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CONTENT

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SIGNIFICANCE OF SURFACE WATER QUALITY FOR BASIN ECOLOGY

SUMMARY

The Council of the European Union and the European Parliament announced the objectives of the "sustainable policy in the water area" in 1996. Following those objectives and recommendations, control of surface water quality from the aspect of sustainable development of the region has been actively executed in Serbia. Water quality monitoring is primarily applied on large water basins, with a focus on small ones as well, especially due to the fact that small basins have a significant role in environmental protection and water supply. This paper presents the significance of regular water control of the River Ralja in Central Serbia. Monitoring was executed in different seasons, and this paper presents results for the period 2011-2012. The aim of this paper is to present the situation of the water basin and point to the significance of surface courses for water supply of the population. Another objective is to point to issues emerging in the phase of using and disposing of used waters, as well as the option of using the River Ralja water for irrigating arable land, in the aim of obtaining healthy and high-quality agricultural produce.

Keywords: monitoring, water quality, living environment, wastewaters.

INTRODUCTION

The battle for water is as old as the life on the Earth. A man certainly first fought for water, and then against floods. That battle for water actually continues today, with a conclusion that it has never stopped. Today's battle is more complex and difficult and will be even harder for future generations. Once sufficient supplies of water for human use could be found in nature, but the situation is different today. There is virtually no water left suitable for use that has not been upgraded by a human action, thus water has become a commodity, a commodity of special significance. It is no longer a raw material only; it is now a subject of work, a tool for work. Not only is it used for physiological needs, but for nearly all technological procedures. Furthermore, it is important to note that

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water has been, is and will always be an irreplaceable food item. (Mihailovic, 2007).

The quality of surface water plays a significant role in developing urban and suburban settlements. In the spatial layout of the territory of Serbia, urban settlements always develop near surface watercourses and accumulations. Water provides many benefits: it ensures drinking water, water for irrigating arable land, food for survival, and a comfortable environment for sports and recreation. This paper presents the data on the water quality of surface watercourses for the basin of the River Ralja, which belongs to the basin of the River Velika Morava, namely the River Danube.

The spring of the streams that create the River Ralja is located at Parcani Rising and Mt. Kosmaj, at the 426 m elevation point, by combining several tributaries. The quality of the spring tributaries waters are ranked as the first Class, but as rural and suburban settlements do not have regulated utility infrastructure, occasional pollution incidents occur along the watercourse and in the spring area of the basin. Wastewaters from these settlements are disposed of in cesspits, which are in most cases drainable and overflowing, and wastewaters from them and cattle farms run off to ditches and streams. As a result, even some streams in the spring area of the basin are polluted with wastewaters. (Milosevic, *et al*., 2014).

Another source of pollution is illegal solid waste landfills, as waste is washed away with rain and poisonous and hazardous substances get to surface courses and ground aquifer waters. (Andjelkovic, *et al*., 2013).

The third source of pollution is pesticides and artificial fertilisers, used in agricultural and fruit production. Individual farmers are often not trained in using these substances properly and sometimes they exceed recommended amounts. In addition, used packaging of these substances is disposed of at non-regulated and unsafe locations (illegal landfills). After using chemical substances, they are washed away with rain and snow to ground and surface watercourses. Packaging of used substances is not recyclable, thus it gets piled up for years, contaminating specific locations, which then become local sources of pollution of the living environment and watercourses. (Djekovic, V., 2004)

The hydrographic network of the River Ralja basin starts in the spring area at the hills of Mt. Kosmaj and Parcani Rising, while from the settlements of Parcani and Ralja, the river runs near Sopot, Mladenovac, in the direction of the settlement of Mali Pozarevac. After that, the watercourse of the River Ralja is regulated in Belgrade-Nis motorway section (Figure 3).

In the central and lower section, the River Ralja runs through the plain agricultural area, settlements: Umcari, Kolari, and Smederevska Ralja. These settlements have no regulated sewage infrastructure and wastewater treatment systems. The River Ralja has no major tributaries in the central section, but has several minor ones and direct runoffs of communal wastewaters. (Andjelkovic, *et al*., 2014).

At around 10 km from the spring, near the settlement of Umcari, the Public Health Institute of Belgrade controls water quality on a quarterly basis. The River Ralja is of exceptional significance for supplying the population in this region with high-quality drinking water and high-quality water for irrigating agricultural and other crops. The relevance of the quality of water for irrigation is evident, as large part of Belgrade is supplied with agricultural produce from this region at green markets, and the quality of water for irrigation generally affects the quality of food and health of the population (Dragovic et. al. 2012).

Very often specific physical-chemical and bacteriological parameters of surface water significantly exceed the maximum permitted concentrations (MPC) for the Class 3 of water quality. It is also found that water samples are occasionally bacteriologically and chemically unsafe. This phenomenon is regularly registered in summer periods, when due to a drought water flow is low, while water demand is high. In these periods, the oxygen regime of the River Ralja water is also disturbed (Table 1).

In the lower section of the River Ralja, landfills of the ironworks in Smederevo represent a special threat for the living environment and ground and surface waters. (Letic, *et al*. 2012)

The Public Health Institute of Pozarevac has been conducting physical and chemical analyses of the River Ralja water 12 times a year (once a month), for an extended period of time (20 years). Constant contamination of surface water causes the pollution of ground (well) waters. Furthermore, the ironworks in Smederevo releases its wastewaters to the River Ralja profile. The concentration of suspended substances is higher than permitted (MPC) for the Class 3 of quality. (Salama *et al*., 2013) The effluent contains a high rate of the following: iron, zinc, ammonia, high five-day chemical oxygen consumption (COC5), as well as phenols, phosphorus, nitrates and nitrites. (Djekovic, V., 2007).

The significance of the River Ralja basin is confirmed by the fact that it is the only watercourse in this part of Central Serbia that connects four municipalities. The River Ralja waters may be a valuable water resource for water supplies for the population, survival of florae and faunae. Therefore, it has been necessary to evaluate the quality of surface waters and define the impact of the waters polluted in such a manner on the environmental potential of the basin. (Drsgovic, S,. et. al.2012)

The aim of the project has been to register polluters, point to pollutions before the ironworks in Smederevo landfills and strive to apply legal and other measures to prevent degradation of such an important area in the vicinity of the capital. As control of environmental pollution is not possible in all periods of a day, during a 24-hour period, it is necessary to define guidelines for efficient protection of the living environment, surface waters and ground waters. In that respect, it is vital to educate the population efficiently and raise public awareness regarding the significance of a healthy living environment and pollution hazards. To conduct this program, education needs to start with the youngest members of the population, including public media as a channel.

Hydrography of the River Ralja Basin

More than a half of the River Ralja watercourse (cca 25km) lies in the direction of Belgrade – Nis motorway, thus the Ralja basin is located between the River Danube basin to the north and the Veliki Lug and Jasenica basins to the south. The terrain of the River Ralja basin is a consequence of forest devastation and activation of erosion processes caused by the movement of material and its occasional retention in the hydrographic network of the watercourse. (Krstic, M., et. al., 2013)

Morphological processes in the river basin and bed have resulted in the erosion and movement of easily degradable material and its transportation along the river. In the zones with a higher drop of the river bed, the bed deepens, the banks are undermined and eroded and high volumes of deposits, transported through the hydrographic network of the basin by the water flow in flooding phases, are created. Moving deposits are comminuted along the watercourse and move mainly as suspended deposits in the central and lower watercourse. (Djekovic *et al*., 2013). A suspended deposit is a natural coagulant and a transporter of a pollution wave along the river. Its rate decreases in a dry period of a hydrological year, and increases with precipitation and higher flows. A rate of diluting the pollution concentration plays an important role in using the River Ralja water for irrigation and water supply. Another issue is emulsified substances emerging in the river bed and not depending particularly on the water flow rate. (Letic *et al*., 2008).

The River Ralja flow rate is characterised by the succession of constant and occasional flows. In a period of heavy rain and storms, the level of specific tributaries and streams rises, they run off their bed and flood the bank zone. There is no practice of monitoring the water level of the River Ralja, but according to previous surveys and traces, it has been concluded that the average annual flow is $Q = 1{,}55 m^3 \cdot s^{-1}$ and the water depth in the profile for this flow rate in the upper and central sections of the basin ranges between 20cm to 30cm. In the previous period, the River Ralja has been used as a receptor of wastewaters from production facilities of various types of industry, primarily foundries and ferrous metallurgy, food industry, agriculture, urban settlements and individual farms. Many production facilities are not operating at the moment, due to the economic crisis, so the quality of water along the basin has improved in a natural manner. (Djekovic, *et al*., 2010).

MATERIAL AND METHODS

This paper is based on the results of a survey on the quality of the living environment of the River Ralja basin in Central Serbia, conducted by the Public Health Institute of Serbia, Public Health Institute of Pozarevac, and some surveys of the paper authors. The total length of the River Ralja is 51km, and the basin area covers 310km2. The River Ralja runs through 4 municipal areas (Sopot, Mladenovac, Grocka and Smederevo). After merging with the River Jezava at the

territory of the municipality of Smederevo, it enters the River Velika Morava, around 5km away before its confluence with the River Danube (Figure 1).

The survey methods presented in this paper are based on field surveys, sampling of water from various profiles of the river course. These activities have established change in the water quality, including the presence of foul smell, as an indicator of environmental pollution. As water supply of all the settlements in the River Ralja basin is so far based on well ground water, and surface water of the River Ralja is used for irrigating agricultural land and accumulation of used up aquifer waters, as well as for the preservation of the ecology of the watercourse and the basin, the quality of surface and ground waters is of exceptional importance. In order to successfully preserve the living environment of the basin, it is necessary to maintain the quality of surface waters.

Figure 1. Position of the River Ralja basin (Izvor: origin)

The significance of agricultural land near large urban centres (Belgrade - Smederevo) has a huge impact on the production and supply of these cities with healthy food. The following methodology was used for surveying the impact of specific parameters of pollution on the quality of the River Ralja water and the quality of ground waters in the basin:

-Experimental survey on the watercourse and in the basin of the River Ralja, with the direction of survey divided in two groups:
-direct definition of quality parameters on selected profiles of the watercourse, from the spring to the confluence;
-registration of point sources of pollution along the river;
-Sampling of water and sediments for physical-chemical and bacteriological analyses.

For further analysis, the entry data consist of results of the direct measurement and, based on the measurement results, the assessment of the impact of local pollutions on the water quality of the River Ralja basin was executed.

The method of direct measurement of change in the water quality was applied, due to releasing communal wastewaters in the river, as well as a volume and concentration of specific polluting elements in the watercourse. Registration

of illegal landfills with industrial and communal waste on the field has resulted in the conclusion that waste and hazardous substances enter the River Ralja surface waters from these locations with precipitation.

This paper presents part of the results of measurements and surveys of the quality of water and sediments conducted in the course of 2011 and 2012. Due to a huge volume of the measurements and analyses, specific data have been selected to present physical-chemical and bacteriological surveys.
the gathered data are presented in the tables in this paper.

RESULTS AND DISCUSSION

Wastewaters in the River Ralja basin

Wastewaters from settlements

The River Ralja basin includes 21 settlements with the population of around 35,000. The residents are mainly engaged in agriculture, fruit and wine growing and cattle breeding. The settlements do not have a system of channelling and draining wastewaters. Instead, wastewaters are disposed of in cesspits, which are mainly drainable and overflowing, or are occasionally emptied, with the effluent content disposed of in the River Ralja or some of its tributaries.

Based on the field surveys, it is concluded that the wastewater volume from these settlements ranges at around 70g ER/day, (ER – equivalent resident), suspended substances (SUS) 65g ER/day, 5-day biochemical oxygen consumption (BOC5) 9g ER/day, nitrogen (N) 0.6g ER/day, phosphorus (P) 0.5g ER/day. (Andjelkovic *et al*., 2013)

The measurements have found that out of the overall volume of used water, around 70% is disposed of in cesspits, which means that around 4,900 m3/day of wastewater come from households in the River Ralja basin, including around 1.715 tons of suspended particles, 1.6 tons of organic waste, 220.5kg of nitrogen and 14.7kg of phosphorus. After filling up cesspits, their content is released to the river bed, as there is no control related to this issue. (Andjelkovic *et al*., 2013)

Accordingly, around 4,900 m3 of water per day is used in the River Ralja basin. That volume of water runs off to surface courses and infiltrates the ground. Used wastewaters are highly polluted with organic matter, nitrates, nitrites and ammonia. In addition, around 2.3 tons of organic matter, 0.64 tons of nitrogen, high volumes of phosphorus, potassium, iron, chlorides, etc., are added to the hydrographic network of the basin on a daily basis. Furthermore, a range of metals in traces is registered, affecting the water and soil quality. (Djekovic *et al*., 2008). The valley of the River Ralja, downstream from mali pozarevac, is located in a close proximity of belgrade - nis motorway and belgrade - mala krsna railway. The river bed is regulated upon the system of land regulation, with occasional protection and stabilisation of bevels and the river bed. (Figure 3).

There are no dykes along the river. Instead, the grade level is brought down, so the regulation is at the same time a system of surface drainage, in case of value and surface sloughing, while the right side of the river valley is protected with the motorway dyke.

Figure 2. Landfills of waste, sediment mud to the River Ralja bed (Photo: V. Djekovic, 2013)

Figure 3. Regulation of the River Ralja with the local enforcement of flow profile (Photo: V. Djekovic, 2012)

Influence of communal wastewaters and small business wastewaters on the quality of surface watercourse

As the river follows the direction of Belgrade – Nis motorway, 11 petrol stations have been built, as well as many car repair shops, car washes, Horeca facilities and motels. It was very difficult in this survey to establish a volume and pollution rate of wastewaters from these facilities that enter the profile of the River Ralja. Central activities in these facilities are executed in late afternoon and night hours. Based on a total number of workforces in these facilities, a volume of sanitary and faecal wastewaters that enters the River Ralja tributaries has been determined. The workforce at petrol stations is around 150, in craft shops 100 and in Horeca facilities 90, or 340 in total. With 50 litres of estimated water consumption per user a day, around $17m^3 \cdot day^{-1}$ of sanitary and faecal wastewaters enter the River Ralja streams and course without any treatment. By washing off spilt petroleum and its derivatives from the working areas of petrol stations, a significant volume of water with a high rate of grease, petroleum and heavy metals enters the river. Chemical oxygen consumption is elevated due to a high rate of chemical compounds in water, while biochemical consumption is very low, resulting in a very small rate of biologically active organisms in water. Most petrol stations have deployed grease and petroleum separators before releasing water to streams, but many of the separators are out of function. A high volume of water, with a high concentration of detergents and suspended matter, from car washes, horeca facilities and motels runs off to the streams. It is difficult the determine the exact volume of these waters, but it is estimated at around 30m3·day-1. Results of the quality water monitoring are presented in the Tables 1, 2 and 6.

Table 1. Monitoring of the River Ralja water quality in the course of 2012 (Source: Original)

Date	t^0C	pH	O_2	$\%O_2$	BOC_5	$KMnO_4$	COC	Susp. part.	Total phosph.
09/04/2012	13.5	8.1	8	76	0.7	23.7	5.9	3	0.128
23/07/2012	19.5	8	3.9	42	1.4	30.7	7.7	39	0.196
20/9/2012	11.3	7.9	4.9	48	3.6	22.2	4.3	25	0.205
12/12/2012	5.3	8.1	6.2	55	1.8	18.2	5.0	9	0.175

Table 2. Results of monitoring of the River Ralja water quality in the course of 2012 (source: original)

Date	Ortho phosph.	Dry residue	Electro conduct.	Alkalinity	Hardness	Fe	NH_3	NO_2	NO_3	TOC
09/04/2012	0.176	920	1230	493.5	517	0.05	0.23	0.017	0.7	6.63
23/07/2012	0.176	920	1230	493.5	517	-	0.34	0.021	1.29	8.54
20/09/2012	0.305	889	1055	392.4	490	0.004	0.45	0.042	0.98	7.97
12/12/2012	0.168	853	1120	385.0	510	0.008	0.29	0.009	0.75	6.28

Table 1 shows that the concentration of oxygen in water is high and that it decreases with an increase of temperature, while chemical oxygen consumption rises, reaching a peak in summer months, when a rate of chemical substances in water is higher (grease, oil, petroleum and its derivatives, detergents). Over time, biochemical oxygen consumption in the lower section of the river decreases, as the content of organic matter in water is reduced, resulting in lower-rate activity of microorganisms and their metabolism.

Table 2 shows that in the course of 2012 all the quality parameters were above the maximum permitted rate for waters of the class ii and iii. Rates of iron, nitrates and nitrites, as well as electrolytic conductivity, rise in the summer months. Dry residue is also increased, indicating to an extent to the presence of mineral particles in water (suspended erosion deposit).

The survey and quality control of recent waters has established a high rate of suspended particles, fluctuating in the course of the year and reaching a peak in the autumn months (October, November and December). The rate of organic matter was below the mpc for the class ii of quality in august and September. It is interesting to note that higher turbidity, caused by the presence of suspended particles, did not result in higher biochemical and chemical oxygen consumption. On the other hand, after heating at 1050c, dry residue is nearly always above 500mg/l, indicating to the origin of suspended particles or (water turbidity) mineral content. The oxygen rate is regularly below the permitted limit for the water of class ii, and the rate of oxygen saturation decreases with a drop of temperature of water and air. Another issue regarding the pollution of surface and recent waters in the lower section of the River Ralja basin is the presence of nitrates and nitrites, pointing to a conclusion that the pollutions are of faecal origin. The presence of heavy metals in water (zinc, iron and lead) points to industrial pollutions, while phenols point to the pollutions which might be of organic origin. Namely, decomposition of organic matter with the reduced presence of oxygen creates hazardous phenol compounds which, even at the upper limit of the mpc, point to the fact that these waters are toxic and are not to be used for water supply. The quality control on 06/11/2011 showed that the sample had a high rate of suspended particles, with metal analyses conducted from a decanted part of the sample. Only total iron was measured in the homogenised sample without decanting, with the result of 4.94 mg/l, which is not presented in the table. At the repeated control in October, on 09/10/2011, it was established that the composite sample had a higher rate of suspended matter, with metal analyses conducted from a decanted part of the sample. Only total iron was measured in the homogenised sample without decanting, with the result of 0.95 mg/l.

In November, on 20/11/2011, an increased rate of suspended matter was registered, and the control of heavy metal content on the homogenised sample without decanting showed the elevated rate of iron of 2.11 mg/l. In December, on 06/12/2011, the rate of iron was not reduced significantly. The sample had a high rate of suspended matter, with metal analyses conducted from a decanted part of

the sample. Only total iron was measured in the homogenised sample without decanting (fe= 2.00 mg/l).

Table 3. Results of testing physical and chemical parameters of recent waters in the course of 2011 (Source: Original)

Testing report				Testing date					
Parameters	Me. unit	MPC	13/8	11/9	9/10	6/11	20/11	6/12	Standard
pH		6.8-8.5	7.9	8.1	7.6	8.2	7.5	7.4	ISO 10523:194
Oxygen O_2	mg^1l^{-1}	>6.0	2	6.5	4.2	6.6	1.3	1.5	SRPS ISO 5814:1994
O_2 Oxy. saturation	%		23	77	50	78	14	17	SRPS ISO 5814:1994
BOC_5	mg^1l^{-1}		7.2	2.5	4.9	2.4	1.1	4.8	SRPS ISO 5814:1994
Consumption $(KmnO_4)O_2$	mg^1l^{-1}		19.5	18.2	21	15.5	25	22	PRI1 P-IV-9a
COC from $KMнO_4$	mg^1l^{-1}	12	4.9	3.0	5.2	3.0	37	34	SRPS ISO6060:1990
Suspend. matter	mg^1l^{-1}	30.0	6	4	225	600	25	11	SMEWW19th m 2540
Dry residue 105^0C	mg^1l^{-1}		650	386	575	379	526	535	SMEWW19th m 2540
Electrolyt. conduct.	µS/cm		930	530	770	550	610	610	ISO7888:1985
NH_4	mg^1l^{-1}		0,09	1.38	0.13	1.43	3.33	3.06	ISO 14911:1998
Nitrites NO_2/N	mg^1l^{-1}	10.0	0.053	0.112	0.036	0.105	0.02	0.02	PPI 1 P-V-32/A
Nitrates NO/N	mg^1l^{-1}	0.05	2.60	1.800	5.80	1.30	1.60	1.70	SMEWW 19th m 4500NO3B
Phenols	mg^1l^{-1}	0.001	<0.001	0.002	0.001	0.003	<0.001	<0.001	SRPS ISO 6439:1997
Arsenic As	mg^1l^{-1}		0.003	0.002	<0.001	0.002	0.008	0.008	EPA 206.3
Iron Fe	mg^1l^{-1}	0.30	0.097	0.590	0.126	0.048	0.293	0.251	EPA200.7 Rev 5
Zink Zn	mg^1l^{-1}		0.002	0.09	0.004	0.183	0.008	0.007	EPA 200.7Rev 5
Lead Pb	mg^1l^{-1}		<0.005	<0.010	<0.010	<0.01	<0.005	<0.005	EPA 2007Rev 5

Based on other parameters, it is concluded that the River Ralja waters are constantly polluted, regardless of a season. The quality of surface water changes abruptly after the entry point of the ironworks in Smederevo collector to the River Ralja. Sudden changes of the pH value confirm that the pollutions are constant, with water turbidity SiO2 increasing abruptly as well (mg1·l-1).

Biochemical oxygen consumption is also related to these changes, pointing to the presence of organic pollution of the river. Before the entry point of

wastewaters from the ironworks in Smederevo, the rate of iron in the sample ranged from 1.08 $mg^{1}\cdot l\text{-}^{1}$ to 15.78$mg^{1}\cdot l\text{-}^{1}$, to increase to 3.07 $mg^{1}\cdot l\text{-}^{1}$ to 52.24 $mg^{1}\cdot l\text{-}^{1}$after the entry point. All testing methods were standardised and in accordance with the standard-defined regular procedure (Tables 3, 4).

Table 4. Results of physical and chemical testing of water in the course of 2012 (Source: Original)

Organoleptic testing:		Visual water colour: (clear, pale yellow, purple)							
No.	Parameter	Meas. unit	MPC	Received value					Method type
				13/04/1	25/05/1	24/06/1	06/07/1	11/08/1	
1	Air temp.	-	-	21.1	28.4	31.4	30.0	32.2	-
2	Water temp.	°C	-	20.8	21.7	21.3	21.4	22.3	1.1.1-S
3	Turbidity	$mg^{1}l^{-}$	-	<10	20	30	800	78	P[1)]-M-P-IV-
4	pH value	-	6.8-8.5	8.0	8.05	8.10	8.05	8.12	P[1)]-M-P-IV-
5	Nitrates (as N)	$mg^{1}l^{-1}$	10.0	0.47	0.266	0.522	1.090	1.95	P[1)]-M-P-V-
6	Nitrites (as N)	$mg^{1}l^{-1}$	0.05	<0.002	0.012	0.003	0.006	0.008	P[1)]-M-P-V-
7	Ammonia ion (as	$mg^{1}l^{-1}$	1.00	0.097	0.233	0.583	0.389	0.455	P[1)]M-P-V-2-
8	Chlorides	$mg^{1}l^{-1}$	-	79.08	98.14	83.48	53.05	54.25	SRPS SO
9	Consumption	$mg^{1}l^{-1}$	-	19.28	19.76	33.19	43.36	45.26	P[1)]-M-P-IV-
10	Iron (Fe)	$mg^{1}l^{-1}$	0.30	0.25	0.25	0.83	1.0	1.26	P[1)]-M-P-V-
11	Detergents - anionic	$mg^{1}l^{-1}$	-	0.030	<0.030	0.127	0.278	0.285	1.1.28-S
12	Phosphates	$mg^{1}l^{-1}$	-	<0.030	0.708	0.795	0.831	0.753	APHA[8)]-
13	Phenols	$mg^{1}l^{-1}$	0.001	<0.001	0.001	<0.001	<0.001	0.002	SRPS IS0 6439:1997-
14	Oxygen (O_2)	$mg^{1}l^{-1}$	>6.0	6.73	4.79	4.65	6.82	4.85	P[1)]-M-P-IV-12/B
15	COC	$mg^{1}l^{-1}$	12	12	17	11	17	17	P[1)]-M-P-IV-10
16	BOC_5	$mg^{1}l^{-1}$	4	10.58	9.16	6.34	17.48	16.95	SRPS ISO 5815:1994
17	Residue from unfiltered water	$mg^{1}l^{-1}$	-	692.0	650.0	776.0	520.0	695	P[1)]-M-P-IV-7
18	Residue from filtered water	$mg^{1}l^{-1}$	1000	652.0	636.0	754.0	490.0	746	VM 11
19	Suspended particles	$mg^{1}l^{-1}$	30.0	40.0	14.0	22.0	30.0	29	P[1)]-M-P-IV-9
20	Sediment matter	ml/l/2h	-	<0.10	0.10	0.10	0.10	0.11	P[1)]-M-P-IV-8

Based on the presented surveys, the total volume of wastewaters from small business facilities is around $50 m^{3}\cdot day^{-1}$. Wastewater that runs off to the surface river profile is exceptionally polluted and has a negative effect on the quality of the River Ralja water and the living environment. Oxygen

consumption (BOC_5) for sanitary (faecal) wastewater is estimated at (O_2) =200g^1. m^{-3}, and for the water from washing equipment and vehicles (O_2) =300g^1 $m^{-3.}$

Below are results of physical and chemical testing of the River Ralja water quality at the measurement point 4 in Radinci, Table 7.

Table 5. Physical and chemical features of the River Ralja water upstream from the entry point of wastewaters from the ironworks in Smederevo (Source: Original)

Organoleptic testing:		Visual water colour: (clear, pale yellow, purple)							
No.	Parameter	Meas. unit	MPC	Received value					Method type
				13/04/1	25/05/1	24/06/1	06/07/1	11/08/1	
1	Air temp.	-	-	21.1	28.4	31.4	30.0	32.2	-
2	Water temp.	°C	-	20.8	21.7	21.3	21.4	22.3	1.1.1-S
3	Turbidity	mg^1l^-	-	<10	20	30	800	78	P[1)]-M-P-IV-
4	pH value	-	6.8-8.5	8.0	8.05	8.10	8.05	8.12	P[1)]-M-P-IV-
5	Nitrates (as N)	mg^1l^{-1}	10.0	0.47	0.266	0.522	1.090	1.95	P[1)]-M-P-V-
6	Nitrites (as N)	mg^1l^{-1}	0.05	<0.002	0.012	0.003	0.006	0.008	P[1)]-M-P-V-
7	Ammonia ion (as	mg^1l^{-1}	1.00	0.097	0.233	0.583	0.389	0.455	P[1)]M-P-V-2-
8	Chlorides	mg^1l^{-1}	-	79.08	98.14	83.48	53.05	54.25	SRPS SO
9	Consumption	mg^1l^{-1}	-	19.28	19.76	33.19	43.36	45.26	P[1)]-M-P-IV-
10	Iron (Fe)	mg^1l^{-1}	0.30	0.25	0.25	0.83	1.0	1.26	P[1)]-M-P-V-
11	Detergents - anionic	mg^1l^{-1}	-	0.030	<0.030	0.127	0.278	0.285	1.1.28-S
12	Phosphates	mg^1l^{-1}	-	<0.030	0.708	0.795	0.831	0.753	APHA[8)]-
13	Phenols	mg^1l^{-1}	0.001	<0.001	0.001	<0.001	<0.001	0.002	SRPS IS0 6439:1997-
14	Oxygen (O_2)	mg^1l^{-1}	>6.0	6.73	4.79	4.65	6.82	4.85	P[1)]-M-P-IV-12/B
15	COC	mg^1l^{-1}	12	12	17	11	17	17	P[1)]-M-P-IV-10
16	BOC_5	mg^1l^{-1}	4	10.58	9.16	6.34	17.48	16.95	SRPS ISO 5815:1994
17	Residue from unfiltered water	mg^1l^{-1}	-	692.0	650.0	776.0	520.0	695	P[1)]-M-P-IV-7
18	Residue from filtered water	mg^1l^{-1}	1000	652.0	636.0	754.0	490.0	746	VM 11
19	Suspended particles	mg^1l^{-1}	30.0	40.0	14.0	22.0	30.0	29	P[1)]-M-P-IV-9
20	Sediment matter	ml/l/2h	-	<0.10	0.10	0.10	0.10	0.11	P[1)]-M-P-IV-8

The conclusion is that the River Ralja waters before the ironworks in Smederevo, namely upstream from the entry point of wastewaters from the ironworks collector, are on the lower limit of the Class II and III of quality.

Concentration of suspended particles, iron, zinc, COC, BOC, phenols and ammonia is above the maximum permitted concentration (MPC). In the survey period, the sampling dynamics was twice a month. Maximum concentrations of suspended particles in the course of 2011 ranged from 160 $mg^{-1} \cdot l^{-1}$ to 498$mg^{-1} \cdot l^{-1}$, of ammonia from 0.09 $mg^{-1} \cdot l^{-1}$ to 3.33.667 $mg^{1} \cdot l^{-1}$, COC from 3 $mg^{1} \cdot l^{-1}$ to 37 $mg^{1} \cdot l^{-1}$, COC_5 from 1.1 $mg^{1} \cdot l^{-1}$ to 7.20 $mg^{1} \cdot m^{-3}$, iron from 0.048 $mg^{1} \cdot l^{-1}$ to 0.59 $mg^{1} \cdot l^{-1}$, zinc from 0.02 $mg^{1} \cdot l^{-1}$ to 0.183 $mg^{1} \cdot l^{-1}$, and phenols from 0.001 $mg^{1} \cdot l^{-1}$ to 0.003 $mg^{1} \cdot l^{-1}$. Increased levels of ammonia, suspended particles, COC and BOC are the consequence of the pollution caused by communal wastewaters from agricultural activities, unresolved issue of drainage and treatment of wastewaters from farms, and a lack of a sewage system.

Table 6. Physical and chemical features of the River Ralja water upstream from the entry point of wastewaters from the ironworks in Smederevo (Source: Original)

	Unit	19/01/2012	11/02/2012	13/03/2012	17/05/2012	14/06/2012	18/07/2012	20/08/2012
TurbiditySiO_2	$mg^{1}l^{-1}$	300	30	800	20	50	60	200
pH value	$mg^{1}l^{-1}$	8.15	8	8.65	8.15	8	7.8	8.05
Nitrates (as N)	$mg^{1}l^{-1}$	2.685	1.323	0.782	0.346	0.457	0.513	0.425
Nitrites (as N)	$mg^{1}l^{-1}$	0.009	0.003	<0.002	0.002	0.003	0.003	0.03
Ammonia ion	$mg^{1}l^{-1}$	0.078	0.097	0.583	0.194	0.233	0.194	0.971
Chlorides (Cl	$mg^{1}l^{-1}$	81.13	72.69	43.33	70.47	77.01	50.83	40.99
$KMnO_4$	$mg^{1}l^{-1}$	44.56	54.05	113.16	23.11	23.26	19.09	33.5
Iron (Fe)	$mg^{1}l^{-1}$	1	0.2	0.67	0.67	0.33	0.33	0.67
Detergents	$mg^{1}l^{-1}$	< 0.030	0.038	0.046	0.27	0.472	0.018	<0.03
Phenols	$mg^{1}l^{-1}$	0.006	0.003	0.002	<0.001	<0.001	<0.001	<0.001
Phosphates	$mg^{1}l^{-1}$	0.521	0.29	-	0.29	0.04	0.726	0.983
Residue from unfiltered	$mg^{1}l^{-1}$	582	652	834	606	626	638	602
Residue from filtered water	$mg^{1}l^{-1}$	522	638	434	588	608	528	566
Suspended	$mg^{1}l^{-1}$	60	14	400	18	18	110	66
Sediment	$mg^{1}l^{-1}$	< 0.10	<0.10	1	<0.10	0.1	0.1	0.3
Oxygen	$mg^{1}l^{-1}$	12	12.23	9.85	7.75	5.17	6.17	6.15
COC	$mg^{1}l^{-1}$	19	31	30	20	22	20	4
BOC_5	$mg^{1}l^{-1}$	10.52	3.07	52.4	7.98	4.69	5.36	22
Copper	$mg^{1}l^{-1}$	< 0.003	<0.003	0.016	<0.003	<0.03	<0.003	<0.03
Zinc	$mg^{1}l^{-1}$	0.101	0.056	0.027	0.031	0.079	0.058	0.043
Chrome total	$mg^{1}l^{-1}$	0.006	<0.006	<0.006	0.006	<0.006	<0.006	<0.006
Cadmium	$mg^{1}l^{-1}$	< 0.002	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002
Lead	$mg^{1}l^{-1}$	< 0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table 7. Results of physical and chemical testing of the River Ralja water in Radinci M – 4 (Source: ZJZ-Srbije)

Organoleptic testing		Unclear, pale yellow			
	Parameter	Meas. unit	MPC	Received value	Method type
1	Air temperature	°C	-	10.5	-
2	Water temperature	°C	-	20.0	1.1.1-S
3	Turbidity (SiO_2)	$mg^1 \cdot l^{-1}$	-	100	1.1.69-S
4	pH value	-	6.8-8.5	8.20	1.1.6-S
5	Nitrates (as N)	$mg^1 \cdot l^{-1}$	10.0	1.617	1.1.52-S
6	Nitrites (as N)	$mg^1 \cdot l^{-1}$	0.0.	0.122	1.1.53-S
7	Ammonia ion (as N)	$mg^1 \cdot l^{-1}$	1.0	1.554	1.1.18-S
8	Chlorides (Cl)	$mg^1 \cdot l^{-1}$	-	49.75	1.1.36-S
9	Consumption $KMnO_4$	$mg^1 \cdot l^{-1}$	-	12.20	1.1.10-S
10	Iron (Fe)	$mg^1 \cdot l^{-1}$	-0.30	1.0	1.1.32-S
11	Detergents anionic	$mg^1 \cdot l^{-1}$	-	0.556	1.1.28-S
12	Phenols	$mg^1 \cdot l^{-1}$	0.001	0.001	1.1.29-S
13	Phosphates	$mg^1 \cdot l^{-1}$	-	0.393	1.1.31-S
14	Residue from unfiltered water evaporation	$mg^1 \cdot l^{-1}$	-	342.0	1.1.7-S
15	Residue from filtered water evaporation	$mg^1 \cdot l^{-1}$	1.000	308.0	1.1.7-S
16	Suspended particles	$mg^1 \cdot l^{-1}$	30.0	34.0	1.1.9-S
17	Sediment matter	$mg^1 \cdot l^{-1} \cdot 2h$	-	0.10	1.1.8-S
18	Oxygen	$mg^1 \cdot l^{-1}$	> 6.0	7.40	1.1.14-S
19	COC	$mg^1 \cdot l^{-1}$	12	26	1.1.11-S
20	BOC_5	$mg^1 \cdot l^{-1}$	4	6.28	1.1.15-S
21	Copper	$mg^1 \cdot l^{-1}$	0.10	0.03	1.1.23-S
22	Zinc	$mg^1 \cdot l^{-1}$	0.20	1.12	1.1.27-S
23	Chrome total	$mg^1 \cdot l^{-1}$	0.10	0.006	1.1.38-S
24	Cadmium	$mg^1 \cdot l^{-1}$	0.005	0.002	1.1.39-S
25	Lead	$mg^1 \cdot l^{-1}$	0.05	0.034	1.1.54-S

A higher rate of iron and zinc is a result of various types of waste and the geological structure of soil. The Table 7 presents the River Ralja water quality in Radinci in the course of 2012. In 2011, out of 17 samples of suspended particles concentration, the concentration of suspended particles was higher than the MPC in 11, ammonia in 1, iron in 10, COC in 15, BOC in 13, zinc in 1, and phenols in 3 samples. It is important to note that other measured parameters were below the MPC, including sulphates, chlorides, phosphates, nitrites, nitrates, sodium, lead, chrome, cadmium, copper, sediment matter, and oxygen. Results of overall analyses are comprehensive and cannot all be presented in this paper. The presence of iron is the consequence of ironworks waste, as well as organic waste which contains iron (blood, waste from slaughter houses, etc.).

Organic waste also results in a higher rate of nitrates, nitrites, ammonium ions and phenols.

Part of results of testing chemical parameters of the River Ralja water quality at the measurement unit M-4 is presented in the Table 7. Testing was executed upon the defined standards. The quality of water is within the legally defined standards for the water of Class II of quality. The measurement unit M-4 is located under the bridge in Radinci.

As presented in the Table 7, there were 7 permanent points (quality monitoring stations) within the execution of the program of surveying the water and sediment quality in the Ralja River basin for establishing sixteen physical and chemical features of the Ralja River water, assumed to often deviate from the legally defined values. Based on the received values, the most frequent deviation from the maximum permitted concentrations (MPC) is registered for the following parameters: dissolved oxygen and % of dissolved oxygen, BOC5, content of suspended articles, COC5, nitrites, iron, phenols, zinc, ammonia ion.

Landfills with solid communal waste in the River Ralja basin

Poor communal waste management is one of the major environmental issues in the Republic of Serbia, mainly resulting from the previous attitude of the society towards waste. High costs, irrational organisation, low service quality and insufficient environmental awareness are the result of an adverse situation in organising waste management. Until several years ago, virtually the only way of managing waste in the Republic of Serbia was disposal at local landfills which, with few exceptions, do not satisfy elementary sanitary and technical and technological conditions. In the previous period, the construction of sanitary landfills has begun in Serbia, and some of them started operating by 2010 (Vranje, Pancevo, Lapovo). (Ministry of Environmental Protection of the Republic of Serbia, 1999)

The national waste management strategy, adopted by the Government of the Republic of Serbia in 2003, represents the foundation for rational and sustainable waste management, including basic EU principles in the area of waste management. The revision of this strategy is in its final phase. The existing landfills – dumps represent sites with a highly negative impact on the living environment. A negative influence on air, ground and surface waters and soil is directly evident. Waste in itself represents a loss of matter and energy, while its collection, treatment and disposal requires a high volume of additional energy

and work. (Water Classification Regulation of the Republic of Serbia "Official Gazette of the RS", no. 5/68)

In the course of 2005, the Environmental Protection Agency implemented the project titled “Innovation of the Landfill Cadastre in the Republic of Serbia”. According to the obtained data, 164 landfills used by municipal public utility companies for waste disposal are registered at the territory of the Republic of Serbia. Out of the total number of municipalities, 15 do not dispose of waste at their territory and use landfills of some other municipality. In 2009, the Sector for Control and Monitoring of the Ministry of Environmental Protection composed a registry of illegal landfills at the territory of the Republic of Serbia. The total number of registered locations is around 4500. In most cases, illegal landfills are located in rural areas and are often formed on the river banks and along roads, mainly at slopes and feet of road dykes. (Figure 4, Environmental Protection Agency, 2008).

Figure 4. Places where the samples were taken. (Source: Original)

Such landfills are often inaccessible for removal. The Environmental Protection Agency compiled data on a volume of communal waste and a number of households that generated that waste in the course of 2006. The obtained data differed significantly among municipalities and it was concluded that those estimates were inadequate in most cases, not reflecting the actual situation regarding waste generation. Volumes of communal waste on an annual basis are calculated based on measuring waste in referential municipalities. Based on the

results of those measurements, it can be concluded that urban population generates 1kg of communal waste per capita a day on average, compared to 0.7kg generated by rural population. Belgrade generates 1.2kg of waste per capita a day. On average, 0.87kg of communal waste per capita a day is generated at the territory of the Republic of Serbia.

Organisation of hazardous waste management in the Republic of Serbia is at a low level and requires and integral approach in all phases – from the moment of generation, to collection, transportation, treatment, to disposal. A similar problem occurs in the area of the River Ralja basin, where there are facilities for treating specific types of waste (car batteries, electronic and electric waste, used oils, used vehicles). There are no facilities for permanent storage of hazardous waste and temporary disposal is mainly executed at the premises of a company generating it, and very often in an illegal manner, in the profile of surface waters (Figure 5).

Figure 5. Various waste materials at the spring section of the river (Photo: Djekovic, 2012)

The adoption of the law on waste management and the law on packaging and packaging waste ("Official Gazette of the RS," no. 36/09) provides a legal framework for establishing an integral system of waste, packaging and packaging waste management.

There are several legal landfills for solid communal waste in the River Ralja basin. However, there are also illegal, non-regulated landfills. The

government of the republic of Serbia adopted the decision on defining the national environmental protection program in Belgrade, on January 21, 2010.

Upon the decision, many activities have been undertaken in the aim of quality control of the living environment, surface and ground water, landfills for solid communal waste. It is established that the volume of communal waste collected per day is 0.87kg per capita. The conclusion is that around 30.54t of communal waste a day is disposed of in the River Ralja basin.

As this is primarily an agricultural and vacation area, the structure of generated communal waste is somewhat different compared to the structure of communal waste in Serbia in general, as a share of organic waste is significantly higher, while a share of paper, textile, glass, polyethylene, plastic, rubber and metal is much lower. Communal waste mainly consists of waste from households, public areas, Horeca facilities, as well as of construction trash and various other types of cumbersome waste. This fact may represent a huge advantage, as this material is biodegradable and its processing can be used for energy production. In the European Union, this type of waste is used in recycling.

Communal waste landfills often include waste of animal origin from households and meat processing and cattle breeding facilities. This waste consists of scrap meat, bones, skin and other animal parts. This type of waste belongs to the category of hazardous waste and is a source of contamination. Its disposal at communal waste landfills is banned. Local administrations of the communities of Ralja, Mala Ivanca and Mali Pozarevac have registries of the position, volume and structure of communal waste, while other local communities do not.

Around 3,000t of non-fermented pomace, consisting of fruit seeds and peel, are disposed of near the River Ralja annually, accounting for 20% of the total volume of grape and around 900t of wine residue, which contain coagulants and wine yeast. Around 6% of wine residue is generated from a total volume of grape. It means that around 3,900t of organic residue is generated annually and disposed of near the River Ralja. In this type of production, stems, pits, rotten fruit represent waste which is mainly disposed of from production facility premises to the River Ralja.

Many waste materials which should be treated separately, such as waste of animal origin in meat processing, are disposed of at illegal landfills (Figure 4). Illegal landfills for waste of animal origin from mini farms, mini slaughter houses, namely, small business facilities, are present in the River Ralja basin, but they vary in size and operate periodically, due to a method of work and technology.

The River Ralja basin includes several mini farms, so manure and other waste from them also pollute the River Ralja via surface waters, due to a lack of sewage systems.

“Ralja" foundry in Ralja (Sopot) did not generate foundry and industrial waste in 2011, while the type of waste material generated in these facilities in the future depends on several factors.

CONCLUSIONS

The tested samples of water, sediments and emulsified matter from the River Ralja lead to the conclusion that the watercourse is characterised by a strong natural ability of self-purification. Despite evidently present point polluters in the overall river basin, the water along the course is ranked as the Class II and III of quality.

Organic matter of protein and carbon hydrate (monosaccharide) origin was present along the entire course, combined with faecal and other pollutions of organic and non-organic origin. Bacteria, as indicators of polysaccharide substances, were not present. The water temperature showed characteristic seasonal fluctuations, which were relatively high, ranging from 5.4°C in January to 23.6°C in July.

Basic physical and chemical parameters were constantly within a range defined for the Class II and III of quality and included: pH value, five-day biochemical oxygen consumption (BOC_5), concentration of dry residue, iron, ammonia ion and nitrates.

Regular fluctuations of the pH value from 7.8 to 8.2 were registered and the water was constantly slightly alkaline, as expected for this type of wastewaters running off to surface watercourses.

The rate of iron was not elevated, even in periods of higher flow, and was balanced throughout 2011 and 2012. The products of protein substance decomposition (ammonia ion, nitrites and nitrates) were constantly present in the River Ralja water, and their concentration fluctuated significantly, depending on a season. Five-day biochemical oxygen consumption was low and relatively stable, ranging from $1.5 mg^{1} \cdot l^{-1}$ O_2 in April 2011 to $52.40 mg^{1} \cdot l^{-1}$ O_2 on 13/03/2012, when the MPC was exceeded significantly. Dry residue concentration ranged from $604 mg^{1} \cdot l^{-1}$ to $889 mg^{1} \cdot l^{-1}$, with high values resulting from strong presence of dissolved salts, except in flooding periods, when it dropped significantly. Ammonia ion concentration fluctuated throughout a year, from $0.20 mg^{1} \cdot l^{-1}$to $1.24 mg^{1} \cdot l^{-1}$, with the July concentration of $1.24 mg^{1} \cdot l^{-1}$significantly above the MPC. Nitrites also fluctuated a lot, from $0.008 mg \cdot l^{-1}$ in December to $0.195 mg \cdot l^{-1}$ in June, with the July rate nearly four times higher than the MPC for the Class II of quality.

Concentration of nitrites, as the final form of protein substance decomposition, largely depended on the rate of oxygen dissolved in water, necessary for their oxidation, and ranged from $<0.5 mg^{1} \cdot l^{-1}$ to $0.9 mg^{1} \cdot l^{-1}$, which were regular rates. The highest concentration of orthophosphates was registered in July – $0.477 mg^{1} \cdot l^{-1}$, when the water level is the lowest, while the minimum was registered in April – $0.06 mg^{1} \cdot l^{-1}$. The oxygen rate decreased significantly at high temperatures, to $2.3 mg^{1} \cdot l^{-1}$ in July, which was almost on a verge of threatening hydrobionts. The deficit was minimal in September – $5.6 mg^{1} \cdot l^{-1}$, while in other samples the concentration ranged from $8.3 mg^{1} \cdot l^{-1}$ to $9.8 mg^{1} \cdot l^{-1}$, corresponding to the Class I. Chemical oxygen consumption was within the defined limits, ranging from $4.3 mg^{1} \cdot l^{-1}$ to $7.7\ mg^{1} \cdot l^{-1}$ O_2, which was below the

MPC and corresponded to the Class II of river waters. The oxygen regime was slightly disturbed, as in the year before.

Concentration of suspended particles was low, ranging from $1mg^{1}\cdot l^{-1}$to $24mg^{1}\cdot l^{-1}$, which was favourable and pointed to the fact that there was no heavy precipitation in the upstream section of the basin prior to the sampling.

Based on the lab testing results, the conclusion is that toxic substances from this group of parameters (detergents, heavy and toxic metals, evaporable phenols and mineral oils) were not present to a high extent in the River Ralja water and did not threaten this watercourse. Such situation was maintained for several years, which was highly favourable from the environmental aspect.

Concentration of chlorides was extremely high - $179mg^{1}\cdot l^{-1}$, most likely resulting from the geochemical structure of soil, as other tested parameters did not point to a significant influence of sanitary wastewaters. Testing of heavy and toxic metals showed only minimal presence of zinc $0.020mg^{1}\cdot l^{-1}$, copper – $0.002mg^{1}\cdot l^{-1}$ and arsenic – $0.003mg^{1}\cdot l^{-1}$, while lead, cadmium, nickel and mercury were not found in measurable concentrations, which was favourable from the eco-toxicological aspect.

Regarding organic micro pollutants, detergents based on ABS substance and mineral oils are not present, while evaporable phenols are barely detectable, which is important and favourable for the preservation of the water life.

These results indicate that the Ralja waters can be used for both supplying cattle with it and irrigation, without concern over potential negative effects, which is relatively rare with small watercourses.

