal activity against 4th larval instar and adults of khapra beetle, *Trogoderma granarium* Everts (Coleoptera:Dermestidae). Also examined were percentage seed damage, seed weight loss and viability of the seeds. Decorticated seeds of groundnut were treated with different concentrations (1, 3 and 5%) of the extracts against the adults while 5% concentration was tested against the larvae. The bioassay showed that the extracts had larvicidal and adulticidal activities which increased with increase in concentration and exposure time. The seed damage data were used to estimate the Beetle Perforation Index (BPI) indicating that *P. biglobosa* seed extract was the most effective with a BPI of 20.14%. The results of this study showed that *C. sinensis* and *P. biglobosa* extracts possess some insecticidal activity which may be associated with the presence of secondary metabolites (saponins, alkaloids, steroids, tannins, cardiac glycosides, flavonoids and phenols in them, This report shows that *P. biglobosa* seed extract could be recommended as a source of insecticidal agent in a pest management technique.

Keywords: *Trogoderma granarium*, plant extracts, insecticidal activity, biopesticides.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is known by many names including peanut, earthnut, goober peas, monkey peas, pig nuts and pygmy nuts (Alabi *et al.*, 2013) and belongs in the family Fabaceae, sub-family Papillionaceae. In Nigeria, it is an important oil, food and forage crop grown by the small-holder farmers in the northern part of the country.

Kernel weight damage in storage has been estimated to range between 10 and 25% under short-term storage period in Nigeria, but it could be higher under long-term storage situations (Musa, 2007). There is no compensation for seed damage caused by stored-product pests as it is the case for damage induced by field pests. In Nigeria, the profitable preservation of groundnut seeds is greatly constrained by *Trogoderma granarium* Everts, thereby creating a challenge of sustainable preservation of the seeds meant for future consumption, planting and

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even trade. *Trogoderma granarium* is a dermestid that can cover the surface of stored grains with crawling larvae capable of turning the grains into powder (Ellis and Hodges, 2007).

The current method of controlling *T. granarium* involves the application of synthetic, broad-spectrum pesticides and fumigants. However, for fumigants to be effective against the pest, high concentration of the chemical must be maintained over the fumigation period to allow penetration into cracks and crevices (Harris, 2006). The use of methyl bromide and phosphine has led to consequent repercussions on the farmers and the environment or even the depletion of ozone layer (Musa, 2007). These synthetic insecticides have also been confirmed to be responsible for development of insect resistance, toxic residues in food, workers' safety and high cost of procurement (Asawalam and Onu, 2014). These problems have necessitated their ban and current research on the use of biopesticides from either plants or micro-organisms that are safe to the farmers and the environment. This study therefore, attempts to contribute to the existing information on the possible effects of ethanolic extracts of plants on *T. granarium*. The objective of the study was to examine the insecticidal activity of sweet orange, *Citrus sinensis* L. and African locust bean, *Parkia biglobosa* (Jacq.) Benth against khapra beetle, *T. granarium*, in stored groundnut.

MATERIAL AND METHODS

Culture of *Trogoderma granarium*

The strain of khapra beetle, *T. granarium*, used for this study was obtained from the culture maintained in the laboratory of the Department of Crop Protection, University of Ilorin, Ilorin, Nigeria. The dermestid was raised on groundnut seeds in 250 ml kilner jars at ambient temperature of $28\pm3^{\circ}\text{C}$ and relative humidity of $68\pm3\%$. Thirty adults of mixed sexes were picked from the existing culture with the aid of pooter (an aspirator), and used to infest fresh and susceptible seeds. The insects were sieved out after two weeks when they would have mated and laid eggs. Fourth larval instar and freshly emerged adults (1-2 days old) were used in this study.

Seed disinfestations

Groundnut seeds (SAMNUT 7) obtained from the Institute for Agricultural Research, Samaru, Nigeria were properly sorted, winnowed, wrapped in a polyethylene bag and then placed in a freezer compartment of a refrigerator for 2 weeks to kill all developmental stages of insect pests. The seeds were certified fit for this study after 2 weeks of equilibration. The moisture content determined on a wet basis using oven dry method was 8.7%.

Plant materials

Fresh leaves of sweet orange, *C. sinensis* were collected from the Park and Gardens of the University of Ilorin, Ilorin, Nigeria while the peels were collected from orange sellers who often regard the peels as waste. The leaves and seeds of African locust bean tree, *P. biglobosa*, were obtained from the tree growing

naturally on the campus. These plant parts were washed thoroughly under running water, air-dried, ground and sieved to obtain fine uniform particle size. The powders were kept separately in airtight plastic containers at prevailing temperature and relative humidity.

Preparation of plant extract

The powdered mass of 50 g each of the *C. sinensis* leaves/peels and *P. biglobosa* leaves/seeds, was extracted by pouring it in 500 ml ethanol to produce a concentration of 10.0%. The mixture of the powder and ethanol was thoroughly shaken and allowed to stand for 48 h before it was filtered through muslin to get the stock solution. The filtrates were transferred to separate beakers and evaporated over a steam bath at 50⁰ C to obtain the pasty extracts. Different concentrations of 1, 3 and 5% were prepared following filtration process and a 2 mm of each concentration was employed in the bioassay.

Experimental procedure

Bioassays were carried out in the Crop Protection Laboratory of the University of Ilorin, Ilorin, at prevailing ambient temperature and relative humidity of 28±3⁰C and 68±3% respectively.

Effect of the extract on larval mortality

The different extracts of *C. sinensis* (leaves/peels) and *P. biglobosa* (leaves/seeds) were applied at 5% concentration to decorticated groundnut seeds in plastic containers (5.0 cm × 8.0 cm) by means of hypodermic syringe. Each container was gently shaken to ensure coating of the seeds with extracts and then allowed to dry for 1 h before infestation. Ten 4th larval instar of *T. granarium* were introduced into each treatment and then covered with muslin fastened with rubber band. Each treatment was replicated three times, including the controls which were seeds either left untreated or treated with 5% ethanol. Dead larvae were counted every 24 h for a total of 96 hours after treatment (HAT).

Effect of the extract on adult mortality

In this investigation, the different extracts of *C. sinensis* (leaves/peels) and *P. biglobosa* (leaves/seeds) were applied at 1, 3 and 5% concentration to decorticated groundnut seeds in plastic containers (5.0 cm × 8.0 cm) by means of hypodermic syringe. Each container was gently shaken to ensure coating of the seeds with the extracts and allowed to dry for 1 h before infestation. Three females and two males of freshly emerged adults (1-2 days old) of *T. granarium* were used to infest the seeds. Dead insects were counted every 24 h for a total of 96 HAT.

Seed weight loss

Percentage seed weight loss was calculated for larvae-infested groundnut at 30 days intervals using the formula:

$$\text{Seed Weight Loss} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

Seed damage assessment

Damage assessment was carried out on the larvae-infested seeds by separating the seeds into damaged (seeds with holes) and undamaged (seeds without holes) groups. Therefore, percentage seed damage was based on differences between the total number of seeds and number of seeds perforated divided by total number of seeds expressed as percentage.

$$\%Seed\ Damage = \frac{Total\ No.\ of\ Seeds - No.\ of\ Seeds\ Perforated}{Total\ No.\ of\ Seeds} \times 100$$

Beetle Perforation Index

Beetle Perforation Index was determined for each treatment concentration for comparison of seed protectant effects using the procedure of Fatope *et al.*, (1995) as shown below:

$$BPI = \% \text{ of treated seeds perforated} \times 100 / \% \text{ of control seeds perforated} + \% \text{ of treated seeds perforated}$$

Seed viability test

The floatation method described by Ehiagbonare and Enabulele (2007) was modified to check the viability of the seeds after treatment with a concentration of each of the extracts. Twenty seeds per replicate were sprayed with 5 ml solution of the plant extracts. Percent viability was recorded at 2 h after treatment. All treatments were conducted in three replicates and the mean values were calculated using the formula:

$$Perforated\ Viability = (S - Sf / S) \times 100$$

where

S = number of treated seeds used/replicate

S_f = number of floating seeds/replicate

Data analysis

Data were subjected to one-way analysis of variance to determine critical difference among treatments. Significantly different ($p=0.05$) means were partitioned using Duncan's multiple range test.

RESULTS AND DISCUSSION

Effect of ethanolic extracts on *T. granarium* adults

The effect of ethanolic extracts of *C. sinensis* (leaves/peels) and *P. biglobosa* (leaves/seeds) on *T. granarium* adults is presented in Table 1. The plant extracts applied at various concentrations had no significant difference ($p>0.05$) on the mortality of *T. granarium* at 24 HAT. However, the extract of *C. sinensis* leaves applied at 5% concentration and *P. biglobosa* seeds applied at 3 and 5% concentrations caused highest mortality which was not significantly different ($p>0.05$) from the mortality in other treatments at 48 HAT. There was no mortality in 1% concentration of *C. sinensis* peels or *P. biglobosa* seeds and the control which was significantly different from the mortality in other treatments.

The extract of *P. biglobosa* seeds applied at 3 and 5% concentrations caused highest mortality of *T. granarium* which was not significantly different ($p>0.05$) from the mortality in 3 and 5% concentrations of *C. sinensis* leaves or *P. biglobosa* leaves at 72 HAT. The least mortality was observed in 1% concentration of *C. sinensis* peels, *P. biglobosa* leaves or *P. biglobosa* seeds which was not significantly different ($p>0.05$) from no mortality in control at 72 HAT. At 96 HAT, the extract of *P. biglobosa* seeds applied at 3 and 5% concentrations again caused highest mortality which was the same as mortality in 5% concentrations of *C. sinensis* leaves. The least mortality was observed in 1% concentration of *P. biglobosa* leaves or *P. biglobosa* seeds which was not significantly different ($p>0.05$) from no mortality in control at 96 HAT.

Table 1: Effect of ethanolic extracts on mortality of *Trogoderma granarium* adults

Extracts	Concentration (%)	Mortality of <i>T. granarium</i> adults (HAT)			
		24	48	72	96
<i>C. sinensis</i> leaves	1	0.00(0.00)	0.33bc (6.6)	1.00bcd (20.0)	1.33bc (26.6)
	3	0.67 (13.4)	1.00abc (20.0)	2.00ab (40.0)	2.33ab (46.6)
	5	1.00 (20.0)	1.67ab (33.4)	2.00ab (40.0)	2.67a (53.4)
<i>C. sinensis</i> peels	1	0.00 (0.00)	0.00c (0.00)	0.33cd (6.6)	1.33bc (26.6)
	3	0.33(6.6)	0.33bc (6.6)	1.00bcd (20.0)	1.33bc (26.6)
	5	0.67(13.4)	0.67abc (13.4)	1.00bcd (20.0)	1.33bc (26.6)
<i>P. biglobosa</i> leaves	1	0.33 (6.6)	0.33bc (6.6)	0.33cd (6.6)	0.67d (13.4)
	3	0.00 (0.00)	0.67abc (13.4)	1.33abc (26.6)	1.67abc (33.4)
	5	0.67 (13.4)	1.00abc (20.0)	1.67ab (33.4)	2.00ab (40.0)
<i>P. biglobosa</i> seeds	1	0.00 (0.00)	0.00c (0.00)	0.33cd (6.6)	0.67d (13.4)
	3	1.00 (20.0)	1.33ab (26.6)	2.33a (46.6)	2.67a (53.4)
	5	0.33 (6.6)	1.33ab (26.6)	2.33a (46.6)	2.67a (53.4)
No extract	0	0.00 (0.00)	0.00c (0.00)	0.00d (0.00)	0.00d (0.00)
SE \pm		0.32	0.32	0.29	0.35
CV (%)		82.0	47.5	24.4	22.1

Values with the same superscript(s) in the same column are not significantly different at 5% level of probability using DMRT.

Figures in parentheses are in percentages

KEY: HAT = Hours After Treatment; CV = Coefficient of Variation; SE = Standard error

Effect of ethanolic extracts on *T. granarium* larvae

The effect of ethanolic extracts of *C. sinensis* (leaves/peels) and *P. biglobosa* (leaves/seeds) on *T. granarium* larvae is presented in Table 2. The *P. biglobosa* seeds caused significantly higher ($P<0.05$) mortality of *T. granarium*

larvae than the mortality in other treatments at 24 HAT. The subsequent mortality values in *P. biglobosa* seeds were not significantly different ($p>0.05$) from mortality in *P. biglobosa* leaves from 48 to 96 HAT. Ethanol-treated groundnut showed increase on larval mortality with increase in exposure period. The ethanol-free treatment had no mortality only at 24 HAT while extract-free treatment had no mortality throughout the exposure period. *C. sinensis* leaves consistently had the least larval mortality during the exposure period.

Table 2: Effect of ethanolic extracts on mortality of *Trogoderma granarium* larvae.

Extracts	Mortality of <i>T. granarium</i> larvae (HAT)				
	Concentration (%)	24	48	72	96
<i>C. sinensis</i> leaves	5	0.33b (3.3)	0.67c (6.7)	1.67cd (16.7)	3.00c (30.0)
<i>C. sinensis</i> peels	5	0.67ab (6.7)	1.33c (13.3)	2.67bc (26.7)	4.67bc (46.7)
<i>P. biglobosa</i> leaves	5	1.33a (13.3)	4.00ab (40.0)	5.00ab (50.0)	6.67ab (66.7)
<i>P. biglobosa</i> seeds	5	3.67c (36.7)	5.33a (53.3)	6.33a (63.3)	7.00a (70.0)
Ethanol only	5	0.00b (0.0)	2.00b (20.0)	3.00bc (30.0)	4.33c (43.3)
No extract	0	0.00b (0.0)	0.00c (0.0)	0.00d (0.0)	0.00d (0.0)
SE±		0.22	0.67	0.71	0.42
CV(%)		22.2	27.0	22.9	9.7

Values with the same superscript(s) in the same column are not significantly different at 5% level of probability using DMRT.

Figures in parentheses are in percentages

KEY

HAT = Hours After Treatment; CV = Coefficient of Variation; SE = Standard error

Seed damage and beetle perforation index

The effect of ethanolic extracts of *C. sinensis* (leaves/peels) and *P. biglobosa* (leaves/seeds) on percentage seed damage caused by *T. granarium* larvae and percentage perforation index of the plant extracts is presented in Table 3. The *P. biglobosa* seed extract caused the least percentage seed damage which was significantly lower ($p<0.05$) than the percentage seed damage in *C. sinensis* leaves at 120 DAI. The untreated seeds (control) had significantly higher ($p<0.05$) percentage seed damage than the seeds treated with the plant extracts. Beetle perforation index for each treatment was proportional to the percentage seed damage recorded. This implies that the seeds treated with the plant extracts had lower damage than the untreated seeds.

Table 3: Percentage seed damage and beetle perforation index of *Trogoderma granarium* larvae.

Extract	% Seed damage (120 DAT)		Beetle Perforation Index (%)
<i>C. sinensis</i> leaves	11.53 ^b		38.07
<i>C. sinensis</i> peels	8.19 ^{bc}		30.39
<i>P. biglobosa</i> leaves	5.70 ^{bc}		23.28
<i>P. biglobosa</i> seeds	4.72 ^c		20.14
No extract SE± 1.58 CV(%) 16.18	18.75 ^a		50.00

SE = Standard error; CV = Coefficient of variation; DAT = Days after treatment

Values with the same letter in the same column are not significantly different at $p=0.05$ using Duncan's multiple range test.**Seed weight loss and viability test**

The effect of ethanolic extracts of *C. sinensis* (leaves/peels) and *P. biglobosa* (leaves/seeds) on percentage seed weight loss caused by *T. granarium* larvae and seed viability is presented in Table 2. The *P. biglobosa* seed extract had the least percentage seed weight loss which was significantly lower ($p<0.05$) than the percentage seed weight loss in other treatments at 30 and 60 DAT; but not significantly ($p>0.05$) different from the percentage seed weight loss in *C. sinensis* peels at 90 DAT. Of all the seeds treated with the extracts, *P. biglobosa* had percentage seed weight loss which was significantly different from the control at 60 DAT. The seed weight loss increased with increase in exposure time. The results also showed that *P. biglobosa* seed extract enhanced better viability of the groundnut seeds than other extracts and the control. It was observed that seeds treated with *P. biglobosa* seed extract had highest percentage viability followed by *P. biglobosa* leaf and *C. sinensis* peel extracts.

Table 4: Percentage seed weight loss caused by *Trogoderma granarium* larvae

Extract	% seed weight loss (DAT)			
	30	60	90	% viability test
<i>C. sinensis</i> leaves	2.36 ^a	6.23 ^a	10.57 ^a	96.7
<i>C. sinensis</i> peels	2.15 ^b	6.10 ^a	10.12 ^c	98.3
<i>P. biglobosa</i> leaves	2.13 ^b	6.08 ^a	10.22 ^b	98.3
<i>P. biglobosa</i> seeds	1.94 ^c	6.02 ^b	10.13 ^c	100.0
No extract	3.34 ^a	6.27 ^a	10.61 ^a	71.7
SE±	0.18	0.12	0.15	
CV(%)	7.50	1.89	1.41	

Values with the same superscript(s) in the same column are not significantly different at 5% level of probability using DMRT

SE = Standard error; CV = Coefficient of variation; DAT = Days after treatment

The four plant parts from *C. sinensis* and *P. biglobosa* were evaluated and found to be effective in causing the mortality of *T. granarium* adults with increase in both concentration and exposure time. The *Parkia biglobosa* seed extract applied at 5% was the most effective in significantly causing mortality of *T. granarium* adults. Barbehenn and Martin (1994) and Barbehenn *et al.* (1996) have stated that tannins can enter the haemolymph of the insect through the peritrophic envelope of the gut. Osarumwense *et al.* (2013) reported the presence of saponins, cardiac glycosides, tannins and flavonoids but they reported the absence of alkaloids in phytochemical analysis of *C. sinensis* peels. *Trogoderma granarium*, a phytophagous beetle, requires protein and other nutrients present in groundnut for optimum development. Shi *et al.*, (2004) had earlier reported that saponins have been extensively used as pesticide.

This study shows that the whole but untreated seeds were severely attacked by *T. granarium* indicating that the insect is a major storage pest of groundnut. Seeds treated with *P. biglobosa* seed extract were the least attacked in terms of having the least BPI of 20.14% and therefore the least damage. It was found that the various concentrations of ethanolic extracts tested were effective in suppressing the damage caused by an introduced insect population. In this study, percentage seed weight loss caused by *T. granarium* in the absence of extracts caused 10.61% within 90 days. The untreated seeds had the least viability in storage compared with 100% viability in the *P. biglobosa* seed extract. The varying degrees of damage recorded in this study are capable of predicting acceptability and marketability of the seeds. It could be reported that when *T. granarium* was left undisrupted it caused significant grain weight loss and damage in the store accompanied by reduction in seed viability.

Also, all treatments caused an increase in the mean number of *T. granarium* larvae that died following application. Extract of *P. biglobosa* seeds was the most effective in causing larval mortality. It caused significantly higher mortality than other extracts from 24 h to 96 HAT. Similarly, larval mortality of *T. granarium* increased with time for all the treatments. Femi-Ola *et al.*, (2008) had earlier reported that aqueous and acetone extracts of *P. biglobosa* caused total mortality within 40-110 min. after application. This study is an index of possible inclusion of plant products in biopesticide formulation against stored-product insects. The extracts have affected 4th larval instar survival, although the rate of mortality varied with extracts. One possible hypothesis that has been given to explain the larvicidal and adulticidal activities of the extracts is the direct toxic effect of some oil constituents (Lienard *et al.*, 1993). The present results indicated that the extracts were effective in reducing damage to groundnut seeds from *T. granarium*.

In the present investigation, the ethanolic extracts of *C. sinensis* (leaf/peel) and *P. biglobosa* (leaf/seed) were capable of suppressing larval and adult populations of *T. granarium*. It was also found that the plant products were effective in reducing losses and damage of stored groundnut seeds caused by an introduced larval population of *T. granarium*. This result might have to be

confirmed or invalidated as the presence of chemical constituents responsible for this activity depends on growing conditions, extraction method, type of solvent and volatilization. It is possible that these factors are responsible for the potential role of these extracts in seed protection. From the results, it was evident that the groundnut seeds suffered potential increase in weight loss and damage as a result of insect pest infestation.

CONCLUSION

The preservation of groundnut seeds is hampered by the attack of *T. granarium*. The study has revealed the appreciable insecticidal activity of *C. sinensis* and *P. biglobosa* extracts against *T. granarium*. Thus there is now empirical validation for the use of plant extracts for the control of the stored-product insect pests. However, *P. biglobosa* seed extract proved to be a more promising control agent for further development as botanical formulation for use against *T. granarium*. The use of *P. biglobosa* seed extract should be encouraged for resource- poor farmers in stored-product protection.

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FLORISTIC COMPOSITION AND ECOLOGICAL ANALYSIS OF THE MIXED FORESTS (BEECH, FIR, SPRUCE) IN THE MANAGEMENT UNIT „BJELASICA“ (BJELASICA Mt, MONTENEGRO)

SUMMARY

Paper dealing with characteristics of the fir and beech association (*Abieti-Fagetum moesiaca* B. Jov. 1976.) and beech, fir and spruce association (*Piceo-Fago-Abietetum* Čol. 1965) on the territory of the management unit „Bjelasica“ (Mt Bjelasica, Montenegro). A total of 45 phytocoenological relevés were analysed, 34 for association *Abieti-Fagetum moesiaca* B. Jov. 1976. and 11 for association *Piceo-Abieti-Fagetum* Čol. 1965. In the beech and fir association significantly more plant species (60) were found compared to the association of beech, fir and spruce (30). Spectrums of life forms and areal types were made for these associations. In the fir and beech association, in spectrum of life forms phanerophytes are dominant (37.28%), followed with hemicryptophytes (35.59%) and geophytes (23.73%). In the second association hemicryptophytes are dominant with 40%, while phanerophytes constitute 36.67%, and geophytes 20%. Within the spectrum of *Abieti-Fagetum moesiaca* areal types the most common is Euro-Asian areal type (33.33%), followed by Holarctic (18.33%) and central-European (16.66%). Within the spectrum of *Piceo-Fago-Abietetum* Čol. 1965 areal types the most frequent are central-European and Holarctic with 21.87% both, but large percent is constituted of Euro-Asian areal type (18.75%).

Keywords: forest plant community, mixed forests, Bjelasica, Montenegro

INTRODUCTION

According to the First national forests inventory of Montenegro (MARD, 2013) fir and beech associations constitute 6.1% of the surface area covered with the forest or 11.8% of the total wood volume. Forests of fir, spruce and beech constitute 4.7% of the surface area or 10.7% of the total wood volume. Annual volume increment of these forests is from 9.6% (fir and beech forests) till 10.3% (fir, spruce and beech forests). Aforementioned forests have great economic value, but they are also significant regarding their ecological aspect.

On the most part of former Yugoslavia forest association *Abieti-Fagetum* s. l. is well noticeable and it represents one of the first associations in Europe that

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

was distinguished and analysed in details (Horvat, 1938). These forests construct climate-regional altitudinal belt within the range of 700/800 to 1700/1800 m altitude. Fir and beech forests on Dinarides represent the most stabile forest type that retains all important natural characteristics in spite of the external influences (Stefanović and Beus, 1991).

Piceo-Fago-Abietetum Čol. 1965 is specific three-dominant association, with equal involvement of all three edificators in natural conditions, and with very different relations in the forests that are managed during the longer period of time. This association is limited at the altitudinal belt (1000-1550 m a.s.l.) where beech and fir achieve optimal growth in height and thickness, but it can descend below at wetter and sheltered places. Historically, it is older forest type compared with beech, beech-fir and spruce-fir forest types; it is closer to the ancestral deciduous-conifer type of forest that was been characteristic for the most part of the mountainous refugium belt from Plješevica to the Rodopi (Horvat, 1950; Jovanović, 1967; Mišić, 1982).

Investigations in mixed forests of fir and beech, and fir, spruce and beech were provided on several localities in Montenegro, only, mostly on Ljubišnja Mt, inside the Biogradska Gora National Park (Čurović and Medarević, 2011; Čurović *et al.*, 2011; Čurović *et al.*, 2012, Čurović *et al.*, 2013) and in the vicinity of Rožaje town (Martinović and Markišić, 2002). This research has included soil and phytocoenological studies, as well as structural, developmental and production characteristics of mixed forests. Investigations of biological and horological spectrums were not been provided, in spite that they are significant indicators of ecological conditions at some locality. Beside the phytocoenological characteristics of mixed forests in management unit "Bjelasica", analyses of those spectrums were done in our paper, with goal to expand the knowledge about these associations. Particularly interesting are data about forest communities of beech, fir and spruce, because investigated area is near to the southern border of distribution of this community and under certain maritime impact (Čurović *et al.*, 2013).

MATERIAL AND METHODS

The investigation of the forests community was carried out during the summers 2014. and 2015. In total, 45 phytocoenological relevés were taken, 34 in association *Abieti-Fagetum moesiaceae* B. Jov. 1976 and 11 in association *Piceo-Abieti-Fagetum* Čol. 1965. Dimensions of all phytocoenological relevés are 5x20 m (100m²). Methodology of phytocoenological studies followed Braun-Blanquet (1964). Phytocoenological relevés are grouped in the table where floristic composition of floors for each association is presented, and the degree of presence is given for each plant species. Identification of collected material was done according to Domac (1994), Pignatti (1982) and Tutin *et al.* (1964-80, 1993), and the nomenclature is in accordance with Euro+Med (2006). Life form classification is performed according to Raunkier, elaborated and updated by Ellenberg and Mueller-Dombois (1967). The categorisation of taxa to floral

element is determined by Meusel et al. (1965, 1978, and 1992) and Pignatti (1982), while grouping of floral elements in areal types is performed according to the plant-geographical classification of Stevanović (1992).

Meaning of used acronyms is given in alphabetical order: Central European (CEUR), Central European Mediterranean (CEMED), Central European mountainous (CEM), Central South European mountainous (CSEM), Euro-Asian (EAZ), Holarctic (HOL), Cosmopolitan (KOSM), Mediterranean-Sub-Mediterranean (MED-SUBMED), Mediterranean Pontic (MED PONT), South European mountainous monttainous (SEM). Presentation of horological and biological spectrum includes species cover, not only their presence in phytocoenological relevés. Syntaxonomic names follow Tomić and Rakonjac (2013).

Study area

Investigation was carried out on the Bjelasica Mt, on their northern slopes, in management unit „Bjelasica“. Surface area of this unit is 9205 ha, while the surface area of the forests and forestland is 5248.4 ha, and the natural tall forests cover 82% of them.



Figure 1. The position of investigated area.

The geological base and hydrology had significant influence during the process of relief forming. The geological groundwork is very heterogeneous, at higher altitudes the carbonate-silicate base prevails, while at the lower altitudes shale and sandstones predominate (Fušić and Đuretić, 2000). The management unit is rich in water flows and springs with steep channels cut deep into the

ground. The ridges, in north-south direction, lay between the water flows. Steep slopes are predominating in the management unit, and 53% of the surface area covered with the forest are on the moderately steep terrains (11-20°) and 43% are on the steep terrains (21-30°). Terrain exposure to the north (N, NE and NW) constitutes 77% of the surface. The elevation of the lowest point in the management unit is 585 m a.s.l., and the highest elevation where the forest outreaches is 1850 m a.s.l.

The climate of the area is continental-mountainous perhumid, with long and cold winters and relatively short and coldish summers. The average annual temperature is 8.6°C, according to the meteorological station in Bijelo Polje (560 m altitude), average annual precipitation is 922 l/m² and the relative humidity is 78%.

RESULTS AND DISCUSSION

The results content floral diversity and structure, spectrums of life forms and spectrums of areal types for fir and beech (*Abieti-Fagetum moesiaca* B. Jov. 1976.) and fir, spruce and beech associations (*Piceo-Abieti-Fagetum* Čol. 1965).