According to the results of bacteria identification, the following bacteria were mainly present in the River Ralja: Esh. Coli, Enterobacter sp., Citrobacter sp. and Pseudomonas aeruginosa. It is evident that this was the case of only recent faecal pollution. Sulphite-reductive clostridia were constantly present in the Ralja water, but their volume per $1dm^3$ of water could not be determined in any of the samples, due to a strong increase of colonies, namely re-swarming of the base, pointing to the conclusion that part of organic matter deposited in the river silt was decomposed in an anaerobic process. The microbiological situation was slightly worse than in 2007.

Based on the testing results, the analysed sample was classified as the Class II of quality upon Kohl. These findings were slightly better than expected, based on results of other tests conducted in September, and did not fully match results on an annual basis. The phosphatase activity index was 1.70, corresponding to the water of the Class II-III.

Resulting from the structural and saprobiological analysis of the bed plankton and faunae and samples of the River Ralja water and sediments in the period of low-level waters, the defined saprobiological status led to the conclusion that the River Ralja waters near Umcari corresponded to the Class II and III of quality in 2011 and 2012.

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TESTING LIVING CAPABILITIES OF SEEDS OF WHEAT VARIETYS FROM BANJA LUKA (COLD TEST)

ABSTRACT

Low temperatures and drought as well as other abiotic factors, have a negative effect on a quality and amount of all grains, including wheat, which could cause great problems in near future considering that grains are foundation of nutrition in a majority of world population. Low temperatures and other stressful factors cause the forming of reactive compound inside the plant, which lead to damaging plant membranes and macromolecules. Water availability and its movement is very important in the process of seed germination, for starting seed grow and cotyledon extension. These processes are largely affected by the lands chemical potential, texture and contact area of seed and land. Two year research (2014-2015) included tracking parameters of seed germination with „cold" test in four varieties of winter wheat selected in the Agricultural institute of Republic of Srpska (Kristina, Bosanka, Orion, Jelena). The seeds used, were three or four years old. The variety Jelena had the largest percent of germination, while the variety Kristina had the longest root, as well as the largest root and coleoptiles mass. The older seeds had worst results compared to the younger ones.

Keywords: wheat, variety, germination, stress, age.

INTRODUCTION

The wheat is the most important grainy plant used in human nutrition and it is the second on the ladder of overall grain production, after the corn. Achieving stable yield does not depend only on agroecological condition of an area and genetic potential of a variety, but also on multiple other characteristics where resilience to low temperatures plays a great part (Mišić et al, 1995). Wheat is an eurotype plant, which means it has a great spread area, and it is considered that, compared to other grains, it has a waste adaptability (Briggle and Curtis, 1987), because of its resistance to cold, that is its capability to withstand temperatures lower then 1-4 °C. Since the seeds represent the beginning of plant production and provide its quality, special attention is dedicated to functioning mechanism of the seeds, regular physiological, biochemical and molecular

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analysis of the seed material, to provide insight into quality and provide solid basics for improving cultivation program, and strengthening control system in the process of seed production (Milošević et al., 2007). Seed quality is a complex feature determined firstly by its germination. Determining germination of seeds by standard germination method (ISTA, 2003.) is carried out in ideal conditions so the results of the test can only apply for optimal field conditions (TeKrony, 1995., Siddique i Wright, 2004.), and average germination often exceeds field growth (Hamman et all., 2002.). Also, the longevity of the seeds influence the vigor (ISTA, 2009).

The goal of this research was to test low and high temperature stress tolerance in the seeds germination period and the influence of the seeds age on germination, root length, coleoptile length, root mass and coleoptiles mass of the genotypes of winter wheat widespread on our fields.

MATERIAL AND METHODS

Research was conducted in 2014 and 2015. with seed of four different varieties of winter wheat (Kristina, Bosanka, Orion, Jelena) produced in 2011. Varieties were selected at Agricultural institute of Republic of Srpska. The seeds were stored three and four years in closed PVC bags in an air – dried facility. The seeds were first sterilized in 96% alcohol and then washed several times in distilled water. Only clean and healthy seeds were used in the experiment. The combination of sand and dirt of certain humidity were put in Petri dishes, and then a hundred seeds of every variety and age were put in it. The experiment was set in four repetitions. The Petri dishes were first stored in the fridge chamber at 4 °C on a 7 days period, while the humidity of the sand and the dirt was monitored. After that, the dishes were put in the dryer at 20 °C, for four days, until the end of the experiment. During the experiment the following parameters were measured, germination – germinated seeds from every Petri dish were counted and expressed in percentages of the full seeds number. Then, ten seeds were extracted from every repetition and length of roots and cotyledons were mesured (the longest root was measured). The measurements were made with elastic measure – tape, and the germ of every germinated seed was measured. The separation of roots and coleoptiles was done with tweezers and they were weighed separately. The mass of fresh roots and coleoptiles was measured with analityc scales (Adventurer). The results were processed by three-factorial analysis of variance (ANOVA) by using the SPSS 4.5 software. The relevance of the treatmants average values was tested with LSD test.

RESULTS AND DISCUSSION

The seeds germination was ranged from 73.7% to 86 % depending on variety and age of the seeds (Table 1). Jelena variety compared to Orion variety

had a much higher, and compared to Bosanka varietly significantly much higher percentage of seed germination.

Table 1. Germination of wheat seeds depending on variety and age

Year / Variety	2014	2015	Average
Jelena	86.0	78.5	82.3
Kristina	83.3	75.8	79.5
Orion	82.6	75.0	78.8
Bosanka	80.0	73.6	76.8
Average	82.9	75.7	79.3
	A	B	AxB
0.05	3.004	1.932	3.724
0.01	4.683	3.012	5.806

By increasing the storage time the seed germination of most agricultures significantly decreased (Saxena and assoc., 1987.; Andrić, 2004.), which is in accordance with our results because the percentage of germination was statistically much higher in 2014. (82.9%) than in 2015. (75.7%). If the seeds are not planted for some reasons, its germination energy and germination decrease in time. The time until the seeds start to lose its germination and i.e. time whet they still can be used for planting and production, depends on species and varieties genetic potential. Physiological aging process of the seeds begins even before sowing, and is continued during harvest, processing, storing (Milošević and assoc.,1996), as well as conservation. Big significance is added to this problem during preservation of germplasma, and appropriate technologies of seed preservation are used, where the most important factors are low temperatures and humidity, while controlling partial oxygen pressure (Ratković, 1996).

The length of the germinating root was highly depending on wheat genotype and seeds age, as well as their interaction. The average root length (Table 2) was the largest at Kristina variety (7.41 cm), and smallest at Bosanka variety (3.96 cm). The seedlings of older seeds had shorter root than seedlings of the younger ones at a level p=0,01 and p=0,05, which is in accordance with research of fodder pea seeds of different ages (Bukvić and assoc., 2007.) and red shamrock (Bukvić and assoc., 2009.).

Coleoptiles length varied between 3.76 and 4.91 cm and differed with high significance depending on variety, age of the seeds and their interaction. The Kristina variety had the longest and Bosanka the shortest coleoptiles. Surprisingly, the older seeds had longer average coleoptiles length then the younger ones.

Figure 1. Germination of Bosanka variety

Figure 2. Germination of Jelena variety

Table 2. Length of the wheat root, coleoptiles and the total length of the seedlings (cm) depending on variety and the age.

Year / Variety	2014	2015	Average
Root length (cm)			
Jelena	7.12	6.84	6.98
Kristina	7.63	7.19	7.41
Orion	6.80	6.40	6.60
Bosanka	4.12	3.81	3.96
Average	6.42	6.06	6.24
	A	B	AxB
0.05	0.0999	0.0645	01238
0.01	0.1889	0.1219	0.2343
Coleoptiles length (cm)			
Jelena	3.76	3.98	3.87
Kristina	4.91	4.78	4.85
Orion	3.96	4.25	4.10
Bosanka	4.12	4.21	4.16
Average	4.19	4.30	4.25
	A	B	AxB
0.05	0.0672	0.0432	0.1044
0.01	0.1270	0.0816	0.1574
Total seedling length (cm)			
Jelena	10.88	10.82	10.85
Kristina	12.54	11.97	12.26
Orion	10.76	13.65	10.70
Bosanka	8.24	8.02	8.12
Average	10.61	10.36	10.49
	A	B	AxB
0.05	0.5832	0.3749	0.7056
0.01	1.1022	0.7085	1.3667

The total coleoptiles length significantly depended on wheat genotype, while the age of the seeds had no statistical impact. Average seedling length was longest at Kristina variety (12.26 cm) and shortest at Bosanka variety (8.12 cm)
Wheat genotype had high significance in total seedlings length, while age of the seeds had no statistical impact.(table 2) Average seedling length was longest at Kristina variety (12.26 cm), and shortest at Bosanka variety (8.12 cm)
Wheat genotype had high significance in the mass of seedlings root, while age of the seeds had no statistical impact. The biggest root mass was found in Kristina variety (0.93 g) while Jelena variety had the smallest root mass (0.73 g).

Table 3. Mass of the root, coleoptiles and total seedlings mass

Year / Variety	2014	2015	Average
Root mass (g)			
Jelena	0.71	0.74	0.73
Kristina	0.97	0.89	0.93
Orion	0.82	0.86	0.84
Bosanka	0.93	0.87	0.90
Average	0.86	0.84	0.85
	A	B	AxB
0.05	0.0334	0.0215	0.0414
0.01	0.0631	0.0406	0.0783
Coleoptiles mass (g)			
Jelena	0.57	0.68	0.63
Kristina	0.95	0.85	0.90
Orion	0.72	0.80	0.76
Bosanka	0.85	0.81	0.78
Average	0.77	0.79	0.78
	A	B	AxB
0.05	0.0220	0.0142	0.0272
0.01	0.0417	0.0268	0.0517
Total seedlings mass (g)			
Jelena	1.28	1.42	1.36
Kristina	1.92	1.74	1.83
Orion	1.54	1.66	1.60
Bosanka	1.78	1.68	1.68
Average	1.63	1.63	1.63
	A	B	AxB
0.05	0.0545	0.0351	0.0675
0.01	0.1031	0.0649	0.1279

Wheat genotype had high significance on coleoptiles mass, while age of the seed had some statistical impact on root mass. Kristina variety had the biggest

and Jelena variety the smallest coleoptiles mass (0.90 g) and (0.63 g) respectively.

Wheat genotype had high significance on total seedlings mass, while the seeds age had no statistical impact (table 3). The Kristina variety had the largest total seedling mass (1.83 g) and the Jelena variety had the smallest (1.36 g)

The influence of low temperatures in the cold test did not have a negative effect on seeds living capability, which was established in previous tests (Vujaković i sar.,2008) that leads to conclusion that physiological damage of the seeds can be caused by extended and inadequate storage as well as by damage caused by drought or frost. The speed at which the seeds germinate can depend on genetic differences in seeds size, seedlings trait and chemical composition. The parameter analysis should be conducted inside one variety, and not between them, because each one can have different sprout growing rates.

CONCLUSION

Based on conducted research of genotype and age influence on winter wheat seeds and seedlings traits, we can conclude that all examined traits were under significant influence of variety, while the age influenced germination, root length, coleoptiles lenght and total lenght and mass of the root. Jelena variety had the largest germination, and Bosanka variety the smallest. Kristina variety had the longest root, the longest coleoptiles and total length of the seedlings, while the Jelena variety had the smallest values. Seeds germination, seedlings root length, as well as total seedlings length were bigger in seeds that were three years old, and seedlings coleoptiles length in four years old seeds. Kristina variety had the biggest root mass, coleoptiles mass and total seedlings mass, while Jelena variety had the smallest values. Bigger root mass vas in the three year old seeds, and coleoptiles seeds mass in the ones that were four years old, while age did not have any influence in total seedlings mass.

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ONE NEW MEMBER OF THE SUBTERRANEAN FAMILY NIPHARGIDAE FROM SPAIN, *NIPHARGUS SPIRITUS*, SP. N. (CONTRIBUTION TO THE KNOWLEDGE OF THE AMPHIPODA 291)

ABSTRACT

From the subterranean waters of the northern Spain, Fuente Aizpara, on the northern slope of Aizpara Mt., Zugarramurdi, Navarra, one new species of the family Niphargidae (Crustacea, Amphipoda, Gammaridea) is described and figured, *Niphargus. spiritus*, sp. n. This species is characterized by presence of scarce number of additional spines on some of pereopod-dactyls, elongated inner ramus of uropod 1 and distal article of uropod 3 in males, long spines on telson, numerous setae along outer margin of dactylus on gnathopods 1 and 2, by presence of only 1-2 setae on maxilla 1 inner plate, etc. The relation between *N. spiritus* and other known species of this genus known from Spain and France is discussed.

Keywords: taxonomy, Amphipoda, new species, *Niphargus spiritus*, Spain.

INTRODUCTION

The genus *Niphargus* Schiödte, 1849 (Amphipoda, family Niphargidae) is widely spread over Europe and Near East, on west to Great Britain and France, but in Spain is present in the northern part of Iberian Peninsula only, mainly towards French border.

The number of known taxa of genus *Niphargus* in Spain is very scarce. Margalef (1952) described a new taxon *Niphargus ciliatus cismontanus*, ssp.n. from Guipuzcoa (Aranzazu: cueva de Guesaltza). G. Karaman mentioned (1986a) *Niphargus delamarei* Ruffo 1954 from Mosquera cave (= Cova de la Mosquera), Beuda, Gerona province, and later (2015a) described *Niphargus notenboomius*, sp. n, and *N. laisi geronensis*, ssp. n.. He cited (2015b) *Niphargus gallicus* Schell., 1935 and *N. delamarei* Ruffo, 1954 for several new Spanish localities. Our recent study of material from Spain, collected by Prof. Dr. Jos Notenboom and other collectors, indicated the presence of some other taxa of this genus in Spain, related to the known species from France. Here is presented the results of study of part of these samples.

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MATERIAL AND METHODS

The studied material was preserved in 70% ethanol. Collected specimens were dissected using a WILD M20 microscope and drawn using a camera lucida. All appendages were temporarily submersed in a mixture of glycerine and freshwater for study and drawing. The body-length of examined specimens was measured from the tip of head to the end of the telson using a camera lucida. All illustrations were manually drawn in ink. After the end of the study, dissected body-parts were submerged in Liquid of Faure and covered by thin cover glass to dry.

Some morphological terminology and seta formulae follow Karaman`s terminology (Karaman G., 1969, 2012b): for the last mandibular palpus [A= setae on outer face; B= setae on inner face; C= additional setae on outer face; D= lateral marginal setae; E= distal long setae] and for propodus of gnathopods 1 and 2 [S= corner spine; L= lateral slender serrate spines; M= facial M-setae; R= subcorner spine on inner face]. Terms “setae” and “spines” are used based on shape, not origin. The studies are provided based on morphological, ecological and zoogeographical data.

TAXONOMICAL PART

Family NIPHARGIDAE

NIPHARGUS SPIRITUS, sp. n.
Figs 1-8.

MATERIAL EXAMINED:
SPAIN: - 84-6/28, Fuente Aizpara, on the northern slope of Aizpara Mt., Zugarramurdi, Navarra, coord.: XN 193917, 320 m, 16.6.1984,, 8 exp. (leg. Notenboom, J. & Meijers, I.).

DIAGNOSIS

Antenna 1 peduncular articles 1-3 progresively shorter; flagellum of antenna 2 longer than last peduncular article; outer plate of maxilla 1 with 7 spines (most of them with one lateral tooth each). Coxae short, coxa 4 without posterior lobe; propodus of gnathopods 1-2 trapezoid, with one S-spine accompanied laterally by 3 L-spines; dactylus of gnathopods 1-2 with row of setae along outer margin; dactylus of pereopods 3-5 with 1-2 strong spines at inner margin. Article 2 of pereopods 5-7 poorly to distinctly lobed; pleopods with 2 retinacula and scarcely setose peduncle; epimeral plate 3 nearly angular.

Uropod 1 in male with inner ramus remarkably longer than outer one and bearing short spines and bunches of setae; uropod 2 inner ramus slightly longer than outer one; uropod 3 with scale-like inner ramus, outer ramus with 2 long articles. In female, inner ramus of uropod 1 is slightly longer than outer one, and uropod 3 distal article of outer ramus much shorter than first article. Telson is with long distal and marginal spines.

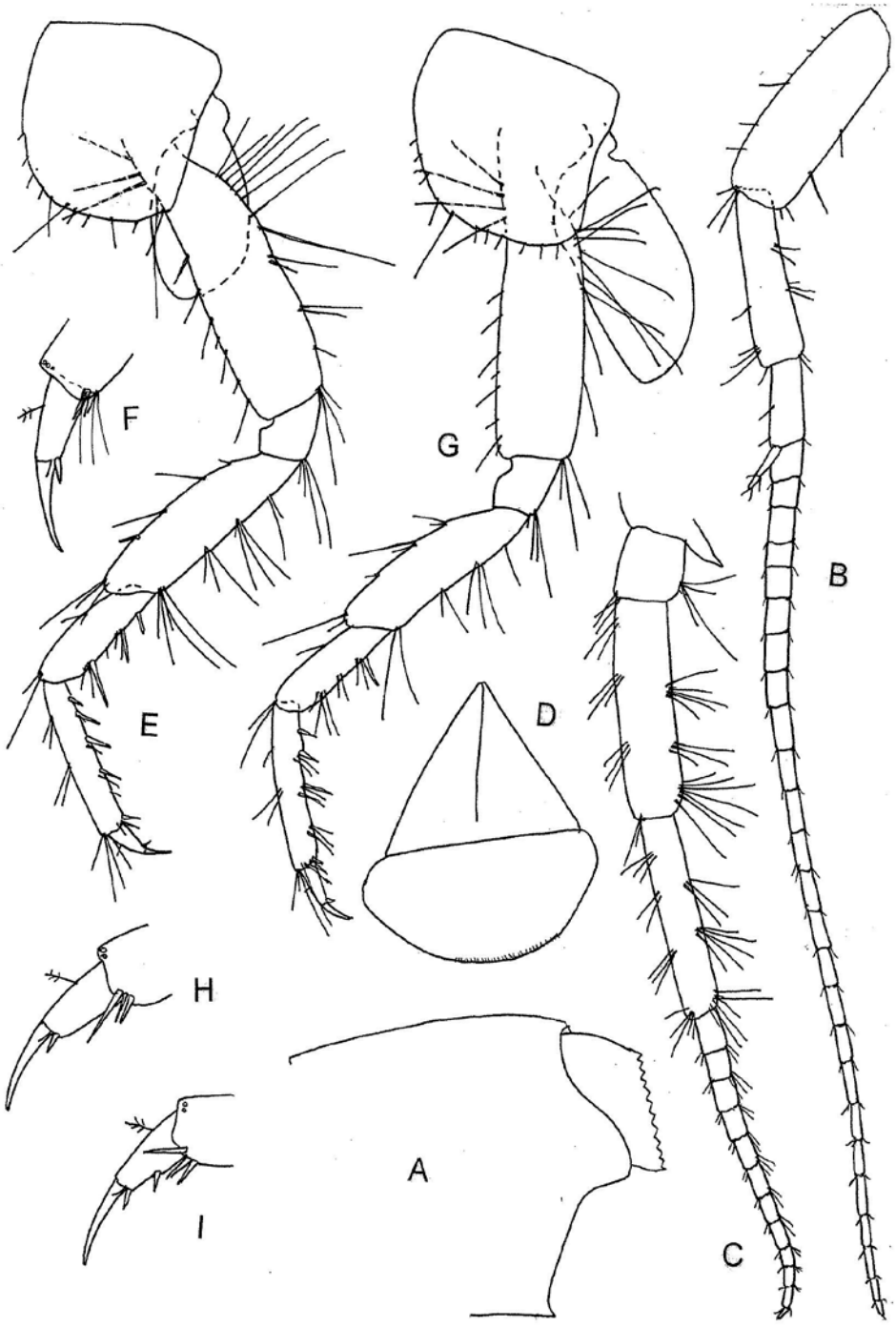

Fig 1. *Niphargus spiritus*, sp. n., Fuente Aizpara, male 12.0 mm (holotype). A= head; B= antenna 1; C= antenna 2; D= labrum; E-F= pereopod 3; G-H-I= pereopod 4.

DESCRIPTION: Male 12.0 mm (holotype): Body moderately slender, metasomal segments 1-3 at dorsoposterior margin with 8-9 setae each (fig. 5D); urosomal segment 1 on each dorsolateral side with 1 seta; urosomal segment 2 on each dorsolateral side with 2 setae, urosomal segment 3 naked (fig. 3E). Urosomal segment 1 on each ventroposterior corner with one short spine (fig. 3E). Epimeral plates 1-2 poorly angular, with marked ventroposterior corner spine-like seta and convex posterior margin bearing a row of short setae; epimeral plate 3 angular, with marked ventroposterior corner by one spine-like seta and almost straight posterior margin bearing a row of short setae (fig. 5D). Epimeral plate 2 with 1 subventral spine, epimeral plate 3 with 2 subventral spines (fig. 5D).

Head with short rostrum and short subrounded lateral cephalic lobes, ventroanterior sinus is developed, eyes absent (fig. 1A).

Antenna 1 slightly exceeding half of body (7:12), peduncular articles 1-3 progressively shorter (ratio: 60:48:23), scarcely setose; peduncular article 3 is not elongated (fig. 1B); main flagellum consisting of 25 articles (most of them with one short aesthetasc); accessory flagellum 2-articulated, shorter than last peduncular article (fig. 1B).

Antenna 2 remarkably setose (fig. 1C); peduncular article 4 poorly longer than article 5 (ratio: 58:55), along ventral margin with 3 bunches of setae (the longest setae poorly exceeding the diameter of article itself), along dorsal margin with 4 bunches of setae (fig. 1C); article 5 along ventral margin with 3 bunches of setae longer than diameter of article itself, along dorsal margin with 4 bunches of short setae; flagellum slender, remarkably longer than last peduncular article (ratio: 85:55), consisting of 12 articles bearing short setae (fig. 1C). Antennal gland cone short (fig. 1C).

Mouthparts well developed. Labrum is much broader than long, with convex distal margin (fig. 1D).

Labium broader than long, inner lobes well developed, outer lobes subrounded distally (fig. 3A).

Mandible is with triturative molar. Right mandible: incisor with 4 teeth, lacinia mobilis bifurcate, serrate, accompanied by 8 rakers (fig. 5C). Left mandible: molar without long seta, incisor with 5 teeth, lacinia mobilis with 4 teeth, accompanied by 7-8 rakers. Mandibular palpus: article 1 naked; palpus article 2 bearing 15 setae (fig. 5A); palpus article 3 falciform, longer than article 2 (ratio: 83:62), at margin with nearly 23-25 D-setae and 6 distal long E-setae (fig. 5A), on outer face with one bunch of 7 facial A-setae, on inner face by 4 groups of B-setae (fig. 5B).

Maxilla 1: inner plate narrow, with 1 distal seta (fig. 3B), outer plate with 7 spines (1-2 spines with 2 lateral teeth, one spine with 3-4 small lateral teeth, 3-4 spines with one lateral tooth (fig. 3B), palpus 2-articulated, with 7 distal setae (fig. 3B).

Maxilla 2: both plates slightly unequal long, bearing distolateral setae only (fig. 3C).

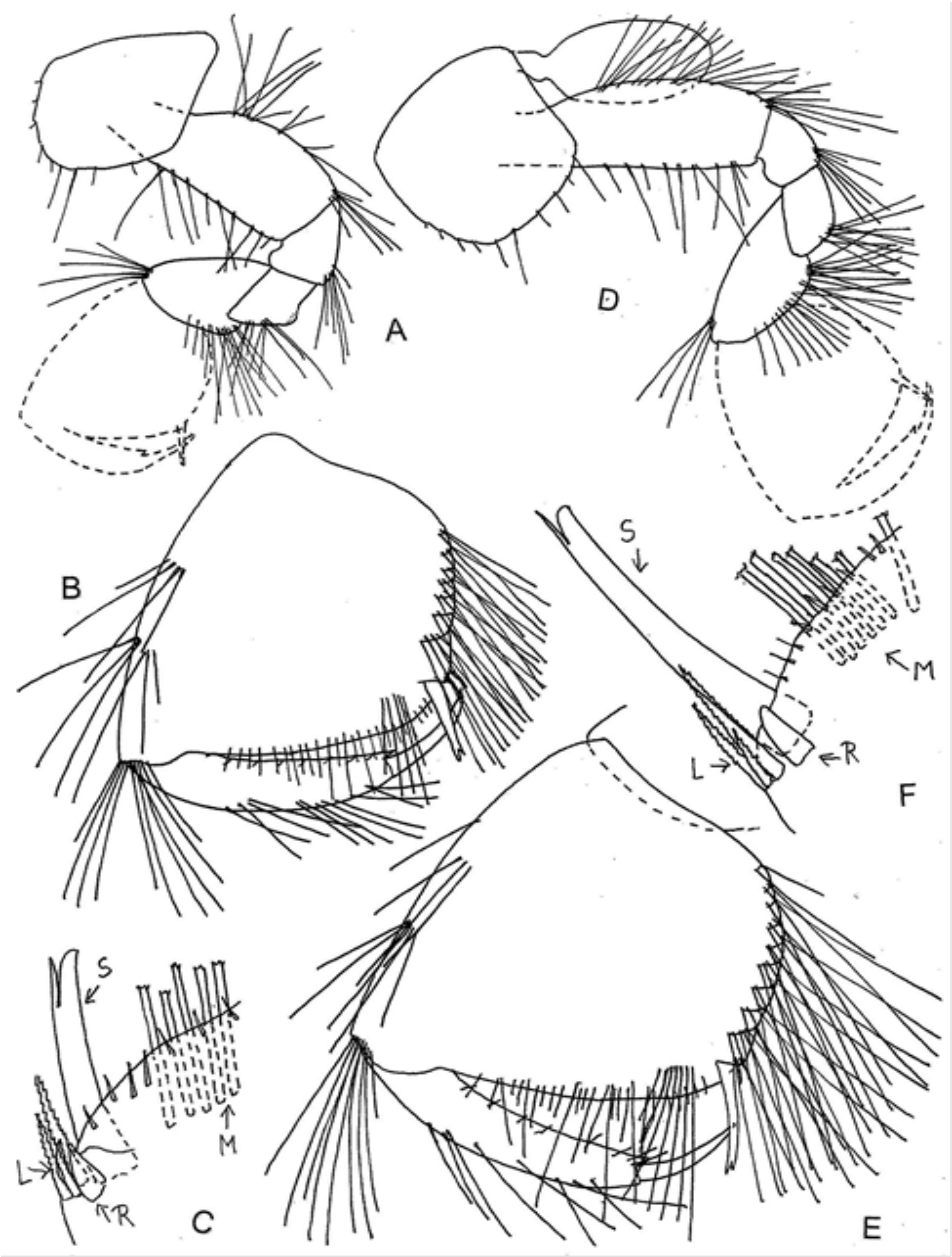

Fig 2. *Niphargus spiritus*, sp. n., Fuente Aizpara, male 12.0 mm (holotype). A-B= gnathopod 1, outer face; C= distal corner of gnathopod 1 propodus, inner face [S=corner spine; L= lateral spines; M= facial M-setae; R= subcorner spine]; D-E= gnathopod 2, outer face; F= distal corner of gnathopod 2 propodus, inner face [S= corner spine; L= lateral spines; M= facial M-setae; R= subcorner spine].

Maxilliped: inner plate short, not exceeding outer tip of palpus article 1, bearing 3-4 distal smooth spines and several setae (fig. 3D); outer plate nearly reaching half of palpus article 2, bearing a row of distolateral smooth spines (fig. 3D); palpus article 3 at outer margin with one median and one distal bunch of setae; article 4 (dactylus) at inner margin with 2 setae near basis of the nail (fig. 3D), along outer margin with one median seta.

Coxae are relatively short. Coxa 1 broader than long (ratio: 47:35), with subrounded ventroanterior corner, bearing several short and one long seta (fig. 2A). Coxa 2 as long as broad, at ventral margin with row of nearly 10 setae of unequal length (fig. 2D). Coxa 3 nearly as long as broad, with subrounded ventral margin bearing row of nearly 10 unequal marginal setae (fig. 1E). Coxa 4 hardly broader than long (ratio: 54:50), along convex margin with row of nearly 12 unequal marginal setae, ventroposterior lobe absent, posterior margin concave (fig. 1G).

Coxa 5 broader than long (ratio: 66:38), anterior lobe almost as long as coxa 4 (fig. 4A). Coxa 6 smaller than coxa 5, broader than long (ratio: 60:33) (fig. 4C). Coxa 7 entire, broader than long (ratio: 55:22) (fig. 4G).

Gnathopods 1 and 2 relatively small, almost as large as corresponding coxa (fig. 2A, D). Gnathopod 1: article 2 along anterior margin with row of long setae, along posterior margin with bunches of long setae (fig. 2A); article 3 at distoposterior corner with one bunch of longer setae (fig. 2A); article 4 with distoposterior bunches of setae; article 5 shorter than propodus (ratio: 34:50), along anterior margin with distal bunch of setae (fig. 2A).

Propodus trapezoid, longer than broad (ratio: 100:80), along posterior margin with 7 transverse rows of setae (fig. 2B); palm slightly convex, inclined nearly half of propodus-length, defined on outer face by one corner S-spine accompanied laterally by 3 slender serrate L-spines and 5 facial M-setae (fig. 2C), on inner face by 1 subcorner R-spine (fig. 2C); dactylus reaching posterior margin of propodus, along outer margin with row of single or paired setae, at inner margin with row of short setae (fig. 2B).

Gnathopod 2 only slightly larger than gnathopod 1. Article 2 along anterior margin with row of long and short setae, along posterior margin with bunches of long setae (fig. 2D); article 3 at posterior margin with distal bunch of setae; article 5 shorter than article 6 (ratio: 45:53), at anterior margin with distal bunch of setae (fig. 2D). Propodus trapezoid, slightly longer than broad (ratio: 107:97), along posterior margin with 9 transverse rows of setae (fig. 2E). Palm slightly convex, inclined nearly half of propodus-length, defined on outer face by one corner S-spine accompanied laterally by 3 slender unequal L-spines and 7 facial M-setae (fig. 2F), on inner face by one subcorner R-spine (fig. 2F). Dactylus reaching posterior margin of propodus, along outer margin with row of single and paired setae, along inner margin with row of short setae (fig. 2E)

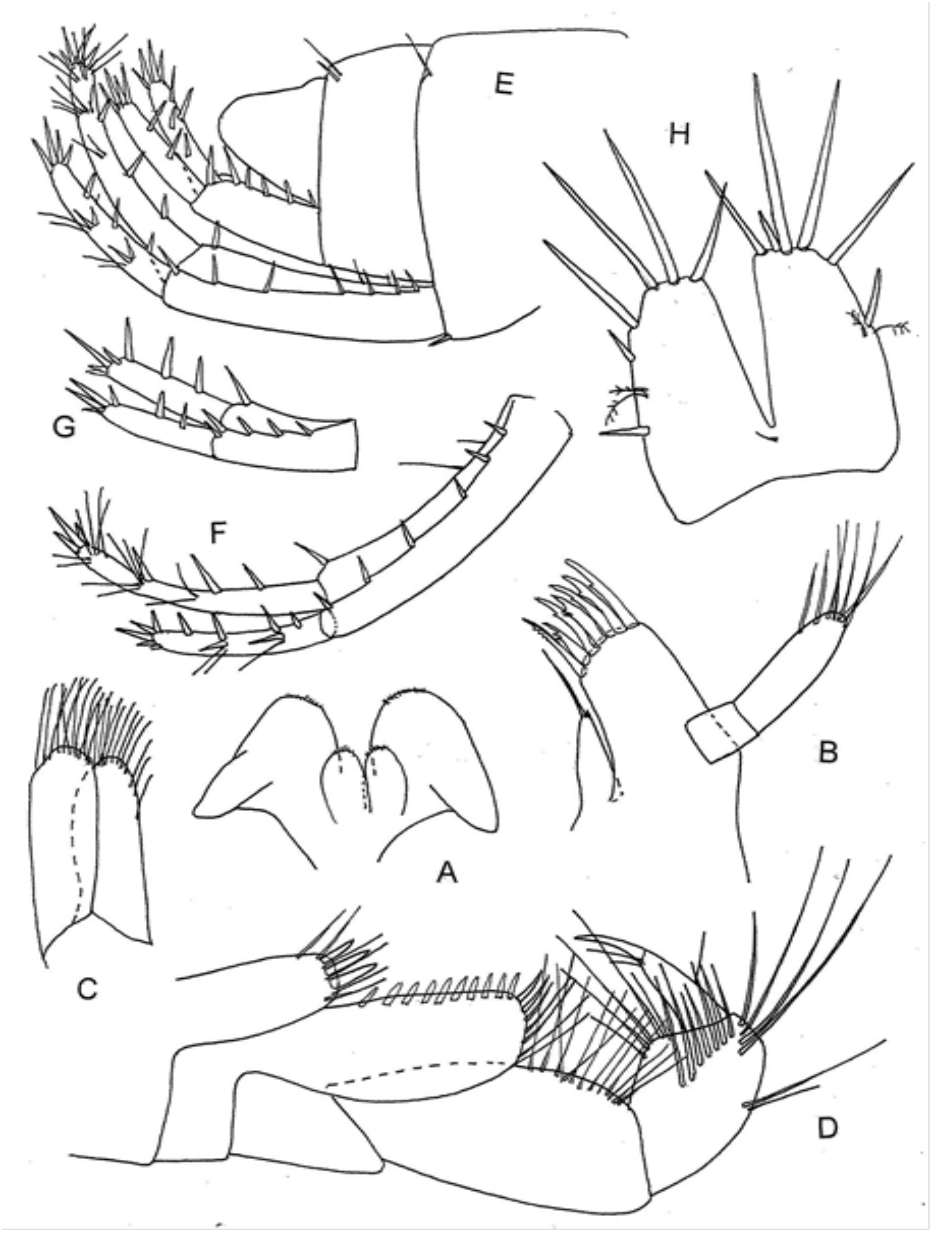

Fig 3. *Niphargus spiritus*, sp. n., Fuente Aizpara, male 12.0 mm (holotype). A= labium; B= maxilla 1; C= maxilla 2; D= maxilliped; E= urosome with uropods 1-2; F= uropod 1; G= uropod 2; H= telson.2; F= uropod 1; G= uropod 2; H= telson.

Pereopods 3 and 4 rather similar to each other, moderately slender. Pereopod 3: article 2 along anterior margin with 4 proximal long setae and 7-8 distal short setae, along posterior margin with proximal long setae and distal short setae (fig. 1E); articles 4-6 of unequal length (ratio: 55:33:44); article 3 along posterior margin with bunch of long setae; article 4 along posterior margin with 4 bunches of setae (the longest setae are much longer than diameter of article itself) (fig. 1E); article 5 along posterior margin with 3 spines mixed with single short setae; article 6 along posterior margin with 5 groups of short spines and setae. Dactylus strong, much shorter than article 6 (ratio: 19:44), along inner margin with one strong spine and seta near basis of the nail, along outer margin with one median plumose seta (fig. 1F), nail hardly longer than pedestal (ratio: 25:23).

Pereopod 4: pilosity of article 2 likes that in pereopod 3; articles 3 and 4 at posterior margin with bunch of longer setae (fig. 1G). Articles 4-6 of unequal length (ratio: 50:34:44); article 4 along posterior margin with 4 bunches o setae (the longest setae are longer than diameter of article itself); article 5 at posterior margin with short spines and setae; article 6 along posterior margin with bunches of short spines and setae. Dactylus much shorter than article 6 (ratio: 19:44), along inner margin with 1-2 strong spines (fig. 1H, I), at outer margin with one median plumose seta; nail nearly as long as pedestal.

Pereopods 5-7 moderately strong, Pereopod 5 remarkably shorter than pereopods 6 and 7; article 2 longer than broad (ratio: 76:46), along anterior margin with several longer spine-like setae, along posterior almost straight margin with nearly 11 short setae, ventroposterior lobe small (fig. 4A). Articles 4-6 of unequal length (ratio: 51:55:62), article 4 along anterior margin with 4 bunches of long setae (the longest setae slightly longer than diameter of article itself), along posterior margin with 3 short spine-like setae (fig. 4A); article 5 at anterior margin with 3 groups of spines and longer spine-like setae, along posterior margin with 3 groups of short spines; article 6 along both margins with bunches of short spines accompanied at anterior margin by single setae. Article 6 is slightly shorter than article 2 (ratio: 76:62), with distal bunch of long setae exceeding half of article 6-length.

Dactylus moderately slender, much shorter than article 6 (ratio: 21:62), at inner margin with one strong spine and seta near basis of the nail, along outer margin with one median plumose seta (fig. 4B); nail shorter than pedestal (ratio: 23:30) (fig. 4B).

Pereopod 6: article 2 much longer than broad (ratio: 90:53), along anterior margin with several longer spine-like setae (fig. 4C), along posterior hardly concave margin bearing nearly 12 short setae, ventroposterior lobe short. Articles 4-6 of unequal length (ratio: 69:75:95), article 4 at anterior margin with 5 bunches of setae, along posterior margin with 4 bunches of spines (fig. 4C); articles 5 and 6 along both margins with bunches of spines mixed with setae. Article 6 is longer than article 2 (ratio: 95:90), with distal bunch of setae much shorter than half of article 6-length (fig. 4D).

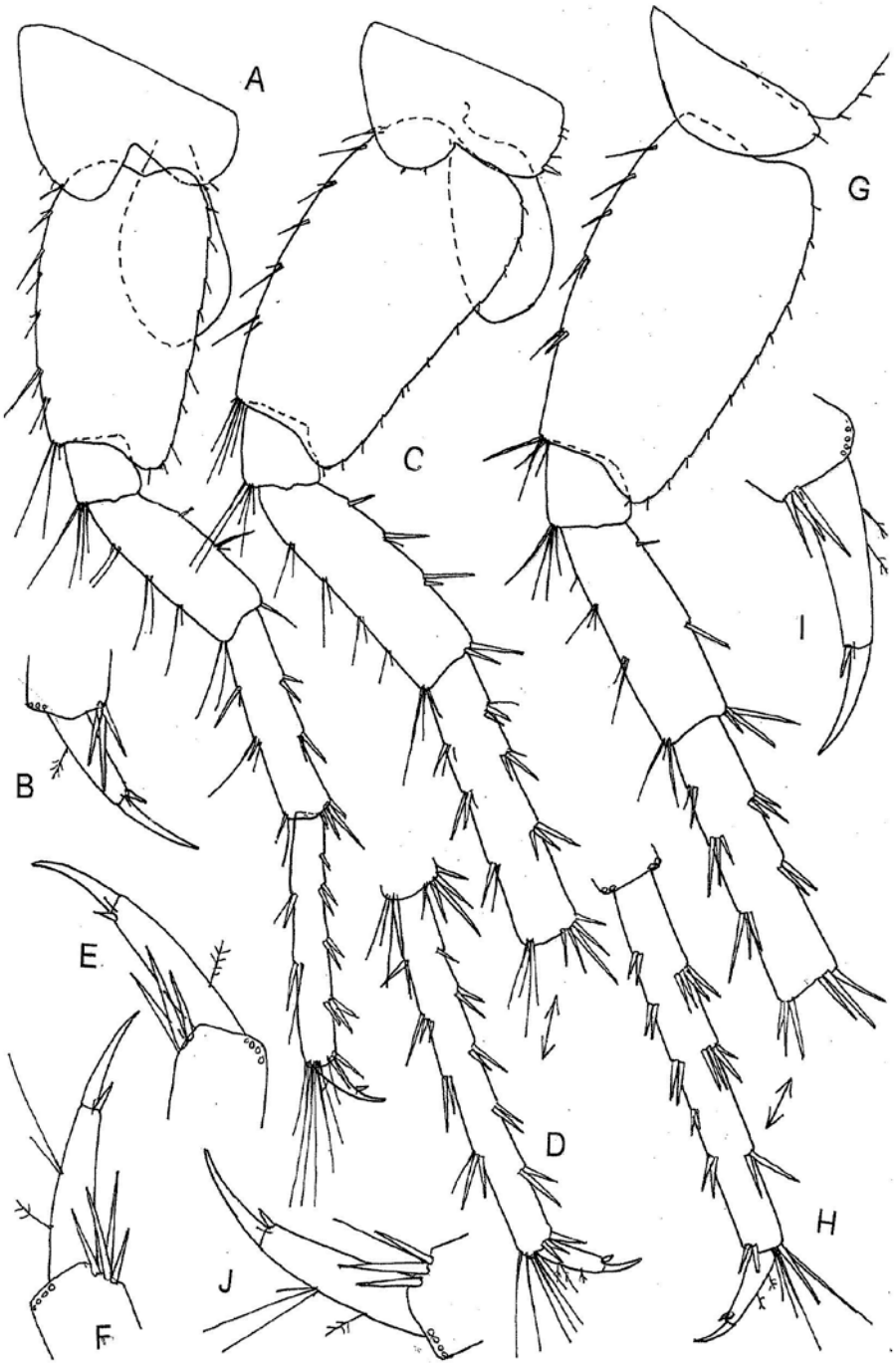

Fig. 4. *Niphargus spiritus*, sp. n., Fuente Aizpara, male 12.0 mm (holotype). A-B= pereopod 5; C-D-E-F= pereopod 4; G-H-I-J= pereopod 7.

Dactylus is moderately slender, much shorter than article 6 (ratio: 30:95), along inner margin with one strong spine and seta near basis of the nail, along outer margin with one median plumose seta (fig. 4E) and 0-1 pair of simple setae (fig. 4F); nail is shorter than pedestal (ratio: 25:50 or 28:46).

Pereopod 7: article 2 longer than broad (ratio: 90:56), along anterior margin with 6 groups of spine-like setae, along posterior slightly convex margin with nearly 13 short setae, ventroposterior lobe short (fig. 4G). Articles 4-6 of unequal length (ratio: 62:73:100); article 4 at anterior margin with bunches of setae, along posterior margin with spines; articles 5 and 6 along anterior and posterior margin with bunches of spines (fig. 4G, H).

Article 6 is longer than article 2 (ratio: 100:90), bearing distal bunch of longer setae (fig. 4H). Dactylus is much shorter than article 6 (ratio: 32:100), at inner margin with one strong spine and seta (fig. 4J), along outer margin with 2 median plumose setae (fig. 4 I) or one plumose seta and one bunch of 3 simple setae (fig. 4J); nail is shorter than pedestal (ratio: 32:48, or 29:50).

Pleopods 1-3 with 2 retinacula each. Peduncle of pleopod 1 with one distoanterior strong seta (fig. 5E); peduncle of pleopod 2 with one distoanterior seta (fig. 5F); peduncle of pleopod 3 with 3 posterior single strong setae (fig. 5G).

Uropod 1: peduncle slightly longer than rami, with dorsoexternal row of spines, on dorsointernal margin with 2 median setae and distal spine (fig. 3E, F); outer ramus bearing 4-5 distal and several lateral short strong spines, as well as 2 lateral bunches of simple setae (fig. 3F)' inner ramus much longer than outer ramus, bearing 4 distal and 3 lateral strong short spines, as well as 2 lateral bunches of simple setae (fig. 3F).

Uropod 2: peduncle with lateral and distal strong short spines (fig. 3G); inner ramus is slightly longer than outer ramus, both rami with 2-3 lateral and 4-5 distal strong short spines (fig. 3G).

Uropod 3 long and slender (fig. 5H); peduncle longer than broad (ratio: 44:17), bearing bunch of distal short spines (fig. 5G); inner ramus scale-like, much smaller than peduncle and bearing 2 distal spines and one simple seta. Outer ramus 2-articulated: first article poorly longer than second article (ratio: 136:122), bearing along both margins several bunches of short spines (fig. 5H); second article slender, along inner margin and tip provided with bunches of simple setae (fig. 5H).

Telson nearly as long as broad, deeply incised (fig. 3H); each lobe with 3-4 long distal spines; 2-3 outer lateral spines are implanted on each lobe; spines at inner margin and face of each lobe absent (fig. 3H).

Coxal gills ovoid, not reaching ventral tip of corresponding article 2 (figs. 1E, G; 2A, D; 4A, C).

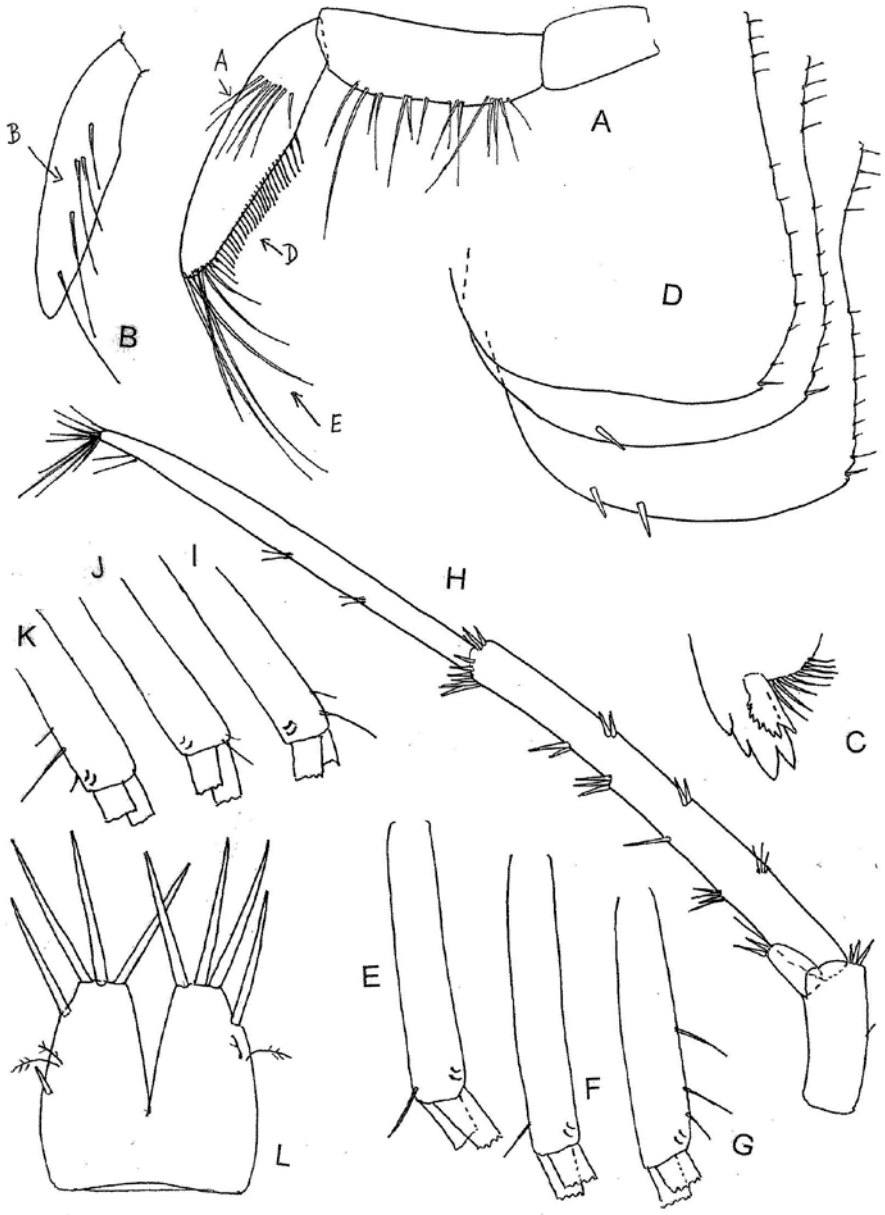

Fig. 5. *Niphargus spiritus*, sp. n., Fuente Aizpara, male 12.0 mm (holotype). A= mandible palpus, outer face [A= A-setae; D= D-setae; E= distal E-setae]; B= distal palpus article of mandible, inner face [B= B-setae]; C= right mandible incisor, lacinia mobilis and rakers; D= epimeral plates 1-3; E= peduncle of pleopod 1; F= peduncle of pleopod 2; G= peduncle of pleopod 3; H= uropod 3.

Female 7.8 mm (paratype). I= peduncle of pleopod 1; J= peduncle of pleopod 2; K= peduncle of pleopod 3; L= telson.

FEMALE 7.8 mm (paratype) with setose oostegites: Body moderately slender, metasomal segments 1-3 with 8-10 setae on each dorsoposterior margin (fig. 7E). Urosomal segment 1 on each dorsolateral side with one seta; urosomal segment 2 on each dorsolateral side with one spine and 0-1 seta; urosomal segment 3 naked.

Epimeral plates are hardly more angular than these in male. Epimeral plate 1 with ventroposterior corner marked by one spine-like seta, posterior slightly convex margin is provided with 5-6 short setae (fig. 7E); epimeral plate 2 with marked ventroposterior corner by one spine-like seta and less convex posterior margin bearing 6-7 short setae. Epimeral plate 3 distinctly angular with marked ventroposterior corner by one spine-like seta, posterior margin slightly inclined and almost straight, bearing several short setae. Epimeral plate 2 with one subventral spine, epimeral plate 3 with 2 subventral spines (fig. 7E).

Head like that in male. Antenna 1 slightly longer than half of body-length (ratio: 49:78), main flagellum with 25 articles. Antenna 2 like that in male, flagellum consisting of 12 articles.

MOUTHPARTS. Mandible: palpus article 2 with 12 setae; palpus article 3 falciform, bearing nearly 23 D-setae, 6 E-setae, one group of 6 A-setae, on inner face appear 5 B-setae (2-2-1).

Maxilla 1: inner plate with 2 setae, outer plate with 7 spines (5 spines with one lateral tooth, one spine with 2 teeth, one spine with 3-4 small teeth).

Maxilliped: inner plate with 3 distal spines; palpus article 3 at outer margin with one median and one distal bunch of setae; article 4 at inner margin with 2 setae near basis of the nail, at outer margin with one median seta.

Coxa 1 broader than long (ratio: 45:37), with subrounded ventroanterior corner and bearing nearly 11 marginal setae (fig. 6A). Coxa 2 is slightly longer than broad (ratio: 50:47), bearing nearly 9 unequal marginal setae (fig. 6C). Coxa 3 longer than broad (ratio: 65:53), bearing nearly 8 marginal unequal setae (fig. 7A). Coxa 4 hardly longer than broad (ratio: 55:53), with nearly 10 marginal unequal setae (fig. 7C).

Coxa 5 broader than long (ratio: 65:43), anterior lobe is only poorly shorter than coxa 4 (fig. 8A). Coxa 6 remarkably smaller than coxa 5, broader than long (ratio: 53:35) (fig. 8D). Coxa 7 entire, broader than long (ratio: 46:22) (fig. 8G).

Gnathopods 1 and 2 relatively small, propodus is nearly as large as corresponding coxa. Gnathopod 1 is only slightly smaller than gnathopod 2, with article 2 bearing a row of long setae along anterior margin and bunches of long setae along posterior margin (fig. 6A). Article 3 at posterodistal corner with one bunch of long setae; Article 5 is shorter than propodus (ratio: 32:48), along anterior margin with one bunch of distal long setae (fig. 6A).

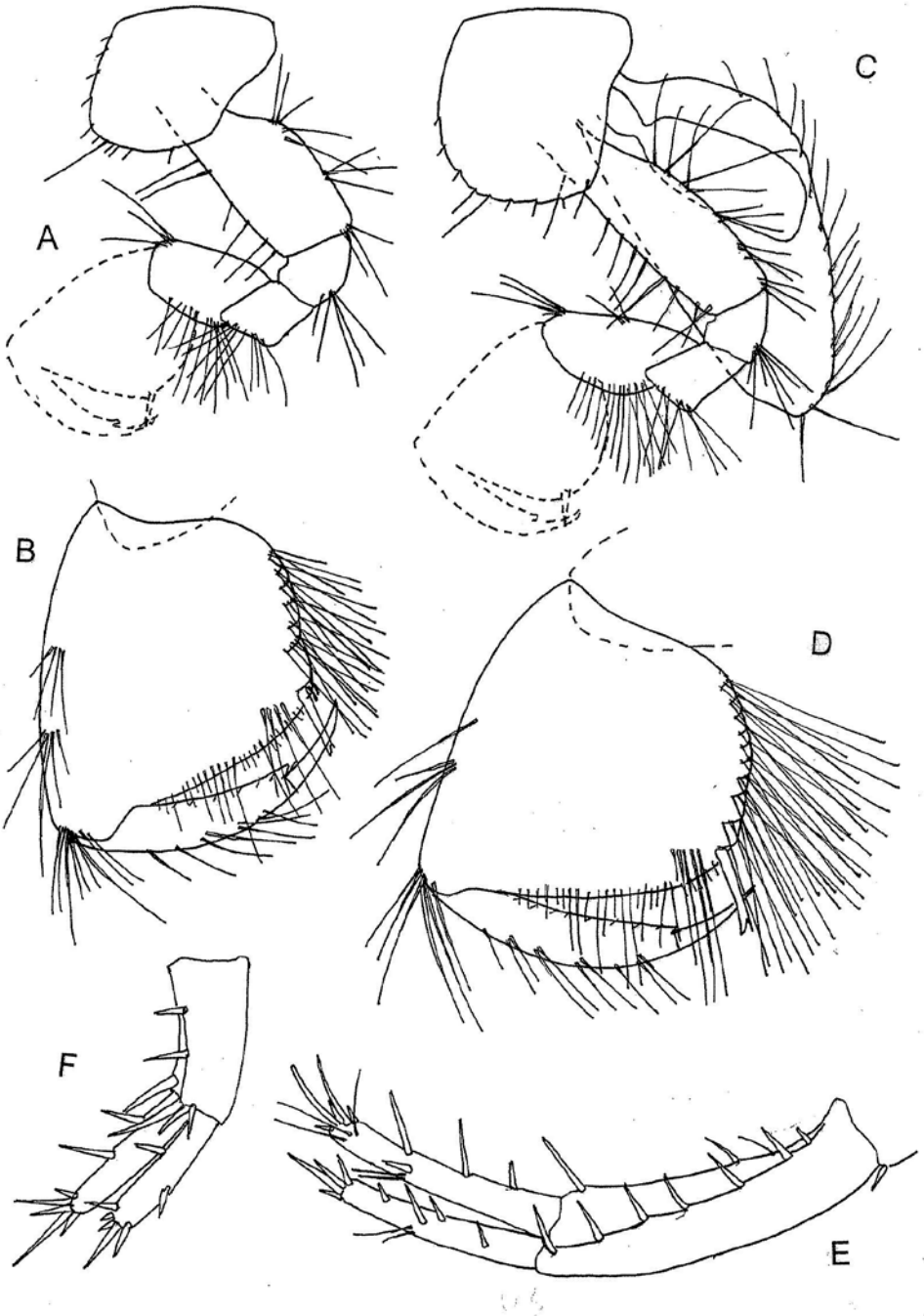

Fig. 6. *Niphargus spiritus*, sp. n., Fuente Aizpara, female 7.8 mm (paratype). A-B= gnathopod 1, outer face; C-D= gnathopod 2, outer face; E= uropod 1; F= uropod 2

Propodus trapezoid, slightly longer than broad (ratio: 85:70), along posterior margin with 6 transverse rows of setae (fig. 6B). Palm inclined nearly half of propodus-length, slightly convex, defined on outer face by one corner S spine accompanied laterally by 3 slender L-spines and 4 facial M-setae, on inner face by one subcorner R-spine. Dactylus reaching posterior margin of propodus, along outer margin are attached 8 setae, along inner margin is attached row of short setae (fig. 6A).

Gnathopod 2: article 2 along anterior margin with row of long setae, along posterior margin with several bunches of long setae; article 4 with posterodistal bunch of long setae; article 5 is shorter than propodus (ratio: 40:45), along anterior margin with 2 bunches of setae (fig. 6C). Propodus trapezoid, slightly longer than broad (ratio: 87:78), along posterior margin with 9 transverse rows of setae (fig. 6D). Palm inclined slightly less than half of propodus-length, convex, defined on outer face by one corner S-spine accompanied laterally by 3 slender L-spines and 5 facial setae, on inner face by one subcorner R-spine. Dactylus reaching posterior margin of propodus, along outer margin provided with 10 single or paired setae, on inner margin with row of short setae (fig. 6D).

Pereopods 3 and 4 are moderately slender. Pereopod 3: article 2 along anterior margins with long proximal setae and short distal setae; along posterior margin appear long setae in proximal part and shorter setae in distal part (fig. 7A); articles 3 and 4 at posterior margin with long setae (the longest setae much longer than diameter of articles themselves). Articles 4-6 of different length (ratio: 55:33:40). Article 4 along posterior margin with 4 groups of setae (the longest setae are much longer than diameter of article itself). Article 5 at posterior margin with 3 bunches of spines and short setae; article 6 along posterior margin with 5 bunches of short spines and setae. Dactylus much shorter than article 6 (ratio: 20:40), along inner margin with 2 strong spines, along outer margin with one median plumose seta (fig. 7B); nail shorter than pedestal (ratio: 32:37).

Pereopod 4: article 2 bearing along anterior and posterior margin several proximal long setae and distal short setae. Articles 4-6 of unequal length (ratio: 50:27:40); article 4 at posterior margin with 3 bunches of setae (fig. 7C); articles 5 and 6 along posterior margin with bunches of short spines. Dactylus much shorter than propodus (ratio: 20:40), along inner margin with 2 strong spines, along outer margin with one median plumose seta (fig. 7D); nail nearly as long as pedestal.

Pereopods 5-7 moderately slender. Pereopod 5 is shorter than pereopods 6 and 7 (fig. 8A, D, G), with article 2 longer than broad (ratio: 67:43), along anterior margin with several spine-like setae (fig. 8A), along posterior margin with nearly 10 short setae; ventroposterior lobe poorly visible. Articles 4-6 of

unequal length (ratio: 48:48:58); articles 2, 3 and 4 along anterior margin with bunches of long setae; articles 5 and 6 along both margins with bunches of strong spines (fig. 8A). Article 6 is slightly shorter than article 2 (ratio: 58:67), with distal bunch of long setae (fig. 8A). Dactylus is much shorter than article 6 (ratio: 21:58), along inner margin with 1-2 spines and one seta (fig. 8B, C), at outer margin with one median plumose seta; nail is shorter than pedestal (ratio: 33:50).

Pereopod 6: article 2 longer than broad (ratio: 86:53), along anterior margin with row of spine-like setae, along posterior margin with nearly 10 short setae (fig. 8D), ventroposterior lobe is short but developed. Articles 4-6 of unequal length (ratio: 63:70:91); articles 3 and 4 along anterior margin with bunches of long setae; article 4 at posterior margin with 3 single spines accompanied by single short setae; articles 5 and 6 along both margins with bunches of strong spines. Article 6 is longer than article 2 (ratio: 91:86), bearing distal bunch of long setae (fig. 8E). Dactylus much shorter than article 6 (ratio: 29:91), along inner margin with one strong spine and seta near basis of the nail (fig. 8F), along outer margin with one median plumose seta; nail shorter than pedestal (ratio: 37:67).

Pereopod 7: article 2 slightly ovoid, longer than broad (ratio: 88:56), along anterior margin with 5 groups of spine-like setae, along posterior margin with nearly 13 short setae and one small spine (fig. 8G), ventroposterior lobe short but developed. Articles 4-6 of unequal length (ratio: 59:66:95); article 4 at anterior margin with spines and setae, along posterior margin with spines; articles 5 and 6 along both margins with strong spines. Article 6 is slightly longer than article 2 (ratio: 95:88), with distal bunch of long setae.

Dactylus is much shorter than article 6 (ratio: 31:96), along inner margin with one strong spine and seta near basis of the nail, along outer margin with one median plumose seta (fig. 8 I); nail shorter than pedestal (ratio: 37:69).

Pleopods 1-3 with 2 retinacula each. Peduncle of pleopod 1 with 2 distal setae along anterior margin (fig. 5 I); peduncle of pleopod 2 with 2 distal setae at anterior margin (fig. 5J); peduncle of pleopod 3 with 3 setae along posterior margin (fig. 5K).

Uropod 1: peduncle longer than rami, with dorsoexternal row of spines; at dorsointernal margin with one spine and one seta (except distal spine) (fig. 6E). Outer ramus is slightly shorter than inner ramus, bearing several lateral and distal strong spines and one lateral pair of short simple setae (fig. 6E); inner ramus with several lateral and distal strong spines and bearing 2 median and 3 distal short simple setae.

Uropod 2: peduncle with lateral and distal strong spines (fig. 6F); inner ramus is slightly longer than outer ramus, both rami with lateral and distal strong spines (fig. 6F).

Uropod 3: peduncle longer than broad (ratio: 44:23), bearing distal spines (fig. 7F). Inner ramus much shorter than peduncle, bearing 3 distal spines (fig. 7F). Outer ramus 2-articulated: first article along outer margin with 3 bunches of strong spines and one bunch of short simple setae, along inner margin with 5 bunches of strong spines; 3 longer plumose setae are attached near bunches of spines (fig. 7F); second article much shorter than first one (ratio: 50:130) bearing along both margins the bunches of simple setae; the tip is pointed and naked

Telson as long as broad, incised nearly 2/3 of telson-length (fig. 5L); each lobe with 3 distal and one outer marginal very long spine; one short spine is attached in the middle of outer margin of one lobe; a pair of short plumose setae is attaches near the middle of outer margin in each lobe (fig. 5L).

Coxal gills not exceeding corresponding article 2 (figs. 6C; 7A, C; 8D. Oostegites are very broad, with marginal setae (figs. 6C; 7A).

VARIABILITY

Maxilla 1 inner plate with 1-2 setae in males and females; 1-2 spines of outer plate provided with 2 lateral teeth. Maxilliped inner plate is with 3-4 distal spines.

The one normal and often one additional spine are present on some of pereopod-dactyls [on pereopod 3, pereopod 4, pereopod 5], but pereopods 6 and 7 were always with only one spine at inner margin. Some specimens are without this additional spine. Evidently, the presence of additional spine on some dactyls of pereopods 3-5 is characteristic for this species, despite the fact that some specimens are without it.

Article 2 of pereopods 5-7 is with well to poorly marked ventroposterior lobe. Urosomal segment 1 in males and females is always with 1 seta only on each dorsolateral side. Urosomal segment 2 on each dorsolateral side in males is with 2 setae or one spine and seta, in females with one spine and one seta, or 2 spines. Telson is with long spines. Females have usually poorly more angular epimeral plate 3 than the males. Peduncle of uropod 1 along dorsointernal margin is with 1-2 median setae or one spine and one seta (except distal spine).

The further discovery of this species in other localities will show the limits of variability of this species.

HOLOTYPE: male 12.0 mm; paratype female 7.8 mm. Holotype and paratypes are deposited in Karaman`s Collection in Podgorica, Montenegro.

DISTRIBUTION: Known from type-locality only.

DERIVATIO NOMINIS. The name “spiritus” arrives from the Latin word “spiritus”, adequate word “spiritus” in English.

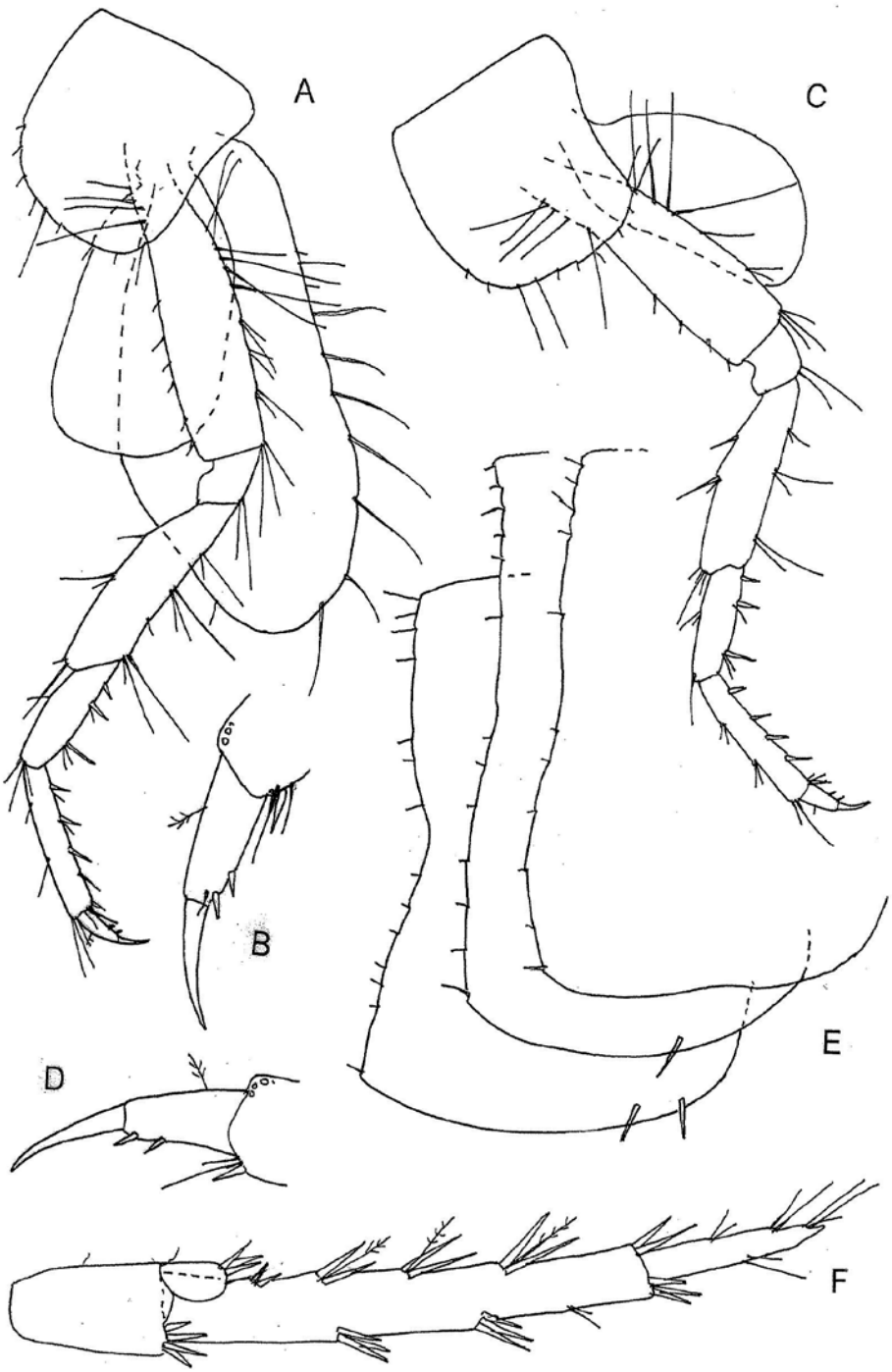

Fig 7. *Niphargus spiritus*, sp. n., Fuente Aizpara, female 7.8 mm (paratype). A-B= pereopod 3; C-D= pereopod 4; E= epimeral plates 1-3; F= uropod 3.

REMARKS AND AFFINITIES

Niphargus spiritus, sp. n. is very close to *Niphargus ciliatus Chevreux*, 1906 [Loc. typ.: Grotte de Meailles, Valee de la Vaire, affluent du Var, France]. As the description of this species was scarce, Ginet (1988 and 1991) redescribed this species more in detail, and based on this description is evident that N. spiritus, sp. n. is rather similar to *N. ciliatus* by various characters: elongated uropod 3, elongated inner ramus of uropod 1 in males, shape of pereopods 3-7 except dactyls, by pleopods with 2 retinacula, etc. But *N. ciliatus* differs from N. spiritus by strong dactylus of pereopods 3-7 bearing higher number of spines each, by inner plate of maxilla 1 bearing 2-4 setae, telson with distal and inner marginal and facial short spines, elevated number of dorsolateral spines on urosomal segments 1 and 2, slightly pointed epimeral plate 3 in male, etc.

Margalef (1952) described new taxon *Niphargus ciliatus cismontanus* from Spain [Loc. typ.: Guipuzcoa (Aranzazu: cueva de Guesaltza] [= *Niphargus cismontanus* Margalef, 1952], provided with strongly angular or almost pointed epimeral plate 3, dactylus of pereopod 7 with 4 spines, maxilla 1 inner plate with 2 setae, outer plate with 8 spines bearing one lateral tooth; telson with short spines along outer and inner margin, face and tip of each lobe.

Margalef (1970) figured partially again this species: epimeral plate 3 poorly angular, nearly like that in our specimens, telson with short spines along inner margin and tip only, and dactylus of pereopod 7 with 6 spines along inner margin.

The large species *Niphargus plateau* (= *elongatus*) Chevreux, 1901 [Loc. typ.: Nantes] differs by dactylus of pereopods 5-7 bearing one seta along inner margin only [no spines], size of male 25 mm; uropod 1 peduncle with dorsointernal row of spines, etc.

Chevreux described (1901: 173) *Niphargus plateau robustus*, nov. var. [Loc typ.: Sare Cave, Basses Pyrenees, France], and cited it also for Beaume-les-Messieurs, Jura; and “gouffre” de Padirac, Lot; grotte de Saint Mesme, Isere; Besse, Puy-de-Dome [= *Niphargus robustus* Chevreux, 1901].

Chevreux figured entire male of this species only, with rectangular epimeral plate 3, mentioning longer coxae, and elongated uropod 3 in males only, with remarks that “this variety don`t differ significantly from *N. plateau*”. On page 232 (1901) he figured uropod 3 of female from Robine with short distal article of outer ramus. France. Ginet (1991) presented several figures of this species: dactylus of pereopod 3 and pereopod 7 with 3 spines along inner margin, inner plate of maxilliped with 5 spines, urosomal segment 2 with 6 spines on each dorsolateral side, etc.

Chevreux & Fage (1925) cited *N. robustus* and figured male 21 mm from Padirac with large gnathopods 1-2 propodus exceeding the width of corresponding coxae, propodus palm inclined over half of propodus-length, and telson bearing short distal and outer marginal spines. Later nobody redescribed this taxon in detail, but based on known taxonomical characters of *N. robustus*, our specimens from Fuente Aizpara are not identic with *N. robustus*.

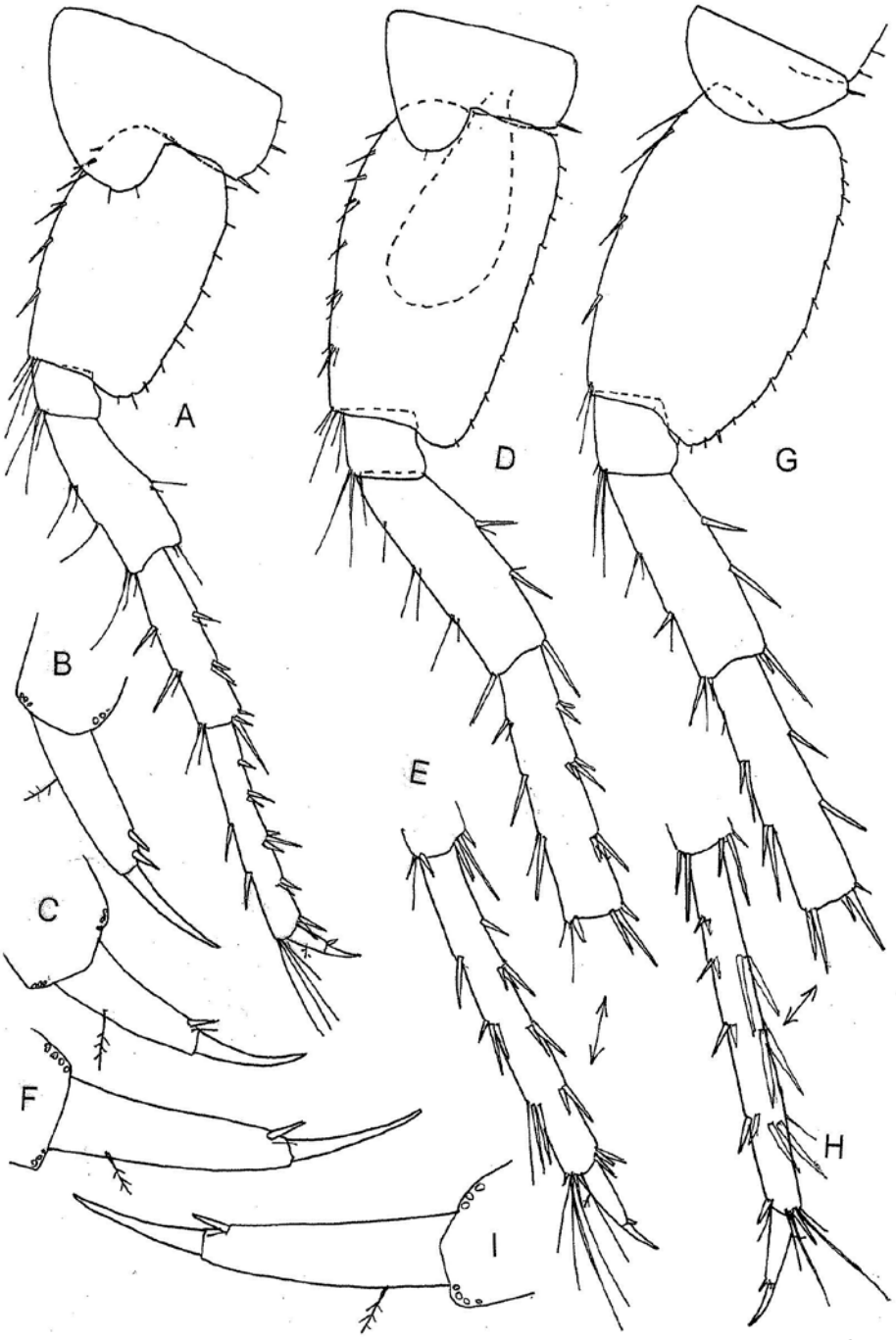

Fig. 8. *Niphargus spiritus*, sp. n., Fuente Aizpara, female 7.8 mm (paratype). A-B-C= pereopod 5; D-E-F= pereopod 6; G-H-I= pereopod 7.

Niphargus virei Chevreux, 1896 [Loc. typ.: Grotte de Arbois, de Beaume-les-Messieurs, de Baume- les-Dames, Jura, France] has long lobed coxa 4, epimeral plates strongly angular to poorly acute, telson with 2 facial and 5-6 distal short spines; maxilla 1 inner plate with 3 setae, dactylus of pereopods 3-7 with one spine, etc.

Niphargus balazuci Schellenberg 1951 [Loc. typ.: Grotte de Colombier in Valon (bank of Ardeche river, Ardeche, France] is characterized by equal rami of uropod 1 in male, inner plate of maxilla 1 with 1-3 setae, dactylus of pereopods 3 and 4 with one spine at inner margin, dactylus of pereopod 5 with 2 spines, and dactylus of pereopods 6 and 7 with 3-4 spines at inner margin, epimeral plates distinctly angular, telson bearing distal and outer marginal spines.

Karaman, G. (2016) described from northern Italy *Niphargus rotundus*, sp. n. [Loc. typ.: Well in Montelupo Albeze, 450 m about sea level (a.s.l.) (Cuneo, Piemonte], species with additional spines on dactylus of pereopods and almost subrounded epimeral plates, but this species differs from *N. spiritus* by numerous distal, marginal and facial short spines on telson, elevated number of spines on dactylus of pereopods 3-7, by stout pereopods 3-7, by large body, etc.

Niphargus sestoputeanus G. Karaman, 2016 [Loc. typ.: Sesta Godano, Passo del Rastrello, 1000 m a.s.l. (N. of La Spezia) is also provided with additional spines on dactylus of pereopods, but differs from our species by elongated inner ramus of uropod 3 in male, by pointed epimeral plates in males and females, by strong dactylus of pereopods 3-7 bearing higher number of spines, by higher number of setae on maxilla 1 inner plate, etc.

Niphargus puteanus Koch, in Panzer, 1836 [Loc. typ.: Weichelmuhle near Ratisbonne (= Regensburg), Germany] was mentioned by various authors from many localities of Europe (often erroneously), because the good description of this species was not published. Stock (1974) redescribed this species from Weichselmühle, Ratisbonne, Germany]. We compared the specimens from this locality with *N. spiritus* from Spain, and *N. puteanus* differs from our species by remarkably higher coxae, by more pointed epimeral plates, by presence of dorsointernal row of spines on uropod 1 peduncle in male, almost quadrate propodus of gnathopod 2, by higher number of setae on maxilla 1 inner plate, shorter spines on telson, etc.

The species *Niphargus rhenorhodanensis* Schellenberg, 1937 [Loc. typ.: Rumingen, N of Lorrach, Germany], redescribed by Ginet (1985), and *N. orbis* G. Karaman, 2013 [Loc. typ.: Calizzano, Alpi Ligure, Rio di Valle, Italy] are rather similar to our species, but both of them differ from *N. spiritus* by absence of additional spines on dactylus of pereopods 3-7. The most of known *Niphargus* species from France are poorly or partially described, and some of its taxonomical characters are still unknown. By this way to establish the real taxonomical relations between newly described taxa and taxa described one century ago, remains very difficult and rather hazardous. The further studies and redescription of known taxa will help in establishing of the real relationships

among various *Niphargus* taxa and understand the limits of variability of each taxon.

CONCLUSION

The fauna of the genus *Niphargus* Schiödte, 1849 (Amphipoda Gammaridea, fam. Niphargidae) is very poorly known from the subterranean waters in Spain, and only several species and subspecies of this genus have been described or mentioned from various localities of Spain. Genus *Niphargus* settled the subterranean waters in northern part of Spain only (Karaman, G. 1986b), replaced in other part of country by members of genus *Haploginglymus* Mateus & Mateus, 1958. The known members of genus *Niphargus* from Spain are related to these of France, but scarce description of many taxa from France made very difficult recognition of real relations among all known taxa in France, and consequently, in Spain also.

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GROWTH CHARACTERS AND YIELD OF DRAGONHEAD IN RELATION TO FE_2O_3 NANO-SCALE FERTILIZER AND SOWING DENSITY

SUMMARY

Dragonhead (*Dracocephalum moldavica* L.) is an annual, herbaceous, balm-scented and spicy medicinal plant of Lamiaceae family. An experiment was carried out during successive season to investigate the response of dragonhead to various plant sowing densities (10, 15, 20 and 40 cm) and iron nano-fertilizer (0, 1, 2 and 3 g lit^{-1}) applications. Iron is a necessary element for plant's growth and plants deficiency or deactivation of iron show with chlorosis of their leaves. Results of ANOVA indicated there is not any significant interaction between nano-fertilizer and sowing density. Iron nano-fertilizer levels had a promoting influence on most of growth traits and accelerated essential oil accumulation. Similarly, wider plant spacing indicated the greatest effect on some growth components. Generally, the third rate of iron nano-fertilizer (2 g lit^{-1}) combined with moderate distance between plants (15 cm) had a favorable effect on most of growth traits. The forth level of sowing density (40 cm) was the best in number of flowering branches, number of secondary branches and stem diameter traits while the second level of sowing density (15 cm) was the best in essential oil yield. Three levels of iron nano-fertilizer (0, 1 and 2 g lit^{-1}) produced long height of first flowering branch while two levels of nano-fertilizer (1 and 3 g lit^{-1}) had high number of secondary branches. The third level of iron nano-fertilizer (2 g lit^{-1}) had high values of total anthocyanins, chlorophyll b and total flavonoid traits. Two levels of iron nano-fertilizer (1 and 2 g lit^{-1}) indicated high magnitudes of chlorophyll a, total chlorophyll, flavonoid 270 nm, flavonoid 300 nm and flavonoid 330 nm traits. It can be advised that medium distance (15 cm) and the second iron nano-fertilizer level (2 g lit^{-1}), could be used for achieving high yield and essential oil performance.

Keywords: *Dracocephalum moldavica;* Essential oil; Nano-scale ferric oxide; yield component**.**

INTRODUCTION

The dragonhead (*Dracocephalum moldavica*), a member of the *Lamiaceae* family, is a grassy annual plant up to 80 cm tall. The origin of the dragonhead is

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reported from southern Siberia and the Himalaya and naturally grows in temperate areas of Europe and Asia (Galambosi and Holm 1989; Galambosi et al. 1989). It contains terpenoids with the odor of molasses, the major constituents of which are citral isomers, flavonoids, iridoids, tannins, and hydroxycinnamic and carboxylic acids (Popova et al. 2008). Dragonhead has some therapeutic properties such as sedative, tonic, antimicrobial, and wound healer and it is widely used in folk medicine as a painkiller and for the treatment of kidney complaints (Hassani, 2006). Extracts of the dragonhead are used against toothache and colds as a poultice against rheumatism, also, it acts as stimulated evolution in female animals (Chachoyan and Oganesyan, 1996). The essential oil of dragonhead ranged from 0.20 to 0.62% (Racz et al. 1978), while Hornok et al. (1990) reported that the essential oil at flowering stage reached 0.74%. Aziz and El-Sherbeny (2003) reported that the essential oil of dragonhead was characterized by a high percentage of oxygenated monoterpenes and the major components were geranial, geranyl acetate, neral and geraniol.

Environmental and agronomical factors have an important effect on the product yield and its components derived from herbs, but it was not possible to fully control these factors but can managed environmental impact with a certain methods. Among the agronomical factors, sowing density has an important role in achieving the proper conditions during the growth period of medicinal plants to achieve optimum yield; also, it is also an important factor in determining productivity (Hassani, 2006). If the planting density is greater, the environmental factors will not be enough for the plant growth and development, while, if it is less, environmental factors are not used more effectively, and leading to a decrease in the performance (Galambosi and Holm, 1991). Hussein et al. (2006) indicated that wider plant distances of dragonhead increased the herbage biomass because individual plants in wider distances had more branches and herbage yield compared to the narrow distances. Sarvari et al. (2013) reported the highest plant height, number of primary shoot, fresh weight and dry weight as well as the highest herbage yield (10.4 ton ha^{-1}), essential oil content (0.46 %) and essential oil yield (44.9 kg ha^{-1}) obtained from 40 cm planting distance.

In medicinal plants it is important to study the response to different fertilizers, since these elements may influence the essential oil content of these species such as dragonhead. Fe deficiency results in substantial yield loss due to stunted plants with pale green or yellow symptoms; also it has negative effect on other physiological activates (Wiersma, 2005). Therefore, most studies have been performed to correct Fe deficiency by applying various seed, soil or foliar Fe chelates or fertilizers. Soils of Mediterranean-type areas generally have high pH and low organic matter, and in consequence, Fe deficiency is one of the most important micronutrient disorders in such areas and Fe status of the soil affects uptake and use efficiency of macronutrients (Fageria, 2001; Rashid and Ryan, 2004; Malakouti, 2008). The employment of nanoparticles in agriculture involves the using of these particles imparting specific beneficial effects to the crops due to potential of nanotechnology for increasing the value of agricultural products

and environmental problems. Using of nanoparticles and nano-powders, researchers can produce controlled or delayed release fertilizers as well as beneficial effect on seedling growth and development (Sheykhbaglou et al. 2010; Kottegoda, et al 2011). Azarpour et al. (2013) reported foliar application of nano-iron had significant effects on saffron yield. Recent studies on nano-particles and nano-fertilizers in most crops has evidenced for enhanced germination and crop performance showing their potential for application in agriculture (Kole et al. 2013). Using of nano-fertilizer causes an increase in nutrients use efficiency, reduces soil toxicity, minimizes the potential negative effects associated with over dosage and reduces the frequency of the application so, nanotechnology has a high potential for achieving sustainable agriculture (Naderi and Danesh-Shahraki, 2013). The aim of this study was to evaluate effects of planting density and iron nano-fertilizer on essential oil percentage and some morphological characteristics of dragonhead.

MATERIAL AND METHODS

The experiment was carried out during successive season of 2014/2015 in the experimental farm, to investigate the response of D. *moldavica* to various plant densities and iron nano-fertilizer application. The experimental design was randomized complete block design with three replicates. Four levels of iron nano-fertilizer (0, 1, 2 and 3 g lit^{-1}) and four plant spacing (10, 15, 20 and 40 cm) were arranged in experimental plots. Synthesized nano particles had been characterized morphologically by scanning electron microscope (Figure 1).

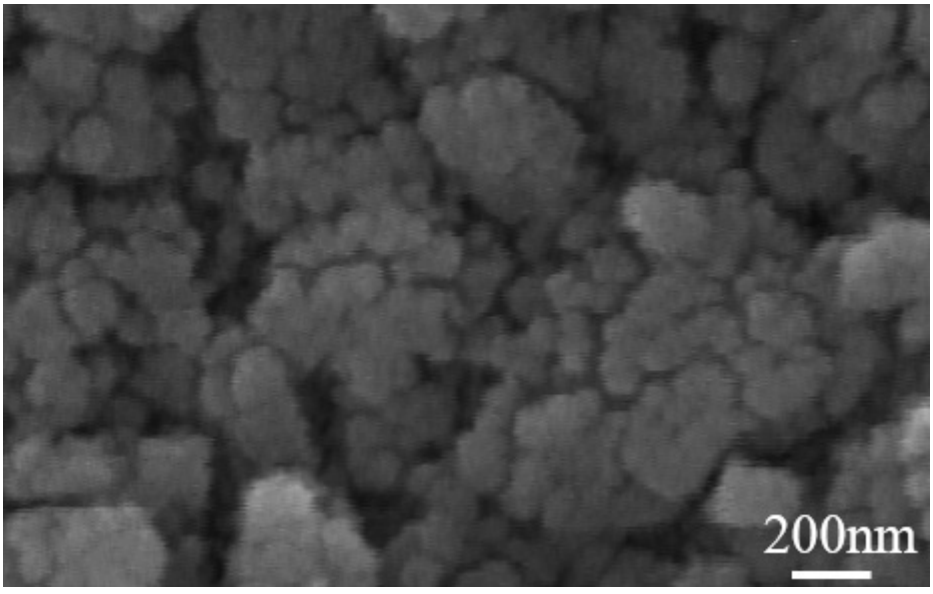

Figure 1. Scanning Electron Microscope (SEM) image of synthesized nanoparticles ferric oxide for iron nano-fertilizer.

The seeds of dragonhead were sown directly in field on 23rd of May. The physical and chemical properties of experimental soil were determined using the methods of Chapman and Pratt (1978) and the data are shown in Table 2. Four levels of iron nano-fertilizer were applied in 23rd of June at vegetative growth period. After two weeks from sowing, the plants were thinned twice, leaving one plant in hills. The plants were collected at full flowering stage in 24th of July, and the following data were recorded for plant growth characters: number of flowering branches (NFB), height of first flowering branch (HFB), Number of secondary branches (NSB), stem diameter (SD), essential oil content (EOC), dry weight kg/ha (DW), essential oil yield (EOY), total anthocyanins (TA), chlorophyll a (CA), chlorophyll b (CB), total chlorophyll (TC), flavonoid 270 nm (F270), flavonoid 300 nm (F300), flavonoid 330 nm (F330), and total flavonoid (TF).Photosynthetic pigments (chlorophyll a and b and total carotenoids mg g^{-1}) of the leaves were determined by AOAC (1990). The resulted essential oil from each treatment was dehydrated over anhydrous sodium sulfate and then subjected to GLC analysis with Varian VISTA 6000 FID model. All data obtained based on two-factor experiment in randomized complete block design was analyzed according to Snedecor and Cochran (1990), means of the traits were compared by least significant difference (LSD) test of Duncan's multiple range test (DMRT) at $p < 0.05$ level by means of the SAS 9.1 statistical software.

RESULTS AND DISCUSSION

According to analysis of variance (Table 1), sowing density was significant for number of flowering branches (NFB), essential oil content (EOC), dry weight kg/ha (DW) and essential oil yield (EOY) at 0.01% probability level while it was significant for height of first flowering branch (HFB), number of secondary branches (NSB) and stem diameter (SD) at 0.05% probability level.

Table 1. Analysis of variance for the measured morphological traits of dragonhead (*Dracocephalum moldavica*).

SOV†	DF‡	NFB	HFB	NSB	SD	EOC	DW	EOY
Block	2	0.2788^{ns}	0.29^{ns}	7.310^{**}	0.314^{ns}	0.0013^{ns}	3775956.8^{**}	17.86^{ns}
Density (D)	3	5.0167^{**}	44.52^{*}	4.427^{*}	4.515^{*}	0.0078^{**}	17656819.8^{**}	193.52^{**}
Fertilizer (F)	3	1.7273^{ns}	36.25^{ns}	5.837^{**}	0.454^{ns}	0.0060^{*}	238818.0^{ns}	20.14^{ns}
D×F	9	1.2420^{ns}	16.30^{ns}	2.531^{ns}	1.248^{ns}	0.0030^{ns}	864817.5^{ns}	11.86^{ns}
Error	30	0.8312	12.69	1.302	1.047	0.0017	599931.9	8.10
CV¶		14.7	24.4	11.8	14.2	15.3	16.2	22.1

† SOV, Source of variation; ‡DF, Degrees of freedom; ¶CV, Coefficient of variation.

**, * and ^{ns} are significant at 1 and 5% probability level and non- significant, respectively.

Traits are: number of flowering branches (NFB), height of first flowering branch (HFB), number of secondary branches (NSB), stem diameter (SD), essential oil content (EOC), dry weight kg/ha (DW), essential oil yield (EOY).

The nano-fertilizer application had significant effect on number of secondary branches (at 0.01% probability level) and essential oil content (at 0.05% probability level). The sowing density × nano-fertilizer interaction was not significant for all of the traits of Table 1, therefore, the mean comparison can be performed on main effects of the factors (sowing density as well as nano-fertilizer). The analysis of variance for the other remained traits (Table 2), showed that sowing density was significant for total anthocyanins (TA) at 0.01% probability level and for flavonoid 330 nm (F330) at 0.05% probability level.

Table 2. Analysis of variance for the pigment flavonoid traits of dragonhead (*Dracocephalum moldavica*).

SOV†	DF‡	TA	CA	CB	TC	F270	F300	F330	TF
Block	2	0.2223^{ns}	0.0054^{ns}	0.0004^{ns}	0.0041^{ns}	1314.4^{ns}	8099.3^{ns}	1937.2^{ns}	27687.3^{ns}
Density (D)	3	2.6075^{**}	0.0044^{ns}	0.0011^{ns}	0.0094^{ns}	707.0^{ns}	8697.4^{ns}	7234.3^{*}	17952.5^{ns}
Fertilizer (F)	3	1.3721^{**}	0.0302^{**}	0.0131^{**}	0.0858^{**}	11452.4^{**}	20632.1^{**}	11251.9^{**}	126263.7^{**}
D×F	9	0.2188^{ns}	0.0062^{ns}	0.0028^{ns}	0.0166^{ns}	3986.5^{ns}	3112.7^{ns}	693.0^{ns}	13257.5^{ns}
Error	30	0.2402	0.0035	0.0017	0.0080	2446.9	3909.4	1916.3	13954.5
CV¶		6.9	10.6	11.8	9.8	19.6	25.3	14.8	14.8

† SOV, Source of variation; ‡DF, Degrees of freedom; ¶CV, Coefficient of variation.
**, * and ^{ns} are significant at 1 and 5% probability level and non- significant, respectively.
Traits are: total anthocyanins (TA), Chlorophyll a (CA), Chlorophyll b (CB), total Chlorophyll (TC), flavonoid 270 nm (F270), flavonoid 300 nm (F300), flavonoid 330 nm (F330), total flavonoid (TF)

Also, application of nano-fertilizer was significant for total anthocyanins (TA), chlorophyll a (CA), chlorophyll b (CB), total chlorophyll (TC), flavonoid 270 nm (F270), flavonoid 300 nm (F300), flavonoid 330 nm (F330) and total Flavonoid (TF) traits (Table 2). Similar to Table 1, the sowing density × nano-fertilizer interaction was not significant for all of the traits of Table 2, thus, the main effects of the sowing density and nano-fertilizer factors can be compared with mean comparison tools.

Mean comparisons were performed via least significant difference (LSD) test for significant effects and via Duncan's multiple range test (DMRT) for non-significant effects. The forth level of sowing density (40 cm) was the best in number of flowering branches, number of secondary branches and stem diameter traits while the second level of sowing density (15 cm) was the best in essential oil yield (Table 3). The height of first flowering branch and essential oil content were high in the first (10 cm), second (15 cm) and third (20 cm) sowing density

while dry weight was high in the first (10 cm) and second (15 cm) sowing density (Table 3).

Table 3.Mean comparison of the measured morphological traits of dragonhead (*Dracocephalum moldavica*) for four levels of sowing density (10, 15, 20 and 40cm)

Density	NFB		HFB		NSB		SD		EOC		DW		EOY	
D1	5.95	B	16.07	A	9.17	B	6.73	B	0.265	AB	5364.3	A	14.22	B
D2	6.21	B	15.54	A	9.39	B	6.89	B	0.282	A	6003.1	A	16.90	A
D3	5.61	B	15.03	A	9.50	B	7.17	B	0.289	A	4533.8	B	12.99	B
D4	7.12	A	11.79	B	10.54	A	8.10	A	0.232	B	3193.7	C	7.36	C

Traits are: number of flowering branches (NFB), height of first flowering branch (HFB), number of secondary branches (NSB), stem diameter (SD), essential oil content (EOC), dry weight kg/ha (DW), essential oil yield (EOY).

There is not any significant differences among four levels of sowing density in chlorophyll a, chlorophyll b, total chlorophyll, flavonoid 270 nm and total flavonoid (Table 4). The first (10 cm), second (15 cm) and third (20 cm) sowing density had the high values for flavonoid 300 nm, while the first (10 cm) and third (20 cm) sowing density had the high values for total anthocyanins and the first (10 cm) and second (15 cm) sowing density had the high values for flavonoid 330 nm (Table 4).

Table 4. Mean comparison of the measured pigment and flavonoid traits of dragonhead (Dracocephalum moldavica) for four levels of sowing density (10, 15, 20 and 40 cm).

Density	TA		CA		CB		TC		F270		F300		F330		TF	
D1	7.65	A	0.538	A	0.349	A	0.889	A	250.0	A	230.8	AB	331.8	A	812.6	A
D2	6.57	BC	0.573	A	0.360	A	0.933	A	259.8	A	274.0	A	295.5	AB	829.3	A
D3	7.31	AB	0.543	A	0.341	A	0.883	A	256.3	A	265.6	AB	279.4	B	801.4	A
D4	6.94	C	0.574	A	0.362	A	0.935	A	242.4	A	218.1	B	280.1	B	740.6	A

Traits are: total anthocyanins (TA), Chlorophyll a (CA), Chlorophyll b (CB), total Chlorophyll (TC), flavonoid 270 nm (F270), flavonoid 300 nm (F300), flavonoid 330 nm (F330), total flavonoid (TF).

Mean comparisons of four levels of iron nano-fertilizer indicated that F4 (3 g lit^{-1}) had the high number of flowering branches (6.5), but it had not any significant differences with F1 and F3 (0 and 2 g lit^{-1}) treatments (Table 5). Three levels of iron nano-fertilizer (F1, F2 and F4) produced long height of first flowering branch while two levels of nano-fertilizer (F2 and F4) had high number of secondary branches (Table 5).