Floristic composition and structure

In the community *Abieti-Fagetum moesiaca* B.Jov. 1976 ten species are recorded in the tree floor, from which only edificators have high level of permanence, while other taxa are sporadically noted. The shrub floor with 19 species is floristically richer, but only youth of edificators have high level of permanence, what was also noted on some other distributional areas of the association (Cvjetičanin and Novaković, 2010; Eremija et al., 2015). In the floor of herbaceous plants 58 species were recorded, and among them, except edificators, the remark level of presence possess *Galium sylvaticum*, *Pteridium aquilinum*, *Lamium galobdolon* and *Rubus ideus*.

All floors of association *Piceo-Abieti-Fagetum* Čol. 1965 are floristically poorer compared with afore mentioned association. But, we must take in mind that the second association covers lesser surface area within the management unit, resulting in fewer number of made phytocoenological relevés during the investigation. The tree floor contents 6 species, while the shrub floor, beside edificators, contents only *Corylus avelana*, presented in small number of relevés. In the tree floor, besides edificators, the high permanence possesses a pioneer species *Betula pendula*. The flora of a ground-floor consists of 31 species, and the greatest permanence possesses *Viola sylvestris* and *Luzula forstei*, along with young specimens of beech and fir. High permanence is shown by youth of pioneer species *Populus tremula*, and this, along with high representing of *Betula pendula* in the tree floor, indicates a certain degree of forest degradation. Forests of spruce, fir and beech in the management unit "Bjelasica" are floristically poorer in all floors, compared to the forests with the same edificators on the mountain Lisina (Bosnia and Herzegovina) and Pešter plateau (Serbia) (Eremija et al., 2015).

Table 1. Floors coverage in associations

	<i>Abieti-Fagetum moesiaca</i> B.Jov. 1976	<i>Piceo-Abieti-Fagetum</i> Čol. 1965.
Tree layer	0,75	0,82
Shrub layer	0,46	0,5
Herb layer	0,38	0,33

The floors coverage in associations is presented in table 1. The coverage is somewhat greater in floors of trees and shrubs in the community *Piceo-Abieti-Fagetum* Čol. 1965, while in the community of beech and fir the coverage is greater in the flora of the ground-floor.

Table 2. Phytocenological data

	Life form	Areal types	<i>Abieti-Fagetum moesiaca</i>	<i>Piceo-Abieti-Fagetum</i>
Number of relevés			34	11
Species			I layer	
<i>Abies alba</i> Mill.	P scap	SEM	V	V
<i>Fagus moesiaca</i> (K. Maly) Czech.	P scap	CEUR	V	V
<i>Picea abies</i> (L.) Karst	P scap	EAZ		V
<i>Betula pendula</i> Roth.	P scap	EAZ	II	IV
<i>Ostrya carpinifolia</i> Scop.,	P scap	MED-SUBMED	I	I
<i>Populus tremula</i> L.	P scap	EAZ	I	
<i>Acer pseudoplatanus</i> L.	P scap	CEMED	I	
<i>Pinus nigra</i> Arnold	P scap	CSEM	I	
<i>Quercus petraea</i> (Matt.) Lieblein	P scap	CEMED	I	
<i>Salix caprea</i> L.	P caesp	EAZi	I	
<i>Pyrus pyrastra</i> Burgsd.	P scap	EAZ	I	
<i>Fraxinus excelsior</i> L.	P scap	CEUR		I
			II 2 layer	
<i>Abies alba</i> Mill.	P scap	CEM	V	III
<i>Fagus moesiaca</i> (K. Maly) Czech.	P scap	CEUR	V	V
<i>Picea abies</i> (L.) Karst	P scap	EAZ	I	V
<i>Corylus avellana</i> L.	P caesp	EAZ	I	I
<i>Betula pendula</i> Roth.	P scap	EAZ	I	
<i>Populus tremula</i> L.	P scap	EAZ	I	
<i>Acer pseudoplatanus</i> L.	P scap	CEMED	I	
<i>Fraxinus excelsior</i> L.	P scap	CEUR	I	

<i>Pinus nigra</i> Arnold	P scap	CSEM	I	
<i>Ostrya carpinifolia</i> Scop.	P scap	MED-SUBMED	I	
<i>Quercus petraea</i> (Matt.) Lieblein	P scap	CEMED	I	
<i>Salix caprea</i> L.	P caesp	EAZ	I	
<i>Pyrus pyraister</i> Burgsd.	P scap	EAZ	I	
<i>Juniperus communis</i> L.	P caesp	HOL	I	
<i>Crataegus oxyacantha</i> L.	P caesp	EAZ	I	
<i>Alnus incana</i> (L.) Moench.	P caesp	HOL	I	
<i>Sambucus nigra</i> L.	P caesp	EAZ	I	
<i>Rosa canina</i> L.	NP caesp	EAZ	I	
<i>Prunus avium</i> L.	P scap	EAZ	I	
			III layer	
<i>Fagus moesiaca</i> (K. Maly) Czech.	P scap	CEUR	V	V
<i>Abies alba</i> Mill.	P scap	CEM	IV	IV
<i>Galium sylvaticum</i> L.	H scap	CEUR	IV	II
<i>Pteridium aquilinum</i> (L.) Kuhn	G rhiz	KOSM	III	II
<i>Lamium galeobdolon</i> (L.) Crantz	H scap	CEUR	III	III
<i>Rubus idaeus</i> L.	NP rept	HOL	III	I
<i>Viola silvestris</i> Lam.	H scap	CEMED	II	V
<i>Luzula forsteri</i> (Sm.) DC	H caesp	HOL	II	V
<i>Rubus fruticosus</i> L.	NP rept	MED-SUBMED	II	II
<i>Oxalis acetosella</i> L.	G rhiz	HOL	II	I
<i>Aremonia agrimonioides</i> (L.) DC	H ros	CSEM	II	I
<i>Galium rotundifolium</i> L.	H scap	CEUR	II	I
<i>Picea abies</i> (L.) Karst	P scap	EAZ	I	III
<i>Betula pendula</i> Roth.	P scap	EAZ	I	I
<i>Populus tremula</i> L.	P scap	EAZ	I	III
<i>Acer pseudoplatanus</i> L.	P scap	CEMED	I	III
<i>Quercus cerris</i> L.	P scap	MED PONT	I	II
<i>Pinus nigra</i> Arnold	P scap	CSEM	I	I
<i>Vaccinium myrtillus</i> L.	Ch frut	HOL	I	I
<i>Fragaria vesca</i> L.	H semiros rept	HOL	I	I
<i>Dentaria bulbifera</i> L.	G rhiz	EAZ	I	I
<i>Prenanthes purpurea</i> L.	H scap	CEUR	I	I
<i>Luzula luzuloides</i> (Lamk.) Dandy&Wilmot	H caesp	EAZ	I	I

<i>Anemone apennina</i> L.	G rhiz	MED-SUBMED	I	I
<i>Euphorbium amygdaloides</i> L.	H scap	EAZ	I	I
<i>Epilobium angustifolium</i> L.	H scap	HOL	I	I
<i>Driopteris filix-mas</i> (L.) Schott.	G rhiz	HOL	I	I
<i>Sorbus austriaca</i> L.	P caesp	CSEM	I	I
<i>Hepatica triloba</i> Gilib.	G rhiz	CEUR	I	I
<i>Daphne blagayana</i> Freyer		SEM	I	I
<i>Fraxinus excelsior</i> L.	P scap	CEUR	I	
<i>Quercus petraea</i> (Matt.) Lieblein	P scap	CSEM	I	
<i>Crataegus oxyacantha</i> L.	P caesp	EAZ	I	
<i>Sambucus nigra</i> L.	P caesp	EAZ	I	
<i>Corylus avellana</i> L.	P caesp	EAZ	I	
<i>Rosa canina</i> L.	NP caesp	EAZ	I	
<i>Driopteris dilatata</i> (Hoffm.) A. Gray	G rhiz	HOL	I	
<i>Luzula sylvatica</i> (Huds.) Gaudin	H caesp	SEM	I	
<i>Galium odoratum</i> (L.) Scop.	G rhiz	CEUR	I	
<i>Anemone nemorosa</i> L.	G rhiz	HOL	I	
<i>Juniperus communis</i> L.	P caesp	HOL	I	
<i>Veronica urticifolia</i> Jacq.	H scap	CSEM	I	
<i>Allium ursinum</i> (L.) Moench.	G bulb	CEUR	I	
<i>Epilobium montanum</i> L.	H scap	EAZ	I	
<i>Polystichum aculeatum</i> (L.) Roth	G rhiz	EAZ	I	
<i>Mycelis muralis</i> (L.) Dum.	H scap	EAZ	I	
<i>Sanicula europaea</i> L.	H scap	EAZ	I	
<i>Hypericum maculatum</i> Crantz	H scap	CEUR	I	
<i>Festuca drymeia</i> Mert.& Koch	G rhiz		I	
<i>Senecium nemorensis</i> L.	H scap		I	
<i>Sorbus aucuparia</i> L.	P caesp	CSEM	I	
<i>Asarum europaeum</i> L.	Ch rept	EAZ	I	
<i>Mercurialis perennis</i> L.	G rhiz	CEMED	I	
<i>Nardus stricta</i> L.	H caesp	EAZ	I	
<i>Ajuga reptans</i> L.	H rept	CEMED	I	
<i>Neottia nidus-avis</i> (L.) L.C.Rich.	G rhiz	EAZ	I	
<i>Pulmonaria officinalis</i> L.	H scap	CEUR	I	
<i>Saxifraga rotundifolia</i> L.	H scap	CSEM		I

Spectra of life forms

The biological spectrum of beech-fir forest has phanerophyte-hemicryptophyte character, whereby the domination of phanerophyte is insignificant (table 3, graph 1). Significant participation of geophytes indicates to the mesophilic character of this association. Considering that the life form of chamaephyta is well adapted to the unfavourable ecological conditions and that it dominates in the opening habitats, no wonder is its low percentage participation in the spectrum of life forms of *Abieti-Fagetum moesiaca*e association.

Table 3: Spectra of life forms in the associations *Abieti-Fagetum moesiaca*e B. Jov. 1976 and *Piceo-Fago-Abietetum* Čol.1965.

Life forms	<i>Abieti-Fagetum moesiaca</i> e		<i>Piceo-Fago-Abietetum</i>	
	%	Number of species	%	Number of species
<i>Phanerophyta</i> (P)	37,28	22	36,67	11
<i>Hemicryptophyta</i> (H)	35,59	21	40,00	12
<i>Geophyta</i> (G)	23,73	14	20,00	6
<i>Chamaephyta</i> (Ch)	3,39	2	3,33	1

Biological spectrum of beech, fir and spruce community is similar as afore mentioned, the difference is in somewhat greater participation of hemicryptophytes in compare to the phanerophytes (table 3, figure 3). Terophytes did not mention in any of associations, and they are rare elements in these woods, they are typical representations of open and warm habitats.

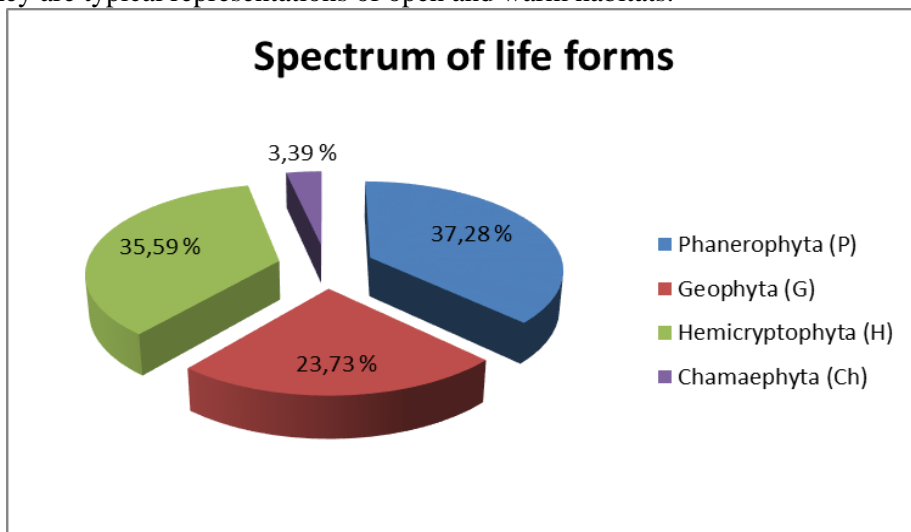


Figure 2. Spectrum of life forms in the association *Abieti-Fagetum moesiaca*e B. Jov. 1976.

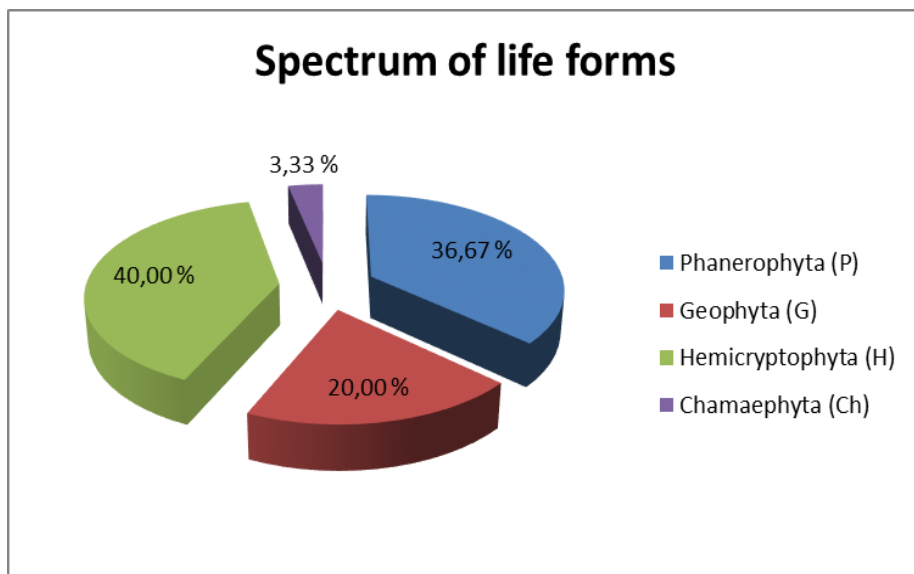


Figure 3. Spectrum of life forms in the association *Piceo-Fago-Abietetum* Čol.1965.

Spectrum of areal types

In the chorological spectrum of community *Abieti-Fagetum moesiaca* B.Jov. 1976 (graph 3) the most represented is Euro-Asian areal type with 33.33%, with mostly eurivalent species with large areale. It is followed by Holarctic areal type with 18.33%, that also containing the species with large areale, and Central-European with 16.66%. The species which areal is mostly Central-European with moderate climate, with smaller part of population that can be spread in the Mediterranean-sub-Mediterranean area, are presented with 8.33% in chorological spectrum (CEMED). Areal types with species that have the most part of their areal on mountainous belts in Central and/or in Southern Europe are presented with 6.66% (CSEM) and 3.33% (CEM). Participation of Mediterranean-sub-Mediterranean areal type is 5%, while cosmopolitan and Mediterranean-Pontic is represented with 1.67% each.

Regarding to the community *Piceo-Fago-Abietetum* Čol. 1965. in the spectrum of areal types (Graph 4), the most represented areal types are: Central European (CEUR) and Holarctic (HOL) with 21.87% each, large percentage has Euro-Asian (EAS), but with remarkably lesser representing compared to the previous community. In mixed community of fir, spruce and beech following areal types are also represented: Mediterranean-sub-Mediterranean (MED-SUBMED) with three species which consist 9.37%, Central-European-Mediterranean (CEMED) with 6.25%, Central-European Mountainous (CEM), Cosmopolitan (KOSM) and Mediterranean-Pontic (MED PONT) with 3.12% each.

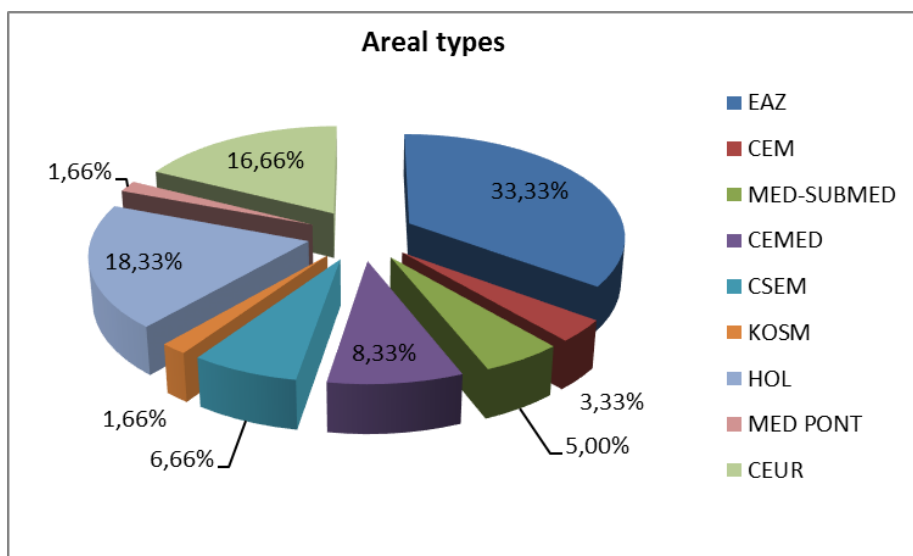


Figure 4. Spectrum of areal types in the association *Abieti-Fagetum moesiacaе* B. Jov. 1976.

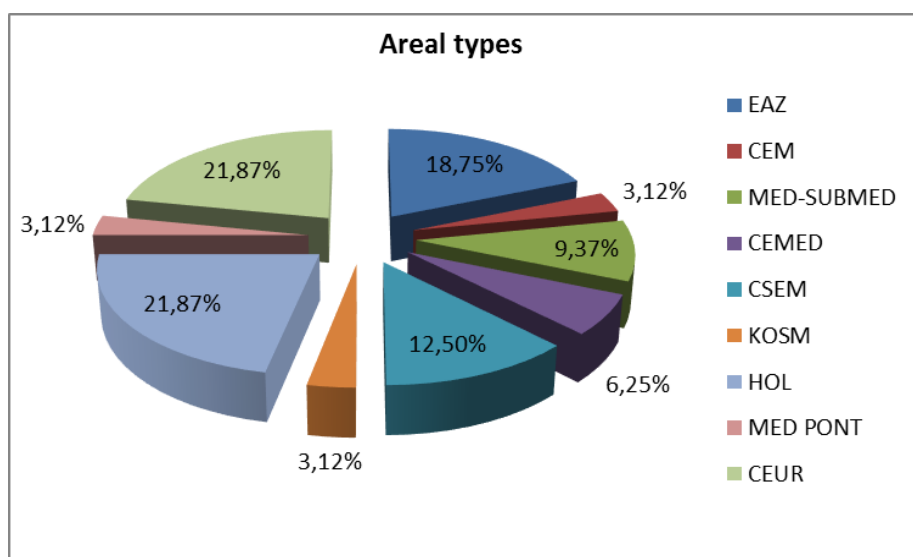


Figure 5. Spectrum of areal types in the association *Piceo-Fago-Abietetum* Čol. 1965.

Horvat (1950) considered that the belt of beech-spruce forests is refugial, because it has smallest changes in compare with other mountainous belts. This belt is situated between warm and dry oak belt, which was markedly changed during the post glaciation (mostly in kserophitisation) and cold and dry sub-Alpine belt, which rapidly changed during the glaciation and cold periods of post glaciation. Beech-spruce belt continuously had the most favourable conditions

for survival, and had conserve large number of species and their cenotic groups during the most critical moments in post tertiary history of our flora and vegetation (Janković, 1984).

Association *Abieti-Fagetum moesiaca* B.Jov. 1976 constitutes climate-regional altitude belt in span from 700/800 to 1700/1800 m a.s.l. across the former Yugoslavia (Jovanović, 1959). In Serbia it is developed as powerful climate-regional belt and covers large areas in different orograph, edaphic and microclimatic conditions, extending in different ranges of altitude on different mountainous belts: on Goč Mt 800-1200 m a.s.l. (Jovanović, 1959; Tomića and Cvjetičanin, 1991; Tomić and Jović, 2000), on Kopaonik Mt 1200-1550 m a.s.l. (Mišić and Popović, 1954), on Stara planina Mt 1200-1600 m a.s.l. (Mišić et al., 1978).

In Montenegro beech-fir forests are developed in the central and southern part of the country, where they constitute belt till 600 m wide (Petrović et al., 2012). They are mentioned on Mediterranean Dinarides, also, where they cover small surface areas, only at northern expositions (Komar, 1995). Regardless to the great economic and ecological importance, systematically investigations of this kind of woods did not provided, and detailed data about its distribution were given only for several mountainous ranges. On a silicate massif of central Bjelasica they extend between 950-1400 m a.s.l. on the northern expositions, while on the southern expositions they form narrower belt between 1200-1550 m a.s.l. On the carbonate massif of north-eastern Bjelasica they cover altitude range 950-1150 m a.s.l. on northern expositions, and 1100 – 1400 m a.s.l. on the southern expositions (Lakušić et al., 1990). On a vertical profile of mountains around Rožaje this type of forest is mostly extended above the belt of beech forest, while on some localities it constitutes first forest belt (Martinović and Markišić, 2002).

Association *Abieti-Fagetum moesiaca* B.Jov. in the management unit „Bjelasica“ is extended in wide range of altitudes, from 800 to 1650 m a.s.l. It covers northern expositions, mostly, that are dominant in the management unit otherwise. Its bedrock is diverse, at higher altitudes it is on the limestone, and on the lower altitudes on the sandstone and shale. The most common lands where this community is developed are brown ground on the limestone, acid and acid-brown grounds.

Mixed deciduous-coniferous community of beech, fir and spruce *Piceo-Fago-Abietetum* Čol. 1965. for a long time was considered as a sub-association of beech-fir association, but opinion that it is separate association prevailed (Mišić and Jovanović, 1983). According to the domination of two edificators (beech and fir), floral content and altitude belt this community is closer to the beech-fir type of wood compared to the spruce type. This community is limited at the altitude belt (1000-1550 m a.s.l.) (Jovanović, 1980).

In the management unit „Bjelasica“ association *Piceo-Fago-Abietetum* Čol. 1965., as well as association *Abieti-Fagetum moesiaca* B.Jov. 1976, dominantly covers northern expositions. It is spread in the belt from 900 to 1700

m a.s.l., resulting in no significant differences within the range of altitudes where associations are present. Due to the northern expositions that are dominates on the investigated area (77% of surface) and higher humidity as result of several water flows, this association can be spread down at the lower altitudes than usually. The spruce is preserved at lower altitudes (below its belt) thanks to the protection provides from fir and beech. Fir determinates lower and upper limits of this wood by its altitudinal and ecological range. Numbered investigations show that the fir has conspicuous individual and group variability, and in spite of limitation on a particular mountain belt, it inhabits ecologically different habitats with tendency to climb in higher, sub-Alpine regions (Fukarek, 1954, Mišić and Popović 1954, Stefanović, 1970, Jovanović, 1980, Mišić and Jovanović, 1983).

Considering that analyses of biological and horological spectrums of before mentioned forests are not provided in Montenegro, we compare obtained results with spectrums of these forests in Bosnia and Herzegovina (Lisina Mt) and Serbia (Pešter plateau) (Eremija *et al.*, 2015). The great similarity is noticed in the spectrum of life forms between forests of the management unit “Bjelasica” and Lisina Mountain. On both localities hemipterophytes are dominant and represented with approximately equal percentage (Bjelasica 40%, Lisina 41%), they are followed by phanerophytes that are somewhat numbered on Bjelasica (Bjelasica 36.67%, Lisina 31%) and geophytes with equal representation (20% each). In the biological spectrum of Pešter plateau the order of life forms is the same, but hemipterophytes significantly dominate (54%) in compare with phanerophytes (21%) and geophytes (14%). So, although the management unit „Bjelasica“ is near to the southern distributional border of communities of beech, fir and spruce, the influence of Mediterranean on biological spectrum is not prominent, because the spectrum is very similar to the same on Lisina, which is located more northern. This indicates that edifiers have strong influence on the microclimate conditions in the community, thus on the biological spectrum, also. Similarly conditions are in the horological spectrum in which, on Bjelasica and Lisina Mt, the species group of Central-European floral elements are dominant, and Holarctic floral elements and species of broad ecological amplitude of the Euro-Asian distribution type have high participation.

CONCLUSIONS

In the management unit „Bjelasica“ analysed associations of mixed deciduous-conifer forests Abieti-Fagetum moesiace B.Jov. 1976 and Piceo-Fago-Abietetum Čol. 1965 dominantly cover northern expositions. Their altitude range of extend is similar: first association from 800 to 1650 m a.s.l., and second from 900 to 1700 m a.s.l. Beech and fir community is floristically richer (60 species) in compare with the beech, fir and spruce community (30 species).

The biological spectrum of the beech-fir forest has phanerophytical-hemipterophytical character, whereby the domination of phanerophytes is insignificant, while in the biological spectrum of second community hemipterophytes are represented in slightly higher percentage in compare with

phanerophyte. In both communities geophytes have significant participation, hamephytes are represented in small percentage and terophytes are absent.

In the horological spectrum of community *Abieti-Fagetum moesiaca* B.Jov. 1976 Euro-Asian areal type is most represented with 33.33%, followed by Holarctic with 18.33% and Central-European areal type with 16.66%. In the spectrum of areal types of *Piceo-Fago-Abietetum* association Čol. 1965 the most represented areal types are: Central European and Holarctic with 21.87% each, Euro-Asian areal type constitutes large percentage (18.75%), but with noticeably lower representing in compare with previous community. Although the management unit „Bjelasica“ is near to the southern distributional border of communities of beech, fir and spruce, the analysis of biological and horological spectrum did not shown significant influence of Mediterranean.

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WINE QUALITY IN MONTENEGRO

SUMMARY

Intensive development of viticulture and winemaking sector is taking place in the last decade in Montenegro. Vineyard areas, the number of wineries and the number of wine types are increasing. In the structure of grape and wine production are still dominant red indigenous Montenegrin varieties, Vranac in composition with Kratošija. Among the producers of grapes and wine dominant position in Montenegro has the company "13. Jul-Plantaže" (94.5% of the total production i.e. quantity of 145.000 hl of all types of wine).

This paper presents results of Montenegrin wine quality analyses in the period from 2007.to 2014. Chemical analyzes were carried out in oenological laboratories of Biotechnical Faculty, as an integral part of the analysis for the market release of wines. Still, dry wines; red, white and rose were analysed.

Results of the analysis showed that wine Vranac from all producers has the highest uniformity in chemical composition. The average content of wine Vranac for the reference period is: 14.0 vol % of alcohol, total extract 28.0 g/l, total acidity 5.2 g/l and pH value of 3.50. This chemical composition is a typical composition for Montenegrin wine Vranac in Montenegro. Wine Kratošija from one producer and Cabernet Sauvignon from two producers were analysed.

White wines that were analysed were: Krstač, Chardonnay and Sauvignon. Among these wines the difference in the chemical composition of wines was recorded, especially in Chardonnay, and it is concluded that the characteristics of white wines are more dominated by production style of different producers than by the influence of variety. The average chemical composition for white wines analyzed is 13.6 vol% of alcohol, total extract 22.0 g/l, total acidity 5.9 g/l and pH value of 3.29. The characteristics of analyzed rose wines are distinguished by the style of production (alcohol content, extract and total acids) and all of them are characterized by elevated alcohol content.

Keywords: red wine, white wine, rose wine, dry wine, chemical composition of wine

INTRODUCTION

Due to the favorable climatic and soil characteristics in Montenegro, vine growing and wine production have a very long tradition that extends to the period

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before the new era. This agricultural activity is associated to wine-growing region of Skadar basin and the Coastal region. Until World War II, the vineyard area in Montenegro was 1.145 ha (Pejović, 1988). The most significant increase in the vineyard area in Montenegro was in period from 1977 to 1982, with new plantations of 1.500 hectares within the project "Ćemovsko polje", property of the company "13.jul-Plantaže". In the last decade vineyard areas and the number of grape and wine producers also have increased. Law on wine published in 2007. and other government measures aimed to promote viticulture and wine production, particularly contributed to this trend. From 2007. to 2012. the vineyard areas were increased for 287 ha. During this period, 505 grape producers were registered. According to the latest MONSTAT records, total area under vines in Montenegro amounted for 2.700 ha at the end of 2014.

In the structure of vineyards in Montenegro, indigenous wine varieties Vranac and Kratošija dominate in the production of red wines, while in the production of white wine Chardonnay and Krstač are predominant. In "13.jul-Plantaže" company, 92% of plantations are wine varieties and 8% are table varieties of grape. The varieties of grapes intended for red wine production occupy 67% of the vineyards, while white varieties occupy 25%. Smaller producers mainly cultivate varieties of grapes used for wine production (94.1%), while table varieties hold only 5.9% of areas. The main grape varieties for the production of red wines are indigenous varieties Vranac and Kratošija, with 91.1% share, followed by Cabernet Sauvignon with 1.9% planted area. The most common variety used in the production of white wine is Chardonnay with 1.1%, followed by Krstač, Smederevka, Sauvignon, Žižak, etc. (MARD).

Wine production in Montenegro was officially registered in 55 wineries. According to EU classification, "13.jul-Plantaže" belongs to medium-size business, with a capacity of 17 million liters per year, and two wineries with a capacity of 0.3 to 10 million liters are small-size businesses.

Technological conditions of production in these companies fully meet internationally accepted standards. For other small producers (family businesses) technology of wine production ranges from the use of modern equipment to the traditional methods of production.

The total quantity of marketed wine in 2013 amounted to 142,559 hl. Company "13.jul-Plantaže" produced 94.5% and small wineries 7,872 hl, or 5.5% of total production. Red wines contributed with the biggest share (71.5%), followed by white wines (25.2%), and rosé wines (3.3%). From the aspect of quality categories, the most abundant are high quality wines with 54.4%, followed by quality wines with 40.4%, while table wines account for 5.1% (MARD).

Numerous of studies on quality of grapes and wine were conducted in Montenegro, and here it will be mentioned only a part of published studies. Ampelographic characteristics of the most important Montenegrin vine varieties in Montenegro described Ulićević (1966).

A detailed review of seven Montenegrin wines with geographical origin was done by Pejović (1987). Raičević et al. (2012) examined the impact of technological processes on the phenol content in wine Vranac. Savić (2013) described the characteristics of the Vranac wine produced from grapes coming from different localities. Pajović et al. (2013) studied the characteristics of nine renowned Vranac wine in a three-year period (2008-2010). Polyphenol profile of wines Vranac, Kratošija and Cabernet Sauvignon, based on the content of anthocyanins and hydroxycinnamic acid was defined by Pajović et al. (2014). Košmarel et al. (2014) examined the impact of the yield in some Montenegrin wines on antioxidative properties. Polyphenolic potential of Vranac, Cabernet Sauvignon and Kratošija was investigated by Pajović et al. (2014b) at 11 localities in Montenegro.

There is an evident trend of increasing vineyards and wine production, especially of small producers in Montenegro recently, which contributed to the increase in the number and types of wines. To our knowledge, studies on the characteristics of "new" wines in Montenegro haven't been done until now. Therefore, the objective of this work is to study and determine the quality of the wine in Montenegro, in recent years, based on their chemical composition. Long term analysis (2007-2014) has been performed, including the most common category of wines - dry wines: red (Vranac, Cabernet Sauvignon, Kratošija), white (Chardonnay, Sauvignon and Krstač) and rose. Based on a detailed analysis of chemical parameters, characteristics of the wine produced in Montenegro were defined.

MATERIAL AND METHODS

This paper presents results of chemical analyzes of Montenegrin wines. Category of still, dry wines that are predominant in the wine production in Montenegro was analysed, and, classified by colour, all three categories: red, white and rose. Analyses were carried out in oenological laboratories of Biotechnical Faculty as an integral part of the controls before wine marketing. Wines were taken by the commission and are representative samples.

Chemical analyzes were performed in the laboratory for wine and decision on quality category was taken by the Commission formed by the Ministry of Agriculture and Rural Development. High-quality wines that can be found in the Montenegrin and wider market are presented.

Table 1 is an overview of the wines analyzed, name of producer-winery, the site from which grapes come from as well as the total annual production of wine. The producers listed release wines on market each year, allowing continuous and referent monitoring.

The following parameters of physicochemical composition of wine were tested: alcohol, extract, total acids, ph, volatile acids and residual sugar in wine. The analyses were performed in accordance with compendium of international methods of wine and must analyses (OIV, 2011).

Table 1: Data on wines analysed

<i>Wine</i>	<i>Period of analysis</i>	<i>Winery/ Producer</i>	<i>Locality</i>	<i>Prod. yearly</i>
Red wines				
<i>Vranac</i>				liters
„Crnogorski vranac“	2007-2013	13.jul-Plantaže	Čemovsko polje, Podgorica	2.000.000
„Vranac Pro-Corde“	2007-2013	13.jul-Plantaže	Čemovsko polje, Podgorica	1.000.000
“Crmički Vranac Sjekloča”	2007-2013	Sjekloča	Limljani, Crmnica, Bar	4.500
“Zenta Vranac”	2008-2012	Vučinić	Rogami, Podgorica	6.000
“Arhonto Vranac”	2009-2012	Krgović	Rogami, Podgorica	7.500
“Vranac”	2007-2013	Biotechnical Faculty	Lješkopolje, Podgorica	10.000
<i>Kratošija</i>				
“Zenta Kratošija”	2009-2014	Vučinić	Rogami, Podgorica	2.000
<i>Cabernet Sauvignon</i>				
“Crnogorski Cabernet”	2007-2013	13.jul-Plantaže	Čemovsko polje, Podgorica	500.000
“Zenta Cabernet Sauvignon”	2007-2012	Vučinić	Rogami, Podgorica	3.000
White wines				
<i>Krstač</i>				
“Crnogorski Krstač”	2007-2014	13.jul-Plantaže	Čemovsko polje, Podgorica	1.000.000
<i>Chardonnay</i>				
“Crnogorski Chardonnay”	2007-2014	13.jul-Plantaže	Čemovsko polje, Podgorica	1.000.000
“Buk Chardonnay”	2010-2014	Rajković	Bukovik, Crmnica, Bar	2.000
“Arhonto Chardonnay”	2012-2014	Krgović	Rogami, Podgorica	7.500
<i>Sauvignon</i>				
“Crnogorski Sauvignon”	2007-2014	13.jul-Plantaže	Čemovsko polje, Podgorica	150.000
Rose wines				
“Crnogorski Rose”	2009-2013	13.jul-Plantaže	Čemovsko polje, Podgorica	150.000
“Zenta Rose”	2010-2014	Vučinić	Rogami, Podgorica	800
“Arhonto Rose”	2010-2014	Krgović	Rogami, Podgorica	8.000

RESULTS AND DISCUSSION

1) Results of quality analysis of red wines

In Table 2. and 2a. results of chemical analysis for the red wines produced in Montenegro: Vranac, Cabernet Sauvignon and Kratošija are presented, and in table 2b average values of quality parameters for all types. In table 2b. mean values of quality parameters according to types and variety of red wines are shown. In table 2., analyzed red wines Vranac are presented.

Wine Vranac

Red wine Vranac is dominant wine and it is produced from the autochthonous Montenegrin variety Vranac, usually in composition with Kratošija (in a significantly lower percentage). For most wine producers registered in Montenegro, prevailing position has wine Vranac in production range. However, here are presented only six wines Vranac, which have a reputation and that can be found in the market, "Crnogorski Vranac", "Vranac Pro Corde", "Crmnčki Vranac Sjekloća", "Zenta Vranac", "Arhonto Vranac" and "Vranac".

Wine „Crnogorski Vranac” is the product of the winery "13.jul-Plantaže". It is produced as high quality and premium wine. In this study (Table 2. are presented only the results of high quality red wines. Thanks to this wine, Vranac has become a recognizable Montenegrin brand, especially in the countries of the region, where it is still highly valued. It is produced in an amount of about two millions litars per year. Table 2. shows the chemical composition of this wine from 2007. to 2013. Density ranged from 0.9915 (2012) to 0.9938 (2008). The alcohol content varied from 12.9 vol % (2009) to 13.7 ol % (2012). Content of the extract ranged from 25.4 g/l (2007) to 29.2 g/l (2008). The content of the total acid is in the expected range for wine Vranac and varied from 4.7 g/l (2008) to 5.8 g/l (2012). pH value varied from 3.45 (2010) to 3.60 (2008). The volatile acid ranged from 0.60 g/l (2007) to 0.95 g/l (2008), and total sulphur content from 32.0 mg/l (2010) to 70.7 mg/l (2013). If we compare average results for the reference period (2007-2013) with a five-year average, reported by Pejović (1987), there is no significant variation in the values of the examined parameters. Pejović (1987) states that the alcohol content is 12.5 vol%, and our studies demonstrated 13.1 vol% averagely; total acid are uniform, averaging 5.1 g/l in both studies; content of the extract, excluding sugars in study of Pejović is 23.87 g/l and in our study is 26.6 g/l; the content of volatile acids is fairly steady and amounted to 0.75 g/l and 0.80 g/l. These data suggest a standard approach in the production technology of this wine for decades. Nevertheless, in our research are evident certain differences in the examined parameters per year, however not significant in the coefficient of variation.

Table 2. shows the data for another wine Vranac from producer "13.jul-Plantaže"- "Vranac Pro Corde". This wine is made by a special, protected technology that provides natural enrichment of wines with proanthocynidins, important antioxidative substances that enhance protection against coronary heart disease, and that is where the name originates from. Around one million liters of this wine are produced in a year. The paper presents the data for the period from 2007. to 2012. Table 2 shows that the density of the wine ranged from 0.9915 (2012) to 0.9942 (2008). The alcohol content varied from 13.5 vol% (2007) to 14.4vol % (2011). The total acid content ranged from 4.6 g/l (2009) to 5.6 g/l (2012), and pH value from 3.45 (2007) to 3.60 (2011). The volatile acid ranged from 0.55 g/l (2010) to 0.95 (2008) and the content of SO₂ from 28.01 mg/l (2008) to 121.42 mg/l (2012).

Table. 2: Chemical composition of red wines Vranac in Montenegro (per vintages)

Vinery	Vintage	Density	Alcohol vol %	Extract g/l	Total acidity g/l	pH	Volatile acidity g/l	Total SO ₂ mg/l
“Crnogorski Vranac”								
Plantaže 13 Jul	2007	0.9930		25.3	5.2	3.50	0.60	64.70
	2008	0.9938	13.4	29.2	4.7	3.60	0.95	69.42
	2009	0.9926	12.9	27.0	4.8	3.59	0.69	39.60
	2010	0.9935	12.9	26.5	5.1	3.45	0.60	32.00
	2011	0.9935	13.3	25.8	4.9	3.55	0.80	47.00
	2012	0.9915	13.7	24.0	5.8	3.53	0.80	60.71
	2013	0.9935	13.4	28.4	5.3	3.58	0.80	70.78
	mean	0.9931	13.2	26.6	5.1	3.54	0.75	54.89
	CV (%)	0.08	2.5	6.70	7.3	1.3	16.9	28.0
“Vranac Pro Corde”								
Plantaže 13 Jul	2007	0.9934	13.5	27.0	4.8	3.55	0.80	39.45
	2008	0.9942	13.8	31.4	4.8	3.50	0.95	44.80
	2009	0.9920	13.4	24.2	4.6	3.60	0.55	28.01
	2010	0.9930	13.7	28.7	4.9	3.50	0.55	34.50
	2011	0.9925	14.3	27.9	4.6	3.47	0.85	55.10
	2012	0.9915	14.0	25.0	5.6	3.45	0.87	121.42
	mean	0.9928	13.8	27.4	4.9	3.51	0.76	53.88
	CV (%)	0.10	2.5	9.5	7.4	1.6	22.4	63.8
Sjekloća								
“Crminički Vranac Sjekloća”								
Sjekloća	2007	0.9915	14.8	27.4	5.5	3.73	0.60	55.04
	2008	0.9920	14.5	27.4	5.2	3.60	0.69	47.36
	2009	0.9930	13.7	28.1	5.4	3.55	0.80	122.88
	2010	0.9912	14.0	28.8	5.7	3.61	0.62	94.72
	2011	0.9920	14.4	27.4	5.4	3.71	0.85	115.00
	2012	0.9915	14.8	27.4	5.8	3.58	0.66	51.20
	2013	0.9925	14.4	28.1	4.7	3.79	0.65	84.03
	mean	0.9920	14.4	27.8	5.4	3.65	0.70	81.46
	CV (%)	0.06	2.8	2.0	7.0	2.5	13.5	38.2

Vučinić

“Zenta Vranac”								
Vučinić	2008	0.9925	14.1	27.9	4.5	3.60	0.66	80.00
	2009	0.9920	13.2	26.0	4.8	3.54	0.60	102.40
	2010	0.9935	12.7	26.3	5.4	3.64	0.85	106.88
	2011	0.9920	14.5	27.6	5.3	3.42	0.70	83.20
	2012	0.9925	15.0	31.3	6.0	3.29	0.72	66.56
	mean	0.9925	14.8	30.0	5.2	3.42	0.70	87.97
	cv (%)	0.12	2.2	14.3	9.6	3.9	9.5	30.9

Krgović

“Arhonto – Vranac”								
Krgović	2009	0.9915	14.3	25.8	4.9	3.55	0.65	87.68
	2010	0.9940	15.0	34.9	5.6	3.25	0.69	68.51
	2011	0.9930	15.0	32.3	5.7	3.38	0.67	69.16
	2012	0.9915	14.8	27.1	4.8	3.50	0.80	126.51
	mean	0.9925	14.8	30.0	5.2	3.42	0.70	87.97
	CV (%)	0.12	2.2	14.3	9.6	3.9	9.5	30.9

“Vranac” Biotehnički fakultet

Biotehnički fakultet	2007	0.9935	14.0	30.2	5.0	3.40	0.80	61.00
	2008	0.9929	14.1	29.1	5.5	3.36	0.60	61.44
	2009	0.9927	14.0	28.9	5.7	3.30	0.68	59.70
	2010	0.9933	13.8	26.1	6.0	3.33	0.70	55.78
	2011	0.9925	14.3	28.4	5.0	3.33	0.79	72.36
	2012	0.9925	14.0	27.9	4.9	3.55	0.62	57.97
	2013	0.9925	14.0	27.9	5.9	3.30	0.65	71.50
	mean	0.9928	14.0	28.4	5.4	3.37	0.69	62.82
	CV (%)	0.04	1.1	4.5	8.5	2.6	11.3	10.4

The six-year average of the parameters for wine "Vranac Pro Corde", compared to the average for wine "Crnogorski Vranac" shows a higher alcohol content of 0.7 vol %, higher extract content for 0.8 g/l and a balanced amount of acid content, pH value of 3.50 and volatile acids 0.75 g/l.

This paper also analyzes the characteristics of the wine Vranac from first registered private wine producers in Montenegro - Winery "Sjekloća". This

producer has built a considerable reputation for the quality of his products, primarily wine "Crnjički Vranac Sjekloča". This wine is produced from the grape variety Vranac, which is grown in locality Limljani (Crmnica, Bar). It is produced in quantities of around litters. The results of chemical analysis for this wine (period from 2007. to 2013.) are shown in table 2. Density varied from 0.9912 (2010) to 0.9930 (2009). The alcohol content ranged from 13.7 vol% (2009) to 14.8 vol% (2007/2012), and the content of the extract from 27.4 g/l to 28.8 g/l. The total acid content ranged from 4.7 g/l (2013) to 5.8 g/l (2012), and we registered high pH value of 3.55 (2009) to 3.79 (2013). The volatile acid ranged from 0.60 to 0.85 g/l, and total sulphur content from 47.36 to 122.88 mg/l.

Wine "Arhonto Vranac" is produced in Rogami locality, in the winery, "Krgović" with modern equipment for production and raw material of exceptional quality, which results in superior quality of this wine. Annual production of this wine is around 7.500 litters. Characteristics of this wine for the period from 2009. to 2012. are given in Table 2. Density ranged from 0.9915 (2009/12) to 0.9940 (2010). The content of alcohol is high and ranged from 14.3 vol% (2009) to 15.0 vol% (2010/11), and the total extract content from 25.8 g/l to 34.9 g/l. Total acid content ranged from 4.8 g/l to 5.7 g/l (2011), and pH value from 3.25 (2010) to 3.55 (2009). Volatile acids were quite uniform 0.65 in 2009 g/l to 0.80 g/l in 2012, and total sulphur content measured was from 68.51 mg/l (2010) to 126.51 mg/l (2012).

Wine "Vranac" from winery of Biotechnical Faculty (former Agricultural Institute) is another wine with a long tradition in Montenegro. Due to scientific research orientation of this institution, this wine was available on market only in bulk under the local name "Institute's wine". In 2007. wine was officially released on the market under the name "Vranac". It is made from grape variety Vranac on locality Lješkopolje, near Podgorica. It is known for a remarkable quality. The chemical composition of this wine is given in table 2, for the period from 2007. to 2013. Density was fairly consistent from 0.9925 in three years (2011/13) to 0.9935 in 2007. The content of alcohol was constant, ranging from 13.8 vol% (2010) to 14.3 vol% (2011). Content of the extract was 26.1 g/l (2010) and 30.2 g/l (2007).

The total acid content varied from 4.9 g/l to 6.0 g/l, and pH value ranged from 3.30 (2009) to 3.40 (2007). Volatile acids were equal, 0.60 g/l and 0.80 g/l and the sulphur content was from 55.78 mg/l to 72.36 mg/l.

Table 2. also presents two recent Vranac wine producers from the localities Rogami in vicinity of Podgorica "Arhonto Vranac" and "Zenta Vranac". Wine "Zenta Vranac" is produced since 2007. in the winery "Vučinić" that has modern equipment for the production and with outstanding quality of grapes produces superior quality wine every year. It is produced since 2008. in an amount of about 6.000 l. Table 2 presents the chemical characteristics of this wine Vranac for the period from 2008 to 2012. Density ranged from 0.9920 (2011) to 0.9935 (2010) and an alcohol content from 12.7 vol% (2010) to 15.0 vol% (2012). In accordance with these values, was the content of extracts ranging from 26.0 g/l to

31.3 g/l (2012). The total acid content also varied from 4.5 g/l to 6.0 g/l (2012), as well as the pH value; 3.29 (2012) and 3.64 (2010). Volatile acids were uniform, from 0.60 g/l (2009) to 0.85 g/l (2010), and the total sulphur ranged from 66.50 mg/l (2012) to 106.88 mg/l (2010).

Table 2a. presents the chemical characteristics of wine Kratošija (one type) and Cabernet Sauvignon wines (two types).

Table. 2a: Chemical composition of red wines Kratošija and Cabernet Sauvignon in Montenegro (per vintages)

Vinery	Vintage	Density	Alcohol vol %	Extract g/l	Total acidity g/l	pH	Volatile acidity g/l	Total SO ₂ mg/l
	KRATOŠIJA WINE							
	“Zenta Kratošija”							
Vučinić	2009	0.9925	15.3	31.3	4.8	3.55	0.65	72.96
	2010	0.9925	13.5	27.0	6.0	3.49	0.65	76.95
	2011	0.9940	15.7	36.5	5.6	3.63	0.80	71.68
	2012	0.9915	15.0	29.2	5.3	3.42	0.69	72.96
	2013	0.9965	15.5	43.1	8.9	3.38	0.75	108.61
	2014	0.9935	12.3	25.8	6.8	3.29	0.57	112.28
	mean	0.9934	14.6	32.2	6.2	3.46	0.69	85.91
	CV	0.18	9.3	20.4	23.8	3.5	11.9	22.9
	CABERNET SAUVIGNON WINES							
	“Crnogorski Cabernet”							
Plantaže 13 Jul	2007	0.9935	13.5	28.7	5.0	3.50	0.75	64.00
	2008	0.9940	13.4	29.6	5.0	3.55	0.95	64.00
	2009	0.9915	13.5	27.5	4.7	3.65	0.72	40.15
	2010	0.9930	13.5	26.9	4.6	3.60	0.60	60.49
	2011	0.9932	13.4	27.4	5.4	3.45	0.60	52.48
	2012	0.9915	13.6	23.7	5.9	3.47	0.66	107.05
	2013	0.9925	13.9	27.4	5.4	3.48	0.75	78.88
	mean	0.9927	13.5	27.3	5.1	3.53	0.72	66.72
	CV	0.10	1.33	6.8	8.7	2.10	16.8	32.0

	"Zenta Cabernet Sauvignon"							
Vučinić	2007	0.9930	13.9	28.4	4.7	3.65	0.88	76.00
	2008	0.9925	14.6	29.2	5.0	3.55	0.75	147.00
	2009	0.9919	14.6	28.9	5.5	3.54	0.80	147.00
	2010	0.9920	13.7	25.3	5.4	3.48	0.85	116.48
	2011	0.9915	15.0	27.9	5.4	3.53	0.72	92.80
	2012	0.9925	13.4	25.8	5.8	3.40	0.71	62.72
	mean	0.9922	14.2	27.6	5.3	3.53	0.79	107.00
	CV	0.05	4.4	6.0	7.9	2.3	8.9	33.5

Wine Kratošija

Although Kratošija is considered as indigenous Montenegrin variety in Montenegro there is only one wine Kratošija. This is the "Zenta Kratošija" produced by Winery also have increased "Vučinić". Table 2a. presents characteristics of this wine for the period from 2009. to 2014. Specific weight ranged from 0.9915 (2012) to 0.9965 (2013). The alcohol content was high and ranged from 12.3 vol% (2014) to 15.7 vol%. The content of the extract varied significantly from 25.8 g/l (2014) to 36.5 g/l (2011). Due to contents of acid ranging from 4.8 g/l (2009) to 8.9 g/l (2013), the pH value varied from 3.29 (2014) to 3.63 (2011). The volatile acid ranged from 0.57 g/l (2014) to 0.80 g/l (2011), and total sulphur content from 71.68 mg/liter to 112.28 mg/l (2014).

Wine Cabernet Sauvignon

Cabernet Sauvignon is the famous French wine dominating worldwide in quantity and quality. In previous years in Montenegro production of this wine has increased due to outstanding performance of wines and grapes. The largest area under vines of this variety, and thus the largest production has company "13.jul-Plantaže". "Crnogorski Cabernet" is produced in the amount of half a million liters per year. The results of chemical analysis for the period from 2007. to 2013. are shown in Table 2. Specific weight ranged from 0.9915 (2009) to 0.9940 (2008). The alcohol content was uniform, from 13.4 vol% (2008) to 13.9 vol% (2013), and the content of the extract varied from 23.7 g/l to 29.6 g/l. The content of total acids ranged from 4.6 g/l (2010) to 5.9 g/l (2012) and pH value ranged from 3.47 (2012) to 3.65 (2009). Volatile acid ranged from 0.60 g/l (2010) to 0.95 (2008), while the overall sulphur content ranged from 40.15 mg/l to 107.1 mg/l.

In Table 2a. are presented characteristics of another wine, Cabernet Sauvignon, called "Zenta Cabernet Sauvignon", which is produced in locality Rogami, in winery "Vučinić". This wine has the highest quality, and is produced

in the amount of about 3.000 liters. Table 3. shows the characteristics of this wine for the period from 2007. to 2012. Specific weight of this wine ranged from 0.9915 (2011) to 0.9930 (2007). The alcohol content varied from 13.4 vol% (2012) to 15.0 vol% (2011), and extract contents from 25.8 g/l (2012) to 29.2 g/l (2008). The total acid content ranged from 4.7 g/l (2007) to 5.8 g/l (2012), and pH from 3.40 (2012) to 3.65 (2007). Volatile acids were equal; 0.71 g/l (2012) and 0.88 g/l (2007), and the total sulphur ranged from 62.72 mg/l (2012) to 147.00 mg/l (2008/9).

Table. 2b: Chemical composition of red wines in Montenegro (average values)

<i>Wine</i>	<i>Vintage (from-to)</i>	<i>Density</i>	<i>Alcohol vol %</i>	<i>Extract g/l</i>	<i>Total acidity g/l</i>	<i>pH</i>	<i>Volatile acidity g/l</i>	<i>Total SO₂ mg/l</i>
<i>Vranac wines</i>								
"Vranac Pro Corde"	2007-2012	0.9928	13.8	27.4	4.9	3.51	0.76	53.88
"Crnogorski Vranac"	2007-2013	0.9931	13.2	26.6	5.1	3.54	0.75	54.89
"Crmički Vranac Sjekloća"	2007-2013	0.9920	14.4	27.8	5.4	3.65	0.70	81.46
"Zenta Vranac"	2008-2012	0.9925	13.9	27.8	5.2	3.50	0.71	87.81
"Arhonto Vranac"	2009-2012	0.9925	14.8	30.0	5.2	3.42	0.70	87.97
"Vranac"	2007-2013	0.9928	14.0	28.4	5.4	3.37	0.69	62.82
mean		0.9926	14.0	28.0	5.2	3.50	0.72	68.20
CV (%)		0.04	4.32	4.55	4.08	3.11	4.50	22.92
<i>Kratišija wine</i>								
"Zenta Kratošija"	2009-2014	0.9934	14.6	32.2	6.2	3.46	0.69	85.91
<i>Cabernet Sauvignon wines</i>								
"Crnogorski Cabernet"	2007-2013	0.9927	13.6	27.3	5.1	3.54	0.74	69.1
"Zenta Cabernet Sauvignon"	2007-2012	0.9922	14.2	27.6	5.3	3.53	0.79	107.0
mean		0.9925	13.87	27.46	5.22	3.53	0.75	86.86
CV (%)		0.04	3.39	0.74	2.27	0.03	6.70	32.79

Wine Vranac from six producers was analyzed and showed fairly uniform characteristics. Density is very uniform and ranges from 0.9920 to 0.9930. The content of alcohol and extract varied from 13.2 vol% and 26.6 g/l in wines "Crnogorski Vranac" to 14.8 vol% and 30.0 g/l for wine "Arhonto Vranac". Total acidity varies from 4.9 g/l in wines "Vranac Pro Corde" to 5.4 g/l in wine

"Vranac" and pH value from 3.37 to 3.65 in wine "*Crmnčki Vranac Sjekloča*". Volatile acids are uniform, ranging from 0.69 g/l to 0.76 g/l, and the total sulphur content was from 53.88 mg/l to 87.97 mg/l. The average contents of wine Vranac in our study, shown in Table 2b. is as follows: the alcohol content 14.0 vol%, extract contents 28.0 g/l, total acidity 5.2 g/l, pH 3.50, volatile acidity 0.70 g/l and total sulphur 68.20 mg/l. It can be concluded that this chemical composition is typical composition of the Montenegrin wine Vranac in Montenegro. The alcohol content in our study (13.2-14.8 vol%) is slightly higher than the results in the survey stated by Pajović *et al.* (2013) - 12.8 to 13.7 vol%, who also studied wine Vranac for 2008-2011 period, whereas alcohol content was from 13.03 to 13.33 vol%. for the period 2008-2011 stated by Raičević *et al.* (2014), and significantly higher than the values (11.15 vol% to 12.40 vol%) cited by Savić (2014) and Pejović (1987) - 12.5vol%. The content of the extract in our survey, from 26.6 to 30.0 g/l, was also higher than in the above mentioned studies. Pajović *et al* (2014) reported values of 25.4 to 27.2 g/l, Savić (2014) 23.85 g/l, and Pejović (1988) 23.82 g/l. Unlike the previous two parameters, total acid content in our studies (4.9 to 5.4 g/l) is consistent with the values of 4.8 to 5.5 g/l measured by Pajović *et al.* (2014), 5.34 g/l which states Savić (2014) and 5.1 g/l stated by Pejović (1988).

The table 2b.also shows wine Kratošija characteristics, as an average, for only one producer "*Zenta Kratošija*". The average content of wine Kratošija is as follows: the alcohol content of 14.6 vol%, extracts content 32.2 g/l, total acidity 6.2 g/l, pH value of 3.46, volatile acidity 0.69 g/l and the total sulphur 85.91 mg/l. Values of alcohol in this study are consistent with the values of 14.2 vol% for 1999, which shows Pajović *et al.* (2002), but higher than the value (11.50 to 13.00 vol%), for the wine produced from this variety reported by Pejović *et al.* (1988).