Table 5. Mean comparison of the measured morphological traits of dragonhead (Dracocephalum moldavica) for four levels of iron nano-fertilizer (0, 1, 2 and 3 g lit^{-1})

Fertilizer	NFB		HFB		NSB		SD		EOC		DW		EOY	
F1	6.46	A	15.98	A	9.24	BC	7.28	A	0.258	B	4769.8	A	12.35	AB
F2	5.68	B	14.07	AB	10.17	A	7.44	A	0.296	A	4916.8	A	14.43	A
F3	6.25	AB	12.38	B	8.88	C	6.98	A	0.271	AB	4825.6	A	13.29	AB
F4	6.50	A	16.00	A	10.31	AB	7.18	A	0.243	B	4582.6	A	11.40	B

Traits are: number of flowering branches (NFB), height of first flowering branch (HFB), number of secondary branches (NSB), stem diameter (SD), essential oil content (EOC), dry weight kg/ha (DW), essential oil yield (EOY).

There is not any significant differences among four levels of iron nano-fertilizer in stem diameter and dry weight. Two levels of iron nano-fertilizer (F2 and F3) showed high values of essential oil content while three levels of nano-fertilizer (F1, F2 and F3) had high amounts of essential oil yield (Table 5). The third level of iron nano-fertilizer or F3 (2 g lit^{-1}) had high values of total anthocyanins, chlorophyll b and total flavonoid traits (Table 6). Two levels of iron nano-fertilizer (F2: 1 g lit^{-1} and F3: 2 g lit^{-1}) indicated high magnitudes of chlorophyll a, total chlorophyll, flavonoid 270 nm, flavonoid 300 nm and flavonoid 330 nm traits (Table 6).

Table 6. Mean comparison of the pigment and flavonoid traits of dragonhead (Dracocephalum moldavica) for four levels of iron nano-fertilizer (0, 1, 2 and 3 g lit^{-1})

Fertilizer	TA		CA		CB		TC		F270		F300		F330		TF	
F1	6.92	B	0.492	C	0.311	C	0.800	C	224.5	B	210.9	B	271.1	C	706.6	C
F2	7.12	B	0.575	AB	0.357	B	0.934	AB	264.1	AB	252.9	AB	308.5	AB	825.6	B
F3	7.59	A	0.611	A	0.392	A	1.003	A	290.3	A	303.2	A	334.6	A	928.1	A
F4	6.83	B	0.550	B	0.353	B	0.903	B	229.7	B	221.3	B	272.6	BC	723.6	C

Traits are: total anthocyanins (TA), Chlorophyll a (CA), Chlorophyll b (CB), total Chlorophyll (TC), flavonoid 270 nm (F270), flavonoid 300 nm (F300), flavonoid 330 nm (F330), total flavonoid (TF).

In this research, the percentage of essential oil ranged from 0.21 to 0.33. The essential oil content of dragonhead show considerable variations due to plant origin, ecological and climatic conditions (Aziz et al. 2010). In Rumania, the percentage of essential oil of dragonhead ranged from 0.20 to 0.62 (Racz et al. 1978), while in Hungary, reported that the essential oil at flowering stage reached 0.74 (Halasz-Zelnik et al. 1988). In Finland, Holm et al. (1978) stated that the maximum percentage of oil of dragonhead was 0.62 during the flowering stage while in Egypt, Aziz and El-Sherbeny (2003) found that the essential oil of this plant, were characterized by a high percentage amounts (0.82 – 0.96). It can be noticed that measured traits of dragonhead were increased with iron nano-fertilizer treatments. These results may be attributed to the role of iron micro-

nutrient, in stimulating metabolic processes, encouraging growth and increasing the synthesis and accumulation of more essential oil in plant tissues. Several investigators mentioned similar results on different plants such as El-Desuki et al. (2001) on sweet fennel, Khalil et al. (2002) on Mexican marigold, Khalil and El-Sherbeny (2003) on mint, and Hussein et al. (2006) on dragonhead, who observed that application of different macro and micro-nutrients significantly improved plant growth characters. This result may be due to effect of iron nano-fertilizer on accelerating metabolism reactions as well as stimulating enzymes. This increment may be due to the effect of iron nano-fertilizer on mass production or/and oil content. Such findings were retrieved by some authors such as El-Masry and Dahab (2001) on pelargonium, and Naguib (2003) on chamomile. Concerning the effect of plant sowing density on essential oil of dragonhead, wider spaces offer ample quantity of nutrients, light and other environmental factors which in turn was reflected on the high amounts of morphological traits and essential oil content.

Concerning the effect of plant sowing density, the obtained results indicated that wide distance decreased most plant characteristics and this might be due to the rapid differentiation of cells in wide spacing than in narrow ones. Wahba and Ezz El-Din (2002) on *Chrysanthemum coronarium* and Hussein et al. (2006) on dragonhead reported similar results, while opposite trends were found by Zayed et al. (2003) on borage plant. Also, number of flowering branches (NFB) was increased with increasing the distance of plants and similar finding was reported by different authors such as Das et al. (1992) on black cumin and Hussein et al. (2006) on dragonhead. Moreover, morphological traits such as dry weight (DW), demonstrated that the widest distance did not result in highest dry weight of herb but some authors found the promotion effect of wider plant spacing on vegetative growth characters such as Belyaonka et al. (1997) on chrysanthemum and Wahba and Ezz El-Din (2002) on *Chrysanthemum coronarium* plants. This promoting effect of the widest distance on growth characters such as number of secondary branches (NSB) and stem diameter (SD) may be due to increment in the amount of nutrients uptake or/and getting more quantity from solar energy for plant.

The effect of the interaction treatments between iron nano-fertilizer levels and plant sowing density was not observed. When calculating the yield performance in a unit area, the narrower spaces will contain high number of plants and consequently more yield will be obtained and the heavier plants obtained in the wider spaces in the same unit area may reach the yield obtained in the narrower distance (Hussein et al. (2006). In the present investigation a positive relationship between increasing plant sowing density (D2 treatment) and essential oil yield was found; the same finding was reported by El-Sherbeny et al. (2005) on ironwort and Sadek et al. (1992) on rosemary (El-Dean and Ahmed, 1997). Data obtained indicated that iron nano-fertilizer application up to 2 g lit^{-1} resulted in increasing chlorophyll a and b, and total anthocyanins, so photosynthetic pigments showed almost similar trend of some other growth traits.

This finding might be reasonable since the improvement of growth traits is mainly a result of stimulation in photosynthetic apparatus that leads to more photosynthesis and food reserve which, the beneficial effects of different fertilizer application on the accumulation of photosynthetic pigments were previously observed by El-Ashry et al. (1997), Khalil et al. (2002) and Khalil and El-Sherbeny (2003). Plant spacing had not the same effect of iron nano-fertilizer application on photosynthetic pigments where wider plant distance dose not causes an increase in amounts of pigments but the promoting effect of wider distance on photosynthetic pigments was reported (Mohamed and Wahba, 1993; El-Sherbeny et al. 2005).

Finally, we found that, most of the measured morphological traits as well as biological pigments (chlorophylls and anthocyanins) were decreased with increasing of plant sowing density. This findings could be due to shading of top leaves and early senescence of the shaded leaves. Application of F3 iron nano-fertilizer (2 g lit^{-1}) is caused to increase of biosynthesis and stability of biological pigments, but had deterrence effects in higher concentrations (F3 or 3 g lit^{-1}). It could be declared that 2 g lit^{-1} of iron nano-fertilizer is the critical level of its application in dragonhead. In conclusion, it could be concluded that under Iran environmental condition, the application of iron nano-fertilizer at level of 2 g lit^{-1} to dragonhead (*Dracocephalum moldavica*) plants cultivated at 15 cm distance between plants is recommended for good plant growth as well as more essential oil content, which lead at the end to improving the productivity of dragonhead.

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ARABLE LANDS DEGRADATION IN THE NORTHERN STEPPE ZONE OF UKRAINE

SUMMARY

The case study is devoted to the agroecological peculiarities of the eroded soils fertility taking into account their genesis and morphology. The researched soils are located on the slopes of different exposure in the subzone of ordinary black mollisols. The laboratory and field observation of the eroded soils was carried out on the base of training farm "Samarsky". The research of quantitative and qualitative composition of physical and chemical qualities 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. On their genesis, the slope soils are represented by the variety of watershed types of black mollisols. These soils are considered as special more arid soils additionally superimposed with erosion process. They represent genetic forms of intrazonal and zonal types. The humus content in the 0-30 cm layer in the plain amounted to 4.1 %, while decreasing on the northern exposure slope by one third, and on the southern exposure slope – twice. The qualitative humus change was observed: the humic acid content decrease when insignificant change of fulvic acids.

Keywords: degradation, soil, slope, genesis, fertility, humus

INTRODUCTION

As it is known, a significant part (8 %) of the mollisoils world reserves is concentrated in Ukraine. The share of these lands in Ukraine is about 36 % [Anisimova et al., 2009]. There is a growing need for crop production and animal husbandry to ensure preservation of fertility for the plain and slope lands. The black soils degradation processes development in Ukrainian Steppe was fixed from the end of the 19 century [Dokuchaev,1983;, Kovda, 1989]. It was shown that rain and melt water have an impact on the soil physical properties. As a result of soil loss the first thing to lose is the silt fraction, soils most valuable part. It was mentioned the fight against erosion is of high priority taking into account the risk of drought. That is why special attention has to be given to the winter soil moisture conservation, the study of washout and leaching processes.

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

The black soils are known to be spread in several countries of the world. Most of the world′s mollisols occur in three regions of the northern hemisphere and one region - the south of the equator, the Parana-La Plata basin of South America. Mollisols are known in other soil classification systems as Chernozems, Kastanozems and Phaeozems and Isohumosols or Black soils (Artigas,2010; Liu X et al.2012). On a world-wide basis, soil scientists generally speak of four main major regions of Mollisols . One is located in the central North America radiating across the central plains of the United States and the southern Canada. The next two regions appear as a discontinuous belt which extends across the southeastern Europe and the central Asia (Liu X et al., 2012). The western belt begins in the sub-humid steppes of the south-central Europe and extends across Russia and into the eastern belt, which is best represented in the Northeast China (Kravchenko, 2009). Mollisols distribution strongly correlates with subboreal geographic belt, humid, semi-humid, and semi-arid zones, forest-steppe and steppe ecosystems, loess sediments. However, their spread is not limited to those areas. The Ukrainian and the Chinese black soils keep together 16.8% of the World Mollisols and play the global role in the food security of both countries and the European-Asian continents in whole. It was established that some territories occupied by black soil in Ukraine and China have common features and problems (Kravchenko, 2009).

The factors affecting the erosion processes development can be divided into two groups: natural and anthropogenic. Natural factors include topography, geological conditions, climate, vegetation and physico-chemical properties of the soil (Spalevic et al., 2012; Spalevic et al., 2015). Rainfall and topography are the natural (abiotic) factors that determine the possibility of flow formation – the direct causes of erosion.

Climatic conditions, in many cases, provoke the erosion processes development. Precipitation has direct impact on the erosion process development. Such climatic factors as temperature, humidity, and winds have an indirect influence. Their indirect impact may occur in soil moisture level and may affect the conditions of runoff formation and erosion manifestation.

The spread of erosive processes are considerably influenced by rugged landscape. The morphological parameters of the relief, which determine the intensity of erosion processes are the depth of local bases of erosion, dismemberment of the territory of the gully network, the size of the watershed, the slope’s length and exposure.

The intensity of soil loss increases with the growth of the length, steepness of slopes, and changes in exposure. The slopes of the opposite exposures vary greatly in the amount of incoming solar radiation, water runoff and soil loss, temperature, water and nutrient regimes of soil. This eventually affects the soil fertility and crop yields (Kharytonov et al, 2004) than the northern ones. Erosion losses of humus in the soils of the southern slopes reach 60 %, and in the northern – only 10 – 20 % of total losses.

In our research we set the following goals: a) to study the genesis of the eroded soil and changes of morphological characters under the abiotic factors impact; b) to see differences in the formation of soil profile genetic horizons fertility 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 training farm located in the Dnipropetrovsk oblast in the northern part of the steppe zone of Ukraine (Kravchenko, 2010). Soil for controlled experiments and laboratory analysis was sampled from the same location. The farm coordinates are: 48°30'N lat. and 35°15' E long. The field station was used for many years as an area for intensive agricultural production and research (Kharytonov et.al.,2004 Kharytonov et.al,2009). It is located far away from the city of Dnipropetrovsk (25-30km) enough to avoid industrial pollution effect (Anisimova et al., 2009).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°, the other two have the slopes up to 3° 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. 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 (Kravchenko, 2010). In keeping with the lower productivity status of the eroded soils, fertilizer was not applied, but the two years of alfalfa just before wheat promoted reasonable growth. 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 (Dokuchaev, 1883, Kovda, 1989, Kravchenko,2012). These are defined as follows (with correspondence to the Ukrainian soil taxonomy system noted in parentheses:

A (H) - upper horizon with very prominent humus accumulation
B_1 (Hp) - upper transition horizon, with considerable humus
B_2 (Ph) - lower transition horizon, with little humus
C (P) – underlying parent material

The A, B1, B2 and C horizons encompassed depths of 0-40, 40-60, 60-80 and 80-100 cm for E0; depths of 0-30, 30-50, 50-70, and 70-100 cm for E1, and depths of 0-30, 30-50, and 50-100 cm for E2 (with the A horizon absent entirely due to erosion).

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 Statistic assessment of eroded soils indexes spatial distribution in ploughed topsoil was made.Particle size determinations were made as follows. First, 1 g of soil was treated with room temperature HCl, which dissolves most carbonates, although a small amount is left bound to cell particles. Upon drying, the dissolved carbonates escape as CO_2, and the loss of weight is recorded. The remaining soil is mixed thoroughly with 1 liter of water and allowed to precipitate. At designated time intervals, aliquots were removed and per cent of specific particle sizes was estimated based on sedimentation of progressively smaller particles. Fractions determined were (diameter in mm in parentheses): medium to coarse sand (0.25-1), very fine to fine sand (0.05 - 0.25), coarse silt (0.01 - 0.05), fine silt (0.005 - 0.01), silt/clay (0.001 - 0.005) and clay (< 0.001). Values for fractions were summed to correspond with USDA definitions of sand (> 0.05), silt (0.002 - 0.05) and clay (< 0.002). Since the silt/clay fraction as measured (0.001 - 0.005) spanned the USDA definition, half of the silt/clay fraction was considered silt and half considered clay, since clay particles smaller than 0.001 were more prevalent than particles of fine silt. Allocating one-half of the silt/clay fraction to each had little influence on the overall proportion of clay vs. silt, since the fraction in question averaged 6% of the total soil weight, and was in no case greater than 10%. Physical clay (clay(FSU)) is a standard definition in the Former Soviet Union (Dokuchaev Soil Institute, 1965), and is the summation of particle sizes less than 0.01 mm diameter (clay and fine silt by USDA classification), plus the per cent lost by HCl pre-treatment, which presumably was fine-particle carbonates. Clay(FSU) is reported here in addition to the USDA categories in order to facilitate comparison of our results with other FSU results (Kharytonov et al., 2004).

Carbonates were determined from the loss of mass that occurred as a result of HCl treatment. A sample of 1g of finely ground soil was mixed with 40 ml of hot 0.1 N HCl d weighed after drying. The loss of mass was expressed as a percentage of the initial 1 g used. A pH meter was used to determine pH after placing 20g of soil in 50 ml of water (pH(H_2O) or 50 ml of 1N KCl aqueous solution (pH(KCl) and mixing thoroughly. Humus concentration was determined by placing 0.1 g of soil in 10 ml of 0.4 N K_2Cr_2O aqueous solution, followed by titration with salt of Moore ($FeSO_4(NH_4)_2SO_4$). Humic acid and fulvic acid were determined by extraction followed by titration.

RESULTS AND DISCUSSION

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 neighboring northward and southward latitudes.

The slopes' soil is a special, more arid soil which is under additional erosion impact. On the one hand eroded soils are formed under very specific conditions, on the other hand zonal conditions are also involved in the process of soil formation. That is why we consider the eroded soil as a separate genetic group of soil zone where they were formed. The main soil genetic group of the steppe are ordinary low humus content loess and loamy like loam. In the Dnipropetrovsk oblast their area is 1 million 280 thousand hectares (Anisimova et al, 2009).

The soil erosion severity, the steepness and aspect of slope greatly affect the soils physical properties. The intensity of many processes of the soil profile formation greatly depends on the soil texture. The silt and clay fractions profile distribution in arable soils of the plain, the slopes of the northern and the southern exposure are shown in the figure 1.

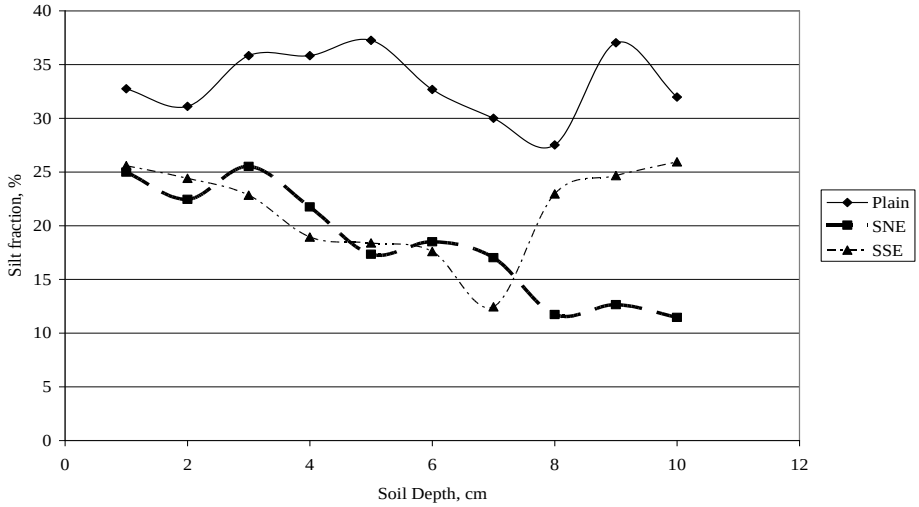

Fig.1. The silt fractions profile distribution in arable soils

The data obtained from the comparative analysis fixed changes in the content of soil particles depending on the environmental conditions. The texture of the arable soil in the plain is defined as silt-heavy loam. The content of fraction < 0.01 mm varies from 52,57 up to 53,96%. The content of silt fraction (< 0.001 mm) was 27,51 – 37,25 % .

The silt content is reduced to 11,46– 25,94 % under the run-off impact in the first meter of soil on slopes. The eroded soil fertility is closely linked to the absorption capacity. It is known that the absorption capacity is directly dependent on the soil texture and the humus content. The absorption capacity data in the arable soils profile are given in the figure 2.

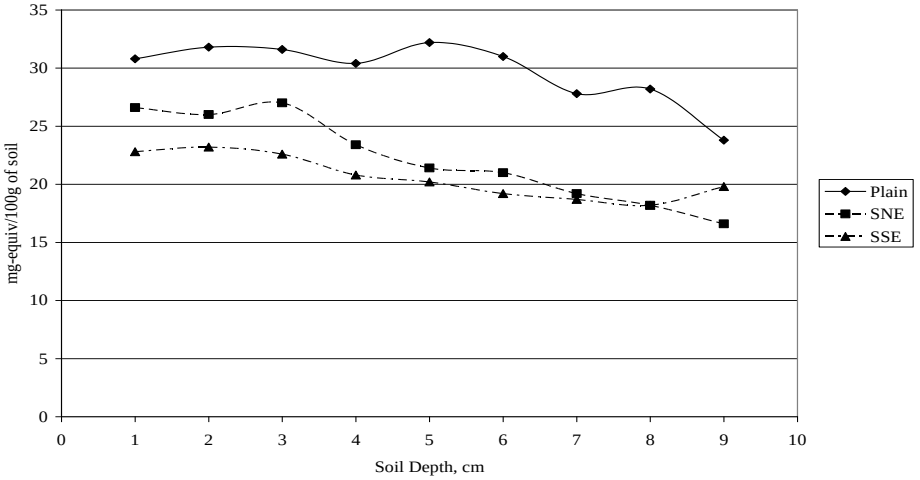

Fig.2. The absorption capacity distribution in soil profile of arable lands

The absorption capacity in the arable layer of 0-30 cm soil profile was at the level of 31.4 meq/100 g of soil. This index decreasing is 18% (on the northern slope) and 37 % (on the slopes of the southern exposure) on eroded arable soils.

It was found that calcium has a large share in the amount of absorbed cations. The calcium content under the influence of runoff decreases with the depth. The maximum content of magnesium is observed in non-eroded black mollisols, especially at the depth of 40-80 cm. The decrease of total amount of absorbed cations (ca+mg) along the soil profile is 42 % (from 292 to 206 meq /100 g of soil). However, non-eroded soils have a high absorption capacity. It is explained by a significant amount of silt fraction.

Next data portion on carbonates distribution in the soil profile of arable lands is shown in the figure 3.the upper soil horizons washout and proximity to the land surface bottom leads to increase of carbonates content the soil surface. That is why the occurrence of carbonates on the slopes of the northern exposure is deeper than on the southern slopes. It was fixed the uptake of the carbonate horizon of medium and severely eroded soils to the surface. In other words, the deterioration of the soil material on these eroded soils dominates in comparison with the flow of the soil formation process. In accordance with data obtained, the content of carbonates in the 0-10 cm layer increased from 0.2 % (plain) to 7.0 % (the slope of the southern exposure), and in the layer of 0-30 cm from 1.4 to 8.8 %, respectively.

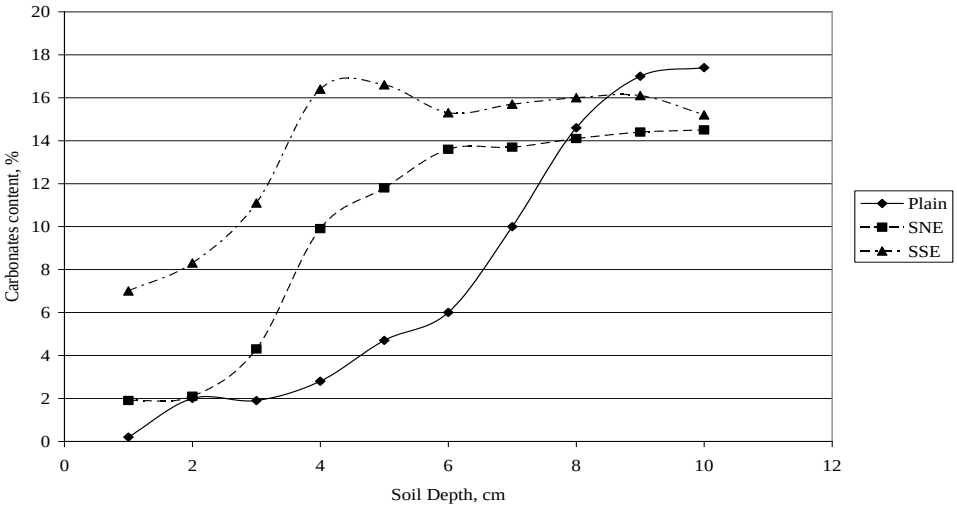

Fig.3. The carbonates distribution in soil profile of arable lands

It should be noted that the amount of carbonates in the layer of 0-10 cm of the northern exposure slope corresponds to the layer of 20-30 cm and the soil slope of the southern exposure of the 50-60 cm layer from the soil profile. These data confirm the regularity of carbonates displacement in the profile. There is a significant difference in their content in non-eroded and eroded to different degrees soils.

The distribution of carbonates in the soil profile allows to make a conclusion about the peculiarities of soils' wetting and the depth of moisture admission. According to calculations the stock of carbonates in the soil layer of one meter thick is washed away from 1262 t/ ha to 1715 t/ ha. It is 1.4 – 1.9 times greater than in non-eroded black mollisols. Thus, the amount of carbonates increases significantly with increasing of erosion severity. This is one of the characteristic features of eroded soils of the steppe landscape. It is known that the soils' structure, their absorbed capacity, the nutrients mobility and the intensity of biological processes are closely related to the pH value. Erosion processes significantly affect the soil pH value. The pH value in the plain arable land in the layer of 0-20 cm thick equals 7.1. This value reaches up to 8,2-8,6 in eroded slope. Soil fertility is a multifactorial and dynamic phenomenon. The level and the ratio of the soil regime is also under the impact of erosion. The buffering capacity of the soil organic matter provides the basis of its fertility, as the most important evaluation criterion.

According to our research of E0 the humus content in the layer of 0-10 cm thick was 4.25 %, 10-20 cm – 4,05 %, 20-30 cm and 3.9 %, declining at a depth of 70-80 cm to 1.4 %, and 90-100 cm to 0.6 %. At the time, as in E1 (the northern exposure slope) these indices were 3,26; 2,9; 2,8; 0.7 and 0.5 %; in E2 (the southern exposure slope) – 2,0; 1,9; 1,8; 0.2 and 0.1% respectively (Table 1).

Table 1.The content and reserves of humus in arable lands.

Soil depth, cm	E0			E1			E2		
	%	t/ha	C, %	%	t/ha	C, %	%	t/ha	C, %
0-10	4.25	51.0	2.50	3.26	39.0	1.90	2.00	24.0	1.18
10-20	4.05	486	2.38	2.90	34.8	1.71	1.90	22.8	1.11
20-30	3.90	46.8	2.29	2.80	33.6	1.65	1.80	21.6	1.06
30-40	2.90	34.8	1.79	2.70	32.4	1.59	1.90	22.8	1.11
40-50	2.20	26.4	1.29	1.50	18.0	0.88	1.10	13.2	0.65
50-60	2.00	26.0	1.18	1.20	15.6	0.71	0.60	7.2	0.35
60-70	1.90	24.7	1.12	0.90	11.7	0.53	0.50	6.5	0.29
70-80	1.40	18.2	0.82	0.70	9.1	0.41	0.20	2.6	0.12
80-90	0.60	7.8	0.35	0.80	10.4	0.47	0.20	2.6	0.12
90-100	0.60	7.8	0.35	0.50	6.5	0.29	0.10	1.3	0.06
0-100		292,1			211,1			124,6	

The reserves of humus in 0-100 cm layer decreased from 292,1 t/ ha (E0) to 211,1 t/ ha (E1) and 124,6 t/ ha (E2) accordingly because of the washout impact. A clear reduction of stocks of humus is observed in the topsoil (0-30 cm): 146,6 t/ ha to 107.4 and 68,4 t/ ha, representing 36 % and 2.1 times.

Data on reduction of soil organic matter (SOM) content and reserves, reflect connection with the relief elements, and the difference in heat and solar radiation. The difference in heat and water regimes on the slopes of different exposure effect biomass production and microbial processes. The observed consequences are: different rates of accumulation, mineralization and humification of the SOM.

As a result of significant water erosion on the slopes of the southern exposure, the soils with lower humus content and humus shortened profile are formed. On the slopes of the northern exposure, where erosion processes are less spread, the depth of the humus profile and the humus content formation is greater than on the southern slopes by 40 %. However, in comparison with the plain soil, the humus content is reduced by 20 % (the northern exposure slope) and 2 times (the southern exposure slope).

Analysis of the humus content of arable soils showed that the northern slope contains humus from 0.4 (90-100 cm layer) to 1.2 % (0-10 cm layer) which is greater than the slope of the southern exposure, and 0.1 – 1 % less than the plain black soil. Significant decrease of humus content (up to 2.0 %) is observed on the southern exposure slope. It can be explained not only by the water erosion impact, but also the soils water-physical properties.

As it is known, the loss of soil humus is under the influence of two processes: humus mineralization and erosion. However, the processes that lead to the soils dehumification on different elements of the landscape are different. Reducing the content of humus in soils in the watershed is solely due to the humus deterioration. But on the slopes it is due to the humus mineralization and

erosion severity. Large humus losses in arable lands occur because of several reasons. Because of the nutrients uptake after harvesting, the arable soils current regime becomes insufficient to compensate the humus losses. Thus, a lower content of humus on arable slopes happens because of conditions for soils formation, the influence of erosion and cultivation technology. The extent of nutrients removal along with the plants biomass depend on the intensity and capacity of the biological cycle, the nature of active roots spread in the soil and detritus location.

The absolute content of humic acids depends on the amount of humus in the soil. So the content of humic acids in eroded soils is 0,670-0,775 %, fulvic acids – 0,348-0,360 % and the ratio of C_{ha} : C_{fa} is 1,82-of 2.23 (Table. 2).

Table 2. Humic and fulvic acids relation in arable soils.

Soil profile	Depth, cm	Total C, %	C_{ha}	C_{fa}	$\frac{C_{ha}}{C_{fa}}$
E0	0-10	2.50	0.775	0.348	2.23
	10-20	2.38	0.670	0.369	1.82
E1	0-10	1.90	0.650	0.347	1.87
	10-20	1.71	0.542	0.352	1.54
E2	0-10	1.18	0.370	0.350	1.06
	10-20	1.11	0.360	0.350	1.03

Different environmental conditions in the slopes of the northern and the southern exposures lead to a decrease in the humic acids quantity up to 0,650-0,542 % and 0,370-0,360 %. The content of fulvic acids is 0,347-0,352 % and the ratio of C_{ha} : C_{fa} decreases to 1.03-1,87. The predominance of humic acids over fulvic acids is due to the activity of a large number of bacteria in the soil microflora. Thus, humic acids are one of the final products of plant residues transformation.

Organic matter (humus) is the main source of nitrogen. Plants that grow on the slopes are poorly spread, their leaves are of light green color. In other words, the nitrogen is a biogenic element whose presence in soil is associated with the processes of humus formation and biochemical activity.

CONCLUSIONS

The research of quantitative and qualitative composition of physical and chemical qualities 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. These soils are considered as special more arid soils additionally superimposed with erosion process. They represent genetic forms of intrazonal and zonal types.

The humus content in the 0-30 cm layer in the plain amounted to 4.1 %, while decreasing on the northern exposure slope by one third, and on the

southern exposure slope – twice. The qualitative humus change was observed: the humic acid content decrease when insignificant change of fulvic acids. On the basis of the results obtained we conclude about a recognized need in application of biological conservation technologies for conditions of arid zones.

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NOTEWORTHY POLYPORES OF PUSHKIN CITY NEAR THE SAINT PETERSBURG (RUSSIA), THE RESERVE OF OLD-GROWTH TREES. 1. *TRAMETES SUAVEOLENS*

SUMMARY

The paper opens a series focused on noteworthy polypores associated presumable with old-growth broadleaf trees in the Pushkin (Tsarskoye Selo) ensemble near St. Petersburg. Some nemoral species complexes are reserved here due to protection of old-growth trees over this area. The species in focus of the present paper is *Trametes suaveolens* (*Polyporaceae*, *Agaricomycetes*), the species with rather uneven taxonomical history. The basionym initially was applied to another fragrant polypore, and its nomenclature was stabilized rather recently, after its neotypification. This is a rare species associated presumable with large willow trees. In some countries (Britain, Montenegro, Finland, Estonia, some regions of Russia) this species is protected. Four habitats of this protected on St. Petersburg species were revealed in the Pushkin city. All these are documented and an enlarged morphological and ecological description of *T. suaveolens* is given. A new form *T. suaveolens* f. *dorsalis* was described.

Keywords: Polyporaceae, polypores, trametoid fungi, parks, old-growing trees, Tsarskoye Selo park ensemble.

INTRODUCTION

The opened series is focused on polypores, associated presumably with old-growth nemoral trees. Such trees are very rare in natural forests of Europe, because of maintaining here the strained forest management. Paradox enough, on some sites of city landscapes (e.g. parks, cemeteries, historical ensembles) there were prerequisites for preservation of old broadleaf trees exemplars. It is known also that during its aging the tree forms an expanding realm of niches, free for settling by various organisms, including the fungi. Noteworthy species of polypores, avoiding of oligotrophic habitats or presented there by leptosomic ecades, can reproduce self-population in association with an old broadleaf trees.

The Pushkin city (known also under name Tsarskoye Selo, Russia) represents a mosaic urban-parks landscape merged into trained hills on edge of the Izhora Plateau near the Saint Petersburg city. As the area of major tourist attraction the Tsarskoye Selo landscape ensemble was included in the list of monuments protected by the UNESCO. The *Picea abies* (L.) Karst. containing

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boreo-nemoral forests characteristic to this intra-zonal Izhora Vegetation Rayon (Alexandrova, Yurkovskaya, 1989) were cut, some additional drying works were carried out and this territory became comparable with those of Central European cities in their environmental plane. The Catherine Park was funded in 1750s, surrounding tree-stands were formed in 1800–1910s and rather wide range of broadleaf trees of the Pushkin parks has endured a bicentennial age. The main stand-formers here are *Quercus robur* L., *Acer platanoides* L. *Fraxinus excelsior* L. and *Tilia cordata* Mill. – all these represent native element of cutted *Picea abies*-boreo-nemoral forests once widespread here. Also, two trees-formed willow species, *Salix alba* L. and *S. fragilis* L. as well as *Alnus incana* (L.) Moench and *A. glutinosa* (L.) Gaertn. were distributed here along the rivers. Additionally, *Tilia platyphyllos* Scop., *Aesculus hippocastanum* L., *Acer rubrum* L. were introduced into park and roadside landings. A lot of nemoral and boreo-nemoral shrubs were landed under bed curtains of the main stand-formers. Within them, *Padus avium* Mill., *Sorbus aucuparia* L., *Malus* spp., *Crataegus* spp., *Acer negundo* L., *Caragana arborescens* Lam., *Corylus avellana* (L.) Karst., *Prunus domestica* L., *Frangula alnus* Mill., *Amelanchier* sp., *Cotoneaster lucidus* Schltdl. are predominant.

The main purpose of this series is enlarged description of ecological polymorphism of noteworthy polypores species founded in Pushkin city (Tsarskoye Selo) territory and documentation of habitats of these organisms.

The fragrant bracket fungus, *Trametes suaveolens*, which opens of the present series, in its current interpretation was described by Fries (1821), whereas its nomenclatural history seems to be uneven. Linnaeus established *Boletus suaveolens* L. (Linnaeus, 1753) with a short diagnosis ("*Boletus acaulis, superne laevis, salicinus*"), habitat information ("Habitat in *Salice*"), and a reference to his polynomial "*Boletus acaulis, superne laevis, salici insidens*" (Linnaeus, 1737) with such characteristic as "Rarius occurrit hic in salicibus et odore suavissimoque pollet". Fries made the new combination *Polyporus suaveolens* (L. : Fr.) Fr. (Fries, 1821), but applied this name to another species, which was characterized as "Pileo carnoso-suberoso azono villoso albo, poris majusculis fuscentibus. Odor gratus, aniseus. Pileus subsolitarius, crassus, azonatus, 2–4 unc. latus. Pori obtusi, opaci, in superficie plana rotundi aequales, in declivi inaequales". Indeed, there are two salicicolous species of trametoid habit, one of them (*Boletus suaveolens* sensu L.) is characterized by even naked ("superne laevis") pale pilei and strong ("suavissimus") odor, another one characterized by white tomentose ("villoso albo") pilei and not strong anise ("gratus") odor. The differences between these diverse species are given, for example, by Ryvarden and Melo (2014), where the names used were *Haploporus odorus* (Fr.) Bondartsev & Singer and *Trametes suaveolens* (L. : Fr.) Fr., respectively.

Later, Fries (1837) described the genus *Trametes* typified by *Polyporus suaveolens* (L. : Fr.) Fr. (Murrill, 1905) and this genus is widely accepted nowadays (Zmitrovich & Malysheva, 2013). However, the Linnaean name *Boletus suaveolens* L. was misapplied to type species of *Trametes* in Friesian

sense and some authors beginning with Donk use the combination *Haploporus suaveolens* (L. : Fr.) Donk in application to the type species of *Haploporus*, but not *Trametes* (Donk, 1971). The consequent sanctioned and correctly applied synonym which substitutes an ambiguous *Trametes*-type would be *Boletus discoideus* Dicks. ("Acaulis planiusculus villosus rugoso-tuberculosus sordide albens poris labyrinthiformibus concoloribus. Nequaqum convenit cum *B. suaveolente* Linn., qui pulvinatus, superficie laevi, nec rugosa aut villosa; poris rotundatis, nec labyrinthiformibus". Cited according to J. Dickson, 1793). It was sanctioned by Fries in Index (1832, p. 57). The corresponding combination *Trametes discoideus* (Dicks. : Fr.) Rauschert was made by Rauschert (1990). However, in 1991 the name *Boletus suaveolens* L. : Fr. was neotypified by Ryvarden (1991), who has selected the specimen from Sweden (Upland, Uppsala, *van der Post 1872*, S) corresponded to *Trametes suaveolens* as neotype. Because the general principle of Nomenclatural Committee was to minimize – and indeed if possible prevent – name changes arising from the change of starting date, which is why names can be typified either on an element in the context of the original protologue or an element in the context of the sanctioning treatment, the Ryvarden's selection in this case was as something best decision (Art. 9.2 and 9.10 of ICBN, Prof. J. McNiell, pers. comm.).

In all the parts of Europe this is rather infrequent species gravitating in its distribution to large and presumably live *Salicaceae* trees. This species is included in a series of European Red lists (Kotiranta & Niemelä, 1996; Evans et al., 2006; Kasom & Miličković, 2006; Parmasto, 2009). In Russia, this species was entered in Red book of Leningrad Region (2000) and Red book of Saint Petersburg (2004). Our research on Pushkin area allows to reveal some populations of this interesting fungus.

MATERIALS AND METHODS

Territory studied. The Tsarskoye Selo (Pushkin) city occupied hilly landscape on border of the Prinevsky Lowland and the Izhora Plateau in 15 kilometers to the south of the St. Petersburg on approx. 59.75°N and 30.36°E crosshairs. The highest areas lye here about 100 m above Sea level. Park zone of the Pushkin (Tsarskoye Selo) ensemble keeps within the area of 704 hectares.

Material collection. A total of thirty kilometer quarters were subject to monitoring since 2012. Old-growth leaf-trees phytosphere was examined regarding existence of the polypore species. The fruitbodies were fixed on photo or collected in the case of necessity and and an existence of sufficient for reproduction quantity of a remained propagative structures of the fungus. In laboratory, – 20 °C followed by frozing of material were carried out. The material is kept in mycological herbarium of the Komarov Botanical Institute of the Russian Academy of Sciences (LE).

Material elaboration. The macroscopic descriptions were based on a study of fresh and dried specimens. Microscopic preparations were mounted from dried material in Melzer's solution, 10% ammoniacal Congo Red and 5%

aqueous solution of KOH, using a LOMO Micmed-6 light microscope. The hyphal system was revealed and described according to updated technique (Zmitrovich et al., 2009). The size of mature spores was measured on 30 spores in distilled water and Melzer's solution. The taxonomic position of the species was based on recent molecular studies (Zmitrovich, Malysheva, 2013; Zmitrovich, Kovalenko, 2015), higher taxa are given according to Hibbett et al. (2014).

RESULTS AND DISCUSSION

Agaricomycetes

Polyporales

Polyporaceae

Trametes suaveolens (L. : Fr.) Fr., Epicr.: 491, 1838. – Bas.: *Boletus suaveolens* L., Sp. Pl.: 1177, 1753 ex Fr., Syst. Mycol. 1: 366, 1821 [ut *Polyporus suaveolens* L. sensu Fr. nec L. (= *Polyporus odorus* Sommerf., 1826)][2]. ≡ *Haploporus suaveolens* (L. : Fr.) Donk, Prok. K. Ned. Akad. Wet. Ser. C 74(1): 20, 1971[3]. − Syn.: *Boletus suberosus* Bolton, Hist. Fung. Append. 3: 162, 1792. – *B. discoideus* Dicks., Fasc. Pl. Crypt. Brit. 3: 21, 1793 sanct. Fr., Index: 57, 1832 [≡ *Trametes discoidea* (Dicks.) Rauschert, Feddes Rep. 101: 643, 1990]. − *Daedalea bulliardii* Fr., Syst. Mycol. 1: 335, 1821. – *Trametes inodora* Fr., Epicr.: 491, 1838. – *Polyporus itoi* Lloyd, Mycol. Writ. 7: 1274, 1924.

Icon.: Bulliard (1787: pl. 310, Fig. A); Sowerby (1799: tab. 288 ut *Boletus suaveolens*); Niemelä (2005: p. 266, Fig. 281); Melo & Ryvarden (2014: p. 419, fig. 323).

Basidiomata annual or perennial, sessile, of trametoid habit, 4–15 cm wide and 2–4 cm thick, triquetrous to cushion-like in section, semicircular in outline, pilei plane or slightly convex; as a rule clustered, but in normal not decurrent and devoid of stipe. Upperside without a differentiated subpellis, subtomentose, villose, of fine bristly, more or less even, but in some specimens scrupose, uniformly white, then cream, then grayish-bloom. Context purely white, rather dense, suberose, homogeneous, with prominent anise odor when fresh, up to 3 cm thick. Margin initially obtuse or bolster-like, then acute, with sterile zone around the hymenophore, in abhymenial surface cream or with grayish shades. Hymenophore as a single tube layer up to 10 mm thick, white to honey-colored or isabelline. Pores normally 0.5–1.5 mm in diam., but in average 1–2 per mm, with thick dissepiments, angular, sinuose in some regions, white, cream to light-coffee, or in some specimens near dissepiments initially becoming grayish-brown (Fig. 1a).

Hyphal system dimitic with sympodially branched sclerohyphae. Generative hyphae 3–5.5 μm in diam., clamped, thin-walled, rarely branched. Sclerohyphae

[2] Neotypus (vide Ryvarden in Syn. Fungorum 5: 230, 1991): Sweden, Upland, Uppsala, *van der Post 1872* (S).

[3] Nomenclatural, but not taxonomical synonymy.

2.5–8.5 μm in diam., thick-walled to subsolid, hyaline, acyanophilic, inamyloid; the branching pattern sympodial, moderate in internal tissues and regular near surfaces, mostly rectangular. Basidia 20–30 × 5–7.5 μm, clavate, 4-spored, with a basal clamp. Cystidia none, but hyphal pegs composed by generative hyphae are regular in the hymenium. Basidiospores 7.5–12.5 × (3)4–4.7 μm, short-cylindrical, in certain projections lacrymoid or amygdaloid, hyaline, thin-walled, inamyloid, acyanophilous.

Figure 1. Typical form of *Trametes suaveolens* growing on *Salix fragilis* (protected population, 59°43’46”N, 30°25’08”E): *a* – basidiome of current season; *b* – wintered basidiome. Scale bar – 1 cm.

Substrata and ecology. *T. suaveolens* grows on living, rarely dry trees, stumps and fallen logs of *Salix* spp. In other parts of Europe the fungus is reported also on *Populus*, *Aesculus*, *Alnus*, *Betula*, *Carpinus*, *Fagus*, *Fraxinus*, *Malus*, *Populus*, *Quercus*, *Salix*, *Tilia*, and *Ulmus* (Ryvarden & Melo, 2014). Causes a white heart-rot.

Figure 2. Typical habitats of *Trametes suaveolens* in the Pushkin city (59°43’46”N, 30°24’22”E). Fruit bodies area is highlighted.

Basidiomata of *T. suaveolens* usually exist during a single season, but even in the boreal zone some fungi which colonize slantwise growing willows over river falls can develop wintering basidiomata capable to resume their active growth after warming. In this case fruit bodies can reach rather sufficient sizes.

The most typical habitat of this fungus is presented by floodlands along rivers where arboriform willows are growing (Fig. 2). In urban landscapes, *T. suaveolens* is associated as a rule with old willows trees.

Distribution. EUROPE: United Kingdom, Norway, Germany, Italy, Georgia, Finland, Russia. ASIA: Russia, Mongolia, Taiwan, China, S. Korea, Japan. NORTH AMERICA: USA, Canada (Global.., 2016).

Table. Records of *Trametes suaveolens* on the Pushkin city (Tsarskoye Selo) territory

N	Coordinates	Locality, habitat	Substrate	Specimen
1.	59°44’42”N, 30°24’19”E	Bufferny Park valley of Kuzminka river	*Salix fragilis*	*T. suaveolens* f. *griseopora* (LE 287618)
2.	59°43’46”N, 30°24’22”E	Bufferny Park valley of Kuzminka river	*Salix fragilis*	*T. suaveolens* f. *dorsalis* (LE 287619)
3.	59°43’46”N, 30°25’08”E	Detskoselsky parkway, public garden	*Salix fragilis*	–
4.	59°42’09”N, 30°24’08”E	Novaya str., public garden	*Salix fragilis*	–

The distribution of this species over Pushkin (Tsarskoye Selo) territory is rather scarce. Only 4 habitats are revealing (Table), and only two deviated specimens were collected, since the populations of *T. suaveolens* are protected on Saint Petersburg territory (see also Fig. 2, 3).

Figure 3. Pushkin city quarter plan with indications of habitats of *Trametes suaveolens* (red circles). The numbers are corresponded to those in Table.

Morphological variability. In a field, the fungus can be recognized due to its rather large basidiomata with plane to convex subtomentose to villose pilei of uniform coloration. Cap zonation or radial color alterations are not characteristic for this species. Only near the margin some grayish shades can be observed in some representatives. The upperside relief normally even, but some obscure ridges or pimples can be expressed, especially when the basidiomes are merged into plant sprouts around basic trunk. The wintering basidiomata bear as a rule stramineous upperside and honey-colored tubes (Fig. 1b). Microscopically, this is rather invariable species, characterized by large basidia and basidiospores. Vegetative hyphae are typical for *Trametes* species. The hyphal system containing both weakly and strongly branched sclerohyphae was described as trimitic (e.g. Ryvarden & Gilbertson, 1993, 1994), but in a reality the binding hyphae represent an apical branches of skeletal dendrites merely, therefore we deal with a kind of dimiticism (Zmitrovich & Malysheva, 2013).

Three forms are known for this species – f. *inodora* Fr. in Pilát, characterized by absence of any odor, f. *gibbosiformis* Nikol. in Bondartsev, characterized by smaller pores 2– 2.5 per mm, f. *griseopora* Komarova, characterized by gray-coffeate shades already in young tubes of the fungus, and f. *dorsalis* ined., characterized by dorsally attached basidiomes with narrow reflexed border. Within aforementioned forms, two last ones were registered in Tsarskoye Selo together with type form and they will be characterized below.

Trametes suaveolens forma ***griseopora*** Komarova, Notulae Systematicae e Sectione Cryptogamica Instituti Botanici nomine V. L. Komarovii Academiae Scientiarum URSS 12: 256–257, 1959.

Icon.: Komarova (1959: p. 256, Fig. 8).

«Basidiomata 2–3.5 × 3–6 × 1–2 cm, with convex milky-white, then yellowish upperside, obtuse margin and snow-white context with slight anise odor. Tubes thin-walled, with gray shades at the margin. Pores roundish-angular, 0.25–0.6 mm in diam., usually 2–3 per mm. The surface of hymenophore varies from light- to intensive mouse-gray, something with brownish tinge. Contextual hyphae of two types: 1) thick-walled to subsolid, rarely branched, 2–6 μm in diam., and 2) thinner-walled, 3–10 μm in diam. Basidia 20–25 × 4.5–6.5 μm, with sterigmata 4–6.5 μm long. Spores hyaline, cylindrical, with prominent base, 7–10.5 × 3–3.5 μm. Found in Minsk Region (Borisovsky district, Borovlyany) on aspen fallen log. October 1954. [without an indication of type specimens attributes – *I. Z.*]. The form described differs from the type one by mouse-gray hymenophoral surface and smaller pores».