Wine Cabernet Sauvignon in Table 2b. is shown as a multi-year average for the two producers and the results are quite uniform. The values of specific weight and extract of these two wines amounts for 0.9922 and 27.6 g/l in wine "*Zenta Cabernet Sauvignon*" and 0.9927-27.3 g/l in wine "*Crnogorski Cabernet*". The alcohol content ranges from 13.6 to 14.2 vol% and is uniform in the content of total acids (5.1 and 5.3 g/l), as well as the pH value (3.54 and 3.53) and the content of volatile acid (0.74 and 0.79). The average content of wine Cabernet Sauvignon in our study, presented in Table 2b is as follows: the alcohol content 13.90 vol%, the content of the extract 27.5 g/l, total acidity 5.2 g/l, pH 3.53, volatile acidity 0.75 g/l and the total sulphur 86.86 mg/l. Values of alcohol content are consistent with the values specified by Avramov (1991) - 12.5 to 14.0 vol% and 14.3% reported by Pajović *et al.* (2009), but significantly higher than the value of 10.9 vol% cited by Pejović *et al.* (1996).

2) Results of white wines quality studies

Table 3 presents the results of chemical analysis for white wines produced in Montenegro; Krstač, Chardonnay and Sauvignon.

Tabela 3: Chemical composition of white wines in Montenegro (per vintages)

	<i>Vintage</i>	<i>Density</i>	<i>Alcohol vol %</i>	<i>Extract g/l</i>	<i>Total acidity g/l</i>	<i>pH</i>	<i>Volatile acidity g/l</i>	<i>Total SO₂ mg/l</i>
Vinery	KRSTAČ WINE							
	“Crnogorski Krstač”							
Plantaže 13 Jul	2007	0.9913	13.7	23.5	4.8	3.50	0.35	112.6
	2008	0.9911	12.9	20.6	5.3	3.40	0.65	97.3
	2009	0.9911	12.9	20.8	5.4	3.45	0.35	92.2
	2010	0.9896	13.1	19.0	5.9	3.15	0.42	85.8
	2011	0.9916	12.9	21.3	6.5	3.15	0.60	121.6
	2012	0.9915	13.2	22.7	6.0	3.19	0.44	198.5
	2013	0.9915	13.1	22.4	5.6	3.29	0.50	107.9
	2014	0.9900	13.4	19.3	6.3	3.14	0.40	112.1
	mean	0.9910	13.2	21.2	5.7	3.28	0.50	116.0
	CV (%)	0.09	2.2	10.5	9.8	4.5	24.0	30.5
CHARDONNAY WINES								
Plantaže 13 Jul	“Crnogorski Chardonnay”							
	2007	0.9902	13.0	22.7	5.4	3.40	0.30	121.6
	2008	0.9905	13.4	20.6	5.2	3.45	0.60	82.0
	2009	0.9905	13.3	20.3	6.1	3.37	0.55	88.3
	2010	0.9900	13.7	20.1	6.8	3.19	0.55	119.0
	2011	0.9915	13.9	25.0	7.1	3.19	0.55	134.4
	2012	0.9915	13.7	24.0	6.9	3.14	0.41	102.5
	2013	0.9915	13.9	25.0	6.0	3.27	0.60	147.4
	2014	0.9900	13.1	18.5	6.7	3.14	0.45	100.5
	mean	0.9907	13.5	22.0	6.3	3.27	0.50	112.0
	CV (%)	0.07	2.6	11.3	11.4	3.8	21.1	20.2
“Buk Chardonnay”								
Buk	2010	0.9883	14.0	21.4	5.8	3.31	0.73	128.0
	2011	0.9900	14.5	22.4	6.7	3.14	0.75	106.2
	2012	0.9900	15.0	24.0	6.7	3.18	0.57	112.6
	2013	0.9900	13.9	20.9	6.6	3.18	0.57	105.8
	2014	0.9910	12.9	20.3	7.2	3.13	0.45	109.7
	mean	0.9899	14.1	21.8	6.6	3.19	0.61	112.46
	CV (%)	0.10	5.6	6.7	7.7	2.3	20.4	8.10

	“Arhonto Chardonnay”							
Krgović	2012	0.9915	15.0	28.1	6.0	3.37	0.51	149.5
	2013	0.9910	14.9	26.3	5.3	3.44	0.80	128.0
	2014	0.9900	13.7	20.1	5.3	3.47	0.55	177.5
	mean	0.9908	14.5	24.8	5.5	3.43	0.62	151.67
	CV (%)	0.08	5.0	16.9	7.0	1.5	25.4	16.4
	SAUVIGNON WHITE WINE							
	“Crnogorski Sauvignon”							
Plantaže 13 Jul	2007	0.9880	13.1	19.6	4.8	3.50	0.50	90.9
	2008	0.9903	13.3	20.3	4.9	3.55	0.60	80.5
	2009	0.9906	13.5	20.3	6.4	3.20	0.40	81.9
	2010	0.9900	12.9	19.9	5.8	3.15	0.51	75.1
	2011	0.9915	13.8	23.7	6.9	3.19	0.80	73.0
	2012	0.9915	13.7	24.0	6.9	3.15	0.45	131.9
	2013	0.9915	14.1	25.3	6.0	3.30	0.60	133.2
	2014	0.9910	13.5	22.4	6.0	3.20	0.45	124.0
	mean	0.9906	13.5	21.9	6.0	3.28	0.54	98.81
	CV (%)	0.12	2.9	10.0	13.4	4.8	23.6	26.6

Wine Krstač

White wine "Crnogorski Krstač" is produced from the indigenous Montenegrin variety Krstač with protected origin (according to the Lisbon Convention). There is only one producer of this wine in Montenegro - "13.jul-Plantaže". Wine is produced as high quality and superior wine. In this study (Table 3) are presented results of superior wine Krstač for the period from 2007. to 2014. The specific weight of this wine varies in years, from 0.9896 (2010) to 0.9916 (2011). The alcohol content ranges from 12.9 vol% (2008) to 13.7 vol% (2007). Extract content ranges, in accordance with the values of the previous two parameters from 19.0 g/l (2010) to 23.5 g/l (2007). Total acid content varies in different harvests; 4.8 g/l in 2007 and 6.5 g/l in 2012 with average value of 5.7 g/l. pH values ranged from 3.14 (2014) to 3.50 (2007). Volatile acid content amounted from 0.35 (2007) to 0.60 g/l (2011), and the total SO₂ from 85.6 (2010) to 198.5 mg/l (2012).

The average alcohol content in wine Krstač in our study (13.2 vol %) significantly differs from the content reported by Pejović (1987) as the average of the five-year tests of the same producer was 11.2 vol%, but it is consistent with recent studies of these wines presented by Košmarel *et al*.

(2013) - 12.8 ol% in the same locality. The total acid content for the eight-year average in our study is 5.8 g/l, which is more than what was presented by Pejović (1987) - 5.0 g/l and lower than the value of the researcher Košmarel et al. (2013) - 6.1 g/l. The average content of total extract in our analysis is 21.2 g/l and it is in alignment with results reported by Pejović (1987) - 19.4 g/l, and Košmarel et al. (2013) - 19.6 g/l showing the same value of extracts without sugar. The differences in alcohol content in previous studies for the five-year period by Pejović (1987), and our eight-year period are explained by different trends in wine production. In eighties (20th century) wines produced had alcohol content up to 12 vol% and in nineties trend of production of wines with higher alcohol content of 13 to 15 vol% was initiated.

Wine Chardonnay

White wine Chardonnay is a famous French wine, which has been successfully produced from the same name variety all over the world and in Montenegro as well (Pejović and Mijović, 2004). In recent years, vineyards with this variety are significantly increasing, and number of producers of this wine. In this study are presented results of the analysis of three reputable producers of this wine (Table 3). Wine "Crnogorski Chardonnay" is produced in largest amounts, about one million liters per year, and has the longest tradition in production since 2003. From 2007. barrique wine is produced. Results of the analysis of high quality wine "Crnogorski Chardonnay", produced with classical methodology by "13.jul-Plantaže ", for the period from 2007 to 2014 are shown in Table. 3. Density of wine ranges from 0.9900 to 0.9915 in tested years. The alcohol content varied from 13.0 vol% (2007) to 13.9 vol% (2013). Extract is content of wine- all non-volatile substances (Daničić, 1988), varies in years from 18.5 g/l 2014 to 25 g/l (2013). The total acid content of the wine "Crnogorski Chardonnay" ranged from 5.2 (2008) to 6.7 g/l (2014), and pH value from 3.14 (2014) to 3.45 (2008). The content of volatile acids varied and ranged from 0.30 to 0.60 g/l. Total SO₂ content ranged from 82 mg/l to 147 mg/l. Given the long tradition and experience of this wine producer ("13.jul-Plantaže") it is suggestible that the differences in the values of the parameters analyzed, with minor values of coefficient of variation, resulted from variable weather conditions in harvest years.

White wine "Chardonnay Buk" from "Buk" winery is produced in Montenegro from grapes grown at the highest altitude (about 700 m), at the location of Bukovik in Crmnica, resulting in specific sensory properties. The total production of this wine is around 2.000 liters per year, with the tendency of producers to increase quantities. Its

characteristics are analyzed since 2010, when production of wine begins. The results are shown in Table 3. Wine "Buk Chardonnay" wines density ranges from 0.9883 (2010) to 0.9910 (2014). The alcohol content varies greatly, from 12.9 vol% (2014) to 15.0 vol% (2012), as well as the content of the extracts from 20.3 g/l (2014) to 24.0 g/l (2012). Due to the geographical location of the vineyard, this wine has a higher total acid content; 5.8 g/l (2010) to 7.2 g/l (2014). pH value was 3.13 in 2014 and 31.3 measured in 2010. Based on the differences in analyzed parameters, we can state a clear impact of harvest. It is known that the 2014. harvest was one of the worst in the last decade and it reflected on the quality of wine produced. Volatile compounds in the wine "Buk Chardonnay" ranged from 0.45 g/l to 0.75 g/l, and the total SO₂ content were quite uniform, from 105.8 mg/l (2013) to 128.0 mg/l (2010).

This paper presents the characteristics of the wine "Arhonto Chardonnay" for a three-year period from 2012 to 2014.

Despite the short period in the market, this wine has good reputation. The analysis results are shown in Table 3. Density of wine ranges from 0.9900 (2014) to 0.9915 (2012). The alcohol content is very high - 13.7 vol% (2014) to 15.0 vol% (2012), and the extract content - 20.1 g/l (2014) to 28.1 (2012). The total acid content ranged from 5.3 g/l to 6.0 g/l (2014), and pH value from 3.37 (2012) to 3.47 (2014). The volatile acidity ranged from 0.5 g/l (2012) and 0.8 g/l (2013), and the SO₂ from 128.0 mg/l (2013) to 177 mg/l (2014). The results of the study for this wine also suggest the effect of unfavourable harvest in 2014 when all parameters measured were lower.

Data presented indicate that alcohol content in the analyzed wine Chardonnay varies from 13.5 vol% to 14.5 vol%, and the acid content from 5.5 g/l to 6.6 g/l.

The results of our study are consistent with results obtained by Avramov (1991); alcohol content from 12.0 vol% to 14.0 vol% and total acid content from 6.0 g/l to 7.5 g/l.

Wine Sauvignon

Wine Sauvignon is another famous French white wine that is widely spread in Italy, Slovenia, Croatia, Romania, Moldova, Serbia and Montenegro, with good productivity results (Bozinović, 2005). Despite its high reputation in the world, only one producer in Montenegro - "13.jul-Plantaže" is producing this wine with name "Crnogorski Sauvignon". Table 3 shows the chemical parameters for an eight-year period (2007-2014). Specific weight of this wine ranges from 0.9880 (2007) to 0.9915 in several years period. The alcohol content ranged from 13.1 vol% (2007) to 14.1 vol% (2013), and the extracts content from 19.6 g/l (2007) to 25.3

g/l (2013). The total acid content varied significantly in the observed period: during 2007. and 2008. amounted for 4.8 g/l and 4.9 g/l, in 2010, 5.8 g/l, and in 2012- 6.9 g/l. The pH ranged from 3.15 (2012) to 3.55 (2008). Volatile acid varied from 0.45 g/l (2014) to 0.80 g/l (2011), and the total SO₂ content from 80.5 mg/l (2008) to 133.2 mg/l (2013).

Table 3a: Chemical composition of for white wines in Montenegro (average values)

Wine	Vintage (from-to)	Density	Alcohol vol %	Extract g/l	Total acidity g/l	pH	Volatile acidity g/l	Total SO ₂ mg/l
Krstač wine								
“Crnogorski Krstač”	2007-2014	0.9910	13.2	21.2	5.7	3.28	0.50	116.0
Chardonnay wines								
“Crnogorski Chardonnay”	2007-2014	0.9907	13.5	22.0	6.3	3.27	0.50	111.9
“Buk Chardonnay”	2010-2014	0.9899	14.1	21.8	6.6	3.19	0.61	112.5
“Chardonnay Arhonto”	2012-2014	0.9908	14.5	24.8	5.5	3.43	0.62	151.7
Mean		0.9905	14.0	22.9	6.1	3.30	0.58	125.4
CV (%)		0.05	3.59	7.34	9.24	3.71	11.55	18.17
Sauvignon white wine								
“Crnogorski Sauvignon”	2007-2014	0.9906	13.5	21.9	6.0	3.28	0.54	98.81
White wine (average)								
Mean (all white wines)		0.9907	13.6	22.0	5.9	3.29	0.54	113.4
CV (%)		0.03	2.99	3.87	3.51	0.35	7.41	11.89

In wine “Crnogorski Sauvignon” the highest variation is measured in the content of total acidity, and therefore in pH value, which is not reflected in the presented coefficient of variation. Values of alcohol content and acidity in eight-year average (13.5 vol% and 6.0 g/l) in our research are consistent with the values of the parameters (12-14 vol% and 5-6.5 g/l) of wine Sauvignon stated by Avramov (1991). Table 3a presents the average values of all analyzed white wines.

Looking at averages (Table 3a.), for all three types of wine: Krstač, Chardonnay and Sauvignon, we see that the specific weight is uniform and ranges from 0.9905 in Chardonnay wines to 0.9910 in Krstač. The content of alcohol and extract varies from 13.2 vol% and 21.2 g/l respectively for wine Krstač to 14.0 vol% and 22.9 g/l for wines Chardonnay. The total acid content

	2010	0.9918	11.4	22.5	6.3	3.10	0.55	160.00
	2011	0.9920	14.8	28.7	6.8	3.05	0.85	92.16
	2012	0.9915	13.3	22.9	5.8	3.28	0.54	94.72
	2013	0.9930	13.0	26.3	6.4	3.21	0.65	94.38
	2014	0.9940	12.5	27.1	7.1	3.25	0.55	97.26
	mean	0.9925	12.99	25.5	6.5	3.18	0.63	107.70
	CV - %	0.10	9.7	10.6	7.5	3.1	21.0	27.2
Krgović	"Arhonto Rose"							
	2010	0.9899	12.9	22.3	5.4	3.35	0.50	112.6
	2011	0.9915	14.1	25.0	6.0	3.17	0.60	122.9
	2012	0.9910	15.0	26.6	5.7	3.21	0.54	118.2
	2013	0.9910	14.5	25.0	5.1	3.29	0.45	115.6
	2014	0.9900	13.6	19.8	6.0	3.33	0.69	169.7
	mean	0.9907	14.0	23.7	5.6	3.27	0.56	127.8
	CV - %	0.07	5.8	11.3	7.1	2.4	16.7	18.6

Characteristics of three rose wines produced in the period from 2009. to 2014. are presented: "Crnogorski Rose", "Zenta Rose" and "Arhonto Rose". The longest tradition in the production of this type of wine and the largest production capacity (about 150.000 litters) has wine "Crnogorski Rose" produced by "13.jul-Plantaže". Chemical characteristics of this wine are presented for the period from 2009. to 2014. Specific weight of the wine ranges from 0.9900 (2010) to 0.9928 (2009). Alcohol content varies slightly, from 12.5 vol% (2014) to 13.0 vol% (2009/10). Extract content ranged from 21.1 g/l (2012) to 25.3 g/l (2009). Total acid content varied significantly from 5.3 g/l (2009) to 6.8 g/l (2014), as well as pH value from 3.00 (2011) to 3.30 (2009). The volatile acid ranged from 0.40 g/l (2014) to 0.70 g/l (2009), and the total SO₂ content was from 69.12 mg/l (2009) to 153.40 mg/l (2012). The average content of the quality parameters of wine in our six-year testing was as follows: alcohol content 13.2 vol%, extract content 21.2 g/l, total acidity 5.7 g/l. In the five-year study of Pejović (1987) describing the same wine was stated: alcohol content 11.50 vol% extract content 24.0 g/l and total acid content 5.3 g/l. From these results, the conclusion is that in the last period changed the style of this wine production. Wine "Crnogorski Rose" has significantly higher alcohol content and acidity, and a lower content of extract nowadays. In table 4. another rose wine is presented, "Zenta Rose", produced at the site Rogami, winery "Vučinić". An overview of the characteristics of this wine, for the period from 2010. to 2014., is shown. Specific weight of wine ranged from 0.9915 (2012) to 0.9940 (2014). The alcohol content varied from 11.4 vol% (2010) to 13.3 vol% (2012), and extract content from 22.5 g/l (2010) to 28.7 g/l (2011). The total acid content ranged from 5.8 g/l (2012) to 6.8 g/l

(2011), and pH from 3.05 (2011) to 3.28 (2012). The volatile acids content measured was from 0.54 g/l (2012) to 0.85 g/l (2011), and the total sulphur ranged from 92.16 mg/l (2012) to 160.0 mg/l (2008/9).

Wine "Arhonto Rose" is another rose wine produced in Rogami, in the winery "Krgović". Annual production of this wine is around 8.000 litters. The characteristics of this wine for the period from 2010 to 2014 are given in Table 4. Density ranged from 0.9899 (2010) to 0.9915 (2011). The content of alcohol was high and varied considerably, from 12.9 vol% (2010) to 15.0 vol% (2012), as well as the total extract content ranging from 19.8.8 g/l to 26.6 g/l.

Table. 4a: Chemical composition of rose wines in Montenegro (average values)

Wine	Vintage (from- to)	Density	Alcohol vol %	Extract g/l	Total acidity g/l	pH	Volatile acidity g/l	Total SO ₂ mg/l
<i>Rose wines</i>								
"Crnogorski Rose"	2009- 2013	0.9916	12.8	22.3	6.0	3.14	0.57	106.64
"Zenta Rose"	2010- 2014	0.9925	13.0	25.5	6.5	3.18	0.63	107.7
"Arhonto Rose"	2010- 2014	0.9907	14.0	23.7	5.6	3.27	0.56	127.8
mean		0.9916	13.3	23.8	6.0	3.20	0.59	114.05
CV (%)		0.09	4.9	6.8	7.5	2.1	6.5	10.5

Table 4a shows the average content by type of wine and the average for rose wines produced in Montenegro.

In the table 4a. are presented average characteristics of analysed rose wines for the observed five/six-year period. Specific weight varied from 0.9907 in "Arhonto Rose" to 0.9925 in "Zenta Rose". Alcohol content measured was 12.8 vol % in "Crnogorski Rose" to 14.0 vol% in "Arhonto Rose". The values of total extract also varied from 22.3 g/l in "Crnogorski Rose" to 25.5 g/l in "Zenta Rose". Acid content also significantly varies among these wines, ranging from 5.6 g/l in "Arhonto Rose" to 6.5 g/l in "Zenta Rose", and therefore the pH value as well, from 3.14 in "Crnogorski Rose" to 3.27 in "Arhonto Rose". Content of volatile acids of 0.57 g/l to 0.63 g/l, and the total sulphur content from 106.6 mg/l to 127.8 g/l are almost uniform for all producers. Average chemical composition of rose wines for the reference period is: 13.3 vol% of alcohol, total extract 23.8 g/l, total acidity 6.0 g/l and pH value 3.20. Yet, from results of analysis of each individual producers it is apparent that three rose wines analysed differ in the most important parameters that affect its properties: the content of alcohol (which determines the strength of the wine), extract (which gives fullness) and total acids (which give freshness). Thus it is concluded that the characteristics of the analyzed rose wines vary in style of production and all of them are characterized by elevated alcohol content.

CONCLUSION

The structure of plantations in Montenegro is dominated by indigenous wine varieties Vranac and Kratošija for the production of red wines, and Chardonnay and Krstač for the production of white wine. Vineyard areas planted have significantly increased in last decade and the number of grape and wine producers, as well as the number of types of wines produced in the viticulture and winemaking sector in Montenegro. This paper presents results of analysis of quality of still, dry wines (red, white and rose) produced in the period from 2007 to 2014.

Red wines analysed were Vranac („*Crnogorski Vranac*”, “*Vranac Pro Corde*”, “*Crmnčki Vranac Sjekloča*”, “*Zenta Vranac*”, “*Arhonto Vranac*” and “*Vranac*”) Kratošija (“*Zenta Kratošija*”) and Cabernet Sauvignon (“*Zenta Cabernet Sauvignon*” and “*Crnogorski Cabernet*”). Among the white wines, wines Krstač (“*Crnogorski Krstač*”), Chardonnay (“*Crnogorski Chardonnay*”, “*Buk Chardonnay*” and “*Chardonnay Arhonto*”) and Sauvignon (“*Crnogorski Sauvignon*”) were analysed. Three types of rose wines were analysed: “*Crnogorski Rose*”, “*Zenta Rose*” and “*Arhonto Rose*”.

The average content for wine Vranac for the reference period was: the alcohol content of 14.0 vol%, extract content 28.0 g/l, total acidity 5.2 g/l and pH value 3.50. It can be concluded that the chemical composition is a typical composition of the Montenegrin wine Vranac in Montenegro. Average chemical composition of the wine Kratošija is as follows: the alcohol content 14.6 vol%, the content of extracts 32.2 g/l, total acidity 6.2 g/l and pH value 3.46. The average content of wine Cabernet Sauvignon in our study was: alcohol 13.90 vol%, total extract 27.5 g/l, total acidity 5.2 g/l and pH value 3.53.

The average content of white wine Krstač for the reference period was: alcohol content 13.2 vol%, extracts 21.2 g/l, total acidity 5.7 g/l and pH value of 3.28. The average content of wine Chardonnay is as follows: 14.0 vol% of alcohol, total extract 22.9 g/l, total acidity 6.1 g/l, pH value of 3.30. Average chemical composition of wine Sauvignon is: alcohol 13.5 vol%, total extract 21.9 g/l, total acidity 6.0 g/l and pH value of 3.28. The listed characteristics of white wines are more dominated by production style of wineries, compared to the variety of grape.

The characteristics analyzed for rose wines vary in production style (alcohol content, extract and total acids) and all of them are characterized by elevated alcohol content. Average chemical composition of wine rose for the reference period was: 13.3 vol%, total extract 23.8 g/l, total acidity 6.0 g/l and pH value of 3.20.

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IMPROVEMENT OF SELECTION EFFICIENCY IN WHEAT GENOTYPES FOR VARIABLE RAINFED ENVIRONMENTS

SUMMARY

Most countries in the world depend primarily on rainfed agriculture for their grain food and there are strong reasons to believe that investments in low-yielding rainfed agriculture could have large impacts on poverty reduction. Most works in crop physiology related to breeding does focus on either yield potential or yield under stressful conditions which may reverse performance in other environments. These finding may explain why past breeding programs have largely not as expected to produce an impact on subsistence agriculture in developing countries. Correlation between the same traits in two environments may be negative or positive, depending on the environment where the experiment was grown. Crop selection in natural rainfall conditions vary in different years with additional stress-managed experiments, particularly when error variance is high and heritability estimate is low, resulted in optimum cultivar selection. These cultivar, yield better than any other available cultivar in high to low rainfall conditions, moreover an economic production under severe drought stress and therefore, increased productivity in a wide range of unpredictable rainfed environments. A researcher can use improved statistical design and analysis techniques, in multienvironments information, and consider secondary traits for making selection decisions. These alternative traits should still be much simpler than the complex genes controlling ultimately yield itself under a wide range of conditions. Earliness, canopy temperature, maintaining high kernel weight and leaf senescence are considered inherent heat and drought tolerance in wheat.

Keywords: Selection environment, Drought, Heat, Secondary traits, Alpha-lattice.

INTRODUCTION

Most of agriculture land area, around 80%, is under rainfed agriculture (FAOSTAT, 2005). The importance of rainfed agriculture varies regionally but produces most food for poor communities in developing countries. Of the 850 million undernourished people in the world, essentially all live in poor, developing countries, which predominantly are located in tropical regions (UNSTAT, 2005).

Almost, 0.70 of variation in wheat grain yield, and the reason for the

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discrepancy between actual and potential yield, was caused by water deficit during the critical period (i.e. 30 days before and 10 days after flowering; Fischer 1985) and high grain filling temperature (Calvin^o and Sadras, 2002). Quantification and qualification of the discrepancies between actual, attainable and potential yield are important to improve productivity of farming systems.

It is important to combine expertise from different disciplines to identify and overcome crop genetic and physiologic limitations in addition to decision for experimental management to yield under unfavourable environments, particularly under heat and drought.

Recent developments include technologies that can lead to improved utilization of genetic resources, improved selection methods, improved statistical analysis, and improved targeting of production environments. They have the potential to allow for the development of new varieties more rapidly, and/or varieties with enhanced productivity in targeted environments (Bernan and Peter, 2007).

Breeding to raise both yield potential and yield further under environmental constraints through improved adaptiveness will be of paramount importance (Araus *et al.*, 2002; Slafer *et al.*, 2007). There is good evidence – in wheat at least – that improved genetic yield potential of cultivars have impact in both favourable as well as marginal agro-ecosystems (Calderini and Slafer 1999; Reynolds and Borlaug, 2006). Although, the spread of modern cultivars into drier areas has been much slower and their impact on yields far weaker than that in favorable climatic areas. The annual gain in genetic yield potential in drought environments is only about half (0.3-0.5%) of that obtained in irrigated, optimum conditions (Timothy *et al.*, 2005). Ever the less, considerable improvement in the adaptation of wheat to dry areas has been made by plant breeders over the last 50 years. The adoption of modern varieties however, has lagged behind that in irrigated areas and the percentage yield advance has been considerably lower (Trethowan and Pfifer, 1999).

An FAO study (FAO, 1998) considered that it would be an error to disregard the potential to increase food production from dryland agriculture because of the difficulties associated with it; where dryland agriculture is inefficient, there is scope for increasing food production by improvement.

Plant improvement is dependent upon the screening of a wide range of germplasm for our major crops in order to identify genetic variation in major traits involved in stress resistance (Lopes and Reynolds, 2010; Richards *et al.*, 2010; Saint Pierre *et al.*, 2010).

The effect of selection environment on the performance of breeding material in a range of environments is a frequently debated fundamental breeding question.

Optimum environment for selection

Three strategies have been considered in relation to the optimum environment for selection (Byrne *et al.*, 1995). Given that the optimum

environment for selection is one which maximizes genetic variation, and hence response to selection in the target population of environments, the first strategy is based on the assumption that these are the characteristics of an environment where growing conditions are optimum or near-optimum. The second strategy assumes that the optimum environment(s) for selection should be as representative as possible of the target population of environments (Blum, 1988). When the breeding program serves a target population of very diverse environments, where genotype by environment interactions are expected to be large, selection should be for specific adaptation (Ceccarelli, 1996) through decentralized selection (Simmonds, 1984; Ceccarelli et al., 1996). The third strategy, the alternate use of optimum and stressed conditions has been used to select genotypes that yield well in both conditions (Calhoun et al., 1994).

Mohammadi and Fathi (2004b) reported that Selection efficiency of barley genotypes for stress conditions was estimated 1.29 in non-stress environment and 1.6 when genotypes were selected in stress conditions for favorable environment.

Identify and characterize dryland wheat regions which affected by heat and drought stress are not well defined even now, and their characterization with a high density of poor farmers will permit more precise targeting of traits to current and anticipated stress profiles.