Ann additional material was founded by us in the Pushkin vicinities growing on fiving willow tree on valley of the Kuzminka river. A short description of additional material follows.

Basidiome consists of two clustered and merged pilei with very obtuse margin, 8.5 × 6.2 × 1–5.5 cm, with decurrent base. Upperside tomentose, white to creamish near the margin, even. Hymenophoral side of the margin is uneven, slightly scrupose and obscurely porose, but mostly sterile, white of milk coffee

colored. Context purely white, with anise odor, thick. Pores anisodiametric, 2–3 per mm on internal and 1–2 per mm on marginal parts, more or less thick-walled, on decurrent part lacerate, combining whitish, milky coffee, mouse-gray and cinnamon coloration (Fig. 4).

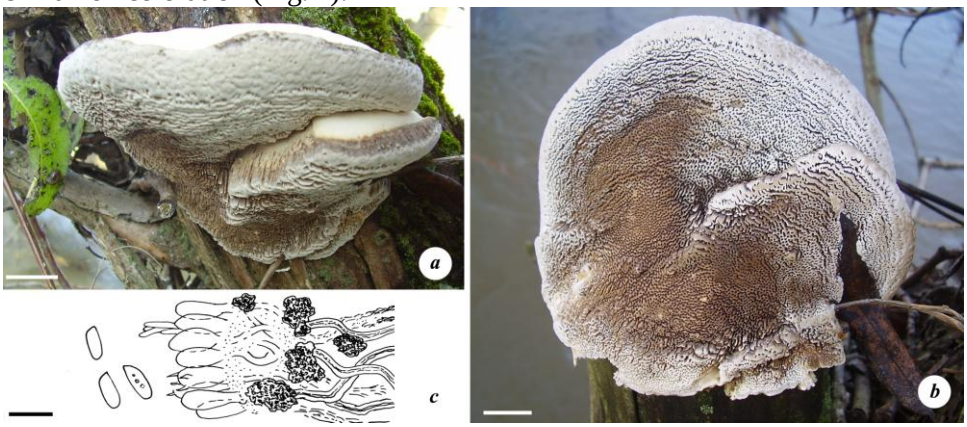

Figure 4. *Trametes suaveolens* f. *griseopora* (LE 287618): *a* – general view, *b* – hymenophore (scale bar – 1 cm), c – encrusted subhymenium and hymenium with basidiospores (scale bar – 10 μm).

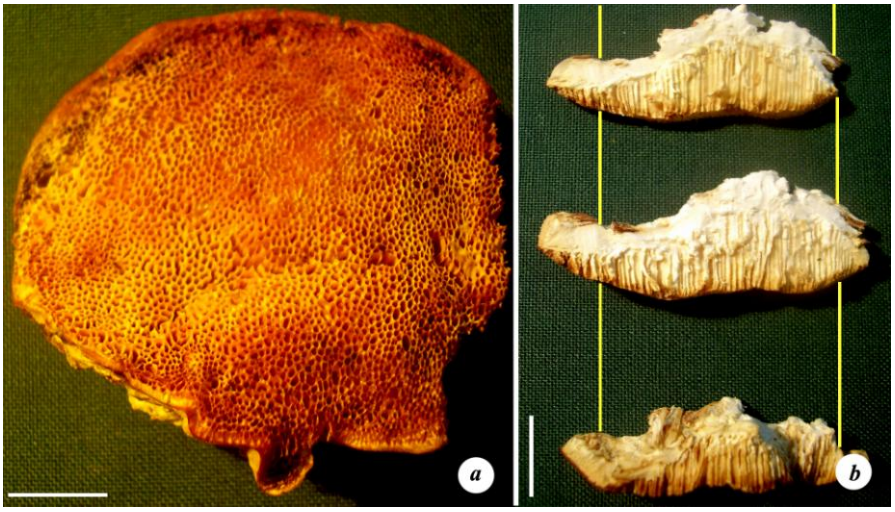

Figure 5. *Trametes suaveolens* f. *dorsalis* (LE 287619): *a* – hymenophore, *b* – cross-sections of basidiome (yellow lines bordering area of substrate attachment). Scale bar – 1 cm.

Generative hyphae 3–5.3 μm in diam., sclerohyphae 2.5–8(9) μm in diam., varying as in a neutral type, but in subhymenium strongly gelatinized and covered with abundant chrysescent resinous-crystalline matter. Basidia 20–28 × 5–7.5 μm. Basidiospores 7.0–11.5 × 3–4.5 μm.

Specimen examined: Russia, Saint Petersburg, Pushkin (Tsarskoye Selo), Bufferny Park valley of Kuzminka river, 59°44’42”N, 30°24’19”E, coll. & det. I.V. Zmitrovich 15.09.2014 (LE 287618).

Trametes suaveolens forma ***dorsalis*** Zmitr. forma nova (MB 817131).

Basidiome as dorsally attached roundish patch 5.5 × 4.5 × 1.7 cm with pileus-like border up to 0.7 cm wide. Upperside subtomentose to hispid, cream, even. Context up to 0.7 cm thick, purely white, with anise odor. Pores anisodiametric, 2–3 per mm, more or less thin-walled, honey-colored, with rufescent margin (Fig. 5).

Generative hyphae 2.5–5.5 μm in diam., sclerohyphae 2.5–8.5 μm in diam., varying as in a neutral type. Basidia 22.5–28.5 × 5–7.5 μm. Basidiospores 7.1–12.0 × 3.5–4.5 μm.

Specimen examined: Russia, Saint Petersburg, Pushkin (Tsarskoye Selo), Bufferny Park valley of Kuzminka river, 59°43’46”N, 30°24’22”E, coll. & det. I.V. Zmitrovich 25.04.2016 (LE 287619).

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EFFECT OF LONG TERM STARVATION ON SOME PRODUCTIVITY TRAITS AND BODY COMPOSITION OF TURKISH STRAIN BROWN TROUT (*SALMO TRUTTA FARIO*) WINTERED IN FLOATING NET-CAGES

SUMMARY

In this research, brown trout had been wintered for 4 months (Between December 2005 and April 2006) in net-cages in Kuzgun Dam Lake (Erzurum-Turkey) without any feeding. Specimens were collected before and after long term fasting in order to investigate effect of starvations on some productivity traits and body composition of fish. Fish were fed daily by commercial trout feed after May 2006. At each sampling time, 15 fish were sampled from square wooden net cages (5*5*8 m) for analysis. Research results showed that 66.10±4.42 g initial mean weight was decreased to 44.99±4.40 g after five months and then fish were recovered and reached to 148.11±13.00 g main weight. Similarly, condition factor had been decreased from 1.19±0.028 to 0.79±0.02 after 5 months and then it was increased as 1.54±0.053 fallowing two months. After wintering, carcass yield was increased from 82.86±0.7% to 84.16±1.40%. Long term starvation did not affect raw protein rate statistically before and after wintering. However, raw fat rate was decreased from 2.67±0.17% to 1.44±0.17% after long term starvation, and then raw fat rate increased up to 3.70 ±0.25% in fallowing months.

Keywords: Brown trout, proximate composition, condition factor, raw protein, raw fat.

INTRODUCTION

In Turkey, the most cultivated finfish are trout species (besides rainbow trout, brown trout strains and other trout species). Undoubtedly, this high amount of production has been contributed and supported by the reservoir and dam lakes used for production and the net-cage farms be established here. Usually, mainly rainbow trout (*O. mykiss*) are grown in fish farms established on the dam lakes. Recently other native trout species besides rainbow trout has also started to culture. According to Ministry of Forrest and Water, there are a total 706 dam

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lakes in Turkey. In dam lakes, climate and water temperature determines the production cycle of cage farms. Trout fry (5-25 g) were stocked in net cages and raised up to 300 g final weight between September and May (during this period water temperature is below 20 °C) in dam lakes which are located on the low altitude. So, summer season is called as dead season in low altitude dam lakes. On the contrary, production season limited between April and November in high altitude lakes such as Kuzgun Dam Lake because of harsh winter and frozen lake surface. Production strategy of high altitude lakes planned as follows. Trout fry (5-10 g) was transferred from hatchery to cage farm in April 2005 and those fry were grown up to 100-250 g until November 2005. And then, grow out trout were (100-250 g) transferred to sea cages (Black Sea) by the farmer. However, transferring of all raised fish is not profitable during short winter eve due to low demand and price.

Effect of long term cold temperature and lack of feed intake on growth depression and proximate composition of brown trout (*S. trutta fario*) is unknown. In this research, a group brown trout were restocked in 5*5*8 m square net cages and wintered between November 2005 and April 2006. Some of the productivity traits and body composition of wintered fish were monitored and aimed to determine recovery and new harvest time of wintered fish.

MATERIAL AND METHODS

Research carried out in Dörtmevsim Trout Farm established on Kuzgun Dam Lake (Erzurum, Turkey). Kuzgun Dam Lake which is an irrigation and energy purposeful dam was built in 1997, in district of Erzurum, at 2115 m altitude. This first private trout fish farm was established on this dam lake. Brown trout (66.11±4.43 g main weight) were stocked in square cages (5*5*8 m) in November 2005 and stocked fish had been wintered for 4 months (from November 2005 to April 2006) without any feeding during the winter season. During this period, surface of lake was frozen; however, water surface of floating net cages sometimes was melted until the end of winter. Water temperature and oxygen were measured as 2-4 °C, 6-9.5 ppm, respectively. After May 2006, water temperature was reached up to 18 °C, and mostly water surface of lake was frozen during this period. When the ice melts on the water surface in April, the fish were begun to be feed routinely. After wintering, samples were collected by monthly (April, May, June) until July 2006. Sampled fish were transferred to Fisheries Faculty Lab (Recep Tayyip Erdoğan University, Rize-Turkey) for checking morphometric data's and proximate quality. Condition factor was calculated as $KF= (W/L^3)*100$ according to Bagenal (1978). Carcass yield (CY) was calculated according to this formula (CY = Carcass weight/ Fish weight*100) after gutting fish. Samples were stored at -18 °C for proximate analysis. Raw fat, raw protein, raw ash, dry matter and moisture were determined according to AOAC.

RESULTS AND DISCUSSION

Research results showed that, initial fish weight had been decreased from 66.11±4.43 g to 45.00±4.40 g after wintering. After the feeding again, wintered fish recovered old weight and reached to 78.84 ± 4.88 g main weight from April to June 2006 (Table 1). Similarly, condition factor decreased dramatically from 1.19±0.03 to 0.80±0.02 after winter season. Condition factor was recovered as 1.55±0.05 after one month. However, carcass yield increased from 82.86±0.40% to 84.16±0.82% at the end of winter. Effect of long term starvation on proximate composition was showed in Table 2. Initial raw fat rate had been decreased from 2.67±0.17% to 1.12±0.18% at the end of winter, and raw fat rate was recovered up to 3.71±0.25% (Table 2). As a result, wintering fish in dam lake caused significant weight loss in fish (26% of weight was lost) and lowered condition factor. The fish regains the weight at the end of first month after starting to feed.

Table 1. Changes of some traits in wintering brown trout (Salmo trutta fario) during experiment.

Sampling Date:		2005 November	2006 April	2006 May	2006 June
Variable	N	12	14	11	11
Weight (g)		66.11±4.43^{a}	45.00±4.40^{b}	78.84±4.88^{a}	148.11±13.00^{c}
Length (cm)		17.58±0.31^{a}	17.73±0.54^{ab}	18.95±0.18^{ab}	21.06±0.66^{b}
Condition Factor		1.19±0.03^{a}	0.80±0.02^{b}	1.15±0.05^{a}	1.55±0.05^{c}
Carcass Yield (%)		82.86±0.40^{a}	84.16±0.82^{b}	75.39±1.40^{c}	83.99±0.45^{b}
Values expressed in the same row with different letter indicate differences are statistically significant (P<0.05)					

Table 2. Changes of body composition in wintering brown trout (Salmo trutta fario) during experimental period.

Sampling time:		2005 November	2006 April	2006 May	2006 June
Variable	N	12	14	11	11
Raw protein (%)		18.81±0.36^{a}	17.03±0.58^{b}	21.82±1.08^{c}	20.14±0.69^{c}
Raw fat (%)		2.67±0.17^{a}	1.12±0.18^{b}	3.71±0.25^{c}	3.02±0.19^{a}
Raw ash (%)		1.22±0.04^{a}	1.06±0.03^{b}	1.30±0.02^{c}	1.31±0.04^{c}
Moisture (%)		74.92±0.95^{a}	77.12±0.55^{b}	76.31±0.84^{ab}	79.56±0.31^{c}
Dry matter (%)		23.15±0.37^{a}	23.54±0.42^{a}	25.05±0.39^{b}	22.79±0.17^{a}
Values expressed in the same row with different letter indicate differences are statistically significant (P<0.05)					

In high altitude, feed abundance and feeding of wild fish are depended on climatic conditions. Fish gain condition when the climate and food are available for sustainable life strategy (Gross et al. 1988). In fish, gained lipids and condition are spent for meeting metabolic usage because of low feed intake in cold winter conditions. (Boivin and Power, 1990). Jørgensen et al. (1997) studied

on seasonal changes of lipid depletion and deposition in Arctic charr. They reported that females and male Arctic charr lost their 80% and 50-55% of total lipid reserves respectively during wintering and spawning. In this study, cultivated brown trout raw fat content was reduced from 2.67% to 1.125% during wintering and fasting period. Protein content of muscle was also reduced from %18.81% to 17.03%. Boivin and Pover (1990) reported that muscle and liver lipid and protein reserves of fasted Arctic char (Salvelinus alpinus) decreased during winter season. Zhang et al. (2007) reported that flesh composition of Sparus macrocephalus was affected with 28 day fasting. Muscle fat content was reduced after 3 days later, and protein content was started to decrease on the 14th day.

Our results showed that long term fasted and wintered brown trout were lost weights and condition (Table 1). Growths was also lowered, and mean length was measured as 17.73 cm after four months. But increased carcass yield was detected. This increase probably caused by empty digestive systems. Thomas et al. (2005) reported slow growth and reduced weight and condition factor in channel catfish which was exposed to cold water and food deprivation.

CONCLUSIONS

In this study, effects of long term wintering and fasting on some productivity traits and proximate composition of Turkish strain brown trout were investigated. Obtained data showed that four months fasting on cold water conditions (water temperature fluctuated between 2-4 °C) caused weight loss and depletion on fat and protein content of fish flesh. However, fish recovered back growth rate and body composition within one month.

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NEW HOST DATA FOR TERRESTRIAL PARASITENGONA OF SERBIA WITH NOTE ON Allothrombium clavatum Saboori, Pešić & Hakimitabar, 2010

SUMMARY

Allothrombium clavatum Saboori, Pešić & Hakimitabar, 2010 was described from Montenegro, based on two damaged specimens. This species was reported for the first time from Serbia on aphid Aphis craccivora. In this paper, new host and metric data are given. Further, on 7 localities, in different parts of Serbia, larvae of three trombidiid mite species on A. craccivora have been found.

Keywords: *Allothrombium clavatum,* new metric data, *Aphis cracccivora,* Serbia.

INTRODUCTION

Fauna of terrestrial Parasitengona of Serbia is poorly known. Hitherto only thirteen species of this fauna have been reported from Serbia: Allothrombium fuliginosum (Hermann, 1804); Abrolophus stanislavae Haitlinger, 1986; A. quisquiliarus (Hermann, 1804); Balaustium nikae Haitlinger, 1996; Erythraeus (Zaracarus) budapestensis Fain & Ripka, 1998; Eutrombidium trigonum (Hermann, 1804); Microtrombidium parvissimum Gabryś, 1999; Charletonia cardinalis (C. L. Koch, 1837) (Mąkol & Wohltmann 2012, 2013; Haitlinger 2007, 2012) and Abrolophus montenegrinus Saboori, Šundić & Pešić, 2012; Leptus eslamizadehi Saboori, 2002; Erythraeus (Erythraeus) mariolae Haitlinger, 1994; Charletonia krendowskyi Feider, 1954; Erythraeus (Erythraeus) serbicus Šundić, Haitlinger, Petanović, Jovičić & Hakimitabar 2015 (Šundić et al., 2015).

Allothrombium clavatum Saboori, Pešić & Hakimitabar, 2010 is new record of fauna terrestrial Parasitengona of Serbia. This species was described from Montenegro, based on two damaged specimens by Saboori, et al., 2010. Scutum was drawn without PL setae and sensillae (S) therefore figure of scutum, new host and metric data are given (Fig. 1, Table 1). Also trombidiid species found on Aphis craccivora Koch, 1854 in Serbia are listed.

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MATERIAL AND METHODS

Total two larvae of mites *Allothrombium clavatum* were collected in Serbia from host *A. craccivora* (Hemiptera: Aphididae) during the June 2013. The specimens of *A. craccivora* were collected in Serbia from alfalfa (*Medicago sativa* L.). Sampling of aphids and larvae of mites was done at different intervals for four years (2011-2014). Aphids were collected directly from plant stems and placed in plastic tubes in 70% ethanol. Samples were labelled and transferred to the laboratory for identification based on morphological characters using a stereoscopic microscope (Bio-Optica, Type: 1000). In the laboratory, mates were separated from the aphids and preserved in 75% alcohol, cleared in Nessbitt's solution and mounted in Faure medium (Walter & Krantz, 2009). All measurements are given in micrometers (μm) and calculated using a Carl Zeiss Axioscope A1 microscope, and Carl Zeiss Axio Imager A2 with differential interference contrast and phase contrast. The terminology and abbreviations follow Makol (2007).

RESULTS AND DISCUSSION

Allothrombium clavatum Saboori, Pešić & Hakimitabar, 2010: 515—519, figs: 1-6.

Material: 2 larvae, Serbia, locality Cerje, Ušće, 29 June 2013.

Distribution: Montenegro, Serbia.

Polyphagous species *A. craccivora* is a widely distributed species that has been reported as a pest of many crops on several continents (Blackman and Eastop, 2000, Berberet et al., 2009), but its origins are clearly in Europe (Blackman and Eastop, 2007). In Serbia, this species has been reported on about 30 host plants (Petrović-Obradović, 2003). *Aphis craccivora* has been recorded as pests of alfalfa in this country (Jovičić et al., 2014).

On 7 localities, in different parts of Serbia, we have found larvae of mites on this aphid (Table 2). Total 33 mites were found on 21 aphids, among them 2 mites *Allothrombium clavatum*, 5 mites *Erythraeus* (*E.*) *serbicus* and 26 mites *Allothrombium fuliginosum* (Hermann, 1804). Most captured specimens of *A. craccivora* were attached by one mite. Maximum 3 mites *Erythraeus* (*E.*) *serbicus* were attached on this host. The most of mites were attached on wingless adults. Most of the mites were located above the thorax of the aphids. Only a few individuals were attached on the abdomen of *A. craccivora*. Mites were present on this aphid in period June-August. The first individuals of mites were collected at the beginning of June at Central Serbia (Belosavci and Donja Šatornja), (Table 2).

Remarks

Morphological variability *A. clavatum* in specimens from Serbia is distinctly differs of *A. clavatum* specimens from Montenegro, especially in metric data of SD, W, AA, ASB, PSB, MA (Table 1). Anterior border of scutum rounded, posterior border of scutum is slightly 3 lobed (in original description posterior border of scutum is more prominent 3 lobed) (Fig.1).

Table 1. Metric data for *Allothrombium clavatum* Sabori, Pešić & Hakimitabar 2010. H-holotype, P-paratype, specimens from Montenegro; S1, S2-specimens from Serbia

	H	P	S1	S2		Holotype	Paratype	S1	S2
IL	329	247	848	630	*or*	6	-	6	7
IW	235	272	548	503	*bs*	7	-	8	9
SD	64	57	84	85	Ta I (L)	40	50	54	58
W	62	62	83	82	Ta I (H)	20	22	22	21
AW	53	45	54	55	Ti I	42	46	35	40
PW	65	-	67	70	Ge I	25	30	22	27
AA	38	-	47	42	Fe I	42	47	43	43
SB	37	32	35	38	Tr I	32	35	28	32
ASB	31	-	46	51	Cx I	50	45	53	52
PSB	33	-	42	40	Leg I	231	253	235	252
MA	17	-	33	33	Ta I (L)	45	43	45	51
AP	26	-	22	29	Ta II (H)	19	17	20	19
AL	28	28	28	33	Ti II	41	37	33	35
PL	-	33	33	43	Ge II	23	25	20	22
AM	32	35	31	31	Fe II	41	40	41	37
S	-	35	37	45	Tr II	34	32	28	23
LSS	53	57	65	59	Cx II	50	52	51	45
HS	22	25	23	21	Leg II	234	229	218	213
SL	-	31	32	33	Ta III (L)	45	50	50	54
SS	25	28	24	25	Ta III (H)	15	16	18	16
DS	22-30	25-28	26-29	27-33	Ti III	44	47	41	42
PDS	20	22	20	21	Ge III	25	26	22	25
1a	32	30	33	35	Fe III	37	40	46	38
1b	27	29	30	32	Tr III	37	40	25	28
2b	32	35	35	30	Cx III	47	50	52	48
3a	31	30	26	26	Leg III	235	235	236	235
3*b*	32	35	25	34	IP	700	735	689	700

Leg setal formula for specimens from Serbia: Leg I: Ta 1ω, 2ζ, 1ε, 13n; Ti 2φ, 1κ, 5n; Ge 2σ, 1κ, 4n; Fe 5n; Tr 1n; Cx 2n. Leg II: Ta 1ω, 1ε, 12n; Ti 2φ, 5n; Ge 2σ, 1κ, 3n; Fe 4n; Tr 1n; Cx 1n. Leg III: Ta 12n; Ti 5n; Ge 2σ, 3n; Fe 4n; Tr 1n; Cx 1n.

NDV= 24 + 6 = 30

Metric data of species from Montenegro and Serbia are given in Table 1.

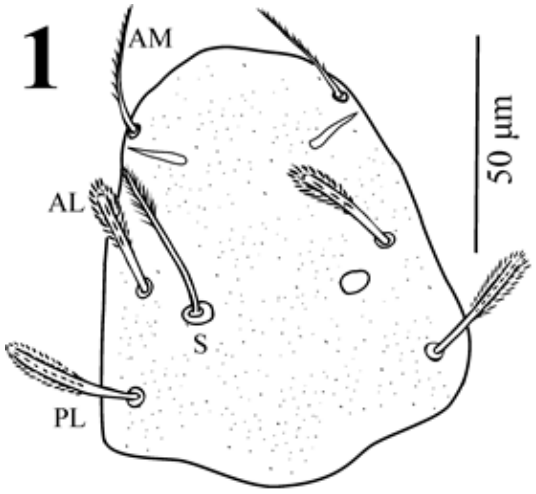

Figure 1. Allothrombium clavatum Saboori, Pešić & Hakimitabar 2010, Scutum

Table 2. Parasitengonae mites found on *Aphis craccivora* in Serbia

Locality name	Geographic coordinates	Mites	Host	Number of mites
Belosavci, Topola	44° 20’ 31’’ N 20° 40’ 58’’ E	*Allothrombium fuliginosum*	*A. craccivora*	2
Braničevo, Golubac	44° 41’ 54’’ N 20° 32’ 29’’ E	*Allothrombium fuliginosum*	*A. craccivora*	4
Cerje, Ušće	43° 29’ 59’’ N 20° 36’ 50’’ E	*Allothrombium clavatum*	*A. craccivora*	2
Donja Šatornja, Topola	41° 11’ 11’’ N 20° 33’ 09’’ E	*Allothrombium fuliginosum*	*A. craccivora*	5
Kotraža, Lučani	43° 41’ 48’’ N 20° 14’ 45’’ E	*Allothrombium fuliginosum*	*A. craccivora*	6
Ovča, Beograd	44º 52’ 49’’ N 20º 32’ 13’’ E	*Allothrombium fuliginosum*	*A. craccivora*	3
Rusko selo, Kikinda	45° 45’ 16’’ N 20° 33’ 47’’ E	*Erythraeus serbicus*	*A. craccivora*	5
Rusko selo, Kikinda	45° 45’ 16’’ N 20° 33’ 47’’ E	*Allothrombium fuliginosum*	*A. craccivora*	6

CONCLUSIONS

Allothrombium clavatum is new record of fauna terrestrial Parasitengona of Serbia. Morphological variability A. clavatum in specimens from Serbia is distinctly differs of A. clavatum specimens from Montenegro. Larvae of three mite species on A. craccivora were found: Allothrombium clavatum, Erythraeus (E.) serbicus and Allothrombium fuliginosum. This is the first investigation of the occurrence of trombidiid mites on this aphid in Serbia.

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YOUNG CONSUMERS' PREFERENCES FOR MACEDONIAN WINE

SUMMARY

Macedonian strategy for viticulture and wine production singles out young consumers as a target group of consumers for further increase of the production value and development of wine culture. Therefore, we aim to determinate the presence of young population on the Skopje Wine Winter festival in 2014 and to understand their preferences towards domestic wine and its quality. We performed structured questionnaires to 420 visitors and segmented young consumers as a focus group. We used conceptual model for analysis of consumer attitudes and Chi2-test in Stata 12.0 and StataCorp 2011, for testing the hypotheses for the consumers' perception of quality wine. The results showed that younger consumers were the most present category on the Wine festival. In general, they do not differ from other age groups in terms of their preferences for domestic quality wine, except that this category showed readiness to pay a higher price for domestic quality wine. The quality perception of domestic wine is satisfactory, which creates an opportunity for the wineries to target and to promote their wine to the new segments of young consumers, committed to purchase domestic wine.

Keywords: young consumers' preferences, domestic wine, quality perception.

INTRODUCTION

Wine industry has a strategic importance for the Republic of Macedonia because of 70% export orientation; growing development trend; high specialization (Balassa index 3.83, Nacka, 2011) and positive trend of investment in sophisticated equipment and marketing skills (CBI, USAID, 2012).

In the next period, an intensive development and wine positioning through building a competitive brand is expected, thus increasing the perception of domestic consumers for quality wines and brands (National Strategy for Viticulture and Wine production (NSVW), 2014-2020).

According NSVW, 2014-2020, the wine market in the country has two consumer groups: middle-aged who have lower purchasing power and consume larger quantities of cheaper wine and younger to middle-aged with higher purchasing power who prefer smaller quantities of high-quality wine. The first group of consumers belongs to the population that normally produces wine for

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personal use and it is not a target group that could influence on raising the domestic production value. Therefore, a growing number of wine producers direct to the second groups of consumers as a focus group for further increase of the production value and development of wine culture (NSVW 2014-2020).

In this regard, the aim of the research is to determinate the presence of young population at the Skopje Winter wine festival in 2014. Additionally, the research focused on understanding the consumer preferences towards domestic wine and their perception of quality, related to future strategies for creating recognizable domestic wine brands. In the following section, the theoretical framework overview the quality perception related to the theory of information and possibilities of avoiding the information asymmetry. The results are presented through analysis of consumers' preferences for domestic quality wine and more detailed analysis for the younger consumers as the most present group at the festival. Finally, by comparing the different age categories, the main discussion and conclusions about younger consumers' preferences for domestic quality wine are drawn.

Theoretical framework

The perception of quality is directly related to the theory of information asymmetry (Wankhade and Dabade, 2010). Factors that lead to information asymmetry and quality uncertainty are associated with:

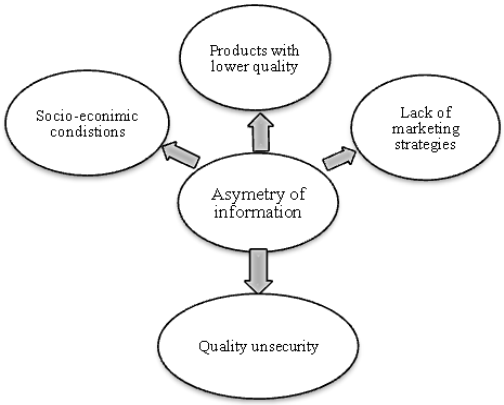
1. Socio-economic situation and culture of the population (education, culture, national economy and global/national legislation) and
2. Marketing strategies (quality, supply chain management, reputation, advertising, guarantee/warranty).

In transition countries and developing countries there is a possibility of more pronounced presence of low-quality products, which can be attributed to the high presence of information asymmetry and low presence of information symmetry (Wankhade and Dabade, 2010).

Often in these countries, a large number of the population is information asymmetric and assume the price of the product as an average of the high-quality or low-quality product. In those conditions, the expected price become an average price, and part of the population takes into account the expected price that is lower than the costs or the price of the high-quality product. This leads to directing asymmetric consumers towards low-quality products. On the other hand, part of the population is information symmetric and recognizes the quality/price ratio. They consider the concept of a higher price for a high-quality product.

The theory of information asymmetry assumes that the market share of

low-quality products is proportional with the growing number of buyers who are information asymmetric. The market share of high-quality products is lower, while at the same time is related to the smaller degree of prevalence of information symmetry.



Picture 1 Mechanism for quality uncertainties
Source: Wankhade and Dabade, 2010

The theory of information considers information asymmetry that appears between the producer and consumer as a reason for market failure. It arises from the producer perspective about product characteristics and consequently the lack of knowledge and identification by the consumer, due to lack of easy access to information (Ackerlof, 1970).

Nelson, (1970) characterizes the agri-food market in three product categories, depending on how the information is transmitted to the consumers:

Table 1 Product categories depending on the access to information

search goods	Consumer is trying to determinate the product quality before he buys, through inspection and research
experience goods	Consumer determinates the product quality after purchase, through use and experience
credence goods	Neither previous inspection nor further use is enough to determinate the quality (e.g. information on chemical and nutritional composition)

Source: Nelson, 1970

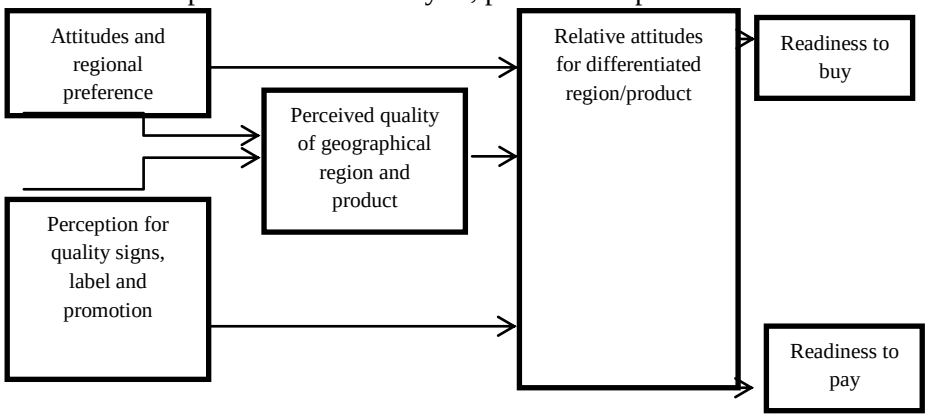
Ari-food commodities often belong to the second category - experience goods, because of the consumer motivation to form their opinion based on product attributes (mostly taste). However, due to differences in consumers' preferences, the product can be classified into various categories, depending on whether the consumer is interested in the price or other the product characteristics. If other characteristics are compared to the price, the product can be classified as experience good. If the consumer is interested in other characteristics of the production process, the product can be classified as credence goods. The difference between the consumers is the basic of market segmentation and the product differentiation strategy of the enterprises (Rangnekar, 2004).

MATERIAL AND METHODS

Data collection and conceptual model

The data collection was performed at the Winter Wine festival in Skopje, 2014, which was a closed type event for segmented group of consumers. The method included "face-to-face" interviews with structured questioners to 420 consumers. The questions contain demographic information and questions about consumer perception of product quality and quality/price ratio (Brewerton and Millward, 2001).

Following the Herman and Teuber method (2011), the questionnaire was based on the conceptual model for analysis, presented in picture bellow.



Picture 2 conceptual model for analysis of consumer attitudes (adapted model of herman and teuber, 2011)

The data were presented in nominal and ordinal scale (likert scale with five level of intensity), statistically processed in stata 12.0.

Testing of the hypotheses

The hypotheses in the research were tested by using chi2-test in stata 12.0, statacorp, 2011. The null hypothesis was tested through several alternative hypotheses, i.e. By crossing of multiple variables corresponding to the hypothesis. Alternative hypotheses show that the results are obtained by the different populations of the sample, while the null hypothesis shows that there is no difference between the different treatments. In this case, the working, or the alternative hypothesis indicated that there is a difference in the types of customers (younger consumers versus older, consumers with higher incomes versus those with lower incomes, consumers with higher education versus those with lower education, etc.) And the perception for the quality of the wine. Otherwise, the null hypothesis asserted that there are no differences between the type of consumers and all differences are observed within the random error.

H 1: younger consumers require quality wine as opposed to other age categories

H 1.1: younger consumers drink better quality wine

H 1.2: younger consumers are well informed about quality wine with geographical origin

H 1.3: young consumers buy wine with higher price

H 1.4: young consumers buy domestic wine

H 1.5: marketing has more influence on young consumers.

RESULTS AND DISCUSSION

The survey was conducted on 420 consumers. Additionally, detailed analysis were performed to the young consumers (18-27 years) which, according NSVW 2014-2020, are targeted group for increasing the value of domestic wine production. The most present age categories of consumers at the Wine festival were those from 18-27 to 28-37 years. The larger percentage of the consumers have personal monthly income from 30 000 to 60 000 MKD (24.1%) and 15 000 to 20 000 MKD (23.4%) and have gained faculty degree (68%).

Table 2 Short descriptive preview of variables with highest percentage of the answers

Variables	Percentage	Variables	Percentage
Most presented: 18-27; 28-37 years	>70%	**Price range:** From 150 to 300 MKD From 300 to 600 MKD From 600 to 1000 MKD	21,8 % 41,2 % 24, 0 %
Income: 15 000-20 000 MKD 30 000-60 000 MKD	23,4 % 24,1 %	**With increase of the income**: Would buy wine with higher price Would buy the same wine	50, 1 % 48,7 %
Higher education	68 %	**Readiness to pay**: Up to 600 MKD Up to 1000 MKD (15 EUR) Over 1000 MKD	25, 8 % 30, 73 % 11,33 %
Frequency of buying wine: Once a week	27,7 %	**Direct relation of quality/price ratio** (Information symmetric population)	71, 3%
Motive: Personal satisfaction	82,5 %	**Importance while buying wine:** Brand Region Country	50 % 26 % 27 %
Type of wine: Domestic wine	86,1 %	Often buy new wines Same wines Rarely buy new wines	51, 0 % 27,4 % 19,9 %
Location: Supermarket (0,75 l)	66,3 %	**How to be improved the domestic consumption?** Better promotion of domestic wines	34,1 %
Region: Tikves wine district Povardaski region	80% 8%		

Additionally, the consumer segment that buys quality wine (table wines with geographical indication, wine with controlled origin and wine with controlled and guaranteed origin) has been selected. These consumers buy wine once a week from the supermarket. Their motive is personal satisfaction.

Consumers are more dedicated to the domestic wines from Tikves wine district and Vardar River Valley wine region. This segment of consumers separates mostly from 300 to 600 MKD for wine (5-10 EUR), but for good quality of wine have showed readiness to pay from 600 to 1,000 MKD (10-15 EUR).

Furthermore, the results include the perception of the consumers about the impact of logos and quality signs, in the process of wine purchase. The intensity of the impact of the specific logo for quality and the awards placed on the bottle, in the highest percentage (32% and 29%) have intermediate level of significance (ranked third out of five levels of significance). The consumers have shown a high awareness of quality, where 38% determinate the importance of quality with the highest level of importance, as opposed to product price.

According the theory of information asymmetry, this result indicates information symmetric population that recognizes the quality/price ratio. In this case, the market share of high quality products is lower and in the same time proportional to the information symmetry (Wankhade and Dabade 2010). In terms of information asymmetry, the theory (Ackerlof, 1970) presents the product risk and consequences of not satisfying the consumer expectations and consequently a tendency for paying a higher price as a quality indicator. This may result in a reduction of the quality supply and extrusion of high-quality products from the market, thus facing unfair competition. The use of appropriate logos may impact the asymmetry, as mechanisms for quality guarantee or building reputation (Ackerlof, 1970). For the segmented consumers present at the Winter Wine festival, the specific logos for quality and awards have a secondary influence. Basically, it proved the intensive impact of the quality logos on the perception of consumers, which justifies their use as marketing tool for building reputation and quality assurance.

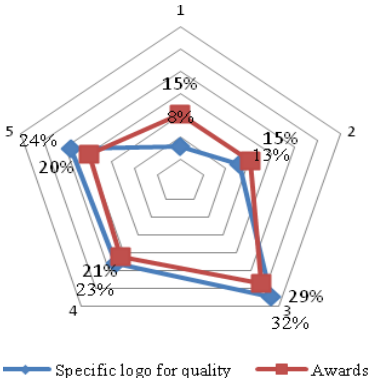


Figure 1 Intensity of the impact of quality sighs in the buying process

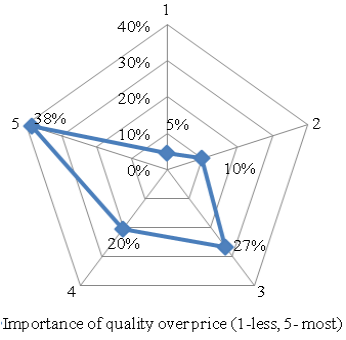


Figure 2 Importance of the wine quality vs. price of the wine

Furthermore, by testing hypothesis, the attitudes and perceptions of young consumers were analyzed, taking into account the consumers' age group from 18-27 years, the socio-economic and the marketing- factors influence.

Table 3. Hypothesis tested for younger consumers 'perspective.

Ho 1. Younger consumers requires quality wine as opposed to other age categories of consumers		
Sub-hypothesis	Ho	Ha
H 1. 1: better quality wine	+	-
H 1. 2: quality wine with geographical origin	+	-
H 1. 3: higher price	-	+*
H 1. 4: domestic wine	+	-
H 1. 5: marketing	+	-

* P<.05; ** P<.01; *** P<.001

The first hypothesis concerned the demand of the younger consumers for better quality wine than other age categories. By accepting the null hypothesis, is confirmed that younger consumers more likely to buy better quality wine with geographical origin, but also other age categories, although in small proportion, are an important category of consumers who buy quality wine with geographical origin. In addition, the null hypothesis is accepted in terms of perceiving the reputation of the geographical region as a sign of quality. It states that the young population, although most present of wine event, do not stand out statistically from the other age categories in terms of awareness of the importance of recognized geographical region and its quality.

The results of the third sub-hypothesis, analyzed the wine purchase with a higher price, by the young population. In this case, alternative hypothesis was accepted with significance level of 0.05, which indicated the difference between the age categories, where younger population has shown readiness to buy quality wine with higher price. In addition, the analysis showed that younger consumers mostly prefer domestic wines, but, the other age categories are also segments that require domestic wine, with geographical origin. Marketing, such as, advertising and promotion of wineries have no particular impact during the wine purchase, neither for the younger consumer segment nor for the other age categories.

CONCLUSIONS

Consumers are the final users that valorize the market value and the quality of the wine. Their perception of the quality of wine and geographical origin is highly influenced on the socio-economic factors, such as level of income and education. Overall, consumers with higher income and higher level of education require better quality wine with geographical origin, taking in to account its importance. Consequently, they are ready to pay a higher price for domestic wine whose quality is defined through the geographical origin. The highest impact on the process of wine purchase has the price of the product and

the perception of the brand. However, the size and homogeneity of the domestic market impact on insufficient commitment of domestic wineries to the domestic consumers. It indicates the relative homogeneity of the domestic market, the lack of different assortment and the low purchasing power of domestic consumers. Consumers are well informed only for two wine regions in the country (Tikves wine distinct and Vardar River Valley region) as well as for the wineries with a long tradition and presence on the market.

The most present category at the Winter Wine festival was the younger consumers, which are defined by the wineries as a potential focus group for increasing the value of the domestic production and development of the domestic market. However, compared to other age categories, younger consumers do not differ much as a separate segment in their demands for better quality domestic wine with geographical origin. The younger consumers differ only in their readiness to pay a higher price for quality domestic wine, which is significant for the development of the wineries' strategies intended for this segment of consumers.

If the National strategy for viticulture and wine production emphasizes the younger consumers as focus group for further increase of the wine culture, then better promotion of domestic wines and education of young consumers is of great importance. This would raise the consumers' awareness and perception of the wine quality, significant for improvement of domestic quality wines consumption.

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YIELD AND QUALITY TRAITS OF SOME FORAGE CROPS CULTIVATED UNDER AGROECOLOGICAL CONDITIONS OF KOSOVA

SUMMARY

The field experiment was carried out in the central part of Kosovo, Prishtina region. The experiment was designed in a CRBD fashion with four replication. The plot sizes were 1.5 x 8 m per plot or 12 m^2. The research comprised 6 treatments of forage mixtures or pure species: *Trifolium pratense + Lolium multiflorum, Lutus corniculatus + Festuca rubra, Lolium multiflorum, Festuca rubra, Trifolium pratense*, *Lotus corniculatus*. Measured traits were, dry matter yield (DMY), mineral composition, acid detergent fiber (ADF), neutral detergent fiber (NDF), Crude Protein (CP), lignin, were determined in this study of each treatments. Our study has revealed that all traits varied significantly ($P<0.01$) among investigated forage species and their combinations.

Keywords: forage, species, yield, mineral composition, NDF, ADF, CP.

INTRODUCTION

The genetic base of sown pastures is very narrow: for example, more than three-quarters of the grass cultivars registered in the European Union are of just six species, and more than half of Lolium perenne and L. multiflorum which account for more than 80 percent of the forage grass seed sold in the EU (Batello et al., 2008). Grasses belong to the family Poaceae (Gramineae)(family names are not written in italic). About 750 genera and 12 000 species, occur in all climatic zones. There are 7 subfamilies: Arundinoideae, Bambusoideae, Centhothecoideae, Chlorideae, Panicoideae, Pooideae (Festucoideae) and Stipoideae (subfamily names are not written in italic). The Poaceae is the fourth largest family of flowering plants after the Orchidaceae, the Compositae and the Leguminosae (family names are not written in italic). (Batello et al., 2008). Alfalfa monoculture is still prevalent in many European countries, where it is the back bone of organic crop-livestock systems and contributes significantly to conventionally managed systems. However, grass-legume mixtures are gaining new interest in Europe and elsewhere, owing to the energy and environmental costs associated with the synthesis and use of nitrogen fertilizer required for grass forage production and the quest for greatest self-sufficiency in feed proteins at

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

the farm and the country levels (Annichiarico, 2014). Many grasses contain ecotypes with a range of ecological adaptation and cultivars have been developed within species to suit very varied conditions. Grasslands represent an important and effective source of energy and proteins to ruminants, and combine high yield stability and draught resistance with low tillage operations and pesticide use and thus leading to good environmental conditions (Rusinovci et al., 2014 and 2015). Legumes are important for the high quality of their forage and their ability to fix atmospheric N, through symbiotic bacteria in their root nodules. There are many agronomic reasons for adding grass to perennial legumes. Grass legume mixtures have long been recognized as having several advantages over legumes or grasses alone. The legume component of a mixture generally increases total forage yield, fixes N from the atmosphere (part of which can be utilized by the grass), and enhances the quality of the pasture or hay, by its higher crude protein content and digestibility (Pirhofer-Walz et al., 2012). However, it is a challenge to manage the mineral supply of ruminants fed on grassland, because mineral concentrations in the herbage are influenced by a number of factors including species composition of the sward (Kuusela, 2006). Kosovo has an area of 10.887 km2 or 1.1 million ha, about 430.00 ha forested or 39.1% and 577.000 ha are agriculture land or 52%. From the total agricultural surface, pastures occupy 166.769 hectares or 28.90%, meadows 860.00 hectares or 14.90% and arable forages 38.000 hectares or 6.59% (MAFRD, 2002).The objectives of the present study were to evaluate the differences for some different cultivated forage crops for different qualitative traits under agro ecological conditions in Prishtina locality.

MATERIAL AND METHODS

Locations, Soils, and Weather

Three field experiments were conducted under reined conditions between 2013 and 2014. The experiment was established at a didactic farm in Prishtina, Faculty of Agriculture and Veterinary, Department of Crop Science located 42° 38' 29" N latitude, 21° 08' 45" E longitude, 570 m a.s.l. The in locality Prishtina is classified as vertisol (black soil).The soil analyses was done at the Norwegian University of Life Sciences, Department of Environmental Sciences, Ås, Norway. Soil characteristics are presented in Table 1.

Table 1 Soil characteristics at locations Prishtina

Location	Depth	pH in H_2O	pH in KCl	Humus (%)	N (%)	mg/100 g soil	
						P_2O_5	K_2O
Pristina	0-30	7.10	6.58	3.01	0.21	4.2	14.9
	30-60	7.05	6.74	2.19	0.19	3.7	11.3
	60-90	7.06	6.42	1.31	0.059	1.7	8.2

The area has an annual rainfall usually in the range of 700-750 mm (HMIK, 2008). Precipitation and temperature data for the Prishtina locations are shown for appropriate years in Table 2. Conditions were droughty with high temperatures especially in the summer time of growing season. Precipitation was considerably above normal at all locations in 2013, and above long term average in 2014. Summer temperatures in this region are sometimes exceeding 35°C resulting in high evapotranspiration (Aliu et al., 2010).

Table 2. Monthly precipitations (mm) and Temperatures (^{o}C) at location Prishtina

Month	Precipitations			Temperatures		
	2013	2014	LTA	2013	2014	LTA*
January	38.7	34.5	59.6	2.3	4.3	-0.2
February	5.8	12.5	18.1	3.6	6.7	1.9
March	70.3	74.5	52.5	5.4	10.2	6.4
April	40.4	52.3	48.1	13.4	13.5	11.5
May	122.3	154.2	126.2	17.4	16.1	16.9
June	55.3	65.5	42.3	20.2	20.4	19.9
July	32.6	68.9	51.6	22.3	22.3	21.5
August	21.2	71.5	32.2	22.9	21.5	21.1
September	56.6	58.5	42.9	15.7	18.3	16.8
October	64.8	48.7	58	13.6	14.1	11.9
November	42.6	49.8	41	8.4	9.2	6
December	15.9	52.3	44.7	1.6	4.6	1.7
Total	566.5	743.2	59.6	12.2	13.4	11.3

*LTA - Long Term Average

Plot Layout and Stand Establishment

The experimental desing was complete random block with four replications. The general formula for these kind of experiment was: 6 treatmenst x 4 replication=24 plots. The species (grasses or their mixtures with legumes) included in experiment were: (1) red clover+Italian ryegrass (Trifolium pratense + Lolium multiflorum), (2) birdsfoot trefoil+red fescue (Lutus corniculatus + Festuca rubra), (3) Italian ryegrass (Lolium multiflorum, (4) red fescue (Festuca rubra), (5) red clover (Trifolium pratense),(6) birdsfoot trefoil (Lotus corniculatus). Aim of the research was to determined the dry matter yield, mineral composition: Iron (Fe), Calcium (Ca), Cupper (Cu), Magnesium (Mg) and Phosphorus (P) and acid detergent fiber (ADF), neutral detergent fiber (NDF),Crude Protein (CP), Lignin . During this year the analyses are conducted only for first cutting. The first cutting term was in the beginning of June, after one year of establishment of plots. The herbage sampling methods was standard. Oven-dried samples of plots were ground and 1 g sample was used for the total nitrogen determination and 0.5 g for ADF and NDF. ADF and NDF were

analyzed by sequential detergent analysis method (Goering and Van Soest, 1970) and total nitrogen by Kjedahl method (AOAC, 1984). Crude protein content was calculated by multiplying total nitrogen with 6.25 constant.The samples were decomposed with concentrated HNO3 at 250°C in UltaClave from Milestone (Milestone microwave Ultraclave III). Samples were diluted in 10 % concentrated HNO3 before analysis. The determination of elements was done on ICP_OES (inductively coupled plasma optical emission spectrometry) with a Perkin Elmer Optima 5300 DV instrument (Perkin Elmer,Inc 2004 Shelton, USA). This analysis was done at The Norwegian University of Life Sciences, Department of Environmental Sciences, Ås, Norway. Statistics were performed by SPSS.version.19, and MS-Excel programme.