Annual precipitation in dryland regions commonly ranges from less than half of average in a dry year to more than twice average in a wet year, which renders the use of averages of little use in planning agricultural and natural resource development. Breeding for these environments, where the frequency, timing, duration and severity of abiotic stresses, such as temperature extremes and drought, are unpredictable and variable, is considered slow and difficult (Passioura, 1986). However, A large body of recent work has demonstrated that new opportunities exist to improve the adaptation of wheat to heat and drought stressed environments (Trethowan and Mujeeb-Kazi, 2008; Rebetzke et al., 2009; Reynolds et al., 2010). The CIMMYT wheat program follows a system of breeding for drought tolerance in which yield responsiveness is combined with adaptation to drought conditions (Timothy et al., 2005). Conventional breeding with a special focus on adaptation to marginal environments provides a necessary baseline in terms of genetic backgrounds into which new traits and their genes can be introduced (CIMMYT and ICARDA, 2011).

Some genotypes are only favorable in one specific environment, like landraces which have been adapted for sever local stresses or bred cultivars which genetically modified for high yield in full irrigation conditions. The optimum variety should have superiority/acceptability in environments with different stress intensities.

A recent review of breeding progress pointed out that selection for high yield in stress-free conditions has, to a certain extent, indirectly improved yield in many water limiting conditions (Cattivelli *et al.*, 2008). But, in several crops and several environments, cultivars or breeding lines selected under optimum conditions, do not perform well under stress, which is common in low-input

agricultural systems where crop production is limited by abiotic stresses (Byrne et al., 1995; Chapman et al., 1997). On the other hand, the genotypes selected for low yield conditions will probably perform better than those released for high yielding environments when grown under very poor environments. But, they have penalties in yield in wet and moderate conditions which have more frequencies in many regions. In the other words, for rainfed areas with variable frequency of drought stress, this type of genotypes with superior performance under most situations and economic production under sever drought stress with low frequency events occurrence.

These genotypes developed through repeated selection cycles under high to low yielding conditions have (1) a higher probability of giving high yields under optimum to low yielding conditions, and (2) a lower probability of giving yields below an economic threshold than genotypes selected in low yielding environments. This type of germplasm has a lower maximum yield when high yielding events occur.

The relative efficiency of indirect versus direct selection can be predicted by the magnitude of the heritabilities and the genetic correlation coefficient. If A is the trait to be improved in environment X by selecting in environment Y, then (Falconer, 1989): $CR/R_x = rG h_y/h_x$ where CR_x is the correlated response in environment X when selection is done in environment Y, R_x is the direct response when selection is done in environment X, rG is the genetic correlation coefficient between A_x and A_y , and h_x and h_y are the square roots of heritabilities of A in the two environments.

The genetic correlation for grain yield between low- and high-yielding environments seems to decrease as stress intensity in the low-yielding experiment increases (Cooper et al., 1997). A major reason for this is the large genotype-by-environment (G×E) interactions that are usually encountered for grain yield among a range of abiotic-stressed target environments over space and times (Fukai et al., 1999).

If rG between yield measured in different environments tends to become negative as the differences between the two environments increase, then selection in one environment tends to become irrelevant to the other environment regardless of the relative magnitude of heritability. This does not preclude that occasionally individual genotypes can be found with a relatively good combination of yield in low-yielding environments and high-yielding environments because estimates of rG are averages for a population of genotypes. However, screening a large number of genotypes only under high-yielding environments implies a high probability of discarding many potentially high-yielding genotypes in low-yielding environments.

A number of studies with barley and other crops (see Ceccarelli, 1989 for a review) suggest that different alleles of the same genetic system have a positive or negative effect depending on the environmental conditions. Falconer (1989) believe that the alleles controlling high grain yield in low-yielding conditions are at least partially different from those controlling high grain yield in high-yielding

conditions. Therefore, selection in high-yielding environments is expected to produce a negative response or no response in low-yielding environments. This may explain why crop varieties bred under high-yielding conditions failed to have an impact in low-yielding agricultural systems.

Screening of breeding materials for grain yield is an expensive procedure and sometimes produces inaccurate results due to the complex genetic nature of yield. The difficulty of selecting for improved adaptation particularly under abiotic stresses makes the use of indirect measures attractive to plant breeders.

Indirect selection

Struggle had been made primarily through the use of empirical breeding approaches by concentrating on yield and yield components in wheat. These traits are genetically complex and are not easy to manipulate.

Many recent works have showed that new opportunities exist to improve the adaptation of wheat to heat and drought stressed environments (Trethowan and Mujeeb-Kazi 2008; Rebetzke et al., 2009; Reynolds et al., 2010). Conventional breeding with a special focus on adaptation to marginal environments provides a necessary baseline in terms of genetic backgrounds into which new traits and their genes can be introduced. However, specific research objectives to identify and accumulate new and appropriate combinations of stress-adaptive traits must follow a systematic approach, since there is still much to learn about how potentially useful traits (and their genes) interact—with each other, with different genetic backgrounds, and across the vast range of environments in which they must be deployed.

Significant genetic progress has been made for grain yield of wheat in the low input rainfed production systems in Australia. This progress has largely resulted from direct selection for yield and broad adaptation, based on the results of multi environment trials, in combination with strategic use of indirect selection for sources of specific adaptation to characterized environmental limitations (Bänziger and Cooper., 2001).

It is possible to predict whether the use of a secondary trait can enhance expected progress in selection by calculating its genetic correlation with yield and heritability. Indirect selection for a single secondary trait results in greater progress for grain yield than direct selection for grain yield when $hGY < |rGhST|$, where hGY and hST are the square roots of the heritabilities of grain yield and the secondary trait, respectively, and rG is the genetic correlation between grain yield and the secondary trait (Falconer, 1989). These secondary traits should not be associated with poor yields in mild stress environments while breeding programs designed for stress-prone environments.

Many traits have been studied for their use in breeding programs for drought tolerance, but only a few are currently recommendable for application in practical breeding programs. For example, CIMMYT (Reynolds *et al.*, 2001), IRRI (Lafitte et al., 2003) and (Blum and Nguyen, 1997) recommend the use of

flowering and maturity dates, changes in stay green (e.g., leaf death score), and low canopy temperature.

Days to heading: Earliness is one of the first attributes optimized by breeding programs (Slafer, 2003) and a major trait related to the adaptation of cultivars to particular areas. It is probably the most effective means to increase yield in regions where grains fill under severe water and heat stress (Passioura, 1996; Slafer and White Church, 2001).

Phenology is the most widely used secondary trait because of ease of measurement and relatively high heritability (e.g., Bänziger *et al.*, 2000). However, this approach has several limitations, for example, in winter grown wheat, confers on genotypes better performance (in terms of yield and stability) in severe to moderate drought environments. But, very early varieties may suffer yield penalty in good seasons. Their sensitive reproduction stages may coincide with late in-season freezing events, then, cause ear infertility and also very early flowering usually increase bird damages.

Canopy temperature: When water evaporates from the surface of a leaf, it becomes cooler canopy temperature is therefore a good indicator of a genotype's physiological fitness. Moreover, leaf cooling contributes to improvement of the photosynthetic activity of leaves and prevents premature ageing. A low value of canopy temperature is indicative of good expression of this trait under heat (Araus *et al.* 2002) and different drought stress conditions (Mohammadi *et al.*, 2012b).

So many research works have demonstrated that root characteristics are important drought adaptive attributes (Manschadi *et al.*, 2008; Reynolds *et al.*, 2007; Christopher *et al.*, 2008). However, root traits are difficult to measure in realistic field conditions (Lopes *et al.*, 2010) and, therefore, cooler canopy temperature has been suggested as a surrogate indicating a genotype's ability to maintain transpiration through access of roots to water deep in the soil profile (Olivares-Villegas *et al.*, 2007; Reynolds *et al.*, 2007).

Measurement of canopy temperature in a field plot is easily, cheaply and quickly (within a few seconds), with a simple infrared thermometer.

Thousand kernel weight: The optimum temperature range for reaching maximum wheat kernel weight is 15-18°C, higher temperatures reduce the duration of grain filling. This reduction is not compensated by the increase in rate of assimilates accumulation and in turn, accelerate maturity and significantly reduces grain weight and yield (Mohammadi, 2001, 2012). Acevedo *et al.* (1991) reported a 4% reduction in grain weight over a range of 17 to 24°C, for each °C increase in mean air temperature during grain-filling.

Wheat genotypes that are able to maintain high individual kernel weight despite heat stress may possess a high level of heat tolerance (Hays *et al.*, 2007; Plaut *et al.*, 2004; Reynolds *et al.*, 1994; Mohammadi, 2012). There is genetic

variability available for such tolerance among wheat genotypes (de Lespinay, 2004; Sharma et al., 2004b and Mohammadi, 2012a).

Leaf senescence: Delayed senescence (stay-green) is considered an important component for sustaining yield potential and in some cases also for sustaining yield under stress during grain filling (e.g. Borrell and Hammer 2000; Sanchez et al., 2002). Often, crop cultivars bred for water-limited environments by selection for yield under stress have a constitutively reduced leaf area. Pathways for constitutive reduction in plant size and leaf area are smaller leaves, reduced tillering, and early flowering. Reduced growth duration is associated with reduced leaf number (Blum, 2004). A crop plant designed for constitutive moderation of water use by the above pathways cannot attain high yield potential.

Leaf senescence is under relatively simple genetic control and can be readily improved by conventional or molecular breeding (Borrell and Hammer, 2000).

Statistical analysis

Experiments conducted under low-yielding conditions have a higher frequency of producing statistically non-significant differences (i.e., $p > 0.05$) or having a large coefficient of error variation for grain yield than experiments conducted under high-yielding conditions. This is because the error variance of grain yield usually does not decrease as much as the genetic variance when moving from high- to low-yielding conditions (Bänziger et al., 1997). Breeders often discard experiments with statistically non-significant genotype effects or large coefficients of error variation and thus do not consider that information when making selection decisions from the results of multi-environment trials. It should be emphasized that neither of these results dictates that there are no real genetic differences among the germplasm units included in the trials, but they do indicate that if the differences exist they will, in most cases, be difficult to detect with a satisfactory level of confidence.

When analyses of yield were conducted within states or regions, the three factor genotype-by-site-by-year interaction was generally found to be the largest component of variance. Within this region, the genotype-by-site interactions are usually the smallest interaction component. Since the three-factor genotype by-site-by-year interaction is usually found to be the largest source of G×E interaction for yield, it is not sufficient to concentrate on only the spatial or temporal aspects of environmental variation in the target population of environments but rather on the interplay of these spatial and temporal dimensions of environmental variation and the influence of this interplay on the yield performance of genotype (Mohammadi, 2011a, Mohammadi et al., 2012a and Mohammadi et al., 2013).

Use of an alpha-lattice design in replicated yield trials of bread wheat at Gachsaran Dryland Agricultural Research Station in 2010-11 under severe heat drought and stress resulted in an average efficiency 15% higher than the

randomized complete block design when average variance was used as the comparison criterion. The results of this study show that alpha lattice design provided smaller standard errors of differences, coefficients of variation and error mean squares as compared to RCBD providing efficiency in comparing different entries/lines. Alpha-lattice was generally most efficient when the C.V.s of the trials were high. It was also slightly more efficient for low-yielding than for high-yielding trials and for rainfed than for irrigated trials. Since the changeover to alpha-lattice designs requires no new major input or changes in present field layout (Yau, 1997).

Alpha lattice has been shown can be more efficient than RCBD in field trials conducted in the UK (Paterson and Hunter, 1983), Yau (1997) in ICARDA and Mc Laren in IRRI. It appears to have the potential to replace RCBD in many trials.

Modern alpha lattice design doesn't suffer from the number of entries and block size. Thus, it has much flexibility field layout. Moreover, It doesn't need definite layout before planting and it is possible to analyze the data of an experiment by alpha lattice design, while, it was planted based on RCBD layout.

YAU, (1997) reported the use of alpha lattice design in international yield trials of different crops and found average efficiency 18 % higher than the RCBD. Alpha lattice is more effective with larger trials than with those involving small numbers of entries.

Managed- stressed experiments

Traditionally, crop improvement and natural resource management were seen as distinct but complementary disciplines. Improved varieties and improved resource management are two sides of the same coin. Most farming problems require integrated solutions, with genetic, management related and socio-economic components.

In developing countries, farmers have traditionally grown landrace cultivars, which are well adapted to serious moisture stress conditions. However, these traditional cultivars are generally poor yielding in "good years" when rainfall is more plentiful. Some researchers believe modern cultivars have consistently out yielded older cultivars, even in the lowest yielding conditions of each particular study (Slafer and Andrade, 1993; Calderini *et al.*, 1995). Based on our experiences, some new improved cultivars such as Zagros and Koohdasht which were released for semitropical dryland regions of Iran, yield the same as or even more than local/landraces cultivars in dry years (with more than 0.7 t/ha in farmers conditions), yet will respond to more favorable moisture and nutrient conditions.

Under a particular pressure of environmental stress, cultivars with high yield potential produce less than certain cultivars that have lower yield potential but seem to be better adapted to stress. For most cereals grown under water-limited conditions the crossover occurs at a yield level of around 2–3 t/ha (e.g. Blum and Pnuel, 1990; Ceccarelli & Grando 1991), which is approximately one-

third of the yield potential. The main reason for a crossover under conditions of variable water supply is an inherent difference among the tested cultivars in drought resistance, beyond difference in their yield potential (Blum, 2005). It seems this border depends on characterization of considered target environments.

Crop selection performed in nurseries with normal natural growing conditions is translated to cultivars with increased productivity in a wide range of growing conditions, from mild (approx. 4-6 t/ha) to moderate stress (approx. 2-4 t/ha) and even more stress (approx. 1-2 t/ha) environments with low probability frequency. However, in selection environments subject to severe drought stress in most years, the situation may change.

When programs selection for yield was performed under low-yielding stress conditions, large differences were seen among different years, locations, and studies in the heritability estimates for yield under stress in a given crop.

Heritability for yield under stress largely depends on 2 key factors: (a) the existence of genes for drought resistance in the population, which are effective in the stress environments under which selection is performed (Blum et al., 2001), and (b) the degree of control over the homogeneity and general stress conditions in the selection nursery (Blum 2005). Because of the difficulty of choosing a few representative selection environments for a target population of environments where low yields may be caused by a number of interacting and varying abiotic stress factors, CIMMYT approached breeding for low input conditions by simulating abiotic stress factors that are important in the target environment and exposing breeding experiments to a clearly defined abiotic stress factor. These selection environments were termed 'managed stress environments' (Bänziger and Cooper 2001).

The use of managed stress environments permitted controlled and quantifiable consideration of the factors that affect breeding progress. Because these trials were established under researcher-managed conditions and thus exploitation of a much larger genetic variance and higher selection intensity than it is usually possible at the advanced breeding stage. Error variance was kept low and heritability was kept high by using a combination of improved statistical design and analysis techniques, choosing fields with rather uniform soil texture and depth, maintaining optimal plant stands, and using well-bordered trials (Bänziger et al., 1995; Lafitte et al., 1997).

Control over the homogeneity and general stress conditions in the selection nursery like weeds, diseases, pests, inherent soil variability, etc. is effective in the stress environments under which selection is performed (Blum et al., 2001). Selection in stress-managed environments does not suffer from these additional problems under stress than in unmanaged environments. Microelement deficiency or parasitic nematodes, whose effects on productivity are severely exacerbated under moisture deficit, confounding potential genetic gains associated with drought adaptation *per se* (Reynolds and Trethowan 2007). Therefore, with the appropriate genetic materials and minimization of the

error variance, heritability for yield under stress can be high and selection effective, particularly, in severe drought stress.

However, as the genetic variance for grain yield is smaller under low input conditions, selection progress is less and breeders may be disappointed when regrowing germplasm selected for improved productivity in the following year, even more so if that year exposes the germplasm to different environmental constraints.

These managed environments may be conducted on research stations or on-farm. In a number of cases this has progressed to the point where these managed environments have been included as additional environments in multi-environments.

Germplasm developed using combined results from managed stress and normal natural environments indeed proved to have a higher yield level and stability, than evaluation across a random sample of trials from the target environments that may include severe drought environments with low heritability accompanied high error variance. It was observed that genotype-by-stress interactions for the particular targeted abiotic stress factor were at their highest, if stress levels were severe, when stress is uniformly severe which is rare in the field. Wheat cultivars tested in a particular set of stressful conditions may not show the same performance in another set (Cooper *et al.*, 1997).

Many scientists have chosen a midway and believe in selection under both stress and non-stress conditions (Fischer and Maurer, 1978; Clarke *et al.*, 1992; Fernandez, 1992). Selection for high yield in an optimum environment is effective because genetic variation is usually maximized and genotype-by-environment interactions are low (Richards, 1996). However, genotypes selected in optimum environments may not yield well in drought stress environments (Mohammadi *et al.*, 2011b). On the other hand, selection under drought stress conditions is often complicated by low heritability of traits, non-uniform testing conditions and large genotype-by-environment interaction.

Several indices have been utilized to evaluate genotypes for drought tolerance based on grain yield in different environments. Rosielle and Hamblin (1981) defined stress tolerance (TOL) as the differences in yield between the stress (Y_s) and non-stress (Y_p) environments and mean productivity (MP) as the average yield of Y_s and Y_p . Fischer and Maurer (1978) proposed a stress susceptibility index (SSI) for cultivars. Fernandez (1992) defined an advanced index (STI = stress tolerance index), which can be used to identify genotypes that produce high yield under both stress and non-stress conditions. The other yield based estimate for drought resistance is geometric mean productivity (GMP). The geometric mean is often used by breeders interested in relative performance, since drought stress can vary in severity in field environments over years (Ramirez & Kelly, 1998). The optimal selection criterion should distinguish genotypes that express uniform superiority in both stressed and non-stressed environments from the genotypes that are favorable only in one environment.

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POSSIBILITY OF PRODUCING ONE-YEAR OLD SEEDLINGS OF THE AUTOCHTHONOUS BRANDY VARIETIES GRAFTING ON THE GENERATIVE ROOTSTOCK IN THE REGION OF NORTH MONTENEGRO

SUMMARY

Plum is the major fruit species in the area of North Montenegro. A study conducted over a period of 4 years in North Montenegro region included in situ identification of autochthonous plum cultivars. Observation and recording of their phenological and pomological traits were performed using IBPGR and UPOV methodologies. Flowering started between 26th March and 12th April and fruit ripening between 13th July (Petrovača) and 18th September (Trnovača). Fruit weight ranged from 6.65 ± 0.235 g to 53.88 ± 0.654 g and stone weight from 0.16 ± 0.003 g to 2.20 ± 0.711 g. The cultivars were classified as being extremely small in terms of fruit size, except for cv. Crvena durgulja (bigger fruit size). Rounded fruit shape and light green ground color were dominant. Skin color ranged from amber to black. Yellow green was a dominant flesh color and medium flesh firmness predominated. The fruits of the above cultivars could be processed, particularly into plum brandy, or they could be used fresh or dried. The selected plum cultivars can be used both in breeding programs and as cultivars for organic plum orchards. This study was made to assess the performance of autochthonous plum cultivars (in situ) and seedling. Producing process consisted of 2 stages: a) initial selection from the population and pomological characterization, b) morphological and quantitative characteristics of one-year old seedlings for autochthonous brandy varieties of plum on Myrobalan seedling (*Prunus cerasifera* Ehrh.).

Keywords: Plum, genetic bases, germplasm, *Prunus domestica* L., *Prunus insititia* L.

INTRODUCTION

Plum is ranked as the second most important fruit tree crop in the temperature climate after apple from the production point of view. It's tasty and good looking fruits have been used extensively during history as fresh or dried fruits, but also processed as jam, marmalade, jelly and brandy. Plums contain health promoting compounds, minerals, vitamins, fibers, and low in calories and among the highest in antioxidant containing foods and for those reasons are beneficial for human consumption (Botu *et al.*, 2012).

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Plum cultivation has a historical tradition, economical, social and cultural implication for the South East of Europe. The European plum (*Prunus domestica* L.) genetic variability in the South East of Europe is large, unique and particularly evident in the characteristics of the fruit, plant and adaptability to different ecological conditions. In Serbia and Romania 80% of the plums go into the production of slivovitz or tuica.

Fortunately, some of plum cultivars and biotypes exhibit tolerance to *Plum Pox Potyvirus* (Botu *et al.*, 2012). As an example, Elisa test that was conducted on cultivar 'Crvena durgulja', proved presence of Sharka (PPV), however it did not affect the fruits. Also, 'Crvena durgulja' showed as very resistant to other pests and diseases. The cultivar 'Crvena ranka' is slightly susceptible to Sharka. The cultivar 'Komperuša', Elisa test showed absence of Sharka (PPV). Also, 'Komperuša' showed as very resistant to other pests and diseases (Botu *et al.*, 2012).

'Požegača' and number of cultivars used for brandy production predominant in the assortment. The Montenegro plum production is characterized by extensive growing technology, low unstable yields, low-quality fruit, PPV-induced problems and a multitude of cultivars. The cultivars include Požegača (35%), foreign standard and introduction newly bred cultivars (15%) and autochthonous (local, primitive) cultivars (50%), and their fruit is typically used for brandy production. Autochthonous plum cultivars are a limiting factor in improving plum production in Montenegro. Nevertheless, they are used as an outstanding source of germplasm and as a genetic basis underlying breeding activities, principally the development of new cultivars, clonal selection (Ogašanić *et al.*, 1994; Milošević, 2000), the development of new plum, apricot and peach rootstocks (Paunović, 1988; Djurić *et al.*, 1998), resistance to economically important diseases (Paunović and Paunović, 1994; Rodrigues *et al.*, 2009) or intensive cultivation (Mratinić, 2000). Similar investigations with focus on identical or similar objectives were also conducted in the other countries of the former Yugoslavia – Serbia (Milošević, 2000), Bosnia and Herzegovina (Buljko, 1977; Jarebica and Muratović, 1977), Croatia (Jelačić *et al.*, 2008) and Slovenia (Usenik *et al.*, 2007). In situ investigations of cultivars derived from *Prunus domestica* L. and *P. insititia* L. in Serbia were conducted by a number of researchers (Paunović *et al.*, 1985; Paunović, 1988; Paunović and Paunović, 1994; Petrović *et al.*, 2002) who defined important biological, pomological and technological traits of both fruit and tree. They reported that the selected cultivars could be used both as breeding programs and as rootstocks, as well as in further disease-related systematic studies under field and laboratory conditions. The main objective of this study was to determine in situ basic biological and pomological traits of some autochthonous plum cultivars derived from *P. domestica* L. and *P. insititia* L. in the area of North Montenegro that could be used as a genetic basis and source of germplasm for future breeding studies and as cultivars for organic plum orchards.

MATERIAL AND METHODS

The investigations were conducted continuously in years 2010, 2011, 2012 and 2013. They involved in situ identification, marking and careful observation of autochthonous plum cultivars (accessions) in the area of North Montenegro.

The researched genotypes or cultivars were selected in Western Serbia (Paunović *et al.*, 1985). Majority of them, eighteen to be exact, derived from *P. domestica* L., while, cultivars 'Trnovača' and 'Turgulja' were derived from *P. insititia* L., (Paunović *et al.*, 1985). The sampled trees were aged 35 ('Plavski piskavac') to 55 years ('Turgulja'). The trees of all the cultivars grew on their own roots.

The study focused on few segments. Very first one included recording of the phenological traits - first flowering, full flowering, end of flowering and harvest date. Phenological characteristics were determined as below: the beginning of flowering was recorded when at least 5% of the flowers bloomed; full flowering was accepted when at least 80% of the flowers bloomed, the end of flowering was determined when 90% of the flowers bloomed and corollas began to fall off, and harvest date was established when the fruits were sufficiently colored and soft to be eaten (Funt, 1998). The other segment comprised pomological, i.e. physical [fruit weight (g), stone weight (g) and fruit size (on a scale of 1-9)] and sensorial traits of the fruit [fruit shape (1-6), ground color (1-5), skin color (0-9), flesh color (1-9), flesh firmness(1-9) and fruit usage (1-4)]. IBPGR and UPOV methodologies were used to describe the cultivars in phenological, pomological and sensorial terms (Zanetto *et al.*, 2002). Measurements included the weight of 25 fruits and as much stones per cultivar. Fruit and stone weights were determined using a Metler 1200 technical scale (range of measurement 0.01-120.00 g, precision ± 0.01 g). The data were subjected to statistical analysis of variance (ANOVA) and means were separated by LSD test at $P < 0.05$ significant level (SAS Institute, 1990).

One-year old seedlings from 20 autochthonous plum cultivars were planted in the nursery and raised seedlings were evaluated for nursery characteristics: plant height (cm), stem diameter (mm), bat take (%) seedling vigor, uniformity and branching. Uniformity was low (grade 1) when coefficient of variation was less than 15%, and high (grade 2) when it was from 15 to 25%.

RESULTS AND DISCUSSION

The data showed that the onset of flowering was recorded in the last five days of March and in the first twelve days of April (Table 1). The earliest onset of flowering was observed in cultivar 'Trnovača' (26.03) derived from *P. insititia* L., and the latest in cultivar 'Dupljanka' (12.04) derived from *P. domestica* L. Among the 20 cultivars examined, 8 (40 %) started to flower at the end of March, and 12 (60 %) during the middle of the first twelve-day period of April. The full flowering stage lasted from 30th March ('Trnovača') to 18th April ('Dupljanka'), and the end of flowering from 7th April ('Trnovača') to 24th April ('Dupljanka').

Flowering lasted 9 ('Turgulja', 'Plavski piskavac', 'Grkaja', 'Kapavac' and 'Komperuša') to 14 days ('Crvena durgulja', 'Mednica', 'Petrovača', 'Belošljiva' and 'Šara').

Table 1. Phenological characteristics of autochthonous plum cultivars in the region of North Montenegro (2010, 2011, 2012, 2013 and average)

Cultivar	location			flowering				harvest
	longitude	latitude	altitude (m)	onset	full	end	duration	Date
Petrovača	19 ° 41' E	41° 01'N	879	25.03.2010	29.03.2010	07.04.2010	13	10.07.2010
				26.03.2011	30.03.2011	11.04.2011	16	14.07.2011
				30.03.2012	02.04.2012	14.04.2012	15	17.07.2012
				27.03.2013	01.04.2013	08.04.2013	12	11.07.2013
				27.03 d	31.03 d	10.04 d	14a	13.07 d
Mednica	19 ° 59' E	42° 70'N	670	27.03.2010	02.04.2010	11.04.2010	15	20.07.2010
				29.03.2011	03.04.2011	13.04.2011	15	22.07.2011
				02.04.2012	06.04.2012	16.04.2012	14	30.07.2012
				31.03.2013	01.04.2013	12.04.2013	12	28.07.2013
				30.03 d	03.04 d	13.04 d	14a	25.07 d
Kapavac	19 ° 29' E	42° 50'N	974	01.04.2010	04.04.2010	10.04.2010	9	25.07.2010
				03.04.2011	06.04.2011	12.04.2011	9	27.07.2011
				06.04.2012	09.04.2012	15.04.2012	9	02.08.2012
				02.04.2013	05.04.2013	11.04.2013	9	31.07.2013
				03.04 c	06.04 c	12.04 d	9c	29.07 d
Grkaja	19 ° 59' E	42° 70'N	670	28.03.2010	03.04.2010	08.04.2010	11	30.07.2010
				30.03.2011	04.04.2011	10.04.2011	11	03.08.2011
				02.04.2012	06.04.2012	14.04.2012	12	06.08.2012
				02.04.2013	03.04.2013	08.04.2013	6	01.08.2013
				01.04 cd	04.04 cd	10.04 d	10c	02.08 d
Crvena ranka	19 ° 43' E	42° 59'N	601	27.03.2010	01.04.2010	08.04.2010	12	04.08.2010
				28.03.2011	03.04.2011	12.04.2011	15	04.08.2011
				01.04.2012	07.04.2012	15.04.2012	14	08.08.2012
				29.03.2013	01.04.2013	09.04.2013	11	04.08.2013
				29.03 d	03.04 d	11.04 d	13a	05.08 cd
Mudara	19 ° 43' E	42° 59'N	601	29.03.2010	04.04.2010	11.04.2010	13	04.08.2010
				31.03.2011	06.04.2011	13.04.2011	13	06.08.2011
				04.04.2012	09.04.2012	15.04.2012	11	10.08.2012
				02.04.2013	05.04.2013	13.04.2013	11	04.08.2013
				01.04 c	06.04 c	13.04 d	12b	06.08 c
Belošljiva	19 ° 52' E	43° 03'N	850	27.03.2010	02.04.2010	12.04.2010	16	05.08.2010
				29.03.2011	02.04.2011	12.04.2011	14	07.08.2011
				04.04.2012	06.04.2012	16.04.2012	12	11.08.2012
				30.03.2013	02.04.2013	12.04.2013	13	05.08.2013
				30.03 d	03.04 d	13.04 d	14a	07.08 c
Crnošljiva	19 ° 20' E	42° 38'N	978	29.03.2010	03.04.2010	11.04.2010	13	06.08.2010
				30.03.2011	03.04.2011	13.04.2011	14	08.08.2011
				02.04.2012	07.04.2012	16.04.2012	14	12.08.2012
				01.04.2013	03.04.2013	12.04.2013	11	06.08.2013
				31.03 d	04.04 cd	13.04 d	13a	08.08 c
Šara	19 ° 57' E	42° 40'N	900	26.03.2010	30.03.2010	10.04.2010	15	10.08.2010
				28.03.2011	31.03.2011	10.04.2011	13	12.08.2011
				31.03.2012	04.04.2012	14.04.2012	14	16.08.2012
				27.03.2013	01.04.2013	10.04.2013	14	10.08.2013
				28.03 d	01.04 d	11.04 d	14a	12.08 c

Metlaš	19 ° 29' E	42° 51'N	984	28.03.2010 30.03.2011 03.04.2012 01.04.2013 01.04 c	03.04.2010 06.04.2011 10.04.2012 05.04.2013 06.04 c	10.04.2010 12.04.2011 16.04.2012 10.04.2013 12.04 d	13 13 13 9 12b	10.08.2010 14.08.2011 16.08.2012 12.08.2013 13.08 c
Crvena durgulja	19 ° 48' E	42° 57'N	870	26.03.2010 28.03.2011 05.04.2012 31.03.2013 30.03 d	01.04.2010 03.04.2011 07.04.2012 01.04.2013 03.04 d	11.04.2010 14.04.2011 15.04.2012 12.04.2013 13.04 d	16 17 10 12 14a	13.08.2010 15.08.2011 19.08.2012 13.08.2013 15.08 c
Plavski piskavac	19 ° 55' E	42° 33'N	940	05.04.2010 07.04.2011 10.04.2012 06.04.2013 07.04 b	09.04.2010 11.04.2011 14.04.2012 10.04.2013 11.04 b	12.04.2010 17.04.2011 20.04.2012 15.04.2013 16.04 c	7 10 10 9 9c	19.08.2010 21.08.2011 25.08.2012 19.08.2013 21.08 b
Turgulja	19 ° 56' E	42° 37'N	910	02.04.2010 04.04.2011 08.04.2012 02.04.2013 04.04 c	06.04.2010 08.04.2011 11.04.2012 07.04.2013 08.04 c	11.04.2010 13.04.2011 16.04.2012 12.04.2013 13.04 d	9 9 8 10 9c	21.08.2010 24.08.2011 28.08.2012 19.08.2013 23.08 b
Obični Piskavac	18 ° 49' E	42° 26'N	858	04.04.2010 07.04.2011 12.04.2012 05.04.2013 07.04 b	08.04.2010 10.04.2011 14.04.2012 08.04.2013 10.04 c	14.04.2010 17.04.2011 22.04.2012 15.04.2013 17.04 c	10 10 10 10 10c	20.08.2010 25.08.2011 27.08.2012 20.08.2013 23.08 b
Komperuša	19 ° 49' E	42° 43'N	850	07.04.2010 09.04.2011 12.04.2012 08.04.2013 09.04 a	11.04.2010 13.04.2011 16.04.2012 12.04.2013 13.04 b	16.04.2010 18.04.2011 22.04.2012 16.04.2013 18.04 c	9 9 10 8 9c	21.08.2010 26.08.2011 29.08.2012 20.08.2013 24.08 b
Mudovalj	19 ° 55' E	42° 33'N	940	06.04.2010 10.04.2011 13.04.2012 07.04.2013 09.04 a	11.04.2010 16.04.2011 19.04.2012 14.04.2013 15.04 ab	19.04.2010 21.04.2011 25.04.2012 19.04.2013 21.04 ab	13 11 12 12 12b	29.08.2010 30.08.2011 05.09.2012 02.09.2013 02.09 b
Dronga	19 ° 55' E	42° 33'N	940	06.04.2010 08.04.2011 12.04.2012 10.04.2013 09.04 a	12.04.2010 17.04.2011 18.04.2012 13.04.2013 15.04 ab	19.04.2010 22.04.2011 24.04.2012 19.04.2013 21.04 ab	13 14 12 9 12b	06.09.2010 07.09.2011 13.09.2012 10.09.2013 09.09 a
Dupljanka	19 ° 59' E	43° 02'N	1180	10.04.2010 12.04.2011 15.04.2012 11.04.2013 12.04 a	16.04.2010 18.04.2011 22.04.2012 16.04.2013 18.04 a	22.04.2010 24.04.2011 27.04.2012 23.04.2013 24.04 a	12 12 12 12 12b	07.09.2010 10.09.2011 14.09.2012 13.09.2013 11.09 a
Jesenska	19 ° 55' E	42° 33'N	940	07.04.2010 11.04.2011 14.04.2012 08.04.2013 10.04 a	13.04.2010 18.04.2011 19.04.2012 14.04.2013 16.04 a	20.04.2010 22.04.2011 26.04.2012 20.04.2013 22.04 a	13 11 12 12 12b	08.09.2010 09.09.2011 15.09.2012 12.09.2013 11.09 a
Trnovača	19 ° 20' E	42° 38'N	979	24.03.2010 26.03.2011 29.03.2012 25.03.2013 26.03 d	26.03.2010 29.03.2011 04.04.2012 30.03.2013 30.03 d	04.04.2010 05.04.2011 12.04.2012 07.04.2013 07.04 d	11 10 14 13 12b	13.09.2010 18.09.2011 20.09.2012 21.09.2013 18.09 a
LSD005 LSD 001				6.58 7.84	3.45 4.54	5.13 6.11	2.15 2.95	7.79 8.32

The harvest period was longer than the flowering period (Gunes, 2003), as it lasted from 13th July ('Petrovača') to 18th September ('Trnovača'). Local plum cultivars began to flower at the end of March or at the beginning of April under the environmental conditions of Serbia (Paunović, 1988; Paunović and Paunović, 1994; Mratinić, 2000; Milošević, 2000). Similar data on the period and duration of flowering of autochthonous plum cultivars were reported by Jarebica and Muratović (1977) and confirmed by the results of this study. Somewhat later flowering under Slovenian conditions was reported by Usenik *et al.* (2007) and early flowering in the Tokat province (Turkey) by Gunes (2003), the reason being environmental, particularly climate effects (Buljko, 1977). In terms of fruit ripening, the results of this study were similar to the ones obtained by Paunović *et al.*, 1985; Paunović, 1988; and Mratinić, 2000. Measurable pomological characteristics of fruit and stone are given in Table 2.

Fruit weight ranged from $6.65 \text{ g} \pm 0.235 \text{ g}$ ('Plavski piskavac') to $53.88 \pm 0.654 \text{ g}$ ('Crvena durgulja'). Jarebica and Muratović (1977) determined that the plum fruit weight ranged from 14.17 to 41.70 g. Jovančević (1977) reported minimum and maximum values of fruit weights of some local plum cultivars, being 5.03 and 23.86 g, respectively. In the study conducted by Petrović *et al.*, (2002), fruit weight of eight local plum cultivars in Eastern Serbia and in the region of Čacak (Western Serbia) ranged from 15.20-26.40 g and from 6.68-36.50 g, respectively (Paunović *et al.*, 1985). According to Mratinić (2000), fruit weight of autochthonous plum cultivars in a broader region of south-western Serbia and Šumadija fell within a range of 6.20-28.00 g with 50% of the cultivars having the fruit weight of 15.00 g.

Similar data for autochthonous plum cultivars were reported by researchers from other countries. In Turkey, for example, Gunes (2003) reported the fruit weight of local plum cultivars in the Tokat province to range from 5.23-25.18 g and from 8.30-29.50 g in the Van province. The results obtained in this study confirmed those provided by the above authors in terms of the high degree of genotypic variability in fruit weight of autochthonous (local) plum cultivars. The cultivars selected in this study were classified as being extremely small in terms of fruit size, whereas the fruits of cultivar 'Crvena durgulja' were the only ones classified as being small (Paunović *et al.*, 1985; Mratinić, 2000; Zanetto *et al.* 2002). 'Crvena durgulja'-fruits are elongated 49 mm long, 44, 10 mm wide, 46, 8 mm thick and weight 60.65 g on average (Botu *et al.*, 2012).

The most dominant fruit shape was rounded in twelve cultivars, followed by ovate - in four cultivars, elliptical - in three cultivars and oblong - in one cultivar ('Grkaja'). Ground color in most of the cultivars was light green (10) and light yellow (6), being yellow in cv. 'Trnovača', cv. 'Dupljanka' and cv. 'Grkaja'. Skin color ranged from white yellow (1) and red (1) and violet (1) and blue (1) and dark blue (1), and to dark violet (2), black (2), mahagoni (4) to red violet (7 cultivars). Flesh color was yellow green in most cultivars (12) and light yellow only in cv. 'Plavski piskavac' and amber only in cv. 'Dupljanka'.

Table 2. Pomological and sensorial characteristics of autochthonous plum cultivars in the Region of North Montenegro (2010, 2011, 2012, 2013 and average)

Cultivar		fruit	fruit	fruit	ground	skin	flesh	flash	use	stone
		weight (g)	size 1	shape 2	colour 3	colour *	colour **	Firmness ***	****	weight (g)
Petrovača	2010	13.24±0.082	1	2	2	5	3	5	2	1.29±0.044
	2011	13.13±0.089	1	2	2	5	3	5	2	1.22±0.038
	2012	12.99±0.069	1	2	2	5	3	5	2	1.10±0.022
	2013	13.08±0.072	1	2	2	5	3	5	2	1.15±0.024
	average	13.11±0.078e	1	2	2	5	3	5	2	1.19±0.032c
Mednica	2010	16.33±0.220	1	4	2	3	5	3	2	1.51±0.079
	2011	16.27±0.230	1	4	2	3	5	3	2	1.47±0.079
	2012	15.99±0.234	1	4	2	3	5	3	2	1.40±0.074
	2013	15.77±0.208	1	4	2	3	5	3	2	1.34±0.068
	average	16.09±0.223e	1	4	2	3	5	3	2	1.43±0.075c
Kapavac	2010	11.95±0.090	1	3	2	8	3	5	2	0.61±0.009
	2011	11.92±0.085	1	3	2	8	3	5	2	0.52±0.006
	2012	11.86±0.081	1	3	2	8	3	5	2	0.55±0.007
	2013	11.79±0.080	1	3	2	8	3	5	2	0.44±0.006
	average	11.88±0.084e	1	3	2	8	3	5	2	0.53±0.007d
Grkaja	2010	14.89±0.782	1	6	4	2	2	5	2	0.99±0.080
	2011	14.80±0.795	1	6	4	2	2	5	2	0.97±0.080
	2012	14.76±0.748	1	6	4	2	2	5	2	0.88±0.074
	2013	14.67±0.787	1	6	4	2	2	5	2	0.76±0.066
	average	14.78±0.778e	1	6	4	2	2	5	2	0.90±0.075d
Crvena ranka	2010	19.45±0.051	1	4	3	3	3	5	1.2	0.74±0.006
	2011	19.42±0.050	1	4	3	3	3	5	1.2	0.81±0.004
	2012	19.25±0.040	1	4	3	3	3	5	1.2	0.59±0.003
	2013	19.08±0.023	1	4	3	3	3	5	1.2	0.50±0.003
	average	19.30±0.041e	1	4	3	3	3	5	1.2	0.66±0.004d
Mudara	2010	35.84±0.311	2	2	3	3	2	5	2	1.91±0.041
	2011	35.60±0.310	2	2	3	3	2	5	2	1.90±0.035
	2012	35.60±0.298	2	2	3	3	2	5	2	1.85±0.033
	2013	35.36±0.277	2	2	3	3	2	5	2	1.7±0.031
	average	35.60±0.299c	2	2	3	3	2	5	2	1.87±0.035b
Belošljiva	2010	14.15±0.318	1	2	3	0	3	3	2	1.12±0.031
	2011	14.12±0.295	1	2	3	0	3	3	2	1.03±0.028
	2012	13.85±0.280	1	2	3	0	3	3	2	0.95±0.022
	2013	13.48±0.307	1	2	3	0	3	3	2	0.86±0.019
	average	13.90±0.300e	1	2	3	0	3	3	2	0.99±0.025d
Crnošljiva	2010	12.95±0.225	1	3	2	7	3	7	2	0.58±0.011
	2011	12.85±0.222	1	3	2	7	3	7	2	0.52±0.011
	2012	12.55±0.217	1	3	2	7	3	7	2	0.49±0.008
	2013	12.73±0.220	1	3	2	7	3	7	2	0.41±0.006
	average	12.77±0.221e	1	3	2	7	3	7	2	0.50±0.009d

Šara	2010	19.22±0.062	1	2	2	4	3	5	2	0.92±0.023
	2011	19.11±0.058	1	2	2	4	3	5	2	0.88±0.021
	2012	18.95±0.055	1	2	2	4	3	5	2	0.78±0.017
	2013	18.80±0.053	1	2	2	4	3	5	2	0.70±0.015
	average	19.02±0.057e	1	2	2	4	3	5	2	0.82±0.019d
Metlaš	2010	18.55±0.088	1	2	3	3	3	7	2	0.80±0.008
	2011	18.44±0.080	1	2	3	3	3	7	2	0.74±0.010
	2012	18.29±0.062	1	2	3	3	3	7	2	0.68±0.007
	2013	18.04±0.054	1	2	3	3	3	7	2	0.58±0.011
	average	18.33±0.071e	1	2	3	3	3	7	2	0.70±0.009d
Crvena durgulja	2010	53.99±0.662	3	3	3	3	5	5	2	2.35±0.722
	2011	53.90±0.659	3	3	3	3	5	5	2	2.25±0.710
	2012	53.84±0.650	3	3	3	3	5	5	2	2.18±0.705
	2013	53.79±0.645	3	3	3	3	5	5	2	2.02±0.707
	average	53.88±0.654a	3	3	3	3	5	5	2	2.20±0.711a
Plavski piskavac	2010	6.69±0.238	1	2	4	7	4	5	2	0.59±0.021
	2011	6.67±0.235	1	2	4	7	4	5	2	0.54±0.020
	2012	6.63±0.229	1	2	4	7	4	5	2	0.48±0.013
	2013	6.61±0.238	1	2	4	7	4	5	2	0.47±0.006
	average	6.65±0.235f	1	2	4	7	4	5	2	0.52±0.015d
Turgulja	2010	22.85±0.325	1	2	2	9	3	5	2	1.66±0.016
	2011	20.15±0.266	1	2	2	9	3	5	2	1.61±0.014
	2012	20.84±0.220	1	2	2	9	3	5	2	1.57±0.012
	2013	19.80±0.073	1	2	2	9	3	5	2	1.52±0.010
	average	20.91±0.221d	1	2	2	9	3	5	2	1.59±0.013c
Obični Piskavac	2010	13.75±0.053	1	2	2	6	3	5	2	0.82±0.006
	2011	13.70±0.046	1	2	2	6	3	5	2	0.74±0.004
	2012	13.55±0.040	1	2	2	6	3	5	2	0.70±0.002
	2013	13.48±0.029	1	2	2	6	3	5	2	0.70±0.004
	average	13.62±0.042e	1	2	2	6	3	5	2	0.74±0.004d
Komperuša	2010	16.99±0.109	1	2	2	7	3	5	2	1.25±0.038
	2011	16.90±0.105	1	2	2	7	3	5	2	1.22±0.035
	2012	16.84±0.103	1	2	2	7	3	5	2	1.15±0.029
	2013	16.79±0.107	1	2	2	7	3	5	2	1.14±0.030
	average	16.88±0.106e	1	2	2	7	3	5	2	1.19±0.033c
Mudovalj	2010	18.62±0.132	1	2	3	3	2	5	2	1.26±0.092
	2011	18.58±0.129	1	2	3	3	2	5	2	1.22±0.087
	2012	18.51±0.128	1	2	3	3	2	5	2	1.17±0.082
	2013	18.49±0.111	1	2	3	3	2	5	2	1.11±0.083
	average	18.55±0.125e	1	2	3	3	2	5	2	1.19±0.086c
Dronga	2010	21.05±0.244	1	2	2	5	3	5	2	1.03±0.19
	2011	20.00±0.240	1	2	2	5	3	5	2	1.03±0.15
	2012	19.89±0.229	1	2	2	5	3	5	2	0.96±0.13
	2013	19.66±0.219	1	2	2	5	3	5	2	0.94±0.13
	average	20.15±0.233de	1	2	2	5	3	5	2	0.99±0.15d

Dupljanka	2010	22.72±0.25	1	4	4	3	6	5	2	1.93±0.495
	2011	22.68±0.23	1	4	4	3	6	5	2	1.87±0.425
	2012	22.62±0.20	1	4	4	3	6	5	2	1.82±0.445
	2013	22.62±0.20	1	4	4	3	6	5	2	1.90±0.395
	average	22.66±0.22d	1	4	4	3	6	5	2	1.88±0.440b
Jesenka	2010	23.80 ±0.267	1	2	2	9	3	5	2	1.90±0.022
	2011	23.56±0.256	1	2	2	9	3	5	2	1.84±0.025
	2012	23.83±0.250	1	2	2	9	3	5	2	1.78±0.023
	2013	24.05±0.231	1	2	2	9	3	5	2	1.72±0.022
	average	23.81±0.251d	1	2	2	9	3	5	2	1.81±0.023b
Trnovača	2010	7.25±0.012	1	2	1	7	2	7	2	0.18±0.004
	2011	7.18±0.011	1	2	1	7	2	7	2	0.14±0.003
	2012	7.25±0.015	1	2	1	7	2	7	2	0.14±0.003
	2013	7.24±0.022	1	2	1	7	2	7	2	0.18±0.002
	average	7.23±0.01f	1	2	1	7	2	7	2	0.16±0.003e
	LSD0.05	4.82								0.26
	LSD0.01	6.39								0.36

IBPGR and UPOV Descriptor List for Plum:

¹ **Fruit size:** 1=extremely small, 2=very small; 3=small, 4=small/medium, 5=medium, 6=medium/large, 7=large, 8=very large, 9=extremely large

² **Fruit shape:** 2 = rounded, 3 = elliptical, 4 = ovate, 6 = oblong;

³ **Ground color:** 1=green, 2=light green, 3=light yellow, 4=yellow, 5=deep yellow

***Skin color:** 0=white yellow, 1=pink, 2=red, 3=red violet, 4=violet, 5=dark violet, 6=blue, 7=mahogany, 8=dark blue, 9=black

****Flesh color:** 1=green, 2=light green, 3=yellow-green, 4=light yellow, 5=yellow, 6=amber, 7=light orange, 8=orange, 9=red

***** Flesh firmness:** 3 = soft, 5 = medium, 7 = firm;

****** Use:** 1 = fresh, 2 = processing, 4 = other (drying)

As for flesh firmness, it was medium in 15 cultivars, firm in three and soft in two cultivars. The fruits of all the cultivars could be used for different types of processing, particularly for plum brandy production (Joshi and Sandhu, 2000). 'Crvena ranka' can be used fresh (Mratinić, 2000). 'Crvena ranka' fruits can be consumed immediately. Fruits are also used to produce an alcoholic drink that is called "Raki" in Albanian (Botu *et al.*, 2012) The autochthonous plum cultivar 'Crvena ranka' is cultivated in the Šumadija area (Serbia) since ancient times as a typical brandy cultivar (Mratinić, 2012). Although it produces excellent quality brandy, it is less and extensively cultivated. The consequence of this type of production is irregular bearing, low yields and small atypical fruits of lower quality. Mratinić (2012) pointed out the study which aim was to determine the influence of necessary agro- and pomo-technical practices such as pruning and fertilizing to improve yields and fruit quality of this cultivar. In cultivar 'Crvena ranka', manure – agrozel combination achieved the highest yields, fruit weight (19.4 g) and fruit quality (17% soluble solid content, 13,25% total sugars and 1.05% total acidity).

Table 3. Morphological and quantitative characteristics of one-year old seedlings for autochthonous brandy cultivars of plum on Myrobalan seedling (*Prunus cerasifera* Erhr.).(2010, 2011, 2012, 2013 and average)

Cultivar		Plant height (cm)	Stem diameter (mm)	Branching	Uniformity	Bud take (%)	Growth of scions (cm)	Uniformity of scions
Petrovača	2010	88.7	8.0	1	2	64	156.2	2
	2011	86.0	7.8	1	2	54	151.6	2
	2012	85.9	7.7	1	2	52	150.2	2
	2013	84.2	7.7	1	2	50	143.6	2
	average	86.2c	7.8c	1	2	55e	150.4d	2
Mednica	2010	120.5	11.5	2	2	90	200.5	2
	2011	121.0	11	2	2	88	196.5	2
	2012	108.5	10.2	2	2	95	198.0	2
	2013	107.6	8.1	2	2	95	197.0	2
	average	114.4a	10.2ab	2	2	92a	198.0a	2
Kapavac	2010	74.0	7.4	4	1	70	192.8	1
	2011	71.5	7.0	4	1	65	190.2	1
	2012	73.2	7.1	4	1	75	192.0	1
	2013	74.1	7.3	4	1	62	206.6	1
	average	73.2c	7.2c	4	1	68d	195.4a	1
Grkaja	2010	113.0	9.8	2	1	85	190.5	1
	2011	115.0	10.0	2	1	90	191.5	1
	2012	104.4	9.0	2	1	89	188.5	1
	2013	114.4	9.2	2	1	88	183.1	1
	average	111.7a	9.5b	2	1	88ab	188.4b	1
Crvena ranka	2010	112.8	10.8	2	1	87	197.4	2
	2011	112.0	10.2	2	1	85	194.5	2
	2012	116.5	9.8	2	1	75	198.0	2
	2013	99.5	8.4	2	1	93	190.1	2
	average	110.2ab	9.8ab	2	1	85b	195.0a	2
Mudara	2010	131.5	12	1	2	91	160.5	2
	2011	133.5	11.8	1	2	95	165.0	2
	2012	129.0	11.4	1	2	98	150.0	2
	2013	120.8	10.4	1	2	96	144.5	2
	average	128.7a	11.4a	1	2	95a	155.0d	2
Belošljiva	2010	111.2	8.6	2	1	75	201.0	1
	2011	110.2	8.2	2	1	78	199.2	1
	2012	107.8	7.8	2	1	68	195.2	1
	2013	103.6	7.4	2	1	59	194.6	1
	average	108.2b	8.0bc	2	1	70c	197.5a	1
Crnošljiva	2010	114.8	9.4	4	1	65	195.4	1
	2011	110.4	9.4	4	1	82	195.2	1
	2012	104.5	9.0	4	1	78	198.4	1
	2013	109.1	9.0	4	1	83	185.0	1
	average	109.7b	9.2b	4	1	77bc	193.5a	1

Šara	2010	126.4	11.5	1	1	80	153.0	2
	2011	121.0	10.5	1	1	72	155.5	2
	2012	120.5	10.2	1	1	74	145.0	2
	2013	118.5	9.8	1	1	74	132.5	2
	average	121.6a	10.5a	1	1	75c	146.5d	2
Metlaš	2010	89.5	8.5	3	1	84	162.0	1
	2011	86.0	7.5	3	1	60	153.5	1
	2012	87.5	7.7	3	1	62	154.5	1
	2013	85.0	7.5	3	1	54	146.0	1
	average	87.0c	7.8c	3	1	65d	154.0d	1
Crvena durgulja	2010	136.8	12.8	1	1	97	208.5	1
	2011	132.5	12.4	1	1	98	206.5	1
	2012	128.5	11.7	1	1	99	202.8	1
	2013	129.8	11.1	1	1	98	202.2	1
	average	131.9a	12.0a	1	1	98a	205.0a	1
Plavski piskavac	2010	54.8	6.5	3	1	38	142.3	1
	2011	54.1	6.5	3	1	48	138.0	1
	2012	52.0	5.9	3	1	42	125.4	1
	2013	52.7	5.1	3	1	52	117.5	1
	average	53.4d	6c	3	1	45f	130.8e	1
Turgulja	2010	93.2	9.0	3	1	79	163.2	1
	2011	92.5	8.5	3	1	76	158.6	1
	2012	89.5	7.8	3	1	74	153.0	1
	2013	90.4	6.7	3	1	79	147.6	1
	average	91.4bc	8.0bc	3	1	77bc	155.6d	1
Obični Piskavac	2010	118.5	10.8	2	1	88	203.2	1
	2011	114.5	10.0	2	1	82	199.5	1
	2012	111.5	9.0	2	1	84	195.0	1
	2013	109.1	8.2	2	1	86	191.1	1
	average	113.4a	9.5b	2	1	85b	197.2a	1
Komperuša	2010	125.5	11.5	2	1	78	195.5	1
	2011	122.0	11.0	2	1	85	190.0	1
	2012	117.5	10.6	2	1	91	175.5	1
	2013	113.0	10.1	2	1	98	159.0	1
	average	119.5a	10.8a	2	1	88ab	180.0b	1
Mudovalj	2010	109.5	9.2	2	2	80	202.5	2
	2011	109.0	9.2	2	2	76	201.5	2
	2012	111.0	9.5	2	2	72	209.0	2
	2013	104.9	8.5	2	2	72	187.0	2
	average	108.6b	9.1b	2	2	75c	200.0a	2
Dronga	2010	124.1	11.0	2	2	78	204.2	2
	2011	118.5	10.6	2	2	87	194.3	2
	2012	116.5	9.5	2	2	92	192.0	2
	2013	116.9	8.9	2	2	95	191.5	2
	average	119.0a	10.0ab	2	2	88ab	195.5a	2
Dupljanka	2010	127.5	11.5	2	1	89	199.5	1
	2011	125.0	11.0	2	1	86	198.0	1
	2012	125.5	11.0	2	1	82	198.2	1
	2013	124.8	10.5	2	1	87	197.4	1
	average	125.7a	11.0a	2	1	86b	198.2a	1

Jesenka	2010	104.2	9.4	2	1	73	177.2	1
	2011	102.3	9.0	2	1	77	174.0	1
	2012	100.5	8.8	2	1	70	170.2	1
	2013	100.2	8.0	2	1	72	172.6	1
	average	101.8ab	8.8b	2	1	73c	173.5c	1
Trnovača	2010	57.8	6.8	3	1	42	132.8	1
	2011	57.2	6.8	3	1	45	132.0	1
	2012	56.8	6.2	3	1	49	131.5	1
	2013	57.8	6.2	3	1	56	132.5	1
	average	57.4d	6.5c	3	1	48e	132.2e	1
LSD0.05		13.1	1.25			0.41	14.02	
LSD0.01		17.4	1.66			0.47	17.94	

Similar data for Serbian autochthonous plum cultivars in terms of pomological, physical and sensorial characteristics were reported by Paunović *et al.*, 1985; Paunović, 1988; Petrović *et al.*, 2002; and Milošević and Milošević, 2012, and data on local cultivars grown in the former Yugoslavia were given by Jovančević, 1977; Jarebica and Muratović, 1977; Usenik *et al.*, 2007; Jelačić *et al.*, 2008. Stone weight ranged from 0.16 ± 0.003 g ('Trnovača') to 2.20 ± 0.711 g ('Crvena durgulja'), which was in similar with the results obtained by Paunović *et al.*, 1985; Paunović, 1988; Paunović and Paunović, 1994; Mratinić, 2000; and Milošević and Milošević, 2012. Those obtained values, particularly those for fruit weight and fruit size, were lower than the ones reported for standard commercial cultivars, both foreign and domestic ones.