RESULTS AND DISCUSSION

The results revealed significantly high difference between investigated variants considering the yield in dry matter (Table 3).The results revealed significantly high difference between investigated variants considering the yield (Dry Weight tha-1). In the second year for the all cuts with higher yield was characterized Italian ryegrass of (5.96, 4.13 and 3.09 tha-1) .The lowest value at all cuts was observed for Birdsfoot -trefoil-red fescue on value (3.15, 2.24 and 1.99 t ha-1).The total value for Italian ryegrass was 13.18 t ha-1, while for combination of Birdsfoot- trefoil-red fescue the total yield was 7.38 t ha-1. The differences between higher and lower total yield were +5.8 t ha-1 or expressed in relative values was 55.39%. During the year 2014 in locality of Prishtina the precipitations were relatively higher and the yield was higher compare with year 2013. Results are presented in Table. 3.

	2012				2013				
Treatment/ Cut	I	II	III	Total	I	II	III	Total	Average
R. clover- It. ryegrass	5.48^{a}	3.75^{b}	2.76^{b}	11.99	6.83^{c}	4.85^{b}	3.11^{c}	14.79	13.39
B. trefoil- Red fescue	3.15^{e}	2.24^{e}	1.99^{c}	7.38	3.94^{f}	3.12^{d}	2.02^{e}	9.08	8.23
Italian ryegrass	5.96^{a}	4.13^{a}	3.09^{a}	13.18	7.39^{a}	5.34^{a}	3.55^{b}	16.28	14.73
Red fescue	4.07^{d}	2.81^{c}	2.17^{c}	9.05	5.12^{d}	3.77^{c}	2.60^{d}	11.49	10.27
Red clover	5.74^{b}	3.82^{b}	2.87^{b}	12.43	7.06^{b}	5.11^{a}	3.81^{a}	15.98	14.21
Birdsfoot trefoil	4.05^{d}	2.62^{d}	2.15^{c}	8.82	4.87^{e}	3.83^{c}	2.59^{d}	11.29	10.05

*Means bearing by the same letter in a column were not significantly different at the $p < 0.05$ level using the LSD test.

From our experiment at all cuts for higher yield was characterized Italian ryegrass with a total yield 14.73 tha-1 , but for lower total yield was obtained at combination Birds foot –trefoil red on value 8.23 tha-1 .The differences between

them were +6.5 tha-1 or expressed in percentage value 55.03%. If compared year 2013 (10.47 tha-1) and 2014(11.81 tha-1) for average total yield the differences were +1.34 tha-1 or 12.02% higher. Concentrations of minerals content varied significantly among investigated species. The maximum average values at the first year for Calcium (Ca) concentration was found in Birds foot trefoil (25.61 g-1 kg-1), while for the second year was at the red clover (10.16 g-1 kg-1) .The difference between them was significant (15.45 g-1 kg-1at level of probability P<0.01). Sultan et al., 2008 have realized different results for Calcium content too, which varied from 0.13 to 0.38%. Also the variations for Iron (Fe) were with small differences. In the first year (2013) the average values at all treatments were 0.24.On higher average values were characterized the Italian ryegrass (0.393 g-1 kg-1). In the second year with higher values was characterized the Birds foot trefoil (0.35 g-1 kg-1). The values of Copper (Cu) and for Potassium (K) were not significantly only for K in the first year of investigation were with differences. Harrington et al., 2006 obtained different results at different forage crops for Ca (0.36 till 1.87) and for Iron (Fe) on value 67 till 167 mg kg-1 . The average values for Zinc (Zn) for both two years investigated were 0.03 and 0.02 mg kg-1. The findings of Sultan et al. (2008) study revealed that Ca content in free rangeland grasses generally increased with maturity .Optimum level of Calcium (Ca) in plants ranged from 0.40 to 0.60% and its level above 1.0% is considered high (Georgievskii, 1982), whereas, Minison (1990) reported Calcium (Ca) level from 0.31 to 1.98% and the mean as 0.63%. Some results was obtained for forage crops for mineral composition with higher variation from Rusinovci et al. (2014). Results are presented in Table.4.

Table.4. Average values for mineral composition (g^{-1} kg^{-1})

	Ca		Cu		Fe		K		Mg		Mn		Zn	
Treatment/years	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
R. clover-It. ryegrass	10.23^{c}	3.72^{b}	0.013^{a}	0.006^{a}	0.156^{b}	0.29^{b}	27.58^{b}	22.75^{a}	2.43^{b}	1.82^{ab}	0.53^{a}	0.063^{a}	0.026^{b}	0.021^{a}
B. trefoil-Red fescue	6.16^{d}	4.06^{b}	0.013^{a}	0.0067^{a}	0.123^{b}	0.28^{b}	25.27^{d}	20.79^{a}	1.63^{d}	1.49^{b}	0.046^{ab}	0.056^{b}	0.023^{bc}	0.016^{ab}
Italian ryegrass	2.47^{e}	7.36^{ab}	0.013^{a}	0.011^{a}	0.393^{a}	0.33^{ab}	20.13^{e}	24.43^{a}	1.15^{e}	1.76^{ab}	0.04^{b}	0.056^{b}	0.01^{c}	0.021^{a}
Red fescue	17.61^{b}	8.91^{b}	0.014^{a}	0.0067^{a}	0.163^{b}	0.31^{ab}	25.27^{d}	24.43^{a}	2.77^{a}	1.01^{b}	0.036^{b}	0.066^{a}	0.026^{b}	0.02^{a}
Red clover	12.03^{b}	10.16^{a}	0.017^{a}	0.0067^{a}	0.183^{ab}	0.25^{b}	30.26^{a}	19.43^{b}	2.5^{b}	1.79^{ab}	0.056^{a}	0.065^{a}	0.026^{b}	0.013^{b}
Birdsfoot trefoil	25.61^{a}	4.11^{b}	0.21^{a}	0.011^{a}	0.393^{a}	0.35^{a}	25.61^{c}	21.77^{a}	2.09^{c}	2.02^{a}	0.053^{a}	0.053^{b}	0.041^{a}	0.021^{a}
Average	12.35	6.39	0.05	0.01	0.24	0.30	25.69	22.27	2.10	1.65	0.13	0.06	0.03	0.02

*Means bearing by the same letter in a column were not significantly different at the $p < 0.05$ level using the LSD test.

Crude protein content is one of the most important criteria for fodder quality evaluation. The forage crude protein contents of the treatments varied

greatly. From results which is considering examined years, red clover-italian ryegrass had the higher cp (11.38%) year 2013 and 12.33% year 2014.while with lowest average values of cp for both years (2013 and 2014) was characterized the birdsfoot trefoil on values (9.88 in year 2013) and 10.08 in year 2014. The differences between years for higher cp were +0.95% higher for year 2014, while the differences between years for lowest values were +0.2% for year 2014. Acid detergent fiber (adf) and neutral detergent fiber (ndf) concentrations are important quality parameters of forages (schroeder, 1994; caballero et al., 1995; henning et al., 1996; assefa and ledin, 2001; albayrak et al., 2011). The highest percent of ndf in 2013 was determined the red fescue (72.63%), while in 2014 it was in the mixture of birdsfoot trefoil-red fescue (66.40%).for adf the higher results were obtained at red fescue 39.57 in year 2013 and for year 2014 was characterized the birdsfoot trefoil on value 38.92.on the other hand, in both years combination red clover-italian ryegrass obtained the higher content of lignin 7.56 (2013) and 6.37 (2014).while for lowest value of lignin was determined at birdsfoot trefoil-red fescue 5.53 in year 2013, and italian ryegrass 5.87 in year 2014. Similar results have been reported by some researchers studied similar mixtures of cereals and legumes under intermediate winter crop system (lithourgidis et al.,2006; kokten et al., 2009; yucel and avci, 2009). Results are presented in table. 5**.**

Table.5. Forage quality parameters (%) of forage crops mixtures in Prishtina location

		Year									
		2012					2013				
	Treatment/ Parameters	CP	CF	NDF	ADF	Lignin	CP	CF	NDF	ADF	Lignin
Location Prishtina	R. clover-It. ryegrass	11.28^{a}	33.8^{ab}	60.11^{bc}	38.89^{ab}	7.56^{a}	12.43^{a}	31.73^{c}	54.48^{bc}	33.05^{c}	6.27^{ab}
	B. trefoil-Red fescue	10.42^{b}	35.2^{a}	64.75^{b}	38.29^{ab}	5.53^{c}	10.51^{c}	34.76^{ab}	66.40^{a}	37.18^{ab}	5.86^{c}
	Italian ryegrass	10.86^{ab}	30.7^{c}	65.44^{b}	35.3^{c}	6.30^{b}	10.27^{d}	33.74^{b}	60.74^{ab}	37.94^{ab}	5.77^{c}
	Red fescue	10.41^{ab}	31.89^{b}	55.82^{c}	39.57^{a}	7.31^{a}	10.50^{c}	35.77^{ab}	59.10^{b}	37.36^{ab}	6.06^{b}
	Red clover	10.69^{ab}	33.22^{ab}	72.53^{a}	37.24^{b}	6.43^{b}	11.03^{b}	36.09^{a}	52.07^{c}	36.47^{bc}	6.24^{b}
	Birdsfoot trefoil	9.78^{c}	32.42^{b}	53.28^{c}	37.35^{b}	6.52^{b}	10.07^{d}	32.21^{b}	60.02^{ab}	38.92^{a}	6.57^{a}

*Means bearing by the same letter in a column were not significantly different at the $p < 0.05$ level using the lsd test.

Notes : cp-crude protein; cf-crude fiber; ndf- neutral detergent fiber; adf- acid detergent fiber

CONCLUSIONS

Weather was the most important factor affecting yield. Forage crops mixtures can obtain several cuts during the year with significant yield but only if species combination is adequate-with adequate grass species, if there is enough

water by precipitation or irrigation and if the plants are cut in proper time. The same was confirmed for mineral composition too with differences . Data obtained in this research may be usefull to practitioners in forage production and utilization, especially when bringing decisions on species composition in new swards establishment. Also, the observed mineral composition, ADF, NDF, CP may provide some data on the need for supplements in livestock nutrition when pastured or fed with investigated forage species.Further research should be based on finding adequate species mixture and their ratio for different cites with specific climate in order to overpass possible losses and ensure feed during the whole year.

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EFFECTS OF WATER DEFICIT AND APPLICATION METHOD, ON DRIP IRRIGATED PEACH

SUMMARY

A 2-year study was conducted to determine the effects of different drip irrigation profiles on the yield quantity and quality of mature peach trees [*Prunus persica* L.]. The irrigation profiles were the combination of emitter location (ground drip or subsurface buried @15cm), emission rate (conventional 4.0 L h^{-1} or low-flow 0.5 L h^{-1}), and water deficiency level (60%, 80%, 100% of ET). The results showed that yield and water use efficiency (WUE) were not affected by the irrigation profiles, while there were some reduction in the yield and improvement in WUE due to water 60% ET deficiency application. On the other hand, some of the fruit quality measures (Total sugars, and maturity index) were affected by the irrigation profile as the low-flow profiles yielded sweeter and more matured fruits. Moreover, the study involved water and salt measurements under each irrigation profile, the conventional drip rate showed more-efficient salt flushing, while the low-flow profiles showed wider lateral movement of water. The best overall treatment under average conditions was the low-flow ground drip with 80% of ET.

Keywords: Low-flow; ultra-low flow drip; drip irrigation system; deficit irrigation; irrigation profiles; peach fruit quality; subsurface drip irrigation.

INTRODUCTION

The peach tree is a deciduous tree that bears a nutritious and aromatic juicy fruit. Although the second part of its binomial name, Prunus persica, refer to Persia; its origin refers to Northwest China where it was grown more than 30 centuries ago. China remains the largest peach producer till now (Faust and Timon, 1995; Zarini, 2014). Peach production has a growing economic importance worldwide, the most considerable economic factors are yield quantity and quality, earlier crop production, less plant stress and reduced yield variability (Cetin et al., 2003; Zarini, 2014). Proper irrigation management is very important for peach to maintain healthy growth and acceptable productivity even in areas with relatively high rainfall (Bryla et al., 2005; Williamson and Crane, 2010).

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Peach is irrigated by almost all the irrigation methods including furrows, borders, micro-sprinklers, and drip irrigation systems, however, the production and growth of the peach trees is significantly affected by the irrigation method especially in the first 3 years of their age (Bryla et al., 2003). Several studies showed that drip irrigation resulted in better fruit quality, greater quantity, and earlier production (Bryla et al., 2005, 2003; Cetin et al., 2003). Additionally, applying drip irrigation using extremely low flow rates (<1.0 L h^{-1}) enhances water lateral-spread, reduces water losses by evaporation and deep percolation, and increase the water use efficiency (Abdou et al., 2010; Gilead, 2002; Mead, 2001). To conserve water, some investigators applied several deficit irrigation strategies and/or irrigation scheduling methods on drip irrigated peach, they reported a significant response to the deficit irrigation strategies and negligible effect of the scheduling methods (Aragüés et al., 2014; Goldhamer et al., 2001). When applied on peach trees, deficit irrigation led to improved fruit quality and increased soluble solid content (Pliakoni and Nanos, 2010), but it lead to yield reduction to some extent (Abrisqueta et al., 2010; Rufat et al., 2010), and increased water use efficiency (Abrisqueta et al., 2010). The aim of this work is to study the effect of dripper flow rate, dripper location, and water deficiency ratio on the yield quantity and quality of drip-irrigated peach trees.

MATERIAL AND METHODS

Study site

The field experiments were conducted in an experimental farm located at Badr City, Northwestern of Cairo, Egypt (30° 30’ 44” N, 30° 27’ 17” E). The experiments were carried out through two successive seasons 2012 and 2013 on seven years old Florida prince peach trees budded onto Nemaguard rootstocks. The trees were planted at distance of 5 m between rows, and 4 m within rows in a sandy soil; the properties of the soil are listed in Table 1. The field was irrigated by water pumped from an 80 m deep well; the chemical properties of it are listed in the same table.

Table 1. Physical and chemical properties of soil and water

Physical properties (*of soil*)							
Soil Depth	Course sand	Fine sand	Silt	Clay	Field capacity[1]	wilting point[1]	Bulk density
cm	%	%	%	%	%	%	$g.cm^{-3}$
0-30	92.8	3.7	2.0	1.5	10	4.8	1.83
30-60	91.5	1.8	0.2	6.5	11	6.3	1.79
60-90	93.1	0.6	0.4	5.9	13	5.5	1.72

*properties are measured in % by volume.

Chemical properties									
pH	EC[2]	Soluble Cations, mg L^{-1}				Soluble Anions, mg L^{-1}			
	$dS.m^{-1}$	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{-}	HCO_3^{-}	SO_4^{--}	Cl^{-}
…of Soil									
8.8	2.80	9.10	9.60	8.61	0.69	-	2.34	12.06	13.6
8.4	0.21	0.82	0.28	0.80	0.20	-	0.73	0.47	0.9
8.8	0.76	1.80	1.28	3.65	0.84	-	1.47	2.50	3.6
…of Water									
6.9	1.634	2.55	1.61	11.9	0.28	-	2.25	2.79	11.3

* EC is measured in the saturation extract

Experimental design

The trees were irrigated using four methods; the methods include two types of emitters; conventional emitters (C, 4.0 L h^{-1}), and low flow emitters (L, 0.5 L h^{-1}), and two locations of the lateral lines; ground drip (G), and subsurface drip (S) at 15 cm depth. The four possible combinations between them are GC, SC, GL, and SL. Each of these combinations were applied using three levels of deficiency 40, 20, 0 % (60, 80, and 100% of the calculated ET_c, {D_4, D_2, and D_0 respectively}). Each treatment combination ($GC \times D_4$ for example) was applied on 6 random trees. The total number of trees was 72 trees (3 deficiency levels × 4 irrigation methods × 6 replications).

Irrigation management and water calculations

The used pumping plant consisted of a centrifugal pump with maximum discharge of 80-m^3 h^{-1} driven by a diesel engine 37 kW with a maximum lift of 20 m. We used two types of emitters; one for the C treatments and one for the L treatments. For the C treatments we used built in emitters with 4 L h^{-1} discharge, while for the L treatments we used 2 L h^{-1} multi-exit emitters (Table 2), with an attached five-way manifold of a female inlet and four outlet barbs with spaghetti tubes installed on each barb emitting 0.5 L h^{-1}

Fiure 1. An illustration of the on-line emitter with a manifold plugin that is used for low flow irrigation. The emitter's outflow is 2 L/h resulting in 0.5 L/h per outlet

For the C treatments 16 emitters were used for each tree placed 0.5 m apart in two laterals around the tree trunk totaling 32 L h^{-1}. While for the L treatments, two multi-exit emitter were used per tree, where the spaghettis encircle the trunk of the tree totaling 8 L h^{-1}. The operating time of the L treatments was always set to be 4 times as the C treatments of the same deficiency level.

To measure the effectiveness of the irrigation methods, we calculated the water use efficiency (WUE), which is the ratio between the yield and the applied water, kg/m^3 , it is a good indicator of the effective water management and successful irrigation practices (Doorenbos and Pruitt, 1977; Kang et al., 2000; Rahil and Qanadillo, 2015).

Orchard management

Irrigation water requirements for peach trees were calculated according to daily climatic data from the local weather station data belonged to the Central Laboratory for Agricultural Climate (CLAC). The water requirements were calculated on daily basis using the FAO-56 methodology (Allen et al., 1998). The reference evapotranspiration (ET_o) values were calculated daily based on measured climatic data, while we took the crop coefficient (k_c) values from the local agriculture extension services. The kc values for the initial, middle, and late stages were 0.48, 0.79, and 0.75 respectively. Due to its rarity in the study region, the active rain is negligible, the seasonal irrigation requirement according to Nakayama and Bucks (2012) recommendations were 5781.4 m^3 ha^{-1} (Table 2)

Table 2. Seasonal water requirements of peach trees.

Growth stage	Month	Reference Evapotranspiration (ET_0)	Crop Coefficient (K_c)	Crop Evapotranspiration (ET_c)	Daily water requirements	Water requirements per
		mm/day		mm/day	L/day	m^3/ha/day
Initial	Jan.	2.4	0.48	1.152	11.5	5.8
	Feb.	3.2	0.48	1.536	15.4	7.7
	Mar.	4.2	0.48	2.016	20.2	10.1
Middle	Apr.	5.6	0.79	4.424	44.2	22.2
	May	6.6	0.79	5.214	52. 1	26.2
	Jun.	7.3	0.79	5.767	57.7	28.9
	Jul.	7.2	0.79	5.688	56.9	28.5
Late	Aug.	6.7	0.75	5.025	50.3	25.2
	Sep.	5.6	0.75	4.200	42.0	21.1
Total water requirements per hectare per season:					5781.4 (m^3/ha/season).	

The irrigation starts at January and ends in September, with 2-3 weekly irrigations. The time of each irrigation depends on the irrigation treatment, the deficiency level, and the average calculated ET_c in this week (which combines weather conditions and phenological stage).

According to the recommendations of the local extension services, the fertilization program was applied to the trees through traditional application methods (no fertigation), and the weeds and pests were controlled.

Soil measurements

To determine soil water content and salt accumulation, we took soil samples manually using a screw auger. At each location, we took twelve samples, (At 4 depths ×3 perpendicular distances). The sampling depths were at locations 0-20, 20-40, 40-60, and 60-80 cm below soil surface, while the perpendicular distances were at 10, 30, 50 cm perpendicular to the lateral line. At the beginning, middle and end of each season, soil samples were taken at four timings; before irrigation, 2, 10, and 18 hours after irrigation. According to the method of Gardner (1986) we measured the gravimetric water content in the samples, then the samples were air-dried and sieved to calculate the electrical conductivity (EC, dS m^{-1}) in the saturation extract (Germaine and Germaine, 2009). The total soluble salts (TSS, ppm) values were calculated by multiplying the EC by 640 (Sonon et al., 2012). The results of water and salts distribution were interpolated using the kriging method (Abramowitz and Stegun, 2012), and then were plotted using Surfer software, (Golden Software v.9).

Crop measurements:

In addition to the yield of each tree that was quantified at the end of each season, additional fruit quality measures were performed on a representative sample of 20 mature fruits from each considered treatment. These measurements taken were: the fruit total weight and pulp weight, dimensions and volume, in addition to some chemical characteristics in juice like the total soluble solids percentage (TSS%) determined using hand refractometer, titratable acidity percentage (TA%, as malic acid, 1% total acidity =10 g malic acid per Liter of juice) following the methods of Cunniff (1999), and the total sugars % was identified by the methods described by Brooks et al (1993) , accordingly, the Maturity index (MI) was calculated as 10×TSS/TA (Conesa et al., 2014). When the MI ratio is high, the overall flavor of the fruit becomes flat, and the fruit becomes tasteless (Shinya et al., 2014). The ripen fruits were taken according to the signs described at Kader (1999).

On the other hand, we have measured some leaf characteristics like the surface area, chlorophyll and NPK contents. The leaf area was measured using 20 mature leaves as the third one of the base of the previously tagged non-fruiting shoots from spring cycle were taken randomly from each replicate at mid-June, then measured by the planimeter. The leaf total chlorophyll content was estimated in the field by using SPAD-502 meter (Minolta Co., and Osaka). To estimate the leaf NPK content, we collected 20 matures leaves, at first week of July each season, from the middle portion of current year shoots of each replicate, leaves were collected to determine macro elements in dry leaf samples, nitrogen percentage was estimated by micro-Kjeldahl method (Steyermark, 1961), phosphorous percentage was determined using atomic absorption spectrophotometer Perken Elmer (Model 3300, USA) according to Chapman and Pratt (1961), and potassium was estimated according to Brown and Lilleland (1966).

Statistical analysis

The obtained results were statistically analyzed using Statistix v.10.0, Analytical Software. Where the factorial linear model was used for analysis of variance. The first factor was the irrigation profile in four levels, and the second factor was the deficiency level in three levels. Means were separated at 0.05 level using Tukey's honest significant difference test (HSD).

RESULTS AND DISCUSSION

Plant results. According to the statistics in Table 3, if a studied property showed only single effects significance, i.e. the interaction DL×IR is not significant, then the results will be discussed according to the single effects' means, Table 4, otherwise the results will be discussed according to the interaction means, Table 5.

Table 3. Statistical significance of the irrigation profile, the deficiency level, and their interaction

	Year 1			Year 2		
	DL	**IR**	**DL×IR**	**DL**	**IR**	**DL×IR**
Yield (kg/tree)	**	**	---	**	---	---
Water use efficiency (kg/m3)	**	**	**	**	---	---
Fruit measures:						
Fruit weight (g)	**	**	**	**	**	**
Fruit height (cm)	---	**	**	---	**	**
Fruit diameter (cm)	**	*	**	**	---	**
Fruit volume (cm3)	---	**	*	**	**	**
Flesh thickness (cm)	**	*	**	**	*	**
Flesh weight (g)	**	**	**	**	**	**
Leaf measures:						
Leaf area (cm^2)	**	**	**	**	**	*
Chlorophyll content (%)	**	**	*	**	**	---
Nitrogen leaf content, N (%)	**	**	**	**	**	**
Phosphorus leaf content, P (%)	**	**	**	**	**	**
Potassium leaf content, K (%)	**	**	**	**	**	**
Juice measures:						
TSS (%)	**	*	---	---	**	*
Total acidity, TA (%)	**	**	---	**	**	**
Maturity index, MI (-)	---	**	---	**	**	---
Total sugars, TSG (g/100cc)	**	**	*	**	**	---

In general, as shown in Table 3. we found that the single effects of both the deficiency level (DL) and the irrigation profile (IR) led to significant differences in almost all of the studied measures in the two years, while the interaction between them showed significance in fruit and leaf measures, but not in the juice measures

Yield and water use efficiency. For the yield, Table 3 showed that the interaction between IR and DL (IR ×DL) is not significant in the two years; hence, we can analyze the single effect of each factor individually. For the water use efficiency (WUE), the table showed that the IR×DL was significant in the first year; hence, we will compare the means of the combination treatments, Table 5, while only the DL is significant in the second year.

The effect of the deficiency level appears on Table 4. The results showed that the increase in water deficiency leads to decrease in yield; the average yield of peach with no deficiency (D0) was 22.7/ 22.4 kg/tree for year 1/ year 2 respectively (22.55 in average). The average values were 22.25 and 20.05 kg/tree for 20% (D2) and 40% (D4) deficiency levels respectively, where there were no significant difference between D0 and D2. The WUE was solely affected by the DL in the second year, where the highest WUE value was the D4 followed by the D2 then D0 (2.91, 2.33, and 1.93 kg/m3 respectively, (50.7%, and 20.7% for D4 and D2 over D0 respectively)

Looking at the irrigation profiles effects, Table 4; we found that in the first year, the low flow treatments (GL and SL) returned higher yields than the conventional treatments (GC and SC), while the effect of the irrigation profile is not significant at the second year.

The interaction between IR ×DL affected only the WUE in the first year, Table 5 showed that the highest WUE was from the GL-D4 treatment combination, with 3.4 kg/m3 followed by the SL-D4 treatment (3.0 kg/m3), while the least WUE was 1.9 kg/m3 from the GC-D0 and SC-D0 treatments. The treatments GL-D4 and SL-D4 were the top in water saving in the second year as well.

Fruit measures. Looking at Table 3, we can notice that excluding the fruit height and volume, all the fruit measures were significantly affected by the DL and by the interaction DL×IR. The heaviest fruit average, Table 5, in the first year was 72.2 g for the control treatment GC-D0, which yielded 85.8 g fruits in the second year with no significant difference of the SC-D0 treatment (89 g). Similarly, the maximum diameter, maximum flesh thickness and weight were obtained at the control treatment GC-D0 in the two years. Furthermore, it is noticed that the fruit weight of the treatments with deficiency applied was significantly less than that of the control treatments in the two years, and the fruit diameter follows the same trend. However, the results showed that regarding the flesh thickness, the D2 treatment had no significant difference than the D0 treatment, while the D4 treatment led to significantly thinner flesh.

Table 4. Means of single effects of the irrigation profile and the deficiency level

	Deficiency level			Irrigation profile			
Year 1	**D0**	**D2**	**D4**	**GC**	**GL**	**SC**	**SL**
Yield (kg/tree)	22.73 a	22.59 a	20.26 b	19.83 C	24.95 A	20.38 C	22.28 B
Water use efficiency (kg/m3)	2.03 c	2.44 b	2.91 a	2.28 C	2.76 A	2.27 C	2.53 B
Fruit measures:							
Fruit weight (g)	70.2 a	65.1 b	65.2 b	68.2 A	67.4 A	64.3 B	67.3 A
Fruit height (cm)	4.81 a	4.81 a	4.72 a	4.80 AB	4.68 B	4.76 AB	4.88 A
Fruit diameter (cm)	5.17 a	5.01 b	4.81 c	4.99 AB	5.02 AB	4.94 B	5.05 A
Fruit volume (cm^3)	52.3 a	54.3 a	53.0 a	57.4 A	56.3 A	43.9 B	55.0 A
Flesh thickness (cm)	1.55 a	1.55 a	1.45 b	1.52 AB	1.55 A	1.48 B	1.52 AB
Flesh weight (g)	62.2 a	56.9 b	57.1 b	60.1 A	59.2 A	56.3 B	59.3 A
Leaves measures:							
Leaf area (cm2)	35.91 a	35.84 a	34.67 b	33.31 C	37.03 A	34.92 B	36.63 A
Chlorophyll content (%)	35.94 a	35.67 a	34.83 b	34.02 C	37.06 A	35.29 B	35.56 B
Nitrogen leaf content, N (%)	2.482 c	2.723 a	2.609 b	2.472 C	2.757 A	2.580 B	2.610 B
Phosphorus leaf content, P (%)	0.163 c	0.201 a	0.183 b	0.111 C	0.238 A	0.192 B	0.187 B
Potassium leaf content, K (%)	1.159 b	1.202 a	1.167 b	1.109 C	1.217 A	1.170 B	1.207 A
Juice measures:							
TSS (%)	10.68 b	11.71 a	11.85 a	11.79 A	11.00 B	11.53 AB	11.33 AB
Total acidity, TA (%)	0.78 b	0.84 a	0.85 a	0.98 A	0.74 C	0.81 B	0.76 BC
Maturity index, MI (-)	13.90 a	14.13 a	14.04 a	12.09 B	14.79 A	14.32 A	14.90 A
Total sugars, TSG (g/100cc)	4.03 ab	4.18 a	3.91 b	3.82 B	4.49 A	3.86 B	3.99 B
Year 2	**D0**	**D2**	**D4**	**GC**	**GL**	**SC**	**SL**
Yield (kg/tree)	22.44 a	21.93 a	19.76 b	20.96 A	20.95 A	21.47 A	22.13 A
Water use efficiency (kg/m3)	1.93 c	2.33 b	2.91 a	2.30 A	2.34 A	2.40 A	2.52 A
Fruit measures:							
Fruit weight (g)	82.2 a	72.6 b	73.2 b	76.1 B	73.7 C	79.2 A	75.0 BC
Fruit height (cm)	5.28 a	5.24 a	5.20 a	5.17 B	5.08 B	5.35 A	5.35 A
Fruit diameter (cm)	5.46 a	5.22 b	5.11 c	5.23 A	5.27 A	5.30 A	5.25 A
Fruit volume (cm^3)	81.7 a	67.0 c	71.8 b	73.8 AB	70.3 B	77.8 A	72.2 AB
Flesh thickness (cm)	1.77 a	1.73 a	1.65 b	1.73 A	1.74 A	1.70 A	1.70 A
Flesh weight (g)	74.1 a	64.5 b	65.1 b	68.0 B	65.7 C	71.1 A	66.7 BC
Leaves measures:							
Leaf area (cm2)	37.69 a	37.29 a	35.78 b	34.83 D	39.31 A	35.85 C	37.69 B
Chlorophyll content (%)	39.70 a	39.48 a	38.36 b	36.89 C	41.25 A	39.11 B	39.47 B
Nitrogen leaf content, N (%)	2.519 b	2.721 a	2.556 b	2.418 C	2.807 A	2.588 B	2.580 B
Phosphorus leaf content, P (%)	0.173 c	0.211 a	0.186 b	0.106 C	0.257 A	0.194 B	0.204 B
Potassium leaf content, K (%)	1.173 b	1.222 a	1.183 b	1.120 D	1.238 A	1.197 C	1.216 B
Juice measures:							
TSS (%)	12.97 a	12.64 ab	12.31 b	12.29 BC	11.58 C	13.09 AB	13.60 A
Total acidity, TA (%)	0.87 b	0.90 b	0.95 a	0.99 A	0.78 C	0.93 B	0.92 B
Maturity index, MI (-)	14.90 a	14.13 a	13.13 b	12.59 B	14.86 A	14.05 A	14.71 A
Total sugars, TSG (g/100cc)	4.09 b	4.64 a	4.12 b	4.04 B	4.84 A	4.10 B	4.16 B

D4, D2, D0: deficiency percent, subscripts refer to 40%, 20%, and 0% deficiency, GC: 4L/h ground drip irrigation, SC: 4L/h subsurface drip irrigation, GL: ground drip irrigation with low flow emitter, SL: subsurface drip with low flow emitter, LSD: least significant difference at p=0.05.

* Means with the same letter for each row, and same group are not significantly different from each other at 0.05 level. Capital letters are used for Irrigation profiles comparisons, while small letters are used for deficiency levels comparisons.

Leaves measures

The surface area of the leaves also affected by the DL×IR interaction, as the largest area occurred at the GL-D2 followed by the SL-D0 in the first year, Table 5, while the largest area in the second year occurred at the GL-D0,2 treatments. The table showed that the low flow treatments (GL-D* and SL-D*, the asterisk means for any deficiency level) result in larger leaf area in the two years.

The chemical analysis showed that the highest chlorophyll content occurred at GL-D* (7-12% higher than the control), while the lowest values were achieved at the GC-D*. Similarly, the treatments GL-D* showed dominance on the N, P, and K contents as listed in Table 5.

Juice measures

The interaction DL×IR was not significant in most of the juice measures as shown in Table 3. The TSS and TA values showed a direct proportion to DL in the first year, Table 4, as the TSS increased with the increase of deficiency. The TSS relationship was not significant in the second year.

Consequently, the maturity index (MI) showed different trends in the first and second years; while relationship is not significant in the first year, it showed significance in the second year with 8-14% less MI when applying the D2 and D4 respectively, Table 4. The IR treatments showed significant effect on the MI as well; the least MI obtained from the GC treatment in the two years, while there were no significant differences between any of the other three irrigation profiles with 12-23% more MI values, Table 4.

The maximum resulted total sugars (TSG) were in the GL-D2 treatment in the first year, 13% more than the control, while the maximum TSG for the second year was obtained at the D2 level of deficiency, 4.6g/100c, which is about 13% more than the D0 and D4 levels. Still, the irrigation profile analysis showed that the GL profile gave the maximum TSG by about 20% more than the conventional treatment GC.

Water and salts distribution in soil

To have a better understanding of the trees reaction to the irrigation scheme we measured the soil water and salts distribution under the orchards at different times. Due to the large number of treatment-combinations and to make it easy to interpret the results, we have combined the water and salts maps into one map per treatment. The maps were stacked so that each water map is in the bottom and the salts map is overlaying it with 40% transparency, Figure 2.

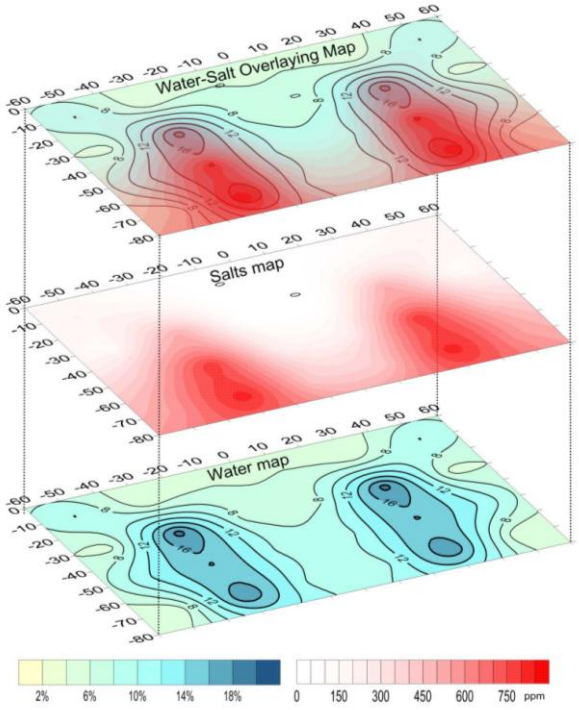


Figure 2. The overlayed maps of salt and water forming a combined map
*: water map units are soil-water content (% by volume), salts map values are TSS (ppm).

The combined maps are shown in Figures 3 and 4 for surface and subsurface treatments respectively. In this paper, we publish only the results of the second year late season measures in for the D0 treatment, the results of the other timings and deficiency levels will be published in a separate paper.

For the ground drip treatments, Figure 3, the conventional drip flow rate appears to flush salts more efficiently than the low flow drip does. It is noticed that the salts appear after irrigation, Figure 3a, b, but the higher flow emitter, a, seems to flush water from the upper 20 cm, while the lower flow rate, b, can hardly flush some salts just around the emitter. The salt flushing process continues with water redistribution, Figure 3c-f, with faster rate at the conventional drip (c, e), and slower at the low flow drip treatments (d, f). At the same token the subsurface treatments act, Figure 4, the conventional treatments showed less salt accumulation than the low-flow treatments. Furthermore, the subsurface treatments, in general, showed less salt accumulation than the ground treatments.

Table 5. Means of the interaction between irrigation profiles and deficiency levels on the studied properties

Year 1	$GC-D_0$	$GC-D_2$	$GC-D_4$	$GL-D_0$	$GL-D_2$	$GL-D_4$	$SC-D_0$	$SC-D_2$	$SC-D_4$	$SL-D_0$	$SL-D_2$	$SL-D_4$
Yield (kg/tree)	21.5 b-d	20.7 c-e	17.3 e	25.1 ab	25.3 a	24.5 ab	20.2 de	22.1 a-d	18.9 de	24.1 a-c	22.4 a-d	20.3 de
Water use efficiency (kg/m3)	1.91 f	2.36 de	2.56 cd	2.13 ef	2.77 bc	3.39 a	1.93 f	2.20 ef	2.67 b-d	2.15 ef	2.42 de	3.01 b
Fruit weight (g)	72.2 a	69.0 a-c	63.4 c-e	71.5 ab	64.8 c-e	65.8 b-e	68.1 a-d	61.9 e	62.9 de	68.9 a-d	64.6 c-e	68.6 a-d
Fruit height (cm)	4.91 ab	4.85 a-c	4.64 bc	4.65 bc	4.75 a-c	4.66 bc	4.89 a-c	4.78 a-c	4.61 c	4.79 a-c	4.87 a-c	4.98 a
Fruit diameter (cm)	5.28 a	5.10 a-c	4.61 e	5.20 a	4.96 b-d	4.89 cd	5.10 a-c	4.87 d	4.85 d	5.12 ab	5.10 a-c	4.92 b-d
Fruit volume (cm3)	53.6 a-c	60.8 a	57.8 ab	57.7 ab	55.3 ab	56.0 ab	41.2 e	44.8 de	45.9 c-e	56.7 ab	56.3 ab	52.1 b-d
Flesh thickness (cm)	1.58 ab	1.55 a-c	1.43 cd	1.64 a	1.54 a-c	1.47 b-d	1.52 a-d	1.51 a-d	1.41 d	1.48 b-d	1.59 ab	1.50 b-d
Flesh weight (g)	64.2 a	60.9 a-c	55.3 cd	63.4 ab	56.6 cd	57.6 b-d	60.2 a-c	53.8 d	54.9 cd	60.9 a-c	56.4 cd	60.5 a-c
Leaf area (cm^2)	34.34 ef	33.54 f	32.06 g	36.2 b-d	37.92 a	37.0 a-c	35.87 cd	34.96 de	33.93 ef	37.3 ab	36.9 a-c	35.68 d
Chlorophyll content (%)	34.9 de	34.1 ef	33.1 f	37.8 a	37.3 ab	36.1 bc	35.3 cd	35.4 cd	35.1 c-e	35.7 cd	35.9 cd	35.0 c-e
Nitrogen leaf content, N (%)	2.448 d	2.457 d	2.512 d	2.497 d	2.935 a	2.837 ab	2.452 d	2.687 bc	2.602 cd	2.529 cd	2.815 ab	2.486 d
Phosphorus leaf cont., P (%)	0.103 g	0.126 f	0.104 g	0.208 c	0.272 a	0.235 b	0.185 d	0.205 cd	0.187 cd	0.157 e	0.199 cd	0.205 cd
Potassium leaf cont., K (%)	1.101 e	1.125 e	1.101 e	1.168 d	1.269 a	1.215 b	1.159 d	1.185 cd	1.167 d	1.209 bc	1.230 b	1.183 cd
TSS (%)	11.14 a	12.10 a	12.13 a	9.67 b	11.34 a	11.99 a	11.07 ab	11.91 a	11.60 a	10.8 ab	11.48 a	11.66 a
Total acidity, TA (%)	0.926 bc	1.037 a	0.988 ab	0.704 e	0.75 de	0.776 de	0.775 de	0.809 d	0.831 cd	0.700 e	0.777 de	0.808 d
Maturity index, MI (-)	12.0 cd	11.9 d	12.3 b-d	13.8 a-d	15.1 a	15.4 a	14.3 a-c	14.7 a	14.0 a-d	15.5 a	14.8 a	14.4 ab
Total sugars, TSG (g/100cc)	3.98 b-e	4.03 a-d	3.45 e	4.50 ab	4.51 a	4.45 a-c	3.67 de	4.11 a-d	3.82 de	3.97 c-e	4.07 a-d	3.94 c-e
Year 2	$GC-D_0$	$GC-D_2$	$GC-D_4$	$GL-D_0$	$GL-D_2$	$GL-D_4$	$SC-D_0$	$SC-D_2$	$SC-D_4$	$SL-D_0$	$SL-D_2$	$SL-D_4$
Yield (kg/tree)	22.2 a-c	20.8 a-c	19.9 bc	21.3 a-c	21.3 a-c	20.2 bc	22.3 a-c	22.1 a-c	20.0 bc	24.0 a	23.4 ab	18.9 c
Water use efficiency (kg/m3)	1.85 f	2.26 d-f	2.80 a-c	1.86 f	2.22 d-f	2.96 ab	1.93 ef	2.37 c-e	2.90 ab	2.08 d-f	2.47 b-d	3.01 a
Fruit weight (g)	85.8 a	75.4 c	67.0 f	79.2 b	70.2 ef	71.6 de	89.0 a	74.6 cd	74.1 cd	74.7 cd	70.3 ef	79.8 b
Fruit height (cm)	5.37 bc	5.17 c-e	4.97 e	4.98 e	5.18 c-e	5.08 de	5.64 a	5.26 cd	5.16 c-e	5.15 c-e	5.34 c	5.58 ab
Fruit diameter (cm)	5.58 a	5.27 b	4.82 c	5.38 ab	5.16 b	5.28 b	5.63 a	5.14 b	5.13 b	5.26 b	5.29 b	5.20 b
Fruit volume (cm3)	82.8 ab	74.2 b-d	64.4 de	77.5 bc	59.2 e	74.1 b-d	93.1 a	68.6 c-e	71.7 b-e	73.4 b-d	66.1 c-e	77.0 b-d
Flesh thickness (cm)	1.81 a	1.75 ab	1.62 d	1.76 ab	1.77 ab	1.70 b-d	1.80 a	1.68 b-d	1.63 cd	1.68 b-d	1.74 a-c	1.67 b-d
Flesh weight (g)	77.7 a	67.3 c	59.1 f	71.3 b	62.2 ef	63.6 de	81.0 a	66.3 cd	66.0 cd	66.4 cd	62.0 ef	71.7 b
Leaf area (cm^2)	35.81 ef	35.24 fg	33.43 h	40.35 a	39.48 a	38.09 b	36.7 c-e	36.4 d-f	34.45 gh	37.91 bc	38.02 b	37.1 b-d
Chlorophyll content (%)	37.3 e	37.2 e	36.2 f	41.6 a	41.4 a	40.8 ab	39.8 c	39.4 c	38.1 de	40.1 bc	39.8 c	38.5 d
Nitrogen leaf content, N (%)	2.456 e	2.403 e	2.396 e	2.613 cd	2.937 a	2.871 a	2.520 de	2.733 bc	2.509 de	2.485 de	2.811 ab	2.446 e
Phosphorus leaf cont., P (%)	0.102 g	0.114 g	0.103 g	0.228 c	0.290 a	0.251 b	0.173 f	0.212 cd	0.196 de	0.188 ef	0.229 bc	0.194 d-f
Potassium leaf cont., K (%)	1.133 e	1.123 ef	1.104 f	1.188 cd	1.291 a	1.234 b	1.179 d	1.234 b	1.178 d	1.191 cd	1.241 b	1.216 bc
TSS (%)	11.9 b-d	12.6 a-c	12.4 b-d	12.69 a-c	11.39 cd	10.65 d	13.0 a-c	13.1 a-c	13.1 a-c	14.26 a	13.44 ab	13.1 a-c
Total acidity, TA (%)	0.905 b	0.941 b	1.122 a	0.778 c	0.770 c	0.792 c	0.890 b	0.959 b	0.948 b	0.920 b	0.923 b	0.931 b
Maturity index, MI (-)	13.2 cd	13.5 bc	11.1 d	16.3 a	14.8 a-c	13.4 bc	14.6 a-c	13.7 bc	13.9 bc	15.5 ab	14.6 a-c	14.1 bc
Total sugars, TSG (g/100cc)	3.78 d	4.48 b	3.85 d	4.54 b	5.18 a	4.80 ab	4.00 d	4.43 bc	3.88 d	4.05 cd	4.47 b	3.96 d

LSD: Least significant difference at p=0.05, D4, D2, D0: deficiency percent, subscripts refer to 40%, 20%, and 0% deficiency, GC: 4L/h ground drip irrigation, SC: 4L/h subsurface drip irrigation, GL: ground drip irrigation with low flow emitter, SL: subsurface drip with low flow emitter.

* Means with the same letter for each row are not significantly different from each other at 0.05 level.

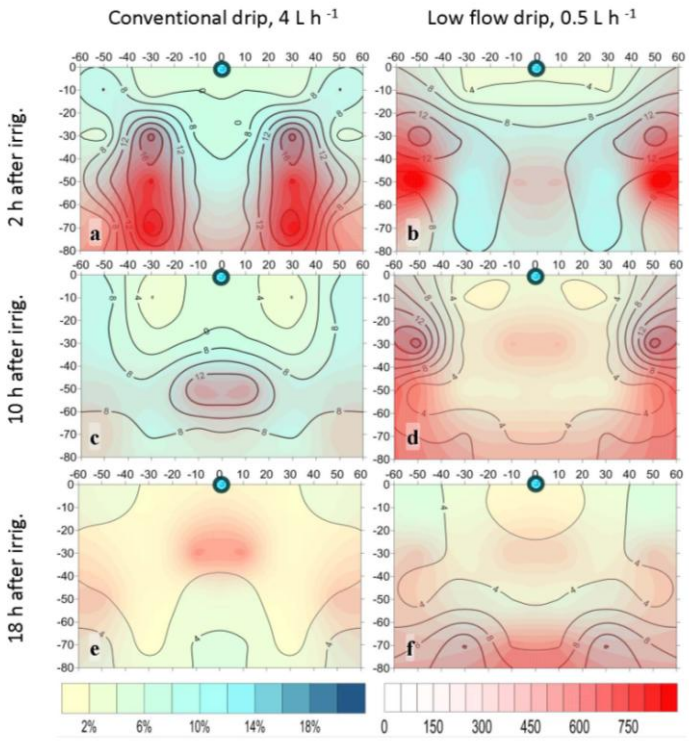
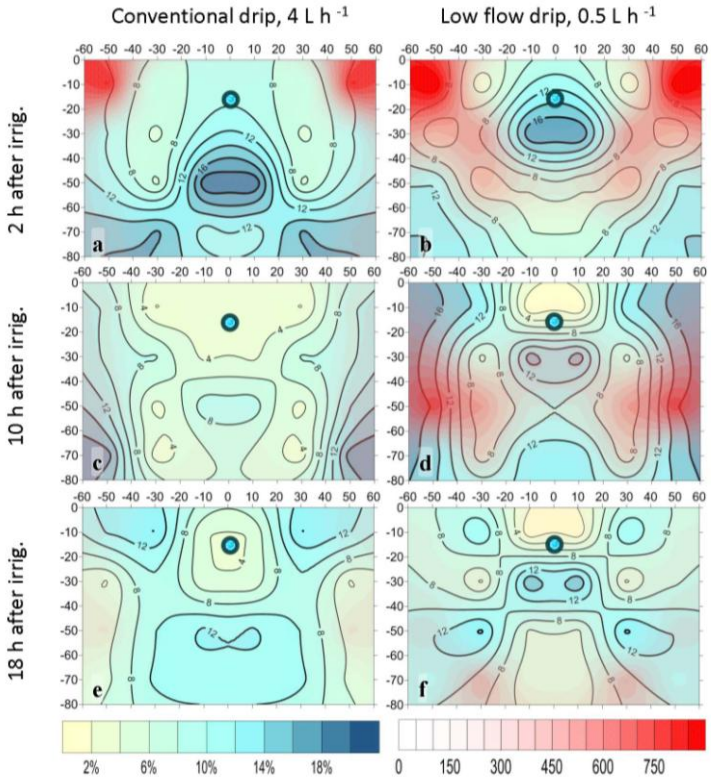


Figure 3. The combined salt and water maps of the conventional and low-flow ground drip irrigation after irrigation in the late season of the second year.
*: water map units are soil-water content (% by volume), salts map values are TSS (ppm).