The fact that substantial climate- and soil-dependent variations could occur in the above traits should be taken into account. Importantly, some cultivars are found to be promising in terms of fruit traits. Almost all the fruits can be processed, particularly into plum brandy, or used fresh ('Crvena ranka'). More importantly, the autochthonous (primitive, local) cultivars or accessions observed in this study can be used as an outstanding genetic basis and source of germplasm in plum breeding aimed at developing new cultivars and rootstocks (Đurić *et al.*, 1998; Esmenjaud and Direlewanger, 2007).

The results of this research show that the plant height, stem diameter, branching and uniformity of one-year old seedlings of autochthonous plum cultivars are genetic characteristics of autochthonous plum cultivars, from which rapid growth and uniformity of scions depend (tab. 3). The plant height of one-year old seedlings of researched autochthonous cultivars of plum was from 53.4 cm (cv. 'Plavski piskavac'), to 131.9 cm (cv. 'Crvena durgulja'). The stem diameter of researched one-year old seedlings of autochthonous cultivars of plum was from 6mm (cv. 'Plavski piskavac'), to 12 mm (cv. 'Crvena durgulja'). The most significant nursery characteristics which must be estimated in selection of autochthonous plum cultivar are ability to propagate, growth-rate, uniformity and compatibility (Vachun, 1995). In most of autochthonous cultivars of plum height and stem diameter at the height of 10 cm above the ground were sufficient for successful grafting in August (tab. 3). The bud take data of researched one-year

old seedlings of autochthonous cultivars of plum was from 48 % ('Trnovača') to 98% ('Crvena durgulja'). The cultivars 'Crvena durgulja' and 'Mudara', whose but take data was 98% and 95%, were also very interesting from the aspect of economic production of one-year old seedlings autochthonous plum cultivars.

Most of the germplasm resources have never been subjected to proper germplasm conservation research work. Many local types of genetic value have already disappeared or will be lost in the next few years without any possibility of recovery. Fortunately genetic resources in sparsely populated and less developed areas of Serbia and Montenegro have been less eroded. The main objective of this work was selection of old autochthonous cultivars with better bio-agronomic characteristics such as uniformity of growth, high productivity, reduction of vigour and adaptation to the pedology-climatic environment.

However, since the results obtained in this study are only preliminary, reliable estimation will be possible only through a multi-disciplinary approach to examining selected cultivars grown in a collection orchard as well as through further findings to be attained under field and laboratory conditions over the next five to ten years.

CONCLUSIONS

The onset of flowering was recorded in the last five days of March and in the first twelve days of April. The earliest onset of flowering was observed in cv. 'Trnovača' (26.03) derived from *P. insititia* L., and the latest in cv. 'Dupljanka' (12.04) derived from *P. domestica* L. Among the twenty cultivars examined, eight (40%) started to flower at the end of March, and twelve (60%) during the middle of the first twelve-day period of April. The full flowering stage lasted from 30 March ('Trnovača') to 18th April ('Dupljanka'), and the end of flowering from 7th April ('Trnovača') to 24th April ('Dupljanka'). Flowering lasted 9 dazs for cultivars 'Turgulja', 'Plavski piskavac', 'Grkaja', 'Kapavac and Komperuša') to 14 days ('Crvena durgulja', 'Mednica', 'Petrovača', 'Belošljiva' and 'Šara').

The harvest period was longer than the flowering period, as it lasted from 13th July ('Petrovača') to 18th September ('Trnovača').

The fruit weight ranged from 6.65 ± 0.235 g ('Plavski piskavac') to 53.88 ± 0.654 g ('Crvena durgulja'). The most dominant fruit shape was rounded - in twelve cultivars, followed by ovate - in four cultivars, elliptical - in 3 cultivars and oblong - in one cultivar ('Grkaja').

Ground color in most of the cultivars was light green (10) and light yellow (6), being yellow in cv. 'Trnovača', cv. 'Dupljanka' and cv. 'Grkaja'. Skin color ranged from white yellow (1), red (1), violet (1), blue (1), dark blue (1) to dark violet (2), black (2), mahagoni (4) to red violet (7 cultivars). Flesh color was yellow green in most cultivars (12) and light yellow only in cv. 'Plavski piskavac' and amber only in cv. 'Dupljanka'.

As for flesh firmness, it was medium in 15 cultivars, firm in tree and soft in two cultivars.

Stone weight ranged from 0.16 ± 0.003 g ('Trnovača') to 2.20 ± 0.711 g ('Crvena durgulja').

All the fruits could be processed, and cv. 'Crvena ranka' could be used fresh. The autochthonous plum cultivars or accessions observed in this study could serve as an outstanding genetic basis and a source of germplasm for plum breeding aimed at developing new cultivars and as cultivars for organic plum orchards.

The results of this research show that the plant height, stem diameter, branching and uniformity of one-year old seedlings are genetic characteristics of autochthonous plum cultivars, from which rapid growth and uniformity of scions depend. From the aspect of production of one-year old seedlings and evaluation of scions, the most interesting autochthonous plum cultivars are 'Mednica' and 'Mudara'.

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ANALYSIS OF THE ENERGY POTENTIAL OF AGRICULTURAL BIOMASS RESIDUES IN MONTENEGRO

SUMMARY

This paper gave an overview of the energy potential of organic remains from agricultural primary production and waste from livestock production in Montenegro. It has been estimated that approximately 9490 tons of dry matter of agricultural plant production residue is available. Intensive livestock farming results in production of 107.675 tonnes of waste.

The estimation of technical energy potential of crop residues amounts about 142.283 GJ (39,56 GWh). The technical potential calculated on the basis of the availability and total animal fund results in total of 298.552 GJ (82,93 GWh) of energy potential. Technical energy potential of agricultural waste is about 440.835 GJ (122,49 GWh) per year. This potential is significantly lower than the theoretical potential.

Keywords: Biomass, technical energy potential, agricultural residues, Montenegro

INTRODUCTION

Agricultural land covers the 230.321,2 ha which means 16,7% of the total land area in Montenegro. The major part of the agricultural land (216.583,4 ha) is characterized as permanent meadows and pastures (grassland), while the area dedicated to the cultivation of various crops (Utilised arable land) was 6.898,4 ha or just 0,5% of the total land in Montenegro in 2014. Montenegrin agriculture is characterized by traditional production, extensive methods of production and fragmented holdings with average size of 5 hectares. Total number of households is about 48.000.

The estimation of the quantities of agricultural residues available for energy production depend on the degree of availability which is different for each crop, varies from year to year and depends on many other factors such as:

- the harvesting method;
- the moisture content;
- the demand of agricultural residues for non-energy purposes (cereal straw, for example, is used for animal feeding, animal bedding, etc.);
- the need for some residues to remain on the soil to maintain the level of nutrients;

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

The main crops producing considerable quantities of field crop residues in Montenegro are potato, maize, wheat, rye and barley. The most important crops on arable land in Montenegro are potato with production of 24.313,1 t in 2014.

The main arboriculture residue resources in Montenegro are vineyards, olives, apples, plums, pears and citrus fruits pruning. Vineyards pruning are the most significant source of biomass to the total crop residues potential. Furthermore, citrus fruits, apples, plums, olives and pears contribute also significantly to the arboriculture residues potential.

The exploitation of livestock residues for energy production through anaerobic digestion process would be feasible only in cases of medium-large scale livestock units. Residues from cattle breeding contribute the highest share to the total potential.

Due its specific natural conditions Montenegro has small areas of arable land that are underutilized, which could be used for energy crops.

There are multiple options for biomass utilization for energy purposes of which various forms of burning prevail. Biogas production by way of anaerobic fermentation is the dominant form of use of livestock residue. From the remaining methods, methyl ester production from bio-oils and alcohol production are the most frequent.

The use of biomass for bioenergy creates new business opportunities in agriculture sector. Bioenergy production can significantly contribute to the development of rural areas and encourage creating new supply chains for biomass feedstock. The creation of new non-food markets for biomass could provide alternative income sources for farmers (EC 2012)

Agricultural residues, may act as important source of renewable energy. Studies assessing the availability of this resource offer little insight on the drivers and constraints of the available potential as well as the associated costs and how these may vary across scenarios (Diaoglou *et al* 2016).

Bilandzija *et al.* (2016) reported on how much biomass could be obtained by pruning of plantations of respective fruit cultures in Croatia. The energy potential of the pruned fruit biomass in Croatia is calculated at 4.21 PJ (1170GWh).

Potential of residual biomass as source of energy in the Czech Republic is about 121PJ/year which equals to 6.8% of primary energy sources used in 2012 (Vavrova *et al.* 2014). In Macedonia (Kanevce *et al.* 2016) the biomass accounted for 6.6% of the primary energy consumption in 2012, and 10.4% in the final energy consumption. The biomass residues potential were calculated on similar way in Albania (Karaj *et al.* 2009) Turkey (Elicin *et al.* 2014), Italy (Chinnici *et al.* 2015) etc. and shows importance of studies for assessment of biomass potential and availability.

MATERIAL AND METHODS

Potential is calculated per each source type. Livestock energy potential (L_{ep}) is calculated based on formula:

$$L_{ep} = N \cdot F \cdot Y \cdot H$$

Where:

N - number of animal species

F - manure generation factor for species [t/head/yr]

Y - coefficient of biogas yield [Nm^3/t manure]

H - heating value of biogas [GJ/Nm^3]

According to Bogunovic *et al.* (2009) and CRES (2010) the technical potential was regarded equal to 30% of the theoretical value.

Table 1. Parameters for energy production from livestock residues.

	waste per animal (t/yr)*	biogas yield (Nm^3/t of waste)	biogas LHV (GJ/Nm^3)
Cattle	1,62	245	0,0216
Pigs	0,30	430	0,0216
Poultry	0,021	450	0,0234

*Source: CRES (2010)

The annual energy for each crop cultivated in Montenegro 2014. (Monstat 2015), is calculated based on the following formula:

$$C = R \cdot P \cdot H$$

R - ratio of residue over main product [t/t]

P - annual production of product cultivated [t]

H- heating value of residue [GJ/t]

The availability factor for arable crop residues is 30% mainly due to technical difficulties in collecting. The availability factor for arboriculture residues is 90%. The factor for olives 50% was estimated based on our previous experience in Greece (Mardikis *et al.* 2004).

RESULTS AND DISCUSSION

Livestock

In rural areas of Montenegro agriculture is the most important sector, also livestock production is most important branch of agriculture.

Montenegro has specific natural conditions with large areas of natural meadows and pastures (210,618.0 ha in 2014), which are suitable especially for extensive livestock production. Livestock sector in Montenegro is composed of small-scale farms in general. More than 58% of the breeding animals are in small farms with 1-3 cows / heifers (MPRR, 2012).

Animal residues are form of biomass that is being well known for energy generation. For the production of biogas very suitable raw material is manure of domestic animals, especially cattle, pigs and poultry. The amount of animal waste residues depends on type and size of animals, number of animal population, domestic animals or confined feedlots and population animal density

for one location (Karaj et al, 2009). In Montenegro is a problem in collection and storage of manure. In order to generate sufficient quantities of manure for potential biogas production, it is necessary to implement adequate system of manure collecting from the facilities. Energy can be derived from livestock residues as long as they are collected in lagoons or large tanks and can be considered feasible only in install livestock systems. Long grazing periods makes it impossible to collect the animal waste, which excluding sheep and goats from such practices during grazing period.

Official agricultural policy in Montenegro is oriented in maximizing the share of medium and big farms as well as increasing the number of animals per household in next period (MPRR, 2012). This will lead to the actualization of the problem of disposal of liquid manure. One of the solutions for the use of this residue will be certainly to use it as energy source.

According to official statistics (MONSTAT, 2015) in Montenegro were 93.550 heads cattle, 22.053 pigs, and 595.675 chickens in 2014. During recent years, positive changes have been recorded in the consolidation of holdings and in an increase in the number of heads. In comparison with last few year (2012, 2013), in 2014 was observed increase number of cattle and pig. Because of that was increase the amount of produced manure and in 2014 it was 107.675t in total.

Table 2. Livestock residues theoretical potential in Montenegro (2014)

	Cattle	Pigs	Chicken	Total
Number of animals*	93.550	22.053	595.675	711.278
Residues (t) (dry)	151.551	6.615	12.509	107.675
Biogas potential (million Nm ³)	37,12	2,84	5,62	45,58
Theoretical Potential (GJ)	802.007	61.448	131.721	995.176
Technically available				30%
Technical Potential (GJ)				298.552

*Source: Monstat 2015.

Presented data in table 2 shows that on the basis residues produced on average per animal and the biogas yield per ton of produced residues, the amount of biogas that could be theoretically produced amounts to 45,58 million Nm³, which is equivalent to 995.176 GJ or 276,437 GWh.

Because of the low technical availability technical energy potential of the livestock residues in Montenegro is just 298.552 GJ (82,931 GWh).

Plant production

Energy potential of field crop residues (32753 GJ) are significantly lower in relation to the production of arboricultural and amounts to only 23% of the total energy potential of 142283 GJ – 39,56 GWh (Table 3.).

Table 3. Agricultural plant production structure in Montenegro and residue quantity in 2014.

Crop	Production	Residues	Degree of Availability	Residues available for energy exploitation		
				Quantity (t)	Energy potential GJ	Energy potential GWh
Total	36621	15767		9490	142283	39,56
Fields	7138	7293		2188	32753	9,11
Wheat	2159	2159	30	648	9331	2,60
Maize	3305	3636	30	1091	16911	4,70
Barley	1147	918	30	275	3988	1,11
Ray	527	580	30	174	2523	0,70
Arboricultural	29483	8474		7302	109530	30,45
Plums	5743	1436	90	1292	19380	5,40
Apple	4900	1715	90	1544	23160	6,43
Pears	915	229	90	206	3090	0,86
Olives	796	812	50	406	6090	1,70
Vineyard	17129	4282	90	3854	57810	16,06

*Source: Monstat 2015.

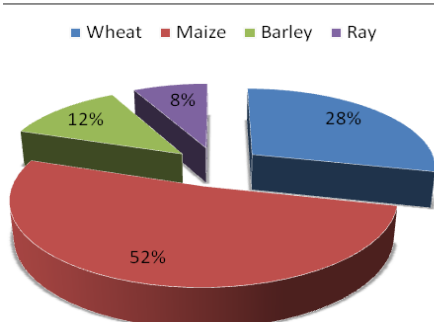


Fig.1. Structure of field crop production

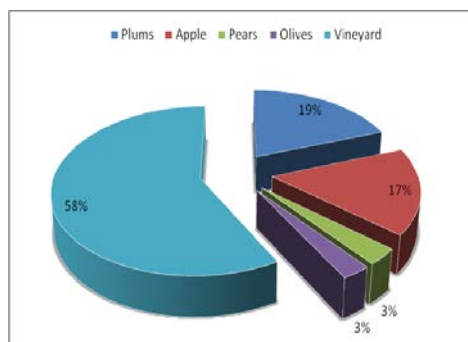


Fig.2. Structure of arboriculture production

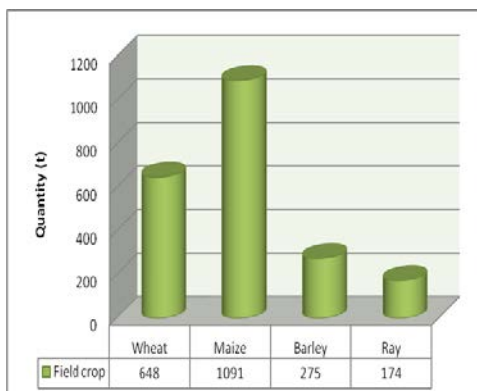


Fig.3. Field crop residue available for energy purposes (in tones)

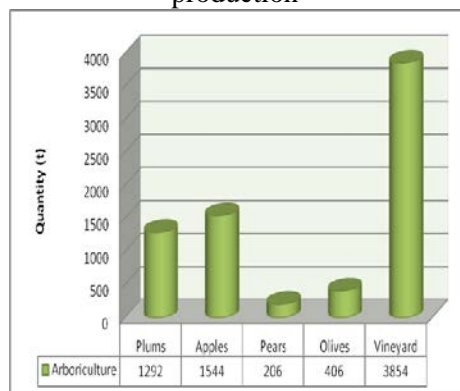


Fig.4. Arboriculture residue available for energy purposes (in tones)

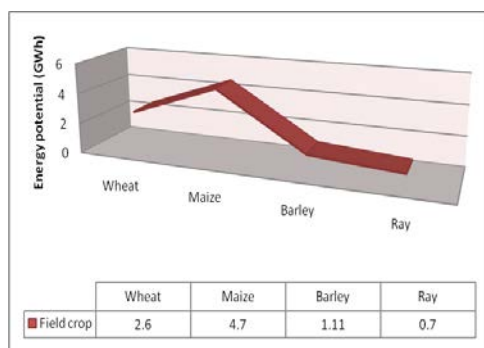


Fig.5. Field crop residue energy potential (GWh)

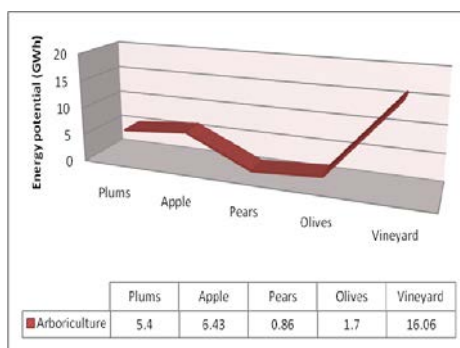


Fig.6. Arboriculture residue energy potential (GWh)

Pruning the orchards are regular agricultural practices. Thermal energy use of biomass residues as pruning is not enough apply in Montenegro because of outdated technology, extensive production and lack of organized collecting, preparing and using of plants remains.

The majority of fruit crops are grown on a small scale without the use of agro-techniques (plowing, fertilizing, cutting, protection from pests and frost, irrigation). In 2014 arboriculture residues amounted 29483 t with energy potential of 109530 GJ (30,45 GWh).

Presented amounts show that fruit growing sector is on very low level. For example in The Republic of Serbia has approximately 600,000 t wood biomass from fruit and grape plantations (Zivkovic et al. 2013).

Of the total arboricultural production, production of olives is 19%, and 58% of the vineyards, and they make one of the most important factors for obtaining biomass. Unfortunately, the use of olive residues is only 50%. The remains of the olives are very important, especially residues from olive oil, which amounts to 80%, because they contain vegetable water, pulp and stones from the olives, and the hard part has a high heating value.

Vineyards pruning are the most significant source of biomass contributing almost by half (40,6%) to the agricultural plant production residues potential.

The overall potential of timber biomass residue from vineyards and orchards is by research of prof. Glavonjic (2012) 2.482 tons per year. The overall potential of agricultural biomass residue is 8.154 tons per year. According The Action Plan for the wood biomass, the total area under vine in Montenegro in 2011 amounted to about 4,5 thousand ha with 18,44 million grapevines stems. Based on the known parameters of the amounts of biomass per hectare in the process of pruning vines, which can be put into operation production of fuel annually from the economic point (profitability) (Cotan and Cavalaglio 2008) in viticulture in Montenegro incurred 12.543 tons of wood waste whose energy value is 37.629 MWh.

Agricultural policy in Montenegro tries to revive fruit production by set of subsidies. Thus, last year is recorded 85ha of new fruit plantations. A noticeable

increase is also in the field of olive growing. In process of creating policy in the sector of plant production should bear in mind the limitations due to climate changes (Curovic and Spalevic, 2010)

However, despite progress in these segments of crop production, the greatest potential for the use of biomass as fuel is seen in viticulture. Montenegro is interesting for the cultivation of vineyards. It is expected increase of land surface under vines in the future. According to presented data from the sector of plant production only Viticulture has the potential of the commercial exploitation of biomass residues for energy purposes. Production of briquettes made of biomass residue from vineyards could be realistic option.

CONCLUSIONS

The theoretical energy content of biomass of Agricultural residues in Montenegro is 440.835 GJ (122,49 GWh) per year.

Currently there is no usage of agricultural land for energy crops or organized usage of agricultural residues for energy production in Montenegro. It is realistic to expect the first examples of the use of residues in the coming period, especially in the sectors of livestock and viticulture. This paper shows that there is potential for utilization of biomass residue as an energy source in these sectors.

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DO RAILWAYS CONTRIBUTE TO PLANT INVASION IN TURKEY?

SUMMARY

Intensive trade, transport and travel activities across different geographic regions are prime source of introduction and spread of alien plant species. The role of roadsides in the long distance transport and spread of alien plants has been well explored whereas, railways are less considered in this context. Therefore, current survey was aimed to determine the role of railways as dispersal corridors for alien plants and weeds along the railways connecting Black Sea and Inner Anatolia regions of Turkey. Surveys were accomplished by randomly stopping at every 8-10 km on the railway tracks. Fifty-eight different plant species belonging to 22 plant families were identified during the study. Majority of the identified plant species (68.9%) were native to Turkey whereas, notable numbers of alien plants which have been established in the country (17.2%) were also recorded during the survey. Moreover, 62% of the identified native plants have been regarded as invasive in number of European countries. Weed Science Society of America have listed 72.4% of the identified plants as weeds and 79.3% are accepted as alien/invasive in different European countries. Maximum number of weeds belonged to Asteraceae family among the 22 identified families. Current surveys present the first report on the presence of common ragweed along railways in Turkey. The results indicate that railways are extensively serving as corridors for introduction and dispersal of weedy and alien plants across the regions in the country. It is therefore recommended that rapid surveys should be urgently conducted along the railways network of the country to record the presence of invasive alien plants.

Keywords: Railways, Dispersal corridors, Alien plants, Survey, Turkey

INTRODUCTION

Weeds and invasive alien plants are one of the chief concerns in natural and agricultural ecosystems due to their undue negative impacts on ecosystem productivity and native biodiversity (Zimdahl, 2007; Ozaslan et al., 2016). Introduction of exotic plants to new regions, their subsequent spread and invasion is the second major threat to native biodiversity whereas, habitat fragmentation and disturbance to the established ecosystems stimulate these processes (Sharma and Raghubanshi, 2010; Kumar and Mathur, 2014). Biotic communities on regional and global scales are awfully affected by habitat fragmentation (Debinski and Holt, 2000; Fahrig, 2003). Habitat fragmentation produces small

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patches which isolate the habitat fragments which act as selective filters for the movement of species across these fragments (Wilcove *et al.*, 1986; Lawton, 1999). Several studies concluded extensive development of infrastructure, especially transport networks as major contributors to plant invasion (Wilson *et al.*, 2009). Highways and railways connect different regions resulting in disturbance of native habitats which accelerate habitat fragmentation (von der Lippe and Kowarik, 2008). Habitat degradation due to highways and railways negatively affect the integrity and survival of native communities (Hansen and Clevenger, 2005; Way and Eatough, 2006; Bangert and Huntly, 2010).

Disturbance to the ecosystems promote introduction and spread of alien plants as, invaders thrive best in disturbed habitats due to their opportunistic nature (Hansen and Clevenger, 2005). Several accidental introductions of alien plants in areas of dense transport system have been well acknowledged (Kowarik and von der Lippe, 2007; von der Lippe and Kowarik, 2008). Similarly, role of vehicles in the transfer of propagates (seeds etc.) of invasive plants have also been cited in some studies (von der Lippe and Kowarik, 2008). Besides, a recent study indicated that railways can better act as corridors for introduction of alien species than habitat disturbance and fragmentation (Bangert and Huntly, 2010). Railways can benefit the invasive plants in dual ways; either by introduction of propagates, or serving as ideal habitats for invasion.

Increased travel is an important source of introduction of exotic plant species of distant exotic origins (Korres *et al.*, 2015a, b). Turkey shares border with many countries in two continents, and have recently increased trade and travel activities with several neighboring countries. Therefore, transports by roads, railways and aerial roots in the country have been increased many folds than past. Black Sea is the most infested region of the country with exotic plants where a rapid survey study identified the prevalence of 81 alien species (Brundu *et al.*, 2011). Black Sea highways are suspected as the source of introduction of many alien plants in the country as, it is a part of route which connects Turkey with Europe through Georgia (Onen, 2015). Large number of alien species observed in the region has also been discussed in the “Invasive Plants Catalogue of Turkey” (Onen, 2015). Although, several surveys have been accomplished in the country to identify the weedy and invasive plants prevailing in different ecosystems (Çaldıran *et al.*, 2015; Önen *et al.*, 2015a, b; Özaslan *et al.*, 2015a, b; Ozaslan *et al.*, 2016), railways have completely been ignored in this regard. Weed surveys are of greater importance in devising management practices for weedy and invasive plants prevailing in a particular region. Moreover, survey studies also help to identify the native and exotic weed flora present at regional and landscape scales (Korres *et al.*, 2015a, b). Therefore, the current survey study was planned to identify the role of railways in the dispersal of weeds with special focus on alien plants along the railway tracks connecting Black Sea and inner Anatolia regions of the country. The results will identify the weed species distributed along the railways and also will be helpful in devising management strategies against weeds as well as invasive plants.

MATERIAL AND METHODS

The surveys were conducted along the railway tracks between Sivas and Samsun railway stations during September 2013. A total of 38 localities were randomly surveyed by stopping at every 8-10 km. The surveyed area is represented in Figure 1.

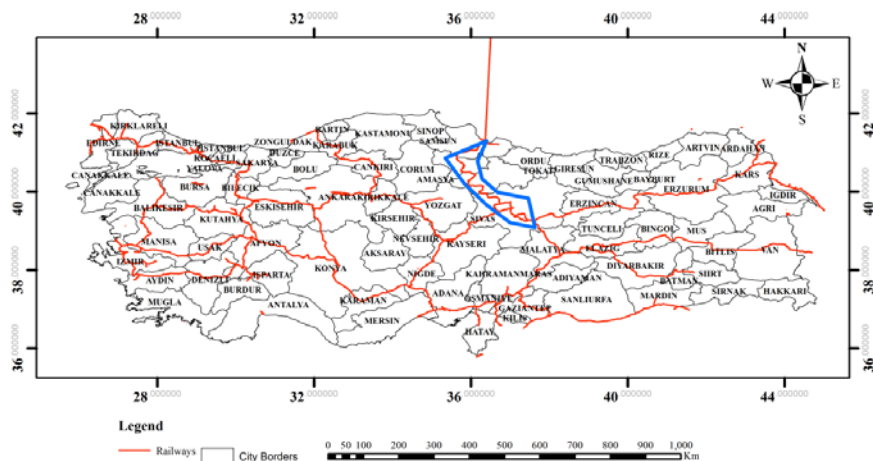


Figure 1. The map of surveyed site

Red lines (Fig. 1.) represent the railways network in the country whereas the blue enveloped area represent the surveyed railwas

An area of 100×100 m area was visually assessed to identify plant species. 1×1 m² quadrates were placed at 5-8 different points, and numbers of plants in the each quadrate were counted at each surveyed location. Herbariums of the plants were made and preserved in herbarium records of the Department of Plant Protection, Dicle University Diyarbakir, Turkey. Plant identification was accomplished following Flora of Turkey (Davis, 1965-1989). The frequency of occurrence, general coverage, special coverage, general density and special density of the plants were individually calculated by following Uygur (1991) and Odum (1971). The equations for calculating these parameters are given as under;

$$\text{Frequency \%} = \frac{\text{Number of surveyed points where a species occurred}}{\text{Total number of surveyed points}} \times 100$$

$$\text{General coverage \%} = \frac{\text{Coverage area of a weed species in surveyed points}}{\text{Total number of surveyed points}}$$

$$\text{Special coverage \%} = \frac{\text{Coverage area of a weed species where it occurred}}{\text{Total number of surveyed points}}$$

$$\text{General density (plants/m}^2\text{)} = \frac{\text{Number of weed species in m}^2\text{}}{\text{Total number of surveyed points}}$$

$$\text{Special density (plants/m}^2\text{)} = \frac{\text{Number of weed species in m}^2\text{ at occurrence points}}{\text{Total number of surveyed points}}$$

Life cycle of the plant, nativity and invasion status in the country as well as in other parts of Europe were searched on different plant databases. Weed Science Society of America (WSSA, 2016), European and Mediterranean Plant Protection Organization (EPPO, 2016), Delivering Alien Invasive Species Inventories in Europe (DAISIE, 2016) and Turkish Plant Data Service (TUBIVES, 2016) were the databases searched during the study. Life forms of the plants were also searched on the Turkish Plant Data Service (TUBIVES, 2016) and Flora of Turkey (Davis, 1965-1989). Plants were separated into different categories such as natives, aliens, alien and established, natives and invasive elsewhere and not identified.