For water flow, we noticed that for the ground drip, Figure 3, the lateral movement of the low flow emitters was higher than that of the conventional emitters, as noticed by comparing the location of the higher-flow zones (12% water content for example) in b vs. a and d vs. c. In comparison, this is not clear for the subsurface drip, Figure 4, but it is observed for subsurface patterns that water intensity below the emitter is higher for low flow patterns. The water pattern figures show an unfamiliar phenomenon, that the surface layer of the ground drip is dryer than the subsurface drip.

Comparing all the salt distribution profiles, we found that the least salt-accumulation occurs at the SC treatment followed by the SL then the GC and finally the GL treatment. All the shown patterns of salts are for D0 treatment, however, salt accumulation problem is worse for the deficit irrigation treatments D2 and D4, data not shown, however, this evidence is supported by the findings of Aragüés et al., (2015).



*: water map units are soil-water content (% by volume), salts map values are TSS (ppm).

Figure 4. The combined salt and water maps of the conventional and low-flow subsurface drip irrigation after irrigation for the second year.

Comparing ground drip to subsurface drip

As we saw in the second year's results, the irrigation profile had no impact on any of the yield measures. This agrees with other investigators (Aragüés et al., 2014; Bryla et al., 2005), where the formers reported that the two drip profiles produced more yield and bigger peaches than furrow and micro sprinkled systems, but that no differences between ground drip (GD) and subsurface drip (SDI) were found. Correspondingly, some studies reported an increase of pears yield due to the GD system than the SDI, (Oron et al., 2002), and others reported a contrary result that they found that SDI produces more yield than GD (Oron et al., 1999). The main factor that led to this contradiction was water salinity, as SDI gave better results when used with saline water (Oron et al., 1999), while GD gave better results when using fresh water (Oron et al., 2002). In this study, we used a moderate salinity water, Table 1, as classified by irrigation indices (Ayers and Westcot, 1985; Nakayama and Bucks, 1991), this may attribute our results that no differences between GD and SDI. However, there may be other

reasons to get the results of SDI and GD to be almost similar, like rain which flushes-out salts (Aloni et al., 2000).

The effect of emitters' flowrate

The dripping rate whether it is conventional or low flow also did not affect the yield neither the fruit size, this agrees with the results of Worthington and Lasswell, (1994). On the other hand, we found that the irrigation profile has a significant effect in the first year; as the yield for GL treatment is the highest, Table 4, then SL followed by SC and GC. This shows that the low flow emitters (L) gave better results than the conventional emitters (C) did, but in the existence of the L emitters, the ground installation is better than the subsurface installation, while with the C emitters, the subsurface installation is better than the ground installation. This may be attributed to some root damage at the depth of subsurface laterals while installation, hence, when we apply low flow emitters to the SDI, water spread around the lateral in the damaged-roots region leading to less benefit of water and nutrients, and hence less yield. On the other hand, when the conventional flow is applied through SDI, water flows deeper by the effect of flux that allows the plant to benefit from extra amount of water. Conversely, on the ground drip treatments, there were no damaged-roots problem, and the roots in the top layer were active and effective to capture each drop of the low flow application that limits the evaporation and runoff losses as opposed to the conventional flow. In this study, the problem of damaged-roots occurred only at the first year of the experiment as reported by also by Burt, (1996). Although the second year's results showed no significance of the irrigation system on the yield quantity, Table 4, but the fruit quality is highly affected by the irrigation profile in the two years, especially the maturity index (MI) and total sugars (TSG). In the two years, the results of the low flow treatments were higher in MI and in TSG, Table 4. The highest MI was achieved at the GL irrigation; however, the values are not statistically different from SL and SC treatments while differs significantly from the control, GC, treatment that gives the lowest values. In addition to the high TSG values as a marketing advantage, the high MI value leads to early marketing, which in turn leads to the advantage of higher selling prices.

The effect of deficiency level

Unlike the D4 deficiency level, the D2 levels did not led to any significant reduction in yield in both years, Table 4, while the WUE is significantly higher in for D_2 than D0. This leads us to recommend using D_2 for its benefits gaining about 20% more WUE with negligible reduction in yield. This agrees with Gunduz et al., (2011) when irrigating every 6 days. Although the D_4 treatment led to 43, 50% increase in the WUE in the two years respectively, agreeing with Verma et al., (2007) and Tejero et al., (2011), it caused 12, 14% yield reduction. If this quantity matters and the water is not scarce, then the D_4 level should not be used (Aragüés et al., 2014; Mounzer et al., 2013; Tejero et al., 2011; Vera et al.,

2013), otherwise, we should recommend using the D_4 level of deficiency because of its water conservation benefits.

In addition to the water reduction effect, water deficiency may cause a side effect increasing the soil salinity (Pedrero et al., 2014) which increases the impact on yield. Furthermore, our results showed that the increase in DL result in higher TA values in fruit juice, and in contrast a lower maturity index values which is a good marketing factor as it reflects sweeter and tastier fruits (Shinya et al., 2014), these results agree with Conesa et al., (2014) who performed similar experiments on mandarin. The results of total sugars analysis reinforces the previous results as D_2 treatment showed up to 13% more sugars than both the D_0 and the D_4 treatments, this means that D_2 treatment produced the sweetest and tastiest fruits than the two other treatments.

Water and salts

As we said that the drippers with conventional flowrate value appears to flush salts more efficiently than the low flow drip does which can be attributed to the relative flux of the conventional flow dripper to the low flow dripper (4 L h^{-1},0.5 L h^{-1} respectively). Additionally, the less salt accumulation, which occur in the subsurface treatments than the ground drip treatments, may be attributed to the emitter's location. The central location of the subsurface emitter helps pushing salts to the edges of the profile in all directions as reported by others (Lamm and Camp, 2007; Oron et al., 1999), rather than the surface dripper that flushes salts mainly downwards.

The increase in the lateral movement of water for the low flow emitters than the conventional emitters may be attributed to the increase in the matric flux component over the gravimetric component of the soil-water movement forces this happens mainly for low fluxes (Gardner and Hsieh, 1959).

The dryness of thee surface layer of the ground drip than that of the subsurface drip could be attributed to the upward water movement from the shallow-buried emitter, or due to the differences between root patterns and special soil variability as reported elsewhere (Olsson and Rose, 1988; Rolston et al., 1991).

CONCLUSIONS

In conclusion, we can say that in this study, DL reduces yield only at D4 level, while it increases WUE, and enhances the fruit quality. Furthermore, the low-flow irrigation enhances water lateral movement, and fruit quality, but it has low ability to flush salts from soil. It can be recommended to implement more studies on the low-flow drip application for different soil textures and crops, it is also recommended to apply the D_2 deficiency level due to its benefits of improving fruit quality, increasing WUE, and for its low impact on yield reduction. Finally, under economic balanced conditions, it is recommended to apply the low-flow ground drip with D_2 level.

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STATUS OF NUTRIENTS IN VINEYARDS OF ĆEMOVSKO POLJE

SUMMARY

The results of investigation of nutrient status in vineyards of Ćemovsko polje are shown in this paper. In order to provide a more reliable basis for the definition of relationships between grapevine (nutrients in leaf petiole – P, K, Ca, Mg, Fe, Mn, Cu and Zn) and soil properties (pH, total carbonates, humus (organic matter), exchangeable Mg and available P, K, Fe, Mn, Zn and Cu), factor and correlation analyses were applied. In the top layer (0 – 30 cm), investigated highly calcareous and alkaline soil had high concentration of nutrients, exceptionally for iron which was on the limit between low and medium level, and for phosphorus on low level. In the underlying soil layer (30 – 60 cm), nutrient contents were low.

The content of Cu was very high in the both soil layer, due to its accumulation through agronomic practice, where Cu as a common ingredient of the plant protection products used especially in vineyards. In average, the level of P, K, Ca, Mg, Zn and Cu in leaf petioles indicated optimal supply of grapevine. However, the deficiency of Fe and Mn was detected. A common cause of chlorosis is a deficiency of these elements, which are of crucial importance for photosynthesis.Four factors determining soil chemical characteristics were identified by factor analysis. They accounted for about 78% of the total variance. The communalities of parameters, considering four factors, varied from about 59% for available K to 85% for humus and exchangeable Mg.

Two main factors represented the statuses of: 1) mutually complementary available fraction of Zn and Mn (positive pole) and pH and carbonate (negative pole), and 2) humus and available Fe. Directly proportionality was found between the content of Mn in petiole and the score of first factor ($p = 0.037$). It means that the status of Mn in grapevine depends directly on the available fraction of Mn in soil (DTPA-Mn), as well as indirectly on the pH value and CaCO3 content. The availability of Fe (DTPA-Fe) depends on the content of humus, since positive statistically significant ($p = 0.015$) correlation was found. The management practices which can influence on availability of Fe and Mn are the increase of organic matter (humus) and modification of the soil pH. The applications of fertilizers containing Fe and Mn through leaves or soil are recommended.

Keywords: soil, petiole, nutrient, deficiency, factor

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INTRODUCTION

Along with climatic conditions, soil characteristics and nutrient supply are important determinants of quantity and quality of yield of agricultural plants (Peuke, 2009). The chemical and physical properties of soil affect the availability of nutrient for plant uptake (Havlin et al., 2005), and in such a way nutrient concentration ranges in plants (Peuke, 2009). In the Mediterranean area, it is estimated that 20–50% of fruit crops are affected by Fe deficiency causing dramatic economic losses (Yunta et al. 2013). Many vineyards in mentioned area are on calcareous soil and therefore due to the deficiency of nutrients under risk of chlorosis. A common cause of chlorosis is a deficiency of Fe and Mn, essential elements, which are the building blocks used to create the thousands of organic compounds that make up plant tissue or drive growth processes. Iron is a component of certain enzymes and proteins that are used during photosynthesis and in respiration. Manganese is an enzyme activator during chlorophyll production and as a structural component of the chloroplasts where photosynthesis occurs (http://www.extension.umn.edu/garden/yard-garden/trees-shrubs/iron-chlorosis/).

The typical symptom of Fe chlorosis is a lack of chlorophyll (yellowing) in the intervenial of youngest leaves during the active growth period and growth depression (Bavaresco et al., 1993; Gruber and Kosegarten, 2002).

The Mn deficiency has little practical effect on vine yields since it appears in late season on older leaves that contribute little to vine function. The symptoms begin on the basal leaves as a chlorosis between the veins (Hamman et al., 1998).

The use of foliar analysis for assessing the nutritional status of plants is an old practice. The aim of study was to reveal the cause of grapevine leaf yellowing and to compare the results of soil and petiole (sampled during full bloom) analyses. In order to avoid shortcomings in the interpretation of simple relationship between nutrients in leaf petiole and soil parameters, the principal component analysis was applied.

MATERIAL AND METHODS

The soil samples (from 0-30 cm and 30-60 cm depth) and leaf petioles of grapevine (from opposite flower cluster, near the middle of the shoot) were taken in 11 localities of Ćemovsko polje. The basic soil parameters were determined by methods widely used in Former Republics of Yugoslavia (Džamić *et al.*, 1996). The content of available Fe, Mn, Zn and Cu (extraction with 0.005 M DTPA) as well as of exchangeable Mg (extraction with 1 M NH_4CH_3COO) in soil were determined by flame atomic absorption spectrophotometry (FAAS).

In acid digest (HNO_3 and $HClO_4$) of dried leaf petioles, P was determined spectrophotometrically, K and Ca flame photometrically, and the other elements by FAAS. The results were processed by means of the SPSS 10.0 Program. The statistical analyses included descriptive (mean and standard deviation), correlation and factor analysis. By factor analysis the original set of 10-correlated soil parameters (pH, humus, total carbonates, exchangeable Mg, available P, K,

Fe, Mn. Zn and Cu), were transformed into a new set of mutually uncorrelated factors according to Topalović *et al.* (2006; 2010).

RESULTS AND DISCUSSION

The descriptive statistics (minimal, maximal and mean values with standard deviation) of soil parameters are given in Tables 1 and 2. In the top layer (0 – 30 cm), investigated highly calcareous and alkaline soil had high concentration of nutrients exceptionally for iron which was on the limit between low and medium level, and for phosphorus on low level.

Table 1. Descriptive statistics for the top soil layer

Soil Layer (0 – 30 cm)	Minimum	Maximum	Mean	Std. Deviation
pH (H_2O)	7.68	7.88	7.77	0.05
Humus (%)	3.52	5.53	4.48	0.66
Total carbonates (%$CaCO_3$)	14.5	45.8	28.2	9.9
P_2O_5 (mg/100 g)	0.9	24.7	6.8	7.1
K_2O (mg/100 g)	12.4	37.5	19.2	6.0
Mg (mg/100 g)	15.7	32.4	23.8	4.8
Fe (ppm)	7.3	22.3	10.7	4.3
Mn (ppm)	3.6	29.1	13.3	7.9
Cu (ppm)	2.3	37.3	11.3	9.9
Zn (ppm)	1.1	8.0	3.7	2.0

In the underlying soil layer (30 – 60 cm), nutrient contents were low. The content of Cu was very high in the both soil layer, due to its accumulation through agronomic practice, where Cu as a common ingredient of the plant protection products used especially in vineyards. Considering organic matter i.e. humus, the top soil layer is on optimal level, since the underlying one has low content.

As shown in Table 3, the average content of P, K, Ca, Mg, Zn and Cu in leaf petioles indicated optimal supply of grapevine. However, the deficiency of Fe and Mn was detected.

Four factors determining soil chemical characteristics were identified by factor analysis (Table 4). They accounted for about 78% of the total variance. The communalities of parameters, considering four factors, varied from about 59% for available K to 85% for humus and exchangeable Mg.

Table 2. Descriptive statistics for the underlying soil layer

Soil Layer (30 – 60 cm)	Minimum	Maximum	Mean	Std. Deviation
pH (H_2O)	7.36	8.32	8.04	0.20
Humus (%)	1.04	3.02	1.85	0.57
Total carbonates (%$CaCO_3$)	57.3	88.6	68.5	7.2
P_2O_5 (mg/100 g)	2.5	12.4	6.4	3.0
K_2O (mg/100 g)	3.5	17.5	7.1	3.6
Mg (mg/100 g)	13.2	22.2	16.8	2.5
Fe (ppm)	3.1	7.8	5.6	1.4
Mn (ppm)	1.9	13.6	6.7	3.6
Cu (ppm)	0.5	14.5	2.6	3.4
Zn (ppm)	0.3	2.7	0.8	0.7

Table 3. Descriptive statistics for the leaf petiole of grapevine sampled in bloom.

Petiole	Minimum	Maximum	Mean	Std. Deviation
P (%)	0.2	0.5	0.4	0.1
K (%)	1.2	3.0	2.0	0.5
Ca (%)	1.7	2.7	2.2	0.3
Mg (%)	0.2	0.7	0.5	0.1
Fe (ppm)	19.3	25.8	22.9	2.3
Mn (ppm)	10.1	35.2	21.4	8.6
Cu (ppm)	7.2	21.1	10.2	3.4
Zn (ppm)	17.8	52.5	34.7	8.9

Table 4. Factor analysis for soil: Eigenvalues, cummulative of the total variance, factor loading of the 4 factors, and communality estimates of the 10 soil parameters

	Factor 1	Factor 2	Factor 3	Factor 4	Commun.
Eigenvalue	2.60	2.28	1.52	1.40	
Cumulative (%)	26.0	48.8	64.0	78.0	
pH (H_2O)	-0.817	-0.171	-0.114	-0.307	0.803
Total carbonates	-0.812	0.108	0.109	0.319	0.784
Zn	0.772	0.311	0.348	0.127	0.829
Mn	0.611	-0.504	0.004	0.363	0.759
Humus	0.099	0.886	0.134	-0.187	0.847
Fe	0.062	0.801	0.149	0.232	0.721
P_2O_5	-0.093	0.370	0.824	0.116	0.838
Cu	0.513	-0.116	0.712	0.025	0.785
K_2O	0.121	-0.082	0.190	0.730	0.590
Mg	-0.070	0.551	-0.339	0.651	0.847

Factor 1 is composed from two complementary groups of parameters. Group I: DTPA-Zn and DTPA-Mn reperesenting available fraction of these elements and group II: soil pH and total carbonates. This is expected, due to fact that the availability of mentioned elements decreases at pH > 6.

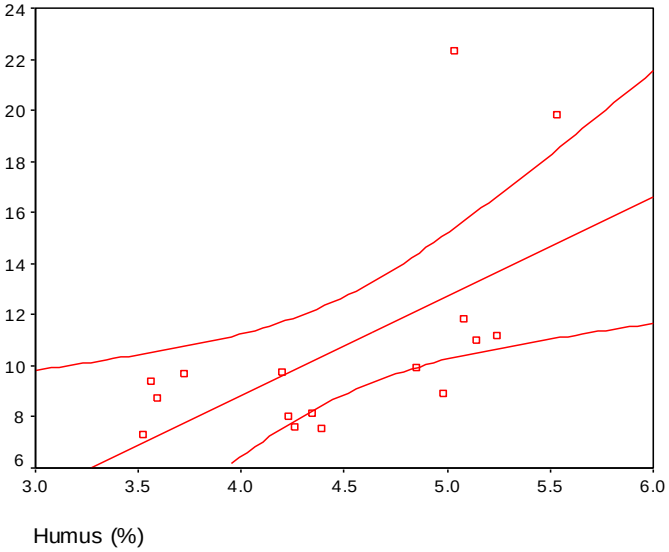

Figure 1. Available Fe vs. humus in top soil layer (R = 0.594, p = 0.015)

Factor 2 is consisting from humus and DTPA-Fe due to associations of Fe with humic substances. Naimely, the availability of Fe (DTPA-Fe) depends on the content of humus, since positive significant correlation was found.

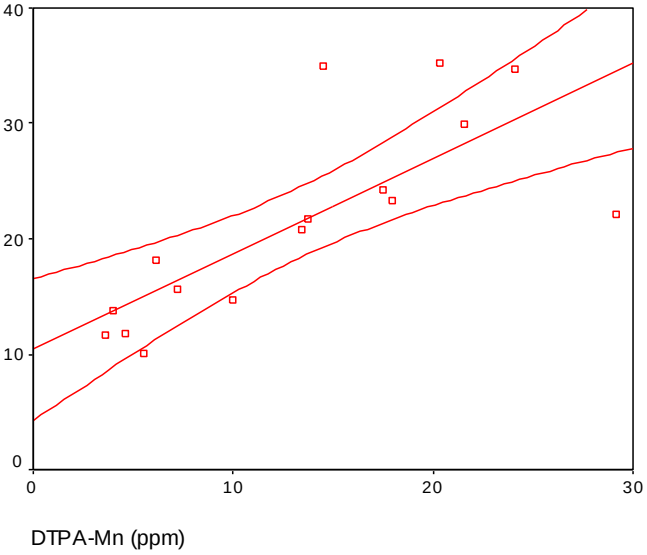

Figure 2. Total manganese in petiole vs. available Mn in top soil layer (R = 0.764, p = 0.001)

Factor 3 includes available P and Cu, and factor 4 is represented by available K and exchangable Mg.

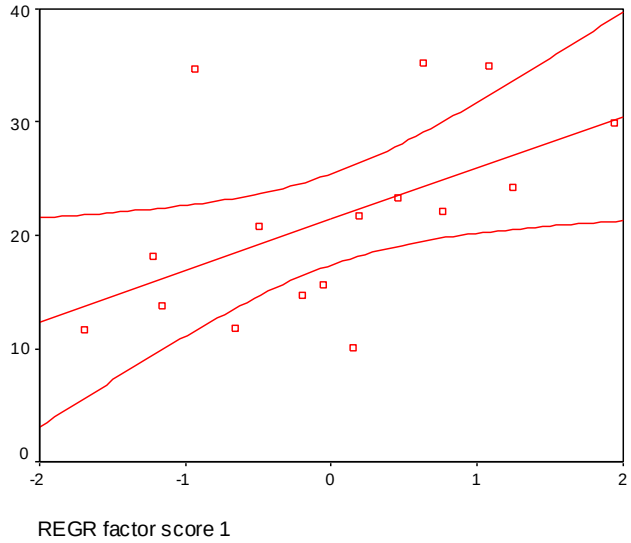

Figure 3. Total manganese in petiole vs. regression factor score 1 for top soil layer (R = 0.532, p = 0.034)

The nutrient uptake by plants can be affected by these factors determining soil chemism. Because of that, the correlations of nutrient contents in leaf petiole of grapevine with the scores of above-mentioned factors were examined. Direct proportionality was found between the content of Mn in petiole and the score of first factor (p = 0.037). It means that the status of Mn in grapevine depends directly on the available fraction of Mn in soil (DTPA-Mn), as well as indirectly on the pH value and $CaCO_3$ content.

The management practices which can influence on availability of Fe and Mn are the increase of organic matter (humus) and modification of the soil pH. The use of Fe and Mn fertilizers is recommended.

Manganese sulfate as a foliar spray at the loose cluster stage has corrected the deficiency in some California vineyards (Hamman et al., 1998). Manganese chelate products have been used as foliar sprays with some success (Fageria et al., 2009). Foliar spray treatments of Fe chelates or of ferrous sulfate result in a temporary correction, at best. If chlorosis is severe and persists, repeated applications at 10 to 20-day intervals may be necessary (Hamman et al., 1998).

Fe chelates (iron(3+) ethylenediamine-N,N'-bis(hydroxyphenylacetic acid i.e. FeEDDHA) and ferrous sulfate are the most commonly used to prevent Fe-chlorosis. The former is substantially more effective than the latter but more expensive and easily leached from the soil (Hamman et al., 1998). The study of Diaz et al. (2010) recommended the use of $Fe_3(PO_4)2{\cdot}8H_2O$ (vivianite) because of its effectiveness (<30% Fe) and long-term fertilizing effect over the other Fe

salts applied to the soil due to the presence of phosphate, which favors its transformation to poorly crystalline lepidocrocite, from which Fe is readily available to plants. Moreover, it is not easily leached from the soil, inexpensive, environmentally safe.

CONCLUSIONS

The results of soil and petiole analysis indicated relatively poor supplying of grapevine with Fe and Mn. The Fe and Mn availability in soil could be controlled by management practices that increase the content of organic matter (humus) and modify the pH value of soil. The applications of fertilizers containing Fe and Mn through leaves or soil are recommended.

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INFLUENCE OF MILK ON THE CONTENT AND TOTAL PROTEIN CHANGES DURING RIPENING WHITE CHEESE IN INDUSTRIAL PRODUCTION

SUMMARY

Sjenica cheese is one of the most famous of the group of white cheese in brine of the Republic of Serbia. Center of the production are mountain plateau surrounding the city Sjenica. It is produced by indigenous technology on individual farms, as well as in industrial conditions of micro dairies. The raw material for the production is fresh whole fat cow and sheep milk, which is made without the application of heat treatment. Because proteins are the most important parameter of chemical composition and milk quality, which affects the course of making cheese, and the quality and overall value of the cheese depends on them, it is necessary to establish their content and the changes during cheese ripening. Research results have shown that the total protein content of 1-day maturity in cow milk cheese was 14.80%, and in sheep milk cheese 17.67%. The differences between two kinds of cheese were statistically highly significant. The total protein content in both kinds of cheese during the first 30 days of ripening uniformly increased and it reached the maximum. After this period the total protein content was found in cow milk cheese 17:54% and 19.07% in sheep milk cheese. These differences were statistically significant. In the last period of 30-45 days of maturity we had in both kinds of cheese a slight reduction in total protein levels. At the end of the ripening period of 45 days, total protein ranged in cow milk cheese 17, 41% and 18.75% in sheep milk cheese. These differences were statistically significant.

Keywords: Sjenica cheese, total protein, ripening

INTRODUCTION

Sjenica cheese is one of the most famous of the group of white cheese in brine of the Republic of Serbia. It is produced by the indigenous technology on individual farms on mountains plateau that surround town of Sjenica, but recently more and more in industrial conditions of mini-dairies. The raw material for production is fresh whole fat cow milk and sheep milk, which process of making cheese starts immediately after milking, without the application of heat treatment.

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The quality and overall value of cheese, depends primarily on the quality and chemical composition of milk, where the highest importance has a dry matter content, fat and protein. Milk from certain types of dairy animals has a specific chemical composition, which is reflected significantly on manufacturing and the chemical composition of cheese.

Of all components of milk the greatest importance, in the production of the vast majority of cheese, belongs to casein. Casein is the most abundant protein in milk which makes 75-80% of total milk protein. Casein is a complex composition and belongs to the group of fosfogliko proteins (Maćej et al., 2007; Jolles, P. and Fiat, A.M. 1979). The technological process of cheese production is based on the specific properties of the casein in the framework of which the most important are the ability of coagulation and gel-forming, ability of the formed gel towards sinerezisu and tendency to proteorizi under the influence of proteolytic enzymes present in cheese. The most important characteristics of casein secure transformation of milk from liquid aggregate state in firm state, concentration of a part of milk dry matter and the formation of the desired sensory properties of the cheese during its ripening.

When coagulation and formation of a gel, casein micelles appear as constitutive elements of the matrix protein, during syneresis due to the increase of acidity, there is a dissociation of colloidal calcium phosphate (CCP), an integral part of the casein micelles in which chunks are depleting the calcium content. Depending on the course of acidity, during the process of making cheese, the proper amount of demineralization casein will be realized, which will regulate mineral composition of cheese (Pudja, 2009).

Numerous factors influence on the primary and secondary phase of milk coagulation and rheological properties of curd, but the most important is the concentration of casein. Coagulation rate is directly proportional to the concentration of casein, and the resulting curd is firmer (Bringe, and Kinsella, 1986).

Mijočinović (2013), states that the firmness of the gel is directly correlated with the content of casein.

Smaller content of casein in milk contributes to formation of curd of less strength, so during its treatment loss of fat from whey is greater and there is a significant loss of protein in the form of cheese dust (Pudja, and Maćej, 1996,; Pudja et al., 1996).

Pudja (2009) points out that in defining the microstructure of cheese; cream cheese dough includes 3 phases, one of which is a protein phase. The protein matrix forms the basis of the structure of the cheese. Mineral complex of cheese is largely incorporated into it, and closely associated with it, so that they are the holders of not only the structure and elasticity of cheese but represent potential and basis for changes during cheese ripening.

The most significant changes during ripening occur in proteins, starting on milk from the time of adding rennet (Fenelon, and Guinee, 2000).

In a series of changes that occur in cheese during ripening, changes on proteins and their products of formation are the most important, so in the literature the term cheese ripening in the strict sense implies changes of proteins. The scope and depth of proteolytic changes are closely related to the technology of making cheese, ripening conditions and the activity of microorganisms, and have a decisive influence on the formation of the sensory characteristics, consistency and rheological properties of cheese (Fox, 1989).

MATERIAL AND METHODS

The experiment was performed in June 2012. All types of cheese were produced in industrial conditions of "Pester" dairy in Sjenica. Basic principles of production, during the production process of cheese, were as follows:

-Milk: Full fat cow and sheep milk.

-Preparing and the process of making cheese: included the draining, filtering and reheating at coagulation temperature . The temperature during the process of making cheese was 32oC, and the time was 50 min.

-Treatment of curd: the curd was cut into cubes - size 5x5cm, and then placed in a strainer. Dewatering was carried out on the table with the application of pressing, and it lasted two hours. After filtering and separation of whey, the clod was formed.

Cheese cutting and salting: clod was cut into slices of 15X15X5cm and 10X10X3cm. The cheese was salted successively with dry, sea salt, during stacking in the packaging.

-Ripening: It was occured in salty whey, and optimal ripening period was 40 days.Research goal: to determine the levels of total protein after making cheese, and then to monitor the dynamics during maturation after 15, 30 and 45 days of ripening.

-Analysis: The analyzes were carried out at the Veterinary-Specialistics Institute of Kraljevo by following methods

-Determination of total nitrogen by Kjeldahl method, using kjeltek device (Carić et al.,2000)

The total protein content was calculated by multiplying the total nitrogen content with a factor of 6.38 (Standard IDF 20-1: 2002). Both kinds of cheese are made in 5 repetitions and the total sample number was 10.

Determination of total nitrogen by Kjeldahl method

Apparatus and accessories:

Analytic scales; - Digestion hot plate where Kjeldahl's flask is placed; - Distillation apparatus; -Erlenmajer, 500 ml; -Menzure, 25, 50, 100 and 150 ml; - Bireta, 50 ml; Substances that facilitate boiling: pieces of porcelain or glass beads for digestion, freshly calcined pieces of pumice for distillation.

Reagents:

Catalyst: crystalline copper sulphate or a mixture of copper sulfate and potassium sulfate, in 1:15 relation;

40% solution of sodium hydroxide;

0.1 mol / l sodium hydroxide solution;

0.05 mol / l of sulfuric acid solution or 0.1 mol / l solution of hydrochloric acid-

Indicator: 0.5% of metilred solution

Procedure:

In Kjeldahl flask's measure 1.5 to 2 g of the cheese, than add 25 ml of concentrated H2SO4, and about 0.5 of a catalyst. Burn it on the digestion hot plates, chill and then add up to 200 ml of water stir and leave to chill again. Erlenmeyer flask to which was added 50 ml of 0.1 mol / l HCl solution or 0.05 mol / l H2SO4 solution, and four drops of indicator solution, place under the condenser so that the condenser tube was immersed in a solution of acid. Before the start of distillation, add about 80 ml of 40% NaOH solution.

The distillate titrated 0.1 mol / l sodium hydroxide solution.

Total nitrogen (UN) in cheese is calculated by the following formula:

UN = (%)

Where is:

C-concentration of HCl (0.1 mol / l);

V1-HCl volume consumed for the binding of ammonia (ml);

-V2 Volume of NaOH consumed for the titration of the distillate (ml);

m-Balanced sample mass of cheese (g)

-Statistical Analysis: The experiment was conducted with two types of milk by 5 repetitions. The analysis included:

-to define the significance of differences of arithmetic means and, also measures of variation

- standard deviation (Sd) and coefficient of variation (Cv). –

To test differences of arithmetic means, we used Student's (t-test) (Stankovic et al., 1989).

RESULTS AND DISCUSSION

Production of white cheese in industrial conditions, unlike traditional production, provides a standard technology and consistent cheese quality. A similar statement gives (Jandric et al.,2015), which state that the opening of a mini-enterprises-small dairies in the area of Peshter, the economy of production and milk processing and product quality improve, as well as the incentive of milk producers and certain sale on the market.

When we talk about the content of protein and its changes, it should bear in mind the fact that the both kinds of cheese are made from milk, which process of making cheese is done without the application of heat treatment, but serum proteins leave the whey and do not participate in the formation of the curd. Additionally, when cheese is produced in this way, the base of protein matrix makes casein, which readily undergoes changes during the ripening. On the other hand, curd processing is simple and there is no application of high temperature, significantly affects the activity of rennet-chymosin and plasmin which in kinds

of cheese produced in this way plays an important role in the process of ripening. The research results are presented in Table 1.

Table 1. Dynamics of total protein during cheese ripening%.

Cheese type	Parametres	Period of maturity(days)			
		1	15	30	45
Cow milk cheese	min	14.16	13.52	15.18	15.69
	max	15.75	15.82	19.20	19.01
	$\bar{x}$ =(n=5)	14.80	15.37	17.54	17.41
	Sd	0.08	0.17	0.23	0.16
	Cv %	3.44	7.31	8.52	6.07
Sheep milk cheese	min	16.14	16.71	18.50	18.11
	max	19.01	19.65	19.84	19.20
	$\bar{x}$ =(n=5)	17.67	18.31	19.07	18.75
	Sd	0.14	0.15	0.07	0.06
	Cv %	5.37	5.45	2.43	2.34

Significance of mean		
	t cal. $\overline{X}_1$ - $\overline{X}_2$	
1	6.25	0.45**
15	1.58	0.16
30	2.24	0.24*
45	2.76	021*

The theoretical value of 't' schedules refer to the number of freedom deegrees (df) 12 and amounts: p <0.05 = 2,179; p <0.01 = 3.05.

Based on the results shown in the table one we had both in cow and sheep milk cheese, during the first 30 days of ripening, increase in total protein, when we recorded the maximum, which is due to an increase in dry matter content of cheese in the same period of ripening.

On the first day of ripening total protein content in cow milk cheese averaged 14.80% and 17.67% in sheep milk cheese. Treatment of the results showed that the differences between the both kinds of cheese were highly significant.

In the further course of maturation it is noticed further increase of the content of total protein in both kinds of cheese, as a result of the water content reduce, and this increase was 0,57% in cow milk cheese, and in sheep milk cheese 0.64%. After 15 days of ripening content of total proteins in cow milk cheese was 15, 37% and 18, 31% in sheep milk cheese. Analysis of the data showed that these differences were not statistically significant.

After a 30-day of maturity further increase of the content of total protein is noticed in both kinds of cheese. This increase in average was 2.17% in cow milk

cheese and in sheep milk cheese 0.76%. After this period of ripening total protein content of cow milk cheese was 17, 54% and 19.07% in sheep milk cheese. These differences between the both kinds of cheese were statistically significant.
At the end of the ripening period of the 45-day total protein content in cow and sheep milk cheese practically remained at the same level as after 30 days of ripening and it was 17,41% in cow milk cheese and 18.75% in sheep milk cheese. Treatment of the results showed that the differences between the cheeses were statistically significant.

Results of experiments confirmed the earlier thesis that there is a mutual dependence between the content of dry matter and total protein in white cheese. A slight decrease in the total protein content of the last 15 days of ripening is the result of crossings part of soluble protein from the cheese into the brine for ripenin.

During cheese ripening, milk proteins are the main substrate for enzymes. Enzyme activity, as well as flow, intensity and the extent of degradation of proteins depend on many factors , where the great importance has salt content in cheese whose effect is inhibitory, which is reflected in lower soluble protein content in cheese (Savic et al., 2015).

The results are consistent and do not differ significantly from the results of other authors for similar types of cheese and these groups: fat cheese Montenegro 17.47% (Adžić et al., 1984); Pljevaljski cheese 19.73% Polimsko- Vasojevićki 18.65% Polimsko- Sjenički 17.15% (Dozet et al., 1996); Domiati 16:42% (Abdel Salam et al., 1993); White cheese-slice 12.15% (Zivic, 1989); Homoljski 17:32% (Jovanovic et al., 2004); Sjenički 14.85%, Zlatarski 16.91%,(Maćej et al., 2006%); 13.79% Sjenički cheese and Sjenica type cheese 15:39% (Savić, 2011); Sjenica type cheese 11.07% (Popović-Vranješ et al., 2011).

CONCLUSIONS

Proteins are one of the most important parameters of the chemical composition of milk and cheese, which affects the speed of enzymatic and physical-chemical phase of milk coagulation, and thus the composition, strength and technological properties of the resulting curd.

Proteins are the basis of the structure of the cheese in which are incorporated other components of milk, especially milk fat and minerals, some of which depend on strength, consistency, rheological and other properties of cheese.

Cheese ripening includes changes in the protein, because the deepest changes occur on them, in other words, proteins serve as a substrate for the enzyme activity during ripening.
Of the many products of ripening, the most important are soluble nitrogen materials, occurred as the result of degradation of proteins, which contribute to the formation of the desired sensory characteristics of cheese.

A type of milk is a very important factor in the chemical composition of any kind of cheese. Sheep's milk, which content is richer with protein, actually

casein, seriously affected on the total protein content in mature cheese. The content of total protein in mature sheep milk cheese amounted 18.75% in average, and in cow milk cheese it was 17:41%. By data processing , these differences were statistically significant.

Due to its rich chemical composition, mainly to proteins, sheep's milk presents original and irreplaceable raw materials for the production of high-protein cheese, for its specific taste, and good organoleptic properties.

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REGIONAL INTEGRATION AND AGRICULTURAL TRADE DEVELOPMENT IN RWANDA: THE CASE OF STAPLE FOODS SECTOR

SUMMARY

This paper investigated the impact of regional integration on the agricultural trade development. Using a literature review, the study showed that the results of common agricultural initiatives aiming at agriculture sector and agricultural trade development have not been convincing due to lack of appropriate mechanisms and institutional actions to operationalize regional agricultural policy and strategy at the national level. The paper also revealed that Rwanda benefited from its accession to EAC, especially in terms of the ease of access to regional markets through the establishment of the Common Market, the Customs Union and the alleviation of some of regional trade barriers for basic foodstuffs and consumer goods. This led to an increased value of its agricultural products exports to neighboring countries. The analysis of the Net Export Index and the Grubel-Lloyd measure for maize, potato and bean revealed that Rwanda is a net importer of maize and a net exporter of potato and bean. For these two staple foods, the results revealed that if Rwanda manages, through policy and institutional actions, to remove or alleviate the bottlenecks that prevent farmers from producing enough for export, it can have a competitive advantage on neighboring countries' markets whose access has been facilitated by its accession to the EAC.

Keywords: Regional integration, agriculture, trade, staple food, Rwanda

INTRODUCTION

The ability of the Rwandan agriculture to deal with increasing competition from trade liberalization and its membership to East African Community (EAC) has significant effects on the macro and micro levels of the national economy. Rwanda's economy is highly depending on agriculture sector which contributes up to 33% in the GDP at the current market prices of 2013 (NISR, 2014). This sector continues to be the leading employer (Alinda and Abbott, 2012) with more than 85% of the economically active population. It is also considered important for national food self-sufficiency, accounting for well over 90% of all the food

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consumed in the country (RDB, 2012). To boost its economy, the country initiated various programs and adopted different policies and strategies. These were developed in line with Rwanda's socio-economic policy document in the form of Vision 2020 (Alinda and Abbott, 2012) which also considers regional economic integration as one of the crucial elements of achieving sustainable economic development (MINECOFIN, 2000). In July 2007, Rwanda officially joined the EAC to form a bloc of five countries with Kenya, the United Republic of Tanzania (hereafter referred to as "Tanzania"), Uganda, and Burundi. It sees its membership to EAC as a way of enhancing initiatives to boost its economy by increasing the share of agricultural production on regional markets. Consequently, the country has revised its trade policy and initiated several agriculture-related programs and strategies to be able to integrate economically in this community, and thus derive economic and social benefits from its membership.

While a lot of domestic and regional political initiatives have been put in place to enable farmers to take advantage of economic benefits offered by the EAC, a major concern arises: is the Rwandan accession to EAC having a substantial impact on the development of regional and cross-border agricultural trade development, especially for Rwandan priority foodstuffs? Therefore, the paper seeks to establish the relationship between regional economic integration, agricultural growth and agricultural sector development in Africa, and analyzes agricultural trade flows between Rwanda and neighbouring and EAC member countries. It evaluates the trade performance of Rwanda, especially for maize, irish potatoes (hereafter referred to as "potato") and bean sectors. The choice of these food crops is based on their importance in the Rwandan agriculture (Musabanganji et al., 2015) and cross-border trade (USAID/EAT, 2013).

MATERIAL AND METHODS

Besides the literature search undertaken by reviewing literature in databases of peer-reviewed scientific publications, books and official publications using the following key words: regional integration, agriculture, staple food, trade, Rwanda, this paper used secondary data on imports and exports retrieved from FAOSTAT website to analyse the level of trade performance for maize, potato, and bean sectors from 2004 to 2013. The analysis of trade performance at the sector level can be carried out by assessing trade indices of competitiveness. Latruffe (2010) presents a list of indicators based on the neoclassical economics which focuses on trade success and which measures competitiveness with the real exchange rate, comparative advantage indices, and export or import indices. According to Frohberg and Hartmann (1997), the use of trade indicators offers two advantages compared to those based on accounting data: (i) the costs of marketing and transport to and from the port of entry are also taken into account, and (ii) demand and supply responses are considered simultaneously. In our analysis, we used the net export index (NEI) and the Grubel-Lloyd (GL) measure for each of the three sectors. The NEI is the

difference between a sector's exports and imports divided by the total value of trade (Banterle and Carraresi, 2007). The GL indicator assesses the health of exports by accounting for the fact that a product is often exported and imported at the same time (Latruffe, 2010). It measures intra and inter-industry trade for a given product. The used indicators in the case of one country trade analysis are given by the following equations:

$NEI_i = \frac{X_i - M_i}{X_i + M_i}$ (1), where *X* are exports; *M* are imports; *i* denotes the sector or product considered. The NEI values lie between -1 (when a country imports only) and 1 (when a country exports only), with a value of 0 in the case of the equality of imports and exports.

$GL_i = 1 - \frac{|X_i - M_i|}{X_i + M_i}$ (2), where *X* are exports; *M* are imports; *i* denotes the sector or product considered. GL measure has a range between 0 and 1, with the value 0 indicating inter-industry trade, while the value 1 indicates an intra-industry trade only.

RESULTS AND DISCUSSION

Regional integration and economic growth

The literature shows that many authors have worked on tracing the impact of the regional integration on a country's economic growth (for example, grossman and helpman, 1995; n'diaye, 2001; van dijk, 2011; tmea, 2014). Reference made to the statement by trademark east africa (tmea): "the success of one nation depends on the success of its neighbors" (tmea, 2014), one can conclude that, to become a developed nation, a country needs its neighbors. This becomes true in the case of regional integration because, for example, when a company produces in a given country, it may not target only local residents as potential buyers but also those from neighboring countries. Intra-region trade, oncemade possibleby regional agreements, also called regional integration agreements (rias), is seen as a leverage of economic development of the countries belonging to the same agreed trade bloc. As asserted by van dijk (2011), the economic rationale of the regional integration is perceived as an important stimulus for trade, investment, and economic development. In this line, n'diaye (2001) states that for african countries which are handicapped by the small size of their markets, deficiencies in basic infrastructure and insufficient financial and human resources, the regional integration can actually speed up globalization as it allows to achieve economies of scale and streamline production processes, making firms competitive in international markets.

As for the trade considered as one of the main targets of the regional integration, grossman and helpman (1995) contend that it is regarded as an important channel for the diffusion of technology, which will, in turn, stimulate long-term growth and development.in addition, farole et al. (2010) confirm that

there exists a strong relationship between a country's trade share and its economic growth performance.

Implications of regional agriculture-led initiatives for agriculture sector and trade development: a focus on some african rias

Although its contribution to countries' economic growth varies from country to country and from region to region, agriculture continues to be the main basis of life for many inhabitants around the world. This reality seems to be more pronounced in developing countries where, as asserted by dowlah (2015), approximately one-third of the population still obtain their livelihoods from agriculture. In this line, the world bank (2015) argues that agriculture sector, which accounts for one-third of the gross domestic product and three-quarters of employment in sub-saharan africa, remains fundamental in the 21st century for eliminating poverty and increasing economic growth.

African countries' leaders are mindful of the role of agriculture sector in alleviating poverty and enhancing the livelihoods of their populations. Aiming at the development of this sector, the leaders have signed many international commitments among which the comprehensive africa agriculture development programme (caadp) was adopted in 2003. This initiative of the new partnership for africa's development (nepad) attracted the most attention of the leaders and their respective countries adhered massively to it. With this program aiming at boosting sustainable agricultural growth throughout the continent, and calling for a minimum commitment of 10% of national budgets to agricultural investment (nepad, 2003), it is not surprising that these countries are putting emphasis on agriculture and agribusiness. One of the nepad principles to create a positive environment for agricultural development is to remove obstacles to cross-border trade and investment, including harmonizing tax and investment codes to promote regional integration. Regional integration may, despite its challenges, be an important way forward for african countries and can be a learning ground for more ambitious global trading if they can resolve the bottlenecks that constrain even the limited existing trade opportunities (nepad, 2003).

Regional initiatives for trade promotion should go together with those focusing on the development of agriculture sector which contributes significantly to the value of imports and exports in sub-saharan africa. Country specific agricultural policy and strategies have been, for a long time, tainted with several critics such as their inappropriateness and failure to address effectively the challenges of agricultural sector at country level (sadc, 2011). Consequently, within the frame of recent efforts to encourage regional integration initiatives in africa since the beginning of the 21 century, numerous projects aiming at implementing common agricultural policies and strategies started (calza bini and boccaleoni, 2010). In this regard, the eac adopted in 2006, the common agriculture and rural development policy. With an overall objective of achieving food security and rational agriculture, this policy was expected to provide a guide in the development of strategies, programs and projects, and a pillar for the

development of a shared regional vision for sustainable development and takes advantage of the opportunities arising from globalization and regional integration (eac, 2006). In sadc , in july 2007, partner-states adopted a regional agricultural policy (rap) to address the low levels of economic growth and investment, the high levels of unemployment and poverty, the lack of competitiveness of regional economies, and the intra-regional and inter-regional economic disparities, all of which negatively impact the sadc region's development, integration and competitiveness (sadc, 2011). In western africa, in january 2005, ecowas , has also adopted a regional agricultural policy with the main objective of contributing in a sustainable way to meeting the food needs of the population, to economic and social development, to the reduction of poverty in the member states, and thus to reducing existing inequalities among territories, zones and nations (eu/ecowas, 2008).

In the staple foods sector too, regional efforts have been put in place in eac to move away from traditional approach to competitiveness in the form of the regional strategy for the staple foods value chain though challenges still remain regarding its implementation (usaid/compete, 2010).

However, despite the efforts that have been put in place at regional level leading to common agricultural policy (cap) or agriculture-related strategies in different african rias, results do not seem to be convincing in many african countries. As evidenced by oxfam international in highlighting the case of sadc-rap, the options for achieving the objectives of promoting actions and supporting the development of regional-level mechanisms and instruments have never been put on the table, and consequently, the promise of a vibrant smallholder sector that enables the realization of rights and supports poverty eradication remains a dream for many smallholder farmers (mutamba and dlamini, 2015). The same applies in eac, where the lack of appropriate application of cap in member countries has been noted. Indeed, according to the study conducted in 2013 by africa lead examining the institutional architecture for food security policy change in eac, the overall performance of the agricultural sector in the five eac partner states has been less than its potential and the overall caadp 6% target agricultural growth rate (usaid/africa lead, 2013). The same study reveals that the agro-industry sector in the eac is stagnant, with little added-value in manufacturing sectors. The main causes associated to this low average performance include: (i) inadequate research, education and skills development; (ii) inadequate financial and budget allocation; (iii) limited institutional capacity at the eac secretariat (and also partner states); and (iv) weak institutional structure at the eac secretariat (usaid/africa lead, 2013).