RESULTS

Fifty-eight different plant species belonging to 22 plant families were identified during the study. Samsun-Tokat railway track hosted higher number of plant species (65.5% of the total observed weed species) compared with Tokat-Sivas track (Figure 2). Among different weedy and invasive plants identified during the survey, 17 species (29.3%) were commonly observed on both railway tracks (Figure 2).

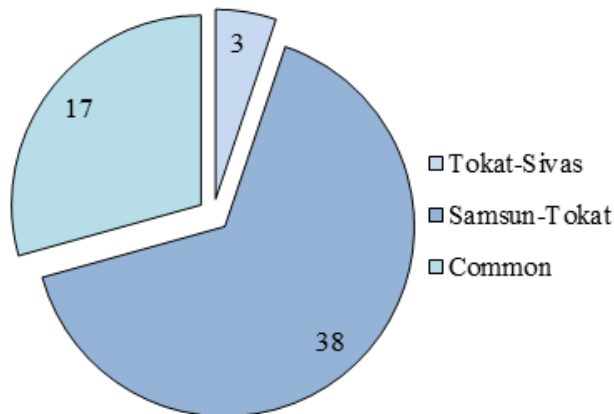


Figure 2. Distribution of different weedy and alien plant species observed during survey on surveyed railway tracks

Highest number of plants observed during the survey belonged to the Asteraceae family (15 plants), followed by Poaceae (10 species) and, Rosaceae and Boraginaceae (3 weed species in each family). Majority of the plant families (13) hosted only one plant (Figure 3; Table 1).

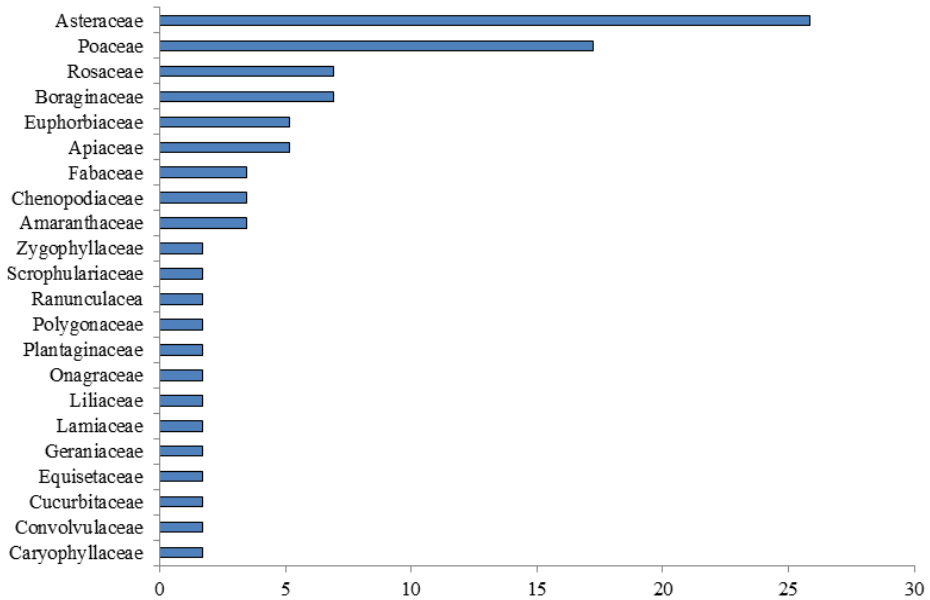


Figure 3. Distribution (%) of the identified plant species in different plant families

Equal number of plants were either annual or perennial (26 plants in each category) while, 6 plants had biennial life cycle (Figure 4; Table 1).

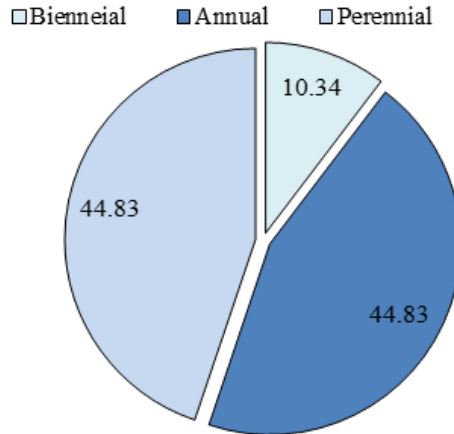


Figure 4. Percentage of the plants recorded during the survey according to their life cycle

Alien species had the minimum contribution to the identified flora during the survey while, the native species were highest in number (Figure 5).

Table 1. List of the plant species observed during surveys with their nativity status, origin, life form, general coverage, specific coverage, general density and specific densities

Weed Species	Family	*Status	EPPO	WSSA	DAISIE	Life Form	Origin	Location ***	GC (%)	SC (%)	GD plants m ⁻²	SD plantsm	FO (%)
Native													
<i>Anchusa azurea</i> P. Mill.	Boraginaceae	N	-	+	+	P	Central & Southern Europe, Mediterranean	1, 2	0.13	0.42	0.16	0.50	31.58
<i>Antirrhinum tinctoria</i> L.	Asteraceae	N	-	+	+	P	Europe & Mediterranean	2	0.18	3.50	0.08	1.50	5.26
<i>Artemisia vulgaris</i> L.	Asteraceae	N	-	+	+	P	Europe & Asia	1, 2	0.47	0.95	0.42	0.84	50.00
<i>Avena sterilis</i> L.	Poaceae	N	-	+	+	A	Mediterranean	2	0.21	2.67	0.32	4.00	7.89
<i>Bromus sterilis</i> L.	Poaceae	N	-	+	+	A	Europe	2	0.05	2.00	0.05	2.00	2.63
<i>Carduus pycnocephalus</i> L.	Asteraceae	N	-	+	+	A	Mediterranean	1, 2	0.34	0.81	0.39	0.94	42.11
<i>Chenopodium album</i> L.	Chenopodiaceae	N	-	+	+	A	Asia, Europe & North America	1, 2	0.16	0.55	0.13	0.45	28.95
<i>Chrozophora tinctoria</i> (L.) Rafin.	Euphorbiaceae	N	-	-	+	A	Mediterranean	2	0.21	0.44	0.24	0.50	47.37
<i>Cirsium arvense</i> (L.) Scop.	Asteraceae	N	-	+	+	P	Europe	1, 2	0.18	0.41	0.11	0.24	44.74
<i>Convolvulus arvensis</i> L.	Convolvulaceae	N	-	+	+	P	Europe & Asia	2	0.32	0.80	0.24	0.60	39.47
<i>Cynodon dactylon</i> (L.) Pers.	Poaceae	N	-	+	+	P	Middle East	1, 2	0.61	0.88	0.79	1.15	68.42
<i>Daucus carota</i> L.	Apiaceae	N	-	+	+	B	East & Central Asia	2	0.11	4.00	0.11	4.00	2.63
<i>Digitaria sanguinalis</i> (L.) Scop.	Poaceae	N	-	+	+	A	Europe	2	0.05	0.50	0.03	0.25	10.53
<i>Dipsacus laciniatus</i> L.	Asteraceae	N	-	+	+	B	Europe & Asia	1, 2	0.16	0.30	0.18	0.35	52.63
<i>Echallium elaterrum</i> A. Rich.	Cucurbitaceae	N	-	-	+	P	Mediterranean	2	0.11	0.44	0.08	0.33	23.68
<i>Echium italicum</i> L.	Boraginaceae	N	-	-	+	B	Italy & Mediterranean	1, 2	0.21	0.36	0.24	0.41	57.89
<i>Epilobium parviflorum</i> (Schreb.)	Onagraceae	N	-	+	+	P	Europe, North Africa, West Asia & India	2	0.05	0.29	0.05	0.29	18.42
<i>Eryngium campastre</i> L.	Apiaceae	N	-	+	+	P	Europe	1, 2	0.11	0.27	0.08	0.20	39.47
<i>Geranium dissectum</i> L.	Geraniaceae	N	-	+	+	A	Europe	2	0.11	0.24	0.08	0.18	44.74
<i>Heliotropium europaeum</i> L.	Boraginaceae	N	-	+	+	A	Europe, Asia & North Africa	2	0.13	0.45	0.11	0.36	28.95
<i>Kochia scoparia</i> (L.) Schrad.	Chenopodiaceae	N	-	+	+	A	Eurasia	1	0.18	0.78	0.08	0.33	23.68
<i>Lactuca serriola</i> L.	Asteraceae	N	-	+	+	B	Europe & Asia	1, 2	0.24	0.50	0.16	0.33	47.37
<i>Lolium perenne</i> L.	Poaceae	N	-	+	+	P	Europe	2	0.13	1.67	0.05	0.67	7.89
<i>Melilotus alba</i> Medik.	Fabaceae	N	-	+	+	A	Central Europe & North-West Asia	2	0.08	3.00	0.05	2.00	2.63
<i>Mercurialis annua</i> L.	Euphorbiaceae	N	-	+	+	A	Europe, Middle East & North Africa	2	0.18	0.58	0.11	0.33	31.58
<i>Onosma</i> sp.	Boraginaceae	N	-	-	-	P	Asia & Europe	2	0.05	0.33	0.05	0.33	15.79
<i>Pastinaca glandulosa</i> Boiss. & Hausskn.	Apiaceae	N	-	-	-	P	Mediterranean	1	0.45	1.42	0.34	1.08	31.58
<i>Phragmites australis</i> (Cav.) Trin. ex Steud.	Poaceae	N	-	+	+	P	Europe	2	0.55	0.91	0.71	1.17	60.53
<i>Plantago major</i> L.	Plantaginaceae	N	-	+	+	P	Asia & Europe	2	0.08	0.33	0.05	0.22	23.68

<i>Rosa canina</i> L.	Rosaceae	N	-	+	+	P	South Europe, North Africa & West Asia	2	0.37	0.64	0.42	0.73	57.89
<i>Rumex crispus</i> L.	Polygonaceae	N	-	+	+	P	Europe & Asia	1, 2	0.16	0.29	0.13	0.24	55.26
<i>Sambucus ebulus</i> L.	Rosaceae	N	-	+	+	P	Europe	2	0.39	0.63	0.29	0.46	63.16
<i>Sanguisorba minor</i> Scop.	Rosaceae	N	-	+	+	P	South Europe & West Asia	1, 2	0.11	0.24	0.13	0.29	44.74
<i>Setaria glauca</i> (L.) Beauv.	Poaceae	N	-	+	+	A	Europe	2	0.16	0.60	0.05	2.00	2.63
<i>Setaria viridis</i> (L.) Beauv.	Poaceae	N	-	+	+	A	Eurasia	2	0.18	0.54	0.16	0.46	34.21
<i>Silene</i> sp.	Caryophyllaceae	N	-	-	-	P	Eurasia	1	0.08	0.43	0.08	0.43	18.42
<i>Sonchus asper</i> (L.) Hill	Asteraceae	N	-	+	+	A	Europe, Asia & North Africa	2	0.05	0.40	0.05	0.40	13.16
<i>Tribulus terrestris</i> L.	Zygophyllaceae	N	-	+	+	A	Mediterranean	2	0.11	0.44	0.13	0.56	23.68
<i>Verbascum songaricum</i> Schrenk Ex Fisch. Et Mey.	Scrophulariaceae	N	-	-	-	B	Iran-Turan	1, 2	0.13	0.42	0.08	0.25	31.58
<i>Vicia sativa</i> L.	Fabaceae	N	-	+	+	A	Mediterranean	2	0.08	0.38	0.05	0.25	21.05
Total	-	-	-	-	-	-	-	-	7.66	40.1	7.05	31.6	-
Aliens Established													
<i>Amaranthus blitoides</i> S.Wats.	Amaranthaceae	A, E	-	+	+	A	North America	1, 2	0.39	0.79	0.42	0.84	50.00
<i>Amaranthus retroflexus</i> L.	Amaranthaceae	A, E	-	+	+	A	Tropical America	2	0.42	0.94	0.34	0.76	44.74
<i>Ambrosia artemisiifolia</i> L.	Asteraceae	A, E	+	+	+	A	North America	2	0.11	1.33	0.08	1.00	7.89
<i>Clematis vitalba</i> L.	Ranunculaceae	A, E	-	+	+	P	United Kingdom	2	0.95	1.71	0.58	1.05	55.26
<i>Coryza canadensis</i> (L.) Cronq.	Asteraceae	A, E	-	+	+	A	North America	1, 2	0.29	0.58	0.32	0.63	50.00
<i>Echinochloa crus-galli</i> (L.) Beauv.	Poaceae	A, E	-	+	+	A	Tropical Asia	2	0.18	0.64	0.13	0.45	28.95
<i>Paspalum distichum</i> L.	Poaceae	A, E	-	+	+	P	Africa & America	2	0.13	5.00	0.13	5.00	2.63
<i>Senecio verididis</i> Waldst. & Kit.	Asteraceae	A, E	-	+	+	A	Russia	2	0.21	0.67	0.16	0.50	31.58
<i>Xanthium spinosum</i> L.	Asteraceae	A, E	-	+	+	A	South America	2	0.11	0.57	0.05	0.29	18.42
<i>Xanthium strumarium</i> L.	Asteraceae	A, E	-	+	+	A	North America	2	0.34	0.68	0.16	0.32	50.00
Total	-	-	-	-	-	-	-	-	3.13	12.9	2.37	10.8	-
Aliens													
<i>Conyza albidia</i> Willd. ex Spreng.	Asteraceae	A	-	-	+	A	South America	2	0.11	4.00	0.05	2.00	2.63
<i>Rubus fruticosus</i> L.	Rosaceae	A	-	+	-	P	North Africa,	2	0.42	0.64	0.50	0.76	65.79
<i>Taraxacum scutigerinosum</i> G. Hagl.	Asteraceae	A	-	-	-	P	Europe	2	0.11	4.00	0.08	3.00	2.63
Total	-	-	-	-	-	-	-	-	0.6	8.6	0.6	5.7	-
Not Identified													
<i>Allium</i> sp.	Liliaceae	NI	-	-	-	P	NI	2	0.05	0.33	0.03	0.17	15.79
<i>Equisetum</i> sp.	Equisetaceae	NI	-	-	-	P	NI	1, 2	0.71	1.00	0.92	1.30	71.05
<i>Euphorbia</i> sp.	Euphorbiaceae	NI	-	-	-	A	NI	1, 2	0.29	0.61	0.11	0.22	47.37
<i>Menisperm</i> sp.	Lamiaceae	NI	-	-	-	P	NI	2	0.13	0.63	0.08	0.38	21.05
<i>Onopordium</i> sp.	Asteraceae	NI	-	-	-	B	NI	2	0.08	0.33	0.05	0.22	23.68
Total	-	-	-	-	-	-	-	-	1.2	2.9	1.1	2.2	-
General Total	-	-	-	-	-	-	-	-	12.7	64.6	11.2	50.5	-

* A, E = Alien plant established in the country. N = Native, NI = Not identified for nativity, ** A = Annual, B = Biennial, *** 1 = Tokat-Sivas railways, 2 = Samsun-Tokat railroad line, + = present, - = absent, GC = General Coverage, SC = Special Coverage, GD = General Density, SD = Special Density, FO = Frequency of occurrence

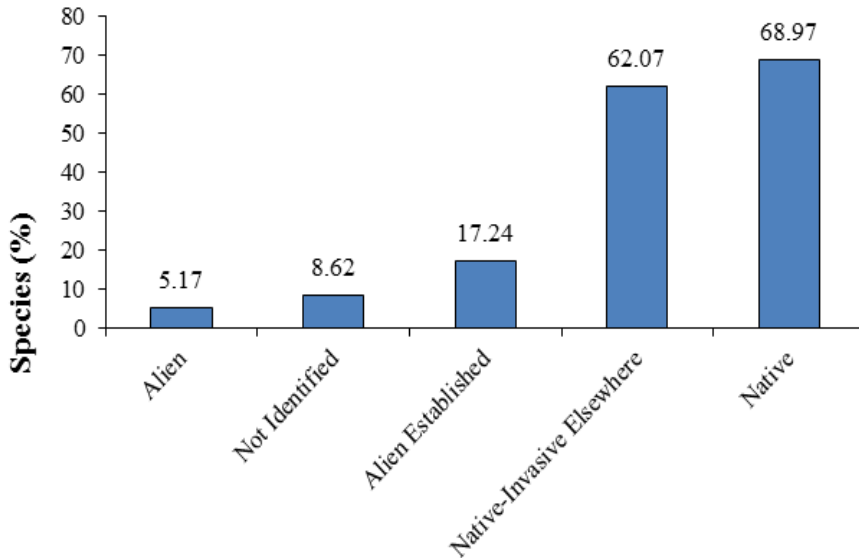


Figure 5. Different categories of weedy and invasive plant species in total floristic diversity of the surveyed region according to their origin and invasiveness

Railways contained considerable number (17.2%) of alien species established in the country. The growth stages of 8.6% species do not allowed exact identification; therefore, their nativity remained anonymous. The frequencies of occurrence of the recorded plant species are represented in Table 1. *Bromus sterilis* (2.6%) and *Cynodon dactylon* (68.4%) were the least and most widespread native plants respectively, during the survey. Similarly, *Paspalum distichum* (2.6%) was the least recorded alien plant which has been established in the country whereas, *Clematis vitalba* was observed in majority of the sampling sites. Moreover, *Ambrosia artemisiifolia* was also observed on the railways first time during this survey (Table 1).

The observed plants were also searched for their weedy and invasive status on different plant database. Only one species (*Ambrosia artemisiifolia*) was listed on the invasive plants lists of EPPO (Figure 5). Weed Science Society of America regards 72.4% of the identified plants as weeds. Similarly, 79.3% of the total plants are regarded as alien/invasive plants in different European countries (Figure 6).

Native plants had the highest general coverage, special coverage, general density and special density followed by alien-established, anonymous and alien plants during the survey (Table 1). Notable coverage areas and densities were recorded for alien-established plants providing sufficient evidence that railways are playing extensive role for the spread of alien plants in the country.

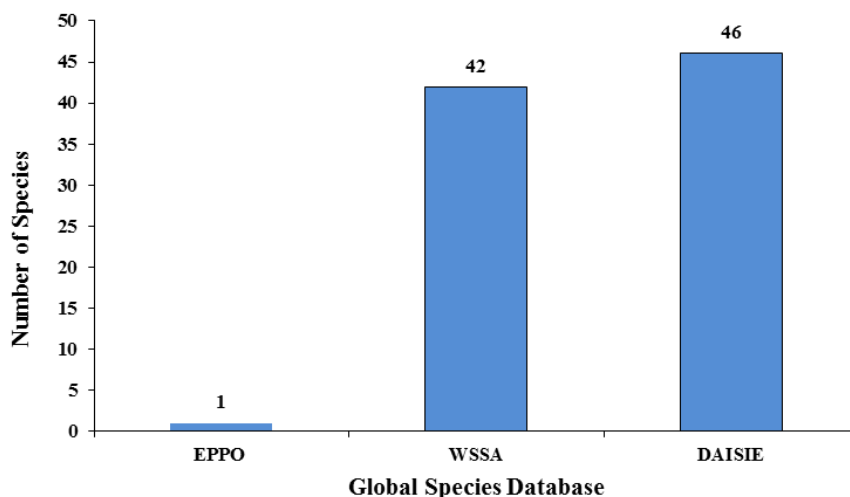


Figure 6. Classification of the identified plant species according to their global importance based on different monitoring and information database for plants

DISCUSSION

Weed surveys allow ecologists and land managers to identify the spatial patterns of plant distribution, help in determining factors affecting weed distribution and assist in devising management practices. Surveys for alien species, particularly of plants have attracted increased attention for inventorying the alien species at regional and spatial scales (Rankins et al., 2005). The role of roadsides in long distance dispersal of weed species has been largely explored (Korres et al., 2015a, b; Ozaslan et al., 2016). However, railways have been ignored for surveying the alien plants.

The results of current survey indicate that railways have notable contribution to plant invasion in Turkey (Table 1, Figure 5). Majority of the identified species were listed as invaders in European plant information databases. The results also specify that transport of the plants from one region to the other can create severe weed infestation problems as the identified species have enormous reproductive and adaptive potentials under different climatic and environment gradients. Therefore, regular inspection and monitoring of the railways throughout the country is immediately needed. Railway tracks surveyed during the current study connect the densely populated regions of the country. Black Sea region have been accepted as the extensively invaded region of the country and many survey studies report infestations of several alien plant species (Burundu et al., 2011; Önen et al., 2013, 2014, 2015a, b). Moreover, majority of the alien plant species observed in the region have been regarded as invasive in the country (Onen, 2015).

Weed species identified during current study have already been reported as widespread in agricultural and natural habitats in different parts of Turkey (Uluğ

et al., 1993; Tepe, 1997; Özer et al., 1999; Özaslan, 2011; Özaslan and Bükün, 2013; Onen et al., 2014, 2015a, b). Maximum frequency of occurrence of weed species was recorded in the Asteraceae family followed by Poaceae family (Figure 3). These families have the maximum contribution towards the weedy flora of the country (Davis, 1965-1989; Düzenli et al., 1993; Özer et al., 1999).

Plant distribution along the roadsides and railway tracks decrease the visibility and cause severe roadside accidents (Cederlund, 2006), fire problems, and infra structure losses (Antuniassi et al., 2004). Infestation along the railways is more worst as it cause traction problems (wheel slipping) and make maintenance of tracks challenging (Antuniassi et al., 2004). Moreover, weeds may obscure rails and switches, making inspections of the railway lines difficult. Keeping in view the adverse effects of weeds on railways, mowing and herbicides have extensively been used to manage the weedy species in the country. Development of weed management approaches requires sufficient weed distribution data at spatial scales (Korres et al., 2015a, b). The current survey also gives valuable information in this regard.

Common ragweed (*Ambrosia artmeisiifolia*)—one of the important allergenic plant— has also been identified first time on railways during the survey. The plant produces the most feared pollens for hay fever suffers (Ozaslan et al., 2016). Widespread presence and invasion of the plant have been recorded along the roadsides and in agricultural fields throughout the country (Onen et al., 2014; Ozaslan et al., 2016). The results indicate that railways can contribute towards the spread of common ragweed across Black Sea and Inner Anatolia regions. Railways have probably transported the seeds of alien species from one place to the other through extensive movement. Moreover, the disturbance caused by railway maintenance might have played a significant role in the invasion of alien plants. The role of railways in propagule transport and disturbance has been reported in several studies (Tikka et al., 2001; Penone et al., 2012; Blanchet et al., 2015).

Differences in frequency of occurrence in the weed species observed during the study are due to the natural climate and landscape variations. Climatic conditions of the area might be most suitable for the frequently faced weeds compared with those of less frequent weed species (Zimdahl, 2007). The variations among the weed species are thought to be the result of the differential adaptive potential to specific soil and climatic conditions prevailing in a particular area. The identified plants during the surveys were distributed alongside and between the railways (Figure 7).

Distribution pattern of weeds indicates that density and coverage area of the weeds can increase with time. The increased densities and coverage of the weed species is suspected to severely interfere with maintenance and other operations at the railway tracks surveyed during the current study. Moreover, presence of invasive plants and extensive invasion potential of these plants can further aggravate the situation.

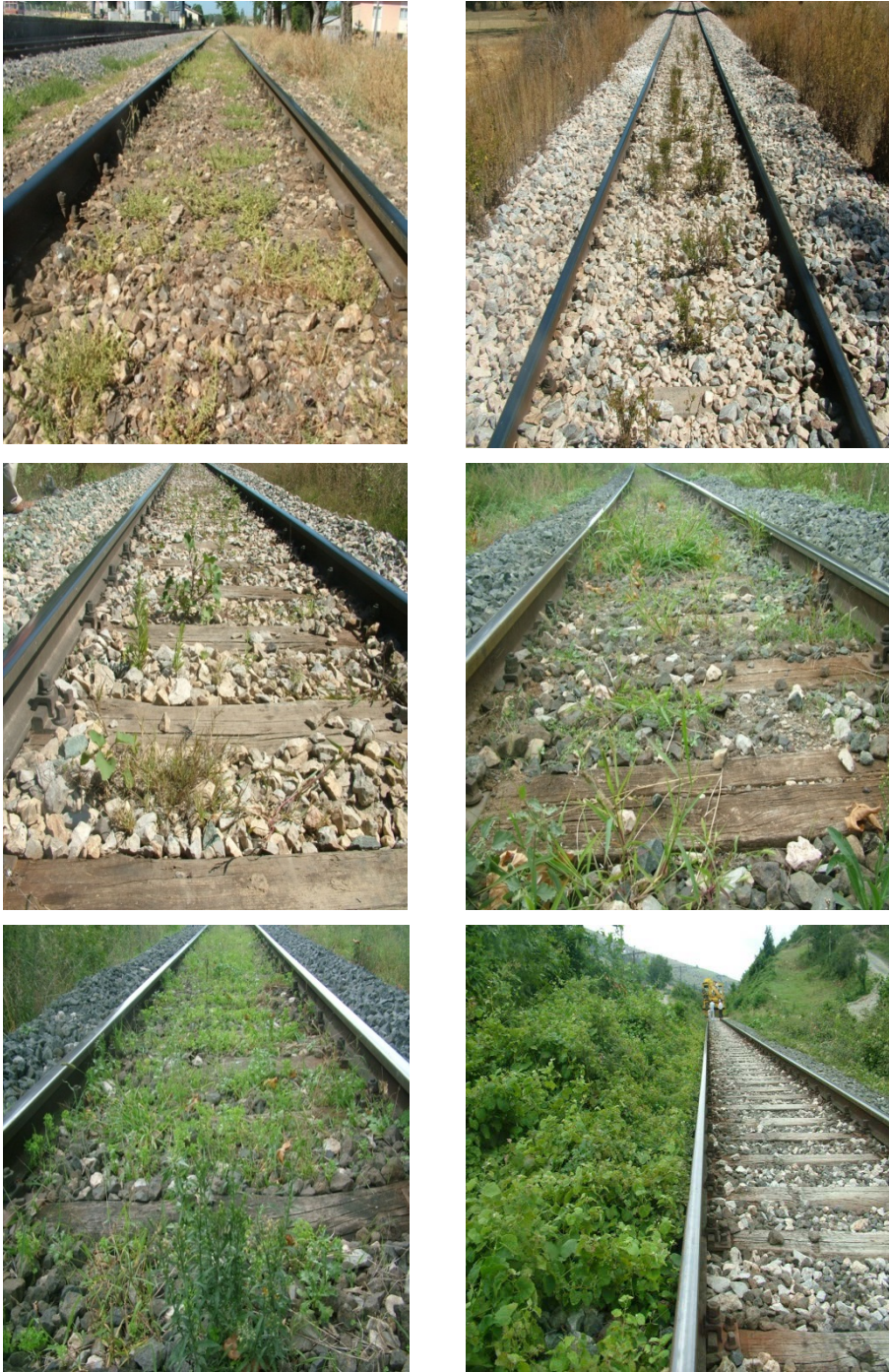


Figure 7. Distribution of some important plant species observed on the railways during survey

CONCLUSION

It is concluded that railways are serving as dispersal corridors for plant invasion in the country. Although current survey was conducted on small portion of the railways network of the country, presence of sufficient number of alien plants suspects that the railways might be playing a major role in the introduction and spread of alien species on regional scales throughout the country. It is therefore recommended that rapid surveys of railways to recognize the current status of plant invasion throughout the country is instantly needed. Moreover, environment and eco-friendly management strategies should be developed to eradicate the existing invasive plants along these railway tracks to avoid heavy losses in the future.

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