With regards to the agricultural trade performance, even though results described in the literature (see van dijk, 2011) reveal a positive impact of regional agreements in most of african regional trade blocs, the effect is not very significant as expected. In support of this view, hartzenberg (2011) states that africa's regional integration record is not impressive. This could be explained by the lack of financial and institutional instruments to apply, at the country level,

policy measures taken within the regional framework. Another cause could be rooted in the fact that, according to hartzenberg (2011), african rias are qualified as ambitious schemes with unrealistic time frames towards deeper integration and in some cases even political union, and are usually neighborhood arrangements.

This situation enables to understand that novel mechanisms need to be developed at country level towards the alleviation of existing challenges in applying and implementing appropriately regional adopted agriculture-oriented actions and interventions. This will lead to the development, in a sustainable way, of their respective agriculture sector characterized by stagnant production coupled with significant demand growth, and poor productivity and limited improvement for key staples (usaid/compete, 2010), and qualified by calza bini and boccaleoni (2010) as the most difficult sector in africa.

Regional agricultural trade and short and long-term gains: the case of Rwanda

Since its creation, the eac has worked on various programs to improve intra-regional trade and, as pointed out by kagira (2011), to stimulate and increase investment in agriculture by targeting intra-regional market. The customs union of the eac was designed to encourage intra-regional agricultural trade (karugia et al. 2009). In this context, tariffs on intra-regional trade have been reduced to zero since 2005 and regional products are protected from external competition through the application of the common external tariff, which lies between 25% and 75% (kagira, 2011). The common market for eac was set up in july 2010, and therefore, trade between the community members was much accelerated with an increase of 50% in less than a year. On the rwandan side, this pushed up the value of exports in 2010 to usd 269 million compared to170 million in 2009.

The analysis of the role of the primary sector in the life styles of the population and in trade between the eac countries shows that actions and strategies to develop this sector are essential in the region. Indeed, this sector continues to be the basis for the sustainable economic growth of the community because the majority of the population of the eac countries lives in rural areas with over 80% of the population depending on agriculture for their daily lives. In addition, 70% of exports in the community member countries are mainly composed by the agri-food or agriculture-related products (onyango, 2010).

Rwanda has an open economy with trade accounting for nearly a third of gross domestic product. Its main trading partners are the european union, eac, and comesa. In 2013, trade with the eac was estimated at 23.8% for imports and 23.3% for exports (nisr, 2014). The 2015 report of the world trade organization (wto) reveals that the share of agricultural products in imports and exports of rwanda were respectively estimated to 16.3% and 30.9% in 2014, and the main export destinations were tanzania, the democratic republic of congo (drc), uganda, kenya and european union (wto, 2015). According to usaid/eat (2013), cross-border and regional trade has considerable potential for the rwandan

agriculture. Their study shows that the eastern drc, inhabited by over two million people who cannot be fed only by local production, has become the main destination of the rwandan formal and informal foodstuffs exports. The main products exported to this area are maize, maize flour, beans and livestock (usaid/eat, 2013). The akagera region of tanzania, burundi, and uganda are also the main importing regions of rwandan agricultural products and constitute the main markets for its agricultural production (usaid/eat, 2013). The integration of rwanda to the eac facilitated its access to these markets and provides rwanda with opportunities to increase the amount of exported agricultural products. According to official rwandan trade statistics, the value of total cross-border trade exports in 2011 was usd 88 million, accounting for 23% of the total exports while the value of cross-border imports over the same period was usd 260 million, with usd 258 million traded formally (usaid/eat, 2013).

Statistics from the national bank of rwanda (bnr) show that for years, the formal foreign trade balance of rwanda with eac countries is negative. Indeed, from 2009 to 2014, the country recorded a considerable trade deficit (bnr, 2014) due to strong demand for intermediate goods and consumer goods because of the continued growth observed in rwanda (usaid/eat, 2013). The overall trade deficit comes from the formal trade while the informal cross-border trade has surplus since 2010. This informal trade consists mainly of agricultural products and livestock, and the main partner countries are the drc (80% of informal exports) and uganda (bnr, 2014; usaid/eat, 2013).

Regarding the distribution of rwanda's formal imports from eac countries, data show that uganda and kenya have the largest shares in imports of rwanda with 50.9 and 30% respectively (nisr, 2014). Although kenya has a large share of imports, available statistics show that their value have gradually decreased over the years, from 36.5% in 2010 to 30% in 2013 (nisr, 2014). Trade with uganda dates back a long time because available figures point out that on average, from 2009 to 2011, 43% of imports from rwanda were dominated by this country (nisr, 2011). As stressed by usaid/eat (2013), this could be explained by the porosity of borders between the two countries that leads to trade flows in the region even in the case of short-term seasonal price fluctuations. The statistics from nisr (2014) reveal that in 2013, the formal exports of rwanda in the eac were mainly intended to kenya with 70.4% followed by tanzania and burundi with 13.5 and 10.9%, respectively. Increased export of rwanda to kenya is likely related to the fact that the selling auction market of the rwandan tea, one of the main export products of the country, is located in mombasa.

Rwandan agricultural trade performance analysis:
A case study of maize, potato and bean sectors

The results reported in table 1 on nei and gl indices reveal different trends for the three food crops. The nei index for the considered period is negative for maize. This indicates that the country is qualified as the net importer of maize. The nei values for potato show that the country has been a net exporter for some

years and as an importer and exporter for other years. The same is observed for the bean whose corresponding net export indices reveal quite a similar pattern except the first two years during which rwanda was the net importer. The gl index values show a quite similar pattern for maize, potato, and bean. Using a threshold of a gl measure of 0.5 (banterle and carraresi, 2007), the findings reveal that rwanda is exhibiting a strong inter-industry trade for many years out of the ten considered for the study period, and this is pronounced more for maize and less for potato and bean.

CONCLUSIONS

This article clarifies the contribution of the regional integration in the development of agriculture and agricultural trade. The literature review showed that, for most African RIAs, the results of common agricultural initiatives aiming at agriculture sector and agricultural trade development have not been convincing due to lack of appropriate mechanisms and institutional actions to operationalize regional agricultural policy and strategy at the country level. The benefits of Rwanda's accession to EAC in terms of agricultural trade with the community are presented and analyzed. The study shows that, notwithstanding the insufficiency of agricultural production for the domestic and regional demand, the country registered substantial gains injoiningthe community. The ease ofaccess to regional markets through the establishment of the customs union and alleviation of some of regional trade barriers is one example among others. This led to increased value of its exports to the community. The analysis of NEI and GL trade performance indicators for maize, potato and bean reveals that Rwanda is a net importer of maize and a net exporter of potato and bean. For these two foodstuffs, it is clear that if Rwanda manages, through policy and institutional actions, to remove or alleviate the bottlenecks that prevent farmers from producing enough for export, it can have a competitive advantage in the neighboring countries' markets whose access is facilitated by its accession to the EAC.

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METABOLIC RESPONSES OF STEPPE FOREST TREES TO ALTIRUDE-ASSOCIATED LOCAL ENVIRONMENTAL CHANGES

SUMMARY

The effect of altitude-associated environmental gradient on leaves metabolic features of *Quercus robur* L. (an oak) and *Fraxinus excelsior* L. (an ash) was investigated in the natural coastal forest at Bellegarde' International Biosphere Reserve in Steppe zone, Ukraine. Decrease in relative humidity contrary to increase in temperature and lighting under the forest canopy were observed on the river steep bank with altitudinal elevation from lower (52 m a.s.l.) to middle (74 m.), and upper (96 m). Responses of tree leaves photosynthetic and antioxidant systems to the environmental local changes were studied by measuring chlorophyll (Chl) content, as well as catalase and peroxidase activities. Decrease in Chl amount in the ash leaves at middle and upper altitudes (17 and 38% compared with lower), along with increase (8% and 13%, respectively) in the oak leaves was found out. Chl content was determined to correlate with light, temperature, and humidity in both leaves of ash (respectively, $r = -0.94$, $r = -0.92$, $r = 0.90$) and oak ($r = 0.95$, $r = 0.93$, $r = -0.90$). Catalase activity grew with increasing altitude in leaves of ash (2 and 2.2 fold compared to lower altitude) and oak (1.2 and 1.4 fold) as well. Contribution of catalase to the total antioxidant enzymes activity enhanced in leaves of both species with increasing altitude. The results confirmed high sensitivity of steppe forest trees even to slight altitude-associated environmental deviations. Data obtained can be used to assess the adaptive potential of woody species to the climate changes aiming towards greater aridity traits and select tree species for planted forest creation as well.

Keywords: Quercus robur *L.*, *Fraxinus* excelsior L., altitude, microclimate, enzymes.

INTRODUCTION

Ecological and economic importance of the forests worldwide necessitates their conservation under climatic changes tending to aridity features enhancement (Bussotti et al., 2015), and determines relevance of trees adaptive capabilities studying. Talbi et al. (2015) suggest the exacerbation of plants

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survival problem with increased drought in numerous areas throughout the world, especially in the arid regions. Bussotti et al. (2015) state that due to increasing temperature and drought southern genotypes are likely to replace forest species in Western and Central Europe. Mokria et al. (2015) agree that namely climatic changes, in particular heat increase, might have contributed to forest dieback in northern Ethiopia. It's noteworthy that El-Hajj et al. (2014) emphasize probable severe consequences of the climatic deviations for the forest ecosystems despite plodding effect and small range of changes.

Natural forest conservation and restoration appears as a significant problem for Ukraine. The percentage of forest land in our country reaches 16.0% (Tkach, 2012). This index is one of the lowest among European countries. It is known that Steppe zone occupies about 40% of Ukrainian territory. The forests distribution is complicated by unfavourable conditions of geographic mismatch for the forest ecosystems. Steppe climate is characterized by the seasonal drought periods accompanied by high temperature and dry winds. Average annual rainfall is 472 mm, while in dry years 250 mm only; the annual amount of evaporation exceeds the precipitation by 2–3 times. Existence of the plant forest communities in the Steppe zone is possible due to geomorphologic diversity which enables formation of special local environmental conditions in the steep rivers banks. The steppe forest ecosystems may be highly sensitive to any environmental deviations under such circumstances. According to Ramirez-Valiente et al. (2015), climate is a major selective force in nature.

Plant responses and adaptation to the environmental conditions are complex biological processes including physiological and biochemical changes (Harfouche et al., 2014; Granda et al., 2014; Parviz, 2016). The mechanism of tree metabolic adaptation to local environmental factors is an important aspect of the overall forest conservation problem. It has been studied intensively during recent years. Thus, Aranda et al. (2015) showed significant physiological differences among six *Fagus sylvatica* populations adapted to specific local water availability. Zadworny et al. (2015) established a strong seasonal variation in nitrogen concentration among roots of oak trees growing in two contrasting soil types. Sperlich et al. (2015) showed differences in photosynthetic potentials and drought-tolerance in sunlit and shaded leaves of four Mediterranean trees. The significant impact of microclimate on transition processes to the generative phase of plant development was described by Bahuguna and Jagadish (2015). Next point is the study of the altitude impact on tree taxa distribution. Rezende et al. (2015) concluded that altitude belongs to major environmental parameters which can be used for developing a forest conservation strategy. The aim of our study - to estimate abiotic factors (local temperature, relative humidity and lighting) impact on photosynthetic and antioxidant systems of coastal forest trees *Quercus robur* L. (an oak) and *Fraxinus excelsior* L. (an ash) associated with the different river slope altitudes.

MATERIAL AND METHODS

Study area

The study was conducted in the territory of Bellegarde' Prisamarsky International Biosphere Reserve located in in the northen part of steppe zone in Dnipropetrovs'k province (47°32'–49°11'N, 33°–33°56'E). Studied areas are located on the southern slope of the Samara river. Observed plots are disposed in right steep bank at lower (52 m a.s.l.), middle (74 m a.s.l.), and upper (96 m a.s.l.) altitude in an old-growth (more than 85 years old) mixed deciduous natural forest. The plant community of the coastal forest is represented by several trees and shrubs with an oak (*Quercus robur* L.) and an ash (*Fraxinus excelsior* L.) dominating along the slope. Both the *Q. robur* and *F. excelsior* are the autochthonous and edificatory species of natural forest. The list of co-dominant species includes a linden (*Tillia cordata Mill*.), two species from *Acer* genus (*Acer platanoides* L. and *Acer campestre* L.), an elm (*Ulmus laevis Pall.)*, and *Corylus avellana* (L.) H. Karst. The forest undergrowth is formed by *Acer platanoides* young trees, with shrub species *Sambucus nigra* L. and *Euonymus europaeus* L.

Data collection

Microclimate data within the studied area were collected during the period of trees vegetation (April–August 2015). Data on air temperature and relative humidity levels under the tree canopy were obtained with the help of a portable weather station equipped by Assmann psychrometer (model 225-5230, Germany). Lighting levels under canopy were measured with a luxmeter (model Y u16, Russia) at the height of 2 m which corresponds to the level of lowest trees branches. Simultaneously, lighting level in open areas at each studied altitude was measured. The leaves of *Quercus robur* and *Fraxinus excelsior* were collected in the mean of May 2015 from 3–5 same-age trees within three groups in each studied area, and frozen.

Data analysis

Chlorophyll content and antioxidant enzyme activities (catalase, guaiacol - peroxidase, and benzidine - peroxidase) were measured with spectrophotometric methods. Chlorophyll content (Chl a, Chl b, and total chlorophyll value) was measured according to the method of Wintermans and De Mots (1965) in the ethanol extracts of tree leaves, and expressed in mcg of chlorophyll per g fresh weight (mcg/g FW).

Antioxidant enzymes activities were determined in the supernatants obtained by centrifugation (15,000 g for 20 min and 4˚ C) of crude extracts (100 mg of fresh leaves homogenized with 0.2 M TRIS-HCl buffer, pH 7.0 contained 0.1% polyvinylpyrrolidone, 250 mM saccharose, and 1 mM $MgCl_2$). Catalase (CAT) activity was evaluated according to Goth (1991) by measurement at 410 nm of optical density of reactive mixture containing 0.2 ml sample, 0.1% H_2O_2, and 4% ammonium molibdate. The result was calculated through the calibration graph and expressed in mM H_2O_2/g FW. Activity of guaiacol-peroxidase (GPOD) was estimated in accordance with Ranieri *et al.* (2001) by detecting

guaiacol oxidation at 470 nm in the reactive mixture containing acetate buffer (pH 6.0), 2 mM guaiacol, 0.2 ml sample, and 0.15% H_2O_2. The result was calculated with consideration of the guaiacol molar extinction coefficient, and expressed in mM guaiacol/g FW. Benzidine-peroxidase (BPOD) activity determination was based on the method proposed by Gregory *(*1966). Optical density change was registered for 1 min at 470 nm after adding 1% H_2O_2 to reactive mixture (acetate buffer, pH 6.0, 0.02 mM benzidine and 0.2 ml sample). The result was expressed in optical units/g FW.

All determinations of air temperature, relative humidity, and lighting (under tree canopy and in open areas) were performed in six replicates at each studied site. All determinations of the biochemical parameters characterizing the oak and ash leaves required five replicates. Data represent mean values and standard deviations (±SD).Differences significance was estimated using Student's t-test ($P<0.05$).

RESULTS AND DISCUSSION

The levels of temperature, relative humidity, and lighting under the forest canopy varied with the altitudinal elevation of the coastal slope, as shown in Table 1.

Table 1. Actinometrical and microclimatic conditions under the coastal forest canopy at the different altitudes of the Samara river bank.

Indices, units	Lower slope altitude	Middle slope altitude	Upper slope altitude
Lighting, (Lx)	1745.0±95.3	2190.0±115.4	4154.0±204.7
Lighting under canopy	3.5±0.2	4.3±0.3	8.2±0.7
Lighting in open area, (%)	3.5±0.2	4.3±0.3	8.2±0.7
Air average temperature, (ºC)	25.5±0.4	26.8±0.4	27.7±0.3
Air average relative humidity, (%)	63.60±1.14	59.90±1.48	55.94±1.49

Increase in lighting reached 1.3 fold at the middle altitude and 2.4 fold at upper altitude compared to the lower ($P<0.05$). Air temperature increase as well as relative humidity reduction under the canopy occurred to have gradient character in the course of moving upwards on the slope. The given study defines these local changes of microclimate and lighting as a conventional enhancing aridity trait with increasing altitude. It is possible to see the plants physiological and biochemical responses to the environmental changes.

Total chlorophyll content and ratio of chlorophyll forms (Chl a/Chl b) varied depending on the altitude-associated microclimatic conditions both in the oak and ash leaves (Table 2). We observed that an increase in temperature and lighting along with decrease in relative humidity upward the slope accompanied by the growth of total chlorophyll content in *Q. robur* leaves at the middle and upper altitudes compared to the lower (respectively, 8% and 13%, $P<0.05$).

Table 2. Effect of altitude associated environmental gradient on chlorophyll content in leaves of coastal forest trees.

Species	Chl a content (mcg/g FW)	Chl b content (mcg/g FW)	Total Chl content (mcg/g FW)	Ratio $\frac{\text{Chla}}{\text{Chlb}}$
Lower slope altitude				
Quercus robur L.	1.95±0.06	0.78±0.02	2.73±0.06	2.50
Fraxinus excelsior L.	2.30±0.08	1.29±0.04	3.59±0.08	1.77
Middle slope altitude				
Quercus robur L.	2.03±0.06	0.92±0.05	2.95±0.06	2.21
Fraxinus excelsior L.	2.15±0.08	0.91±0.05	3.06±0.08	2.38
Upper slope altitude				
Quercus robur L.	2.11±0.07	0.97±0.03	3.08±0.07	2.18
Fraxinus excelsior L.	1.85±0.07	0.75±0.03	2.60±0.07	2.46

At the same time, reduction in chlorophyll amount (17% and 38% at the middle and upper altitudes compared to the lower, $P<0.05$) was found in leaves of F. excelsior. The results obtained are consistent with the notion (Ramirez-Valiente et al, 2015) that photosynthesis is one of most sensitive process to the environmental stresses. In particular, intensity of photosynthetic pigments biosynthetic pathway depends on lighting and moisture. In oak leaves, accumulation both of chlorophyll a and chlorophyll b contributed to the increase in total chlorophyll amount with altitudinal elevation. However, decrease in the ratio Chl a/Chl b indicates the heightened Chl b accumulation. In contrast, more significant reduction of chlorophyll b content was observed in ash leaves at the middle and upper altitudes, thereby increasing the ratio Chl a/Chl b. According to Caudle et al. (2014), high Chl a/Chl b ratio is an index of plant adaptation to drought. Thus, we assumed that the Quercus robur trees showed higher adaptability to relative humidity reduction, as well as complex environmental changes tending to enhance the aridity traits uphill the slope. Correlation analysis revealed strong positive coefficients between changes of Q. robur leaves total Chl content and temperature ($r = 0.92$) together with lighting ($r = 0.86$), whereas the interaction with relative humidity changes was negative ($r = –0.86$). On the contrary, leaves of F. excelsior showed a positive relationship between humidity and Chl content ($r = 0.92$), while correlation was getting negative in the course of temperature ($r = –0.89$) and lighting ($r = –0.90$) changes uphill. Hereof, the rate of Chl biosynthesis in oak leaves was stimulated by an increase in temperature and light despite the decrease in relative humidity; at the same time, Chl accumulation in ash leaves was oppressed due to environmental changes. These conclusions resonate with data by Caudle et al. (2014), according to which drought-tolerant species are able to maintain high intensity of photosynthesis and

protect photosystem II during dry periods. In addition, our results coincide with data by Rajsnerova et al. (2015) on significant growth of the total Chl content in the lower leaves of Fagus sylvatica canopy just at the upper altitude and the highest light intensity.

The different changes in catalase and peroxidase activity followed the altitude- associated modifications of temperature, lighting, and relative humidity levels both in the leaves of *Q. robur and F. excelsior* (Table 3).

Table 3. Effect of altitude-associated environmental gradient on the antioxidant enzymes activity in leaves of coastal forest trees.

Species	GPOD activity (mM guaiacol/g FW)	BPOD activity (optical units/g FW)	CAT activity (mM H_2O_2/ g FW)
Lower slope altitude			
Quercus robur L.	0.19±0.02	6.07±0.17	13.22±0.61
Fraxinus excelsior L.	9.91±0.14	1.52±0.06	1.42±0.29
Middle slope altitude			
Quercus robur L.	0.26±0.02	4.36±0.20	15.53±1.72
Fraxinus excelsior L.	9.61±0.11	0.99±0.06	2.90±0.19
Upper slope altitude			
Quercus robur L.	0.09±0.02	1.64±0.11	17.91±1.44
Fraxinus excelsior L.	3.41±0.09	1.15±0.04	3.11±0.29

Significant decrease in BPOD activity was observed in oak leaves at the middle and upper altitudes compared to lower (1.4 and 3.7 folds respectively, $P<0.05$), while activity of GPOD tended to enhance at the middle altitude and decrease at the upper (1.3 and 2.2 folds respectively, $P<0.05$). In ash leaves decrease in BPOD activity was insignificant, whereas GPOD activity declined 2.9 folds ($P<0.05$) at the upper altitude compared to lower. Halliwell (2006) proclaims plant peroxidase functions to be associated with eliminating the excessive hydrogen peroxide accumulated during various physiological processes. Therefore, the results obtained indicate significant metabolic adjustment in oak and ash leaves in the course of slope altitude elevation and enhancing aridity. Probably, changes may affect the accumulation of phenols and sugars, since Allison and Schultz (2004) emphasized the peroxidases appear to play the important role in these metabolic pathways.

The catalase activity at the middle and upper altitudes exceeded the lower level both in the oak (1.2 and 1.4 folds respectively, $P<0.05$), and ash of leaves (respectively 2 and 2.2 folds, $P<0.05$). Together, the results showed increase in catalase proportion in the total antioxidant activity of tree leaves with growing altitude. This proportion in oak leaves varied from 68% at lower altitude to 77% at the middle and 91% at the upper. The catalase contribution in the leaves of ash was 11%, 22%, and 41% of total antioxidant activity respectively at the lower, middle and upper altitudes. Strong positive correlation between the catalase activity and lighting together with temperature was revealed in the leaves of oak ($r = 0.83$ and $r = 0.93$ respectively) and ash ($r = 0.78$ and $r = 0.82$). Thus, our data

are consistent with the conclusion of Mhamdi et al. (2010) about high plant catalases sensitivity to light. In the whole, the above data showed a substantial growing catalase involvement in the antioxidant processes in leaves of .Q. robur and F. excelsior with increasing altitude and enhancing aridity traits. This conclusion is consistent with Queval's et al. (2007) opinion on the important plant catalase role in hydrogen peroxide elimination during photosynthesis as well as photorespiration stimulated by solar radiation and high temperature. Besides, the results obtained coincide with data by Mhamdi et al. (2010) that catalases are highly activated enzymes. It is an integral part of plant defense system. So, enchanting antioxidant protective capacity associated with catalase activation was revealed both in oak and ash leaves with increasing altitude of the slope and enhancing aridity. Comparing results of the our study with data by Rajsnerova et al. (2015) on the beech leaves metabolic changes due to significant altitude difference (400–1100 m), it is reasonable to mention high sensitivity of Quercus robur and Fraxinus excelsior leaves to the environmental changes, associated with smallest altitudinal elevation.

CONCLUSIONS

Results of present study confirmed the hypothesis of a high sensitivity of Quercus robur L. and Fraxinus excelsior L. metabolic processes to even small environmental differences with increasing altitude. Decrease in relative humidity along with increase in temperature and lighting under the forest canopy were assessed as a conventional increase in aridity up the slope. Significant catalase activation together with increasing enzyme contribution to the total antioxidant capacity was common trait both in oak and ash leaves. Species - determined differences in the metabolic changes were manifested in the variations of photosynthetic process. Accumulation of chlorophyll in oak leaves was stimulated by an increase in temperature and light in spite of the decrease in relative humidity; and it declined in ash leaves due to environmental changes. The results of study may be useful for assessing adaptive capacity of woody species to increased aridity, and species selection for further creation of forest plantations in the Steppe zone.

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EFFECT OF DIFFERENT FERTILIZATION REGIMES ON THE WEED COMMUNITY IN SWISS CHARD

SUMMARY

Weed community of Swiss chard was studied in plots with different fertilization regimes. Fertilization doses correspond to 50% (F50), 100% (F100) and 150% (F150) nutrient demand of Swiss chard. Sinousia consists of 29 species, out of 26 genera and 17 families, among which dominant are: *Sorghum halepense, Solanum nigrum, Kickxia spuria,* and *Atriplex patula*. The analysis of the biological spectrum indicates the dominance of therophytes with 75.8%, while hemicryptophytes contribute the spectrum with 17.2%. Considering the effect of fertilizers on the crop growth and weediness, general conclusion is that fertilizers benefit both the crop and weeds, but increased doses of fertilizers had better effect on the crop yield than on weediness. The average crop yield was highest in the regime F150, as well as total weediness F150. However, analysing the species abundance, cover per single plot and crop yield in each regime of fertilization, some deviations are noticed. Such as, in the plots No 10 (belongs to F100) and No 17 (belongs to F150) the total weed cover was 10%. In eight plots which belongs to F50 the weed cover exceeded this value.

Keywords: weed community, fertilization, Swiss chard

INTRODUCTION

Fertilisation is considered as one of the powerful tools in managing weeds (Liebman and Mohler, 2001), with precondition of exact choise of time and way of application of the fertilizers (Blackshaw et. al., 2004). In general, process of fertilization increases total biomas production in filed, but not exclusively crop production. Some studies shown that weeds might be more effective than crops in capturing nutrients added as fertilizers (Santos et al., 1998; Blackshaw et al., 2003), while in others crops are more effective (Dusky et al., 1996; Dhima and Eleftherohorinos, 2001).

Alhtough in Mediterraena cuisine Swiss chard is frequently used, in the global market it is treated as one of the neglected vegetable species, which production area is not commercially important (Pokluda and Kuben, 2002). Similar situation is present in Montenegro. The crop is grown only in Zetsko-

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

Bjelopavlićka plain on rather small areas and mostly distributed in the southern part of the country, where the tradition in its consumption still exists (Knežević et al., unpublished data).Taking into account its good nutritional values (Santamaria et al., 1999a, 1999b) and medicinal properties – the extract of this plant when administered by gavage may reduce blood glucose levels by regeneration of the B cells (Bolkenta et al., 2000), its production might increase in the future. Up to know research on Swiss chardwere focused on morphological characteristics, yield, content of mineral elements and nutritional quality (Malý et al., 1998; Santamaria et al., 1999a, 1999b; Pokluda and Kuben, 2002; Smith et al., 2001; Miceli and Miceli, 2001), salt tolerance (Shannon et al., 2000), while weed research were rather neglected. The aim of this research is to determine the dynamics of weed infestation in Swiss chard cultivated in crop rotation with different levels of mineral fertilizing.

MATERIAL AND METHODS

The Swiss chard was grown in the field of Green House Jovović company, in Bjelopavlićka Plain, site Sige (42.553191° N latitude and 19.149113° E longitude). The soil is intesively used for vegetable production, has high content of total carbonate and alkaline reaction (Knežević et al, 2014). The results of soil analysis (samples taken before fertilization) are shown in Table 1.

An experiment with randomized design was conducted in the period March-July 2014. The experimental field contained 28 plots - nine plots treated with three different level of fertilization (labelled as F50, F100 and F150) and one control plot without fertilization and irrigation (C). Plot area was 10.5 m^2. Fertilization doses corresponded to 50%, 100%, and 150% of commercial recomendations for Swiss chard, which were 100-120 kg/ha N, 80-120 kg/ha P_2O_5, and 100 kg/ha K_2O per season. Fertilization amounts of NPK fertilizer 11:11:21 were 200 kg/ha, 400 kg/ha, and 600 kg/ha. Additional nutrient fertilization, in amounts 35 kg/ha, 70 kg/ha, and 105 kg/ha for various treatments, was applied by watering with the same volume of appropriate solution twice during vegetation. The solution was prepared by dissolving of NPK 24:8:16 and NPK 15:30:15 in ratio 2:1. Irrigation amount of 60 mm of water was applied uniformly at all plots, excluding control plot, during the season.

The investigation of the weed community was carried out in the second half of May and early June 2014. Herbicides were not used in this period. In each plot all weed species, weed density (number of individuals per m^2), the cover of single weed species and total weed cover were recorded.The cover is estimated with original cover-abundance scale of Braun-Blanquet extended to a combined cover-abuncance scale by Barkman *et al*. (1964). Total weed cover is given in percents (%). Weed species were identified by Domac (1994). Life form classification is performed according to Raunkier, elaborated and updated by Ellenberg and Mueller-Dombois (1967) and, for our circumstances, modified by Stevanović (1992).

Table 1. Soil parameters (before fertilization)

Label of plot	pH (H_2O)	pH (KCl)	$CaCO_3$ (%)	Total N (%)	Humus (%)	Available P_2O_5 (mg/100 g)	Available K_2O (mg/100 g)	EC (µS/cm)
1F100	7.60	7.04	3.3	0.278	5.89	6.2	23.0	155.0
2F50	7.55	7.02	2.8	0.263	6.10	4.9	22.0	158.2
3F100	7.70	7.17	2.3	0.252	5.74	4.2	23.4	158.8
4F100	7.70	7.20	4.7	0.248	5.50	3.8	20.6	145.8
5F50	7.70	7.21	21.4	0.254	5.77	5.0	21.3	156.1
6F150	7.77	7.25	21.9	0.236	5.21	6.8	19.9	146.0
7F150	7.76	7.27	10.9	0.255	5.95	5.3	23.0	167.9
8F150	7.88	7.35	21.4	0.245	6.10	5.8	22.3	163.3
9F50	7.91	7.35	6.7	0.236	5.86	3.8	21.8	150.3
10F100	7.93	7.34	21.9	0.228	5.95	9.4	18.7	150.7
11F50	7.90	7.32	21.9	0.222	5.06	20.1	18.7	150.1
12F100	7.87	7.31	23.8	0.216	4.91	21.6	16.2	145.4
13F100	7.95	7.38	26.2	0.206	4.94	16.6	19.2	155.4
14C	7.94	7.40	26.2	0.216	4.73	7.6	20.4	153.4
15F50	7.79	7.20	3.8	0.280	6.25	7.9	32.7	158.8
16F150	7.77	7.23	2.8	0.291	6.19	11.6	32.7	157.4
17F150	7.77	7.27	3.3	0.284	6.01	12.5	30.4	152.4
18F150	7.75	7.28	4.7	0.285	5.92	11.2	30.4	158.2
19F50	7.93	7.35	6.7	0.284	5.53	10.3	32.7	166.3
20F100	7.97	7.38	11.9	0.271	5.39	8.0	23.0	157.6
21F50	7.91	7.37	19.0	0.263	5.06	5.3	22.0	161.6
22F100	7.90	7.41	30.9	0.237	5.15	6.1	20.6	149.5
23F100	7.78	7.21	38.1	0.208	4.85	8.3	19.2	154.6
24F50	7.80	7.22	38.5	0.237	4.79	9.4	17.3	148.2
25F150	7.91	7.35	39.0	0.229	3.50	13.8	16.9	149.1
26F150	7.88	7.35	35.7	0.224	2.60	18.8	17.6	154.6
27F150	8.00	7.46	32.4	0.227	2.93	12.7	18.7	158.6
28F50	8.00	7.48	33.3	0.227	3.05	8.2	18.5	153.7

The descriptive statistical analysis (minimum, maximum, mean and standard deviation) of the data was performed by SPSS.

RESULTS AND DISCUSSION

Floristic composition and taxonomic spectrum.

The structure and number of weed species found in the Swiss chard during investigation are shown in Tables 2, 3 and 4. Weed sinousia of Swiss chard in Montenegro consists of 29 species, out of 26 genera and 17 families. Families *Poaceae* and *Scrophulariaceae* are dominant and represented with 4 species each, *Fabaceae* with 3, *Asteraceae, Chenopodiaceae, Euphorbiaceae,* and *Geraniacaae* with 2 species each, while others have one representative each.

Such taxonomical spectrum differs from the one of the sinousia of other crops investigated in Montenegro: i) maize- *Poaceae* (4 species) *Polygonaceae* (3 species), *Brassicaceae, Chenopodiaceae,* and *Plantaginaceae* (2 species each), *Amaranthaceae, Caryophyllaceae, Convolvulaceae, Fabaceae, Lamiaceae, Scrophylariaceae* (1 species each) (Jovović, 1998); ii) potato- *Asteraceae* (8 species), *Lamiaceae* (5 species), *Fabaceae, Poaceae, Polygonaceae* (4 species each), *Scrophulariaceae* (3 species), *Brassicaceae, Caryophyllaceae, Chenopodiaceae, Euphorbiaceae* (2 species each), *Amaranthaceae, Apiaceae, Boraginacea, Convolvulaceae, Equisetaceae, Geraniaceae, Primulaceae, Rosaceae, Rubiaceae, Solanaceae, Violaceae* (1 species each) (Stešević and Jovović, 2005). Speaking in the term of dominant species in Swiss chard sinousia the most abundant were *Sorghum halepense, Solanum nigrum, Kickxia spuria* and *Atriplex patula,* while in maize crop *Chenopodium album, Amaranthus retroflexus* and *Polygonum aviculare* prevailed (Jovović 1998). In the potato crop dominant weeds were *Convolvulus arvensis, Anthemis arvensis* and *Sonchus arvensis* (Stešević and Jovović, 2005).

On the plots with treatmen F50 and F150 number of recorded species is 27, while tretment F100 has 26 species. Average number of species per treatment is: C – 15, F50 – 14.7, F100 – 14.1 and F150 – 15.1. The most abundant species in sinousia are *Sorghum halepense, Solanum nigrum, Kickxia spuria* and *Atriplex patula.* They comprised 67.3% of the total weediness. Ranking of dominant species (number of individuals per $m^{2)}$ differs between the treatments: in the C are *A. patula* (13,3/m^2) and *S. halepense* (10,5/m^2), in the F50 are *S. halepense* (12,8/m^2), *K. spuria* (4,1/m^2) and *S. nigrum* (2,6/m^2); in the F100 are *S. halepense* (12,9/m^2), *K. spuria* (3,8/m^2) and *A. patula* (3,4/m^2); and in the treatmen F150 are *S. halepense* (15,8/m^2), *S. nigrum* (6,9/m^2) and *A. patula* (2,9/m^2) (tab. 2, 3, 4). Although changes in the relative abundances of weeds caused differences in the community composition between plots without (C) and with different fertilizing regime (F50, F100 and F150), species composition was quite similar.

Additional remark considering floristic composition of sinousia is that up to this research *A. patula* and *K. spuria* were not consideres as troublesome agricultural weeds in Montenegro (Jovović, 1998; Stešević and Jovović, 2005; Caković *et al*., 2012).

Life forms.

The analysis of the total biological spectrum of the flora indicates the dominance of therophytes with 75.8%, the most dominant of which were T scap terophytes (51.7%). Hemicryptophytes contribute 17.2% to the biological

spectrum, and geophytes (rhizomatous) 7%. The spectrum of life forms fits into the general biological spectrum of the flora of Bjelopavlići plain (Caković *et al.*, 2012). Considering the geographical position of this area and the environmental conditions that are under specific Mediterranean influence, and also bearing in mind that the use of cropping practices works in favour of the dominance of annual life forms (Armesto and Vidella, 1993), the predominance of therophytes in the biological spectrum is expected.

In the biological spectrum of vegetation participation of life forms is a bit different: annuals are dominant life form with 56,7 %. They are followed by geophytes with 41,8 % while hemicriptophytes participate the spectrum with 1,5 %. In the different treatments the biological spectrum of vegetation did not show significant variation

Weed density and weed cover.

Primary role of the nutrients is to promote crop growth, but very often it also benefit weeds (Di Tomaso, 1995). In our experiment the average crop yield was highest in the F150 (11.19±2.88SD kg/plot) > F100 (9.11±1.85SD kg/plot) > F50 (8.21±2.25SD kg/plot), as well as total weediness F150 (19.33%) > F100 (15.4%) > F50 (14.8%) > C (7%). Considering average weed density the decreasing order was a bit different F150 (41.3 ind/m^2) > C (36.9 ind/m^2) >F100 (33.7 ind/m^2) >F50 (32.3 ind/m^2). It is explained with the fact that number of individuals and the cover are not obligatory in positive correlation. Size of individuals recorded at the control plot was significantly smaller comparing to the fertilized ones, thus the cover value was lower. However, analysing the species abundance, cover per single plot and crop yield in each regime of fertilization, some deviations are noticed (tab. 2, 3, 4), such as, in the plots No 10 (belongs to F100) and No 17 (belongs to F150) the total weed cover was 10%. In eight plots which belongs to F50 the weed cover exceeded this value. Or the yield in plot 6 (belongs to F150) was rather low (6.95 kg). Due to the fact that such deviations is not possible to explain with biotic factors, such as competition between weed and crop, the answer migh be hidden in soil properties. Thus the future research should also include detailed soil analysis per each plot.

Nevertheless, when speaking about the weedeness degree of dominant species, following remarks are given. In the case of *Sorghum halepense*, it is shown that fertilizers increases the crop weediness, from 10.48 ind/m^2 in the control, to 23.52 ind/m^2 the treatment F50, to 121.72 ind/m^2 was in the treatment 100 and 149.11 ind/m^2 in the treatment F150 (tab. 2, 3, 4). Study of Dražić and Konstantinović (1996) showed the same regularlity, when nitrogen fertilizers are

applied. Some other weeds from the sinousia like *Amaranthus retroflexus, Portulaca oleracea, Polygonum aviculare, Setaria viridis* etc. reacted the same way. This is also pointed out by Efthimiadoua *et al*. (2012) and Papastylianou *et al*. (2014). Opposite to this case, fertilizers can reduce crop weediness. Such case is reported for *K. spuria* (Salat *et al*. 2014). In our study treatment F50 caused rapid increase of weeding degree (from 2.10 ind/m^2 in control to 37.40 ind/m^2), while treatments F100 and F150 had opposite effects. The weedeness decreased from 35.74 ind/m^2 in F100 to 19.14 ind/m^2 in F150. It could be explained with the fact that F150 plots has bigger cover of aboveground part of the Swiss chard and taller weeds as *S. nigrum* and *A. patula*, thus the light conditions are infavorable for development of *Kickxia spuria.* Study of Lo Bianco (2007) and Puhui *et al*. (2011) pointed that fertilizing had no significant effect on plant biomass of either *A. patula* or *S. nigrum*. Our study did not show any clear pattern. In the case of *S. nigrum*, not even one induvidual was recorded on the control variant, while on F50 weeding degree was 23.53 ind/m^2, on the F100 it was 4.38 ind/m^2 and on the F150 it was 65.63 ind/m^2. Weedeng degree of *A. patula* increased from 13.3 ind/m^2 in C to 18.10 ind/m^2 in F50 to 32.09 ind/m^2 in F100 and than decreased on 26.19 ind/m^2 in F150.

Some studies shown that increase in the dose of fertilizer has positive effect on weed density, but negative on yield (Knežević *et al*., 2008). In our research increased doses of fertilizers had better effect on the crop yield than on weediness; this results inicated that swiss chard are better competitors for nutrients than are weed. As it is presented in table 4, the plots 16 and 17 had higher crop yield (16.05 kg and 15.05 kg, respectively) compared to other plots with the same regime of fertilization (F150), and in the same time lower weed cover (12% and 10%, respectively). Soil analyses conducted before the fertilization have shown that plots 16 and 17 has higer content of humus and nitrogen in comparison to other plots with the same regime of fertilization (Kneževic *et al.*, unpublished data). In such conditions, Swiss chard grows faster than weeds, thus it overcompetes them. Nevertheless, one plot in regime F150, the plot 6 had rather low yield of 6.95 kg, but this can not be explained with better weed competitivity, while the weed cover was only12%. However in this plot was recorded the lowest value of electical conductivity of soil, as a parameter of amount of salt in the soil, as well as relatively lower content of available macronutrients P and K. As it is concluded before, the answer migh be hidden in soil properties, thus the future research should also include more detailed soil analysis per each plot.

Table 2. Weed density and weed cover in the plots in the regim F50 (50%). n – average No of individuals per m^2. c – weed cover in the plot

Taxon Family Life form	Plot 2 n c	Plot 5 n c	Plot 9 n c	Plot 11 n c	Plot 15 n c	Plot 19 n c	Plot 21 n c	Plot 24 n c	Plot 28 n c	Total No of individuals in all F50 plots	Control n c	Total No of individuals in control
Anagalis arvensis Primulaceae v-a Mi-Mes T scap					0.29 +	0.19 +	0.38 +	0.67 1	0.57 1	22	2 1	20
Amaranthus retroflexus Amaranthaceae a-aut Mes-Alt T scap						0.29 +	0.48 +	1.33 1		22		0
Atriplex patula Chenopodiaceae aut Meg-Alt T scap	0.57 1	4.10 1	4.19 1	3.24 1	0.38 +	4.67 1	0.10 r	0.19 +	0.67 1	**190**	13.33 2a	**133**
Capsella bursa-pastoris Brassicaceae n-aut Mi-Meg T semiros		0.10 r	0.19 +		0.29 +	0.57 1	1.62 1	1.43 1	1.33 1	58	0.38 +	4
Chaenorrhinum minus Scrophulariaceae v-aut Mi-Mes T scap			0.10 r		0.10 +	0.10 r	0.10 r	0.10 r	0.19 +	7		0
Chenopodium album Chenopodiaceae a-aut Meg-Alt T scap						0.19 +	0.29 +			5		0
Convolvulus arvensis Convolvulaceae a SG herb rhiz		0.10 r	0.67 1	0.10 r	0.57 1	0.57 1	0.76 1	0.38 +	1.14 1	45	1.33 1	13
Cynodon dactylon Poaceae a-aut Mes-Mac H caesp						0.10 r		0.19 +		3		0
Euphorbia helioscopia Euphorbiaceae v Mi-Mes T scap	0.40 +		0.10 r			0.19 +		0.10 r		8	0.38 +	4
Euphorbia maculata Euphorbiaceae v-aut Mi-Mes T rept	0.30 +				3.81 1	0.86 1	0.48 +	0.29 +	0.38 +	64	0.48 +	5
Geranium columbinum Geraniaceae v-a Mes T semiros					0.10 r			0.29 +		4		0

Taxon Family Life form	Plot 2 n c	Plot 5 n c	Plot 9 n c	Plot 11 n c	Plot 15 n c	Plot 19 n c	Plot 21 n c	Plot 24 n c	Plot 28 n c	Total No of individuals in all F50 plots	Control n c	Total No of individuals in control
Geranium dissectum Geraniaceae v-a Mes T semiros	0.70 1	0.29 +	3.33 1	3.62 1		0.29 +			0.19 +	88	3.14 1	31
Kickxia spuria Scrophulariaceae a Mes-Meg T scap	0.45 +	7.62 2a	0.48 +	1.52 1	2.57 1	4.67 1	11.14 2a	7.43 2a	1.52 1	**392**	2.10 1	21
Lotus corniculatus Fabaceae v-aut Mes H scap										0.00	0.10 r	1
Mentha longifolia Lamiaceae a Mes-Meg H scap	1.48 1						1.05 1	1.52 1		42		0
Medicago lupulina Fabaceae v Mes-Mac T scap	0.48 +				0.29 +	0.57 1	0.19 +			16	0.19 +	2
Plantago lanceolata Plantaginaceae v-a Mi-Meg H ros						0.29 +		0.10 r		4		0
Poa anua Poaceae n-a Mi-Mes T caesp										0		0
Polygonum aviculare Polygonaceae a-aut Mi-Mes T rept	3.90 2a	1.05 1	0.48 +	1.14 1	2.19 1	0.95 1	1.14 1	1.24 1	2.38 1	152	0.76 1	8
Portulaca oleraceae Portulacaceae a Mes T scap					0.48 +				0.29 +	8		0
Trifolium repens Fabaceae v-aut Mi-Mes H rept					0.19 +		0.19 +	0.48 +		9		0
Senecio vulgaris Asteraceae n-v Mes-Meg T scap	0.57 +	0.57 1		0.10 r	0.10 r		0.10 r	0.19 +	1.14 1	29	0.19 +	2

Taxon Family Life form	Plot 2 n c	Plot 5 n c	Plot 9 n c	Plot 11 n c	Plot 15 n c	Plot 19 n c	Plot 21 n c	Plot 24 n c	Plot 28 n c	Total No of individuals in all F50 plots	Control n c	Total No of individuals in control
Setaria viridis Poaceae a-aut Mes-Mac T scap							0.29 +	0.29 +	0.29 +	9		0
Solanum nigrum Solanaceae a-aut Mes-Mac T scap					1.05 1	4.38 1	3.14 1	6.10 1	8.86 2a	**247**		0
Sonchus asper Asteraceae v-a Mes-Alt T scap					0.29 +	0.38 +	0.29 +	0.57 1		16		0
Sorghum halepense Poaceae a-aut Mes-Meg G rhiz	5.52 2m	7.62 2m	8.10 2m	6.67 2m	7.71 2m	19.52 2a	25.71 2a	19.33 2a	15.24 2a	**1212**	10.48 2a	**105**
Stellaria media Caryophyllaceae n-aut Mi-Meg T rept									0.10 r	1	0.19 +	2
Veronica arvensis Scrophulariaceae v-a Mi-Mes T scap					7.24 1	6.38 2m	2.19 1	2.10 1	0.48 +	193		0
Veronica persica Scrophulariaceae n-aut Mi-Mes T scap	2.67 1	0.67 +	1.90 1	2.38 1	2.86 1	1.33 1	2.10 1	2.57 1	3.33 1	208	1.90 1	19
Total No of individuals per m^2	17.04	22.09	19.5	18.7	30.7	46.47	51.7	46.85	38.1	∑ 3054	19.33	∑ 370
No taxa	11	9	10	8	18	19	20	21	17		15	
Weed cover (%)	12	12	12	10	17	17	20	17	17		7	
Swiss Chard yield (kg of aboveground part)	9.95	8.55	5.35	4.75	12.05	8.75	8.45	9.05	7.07		4.65	

Table 3. Weed density and weed cover in the plots in the regim F100 (100%). n – average No of individuals per m^2. c – weed cover in the plot

Taxon **Family** **Life form**	**Plot 1** **n** **c**	**Plot 3** **n** **c**	**Plot 4** **n** **c**	**Plot 10** **n** **c**	**Plot 12** **n** **c**	**Plot 13** **n** **c**	**Plot 20** **n** **c**	**Plot 22** **n** **c**	**Plot 23** **n** **c**	**Total No of individuals in all F100 plots**	**Control** **n** **c**	**Total No of individuals in control**
Anagalis arvensis Primulaceae v-a Mi-Mes T scap							0.29 +	0.67 1	0.67 +	16	2 1	20
Amaranthus retroflexus Amaranthaceae a-aut Mes-Alt T scap							5.71 2m			57		0
Atriplex patula Chenopodiaceae aut Meg-Alt T scap	1.84 1	1.20 1	2.10 1	1.14 1	7.24 2a	13.9 2a	3.52 1	0.48 +	0.67 +	**320**	13.33 2a	**133**
Capsella bursa-pastoris Brassicaceae n-aut Mi-Meg T semiros	0.40 +	0.30 +	0.32 +	0.30 +	0.29 +	0.38 1	0.57 1	2.67 1	3.24 1	84	0.38 +	4
Chaenorrhinum minus Scrophulariaceae v-aut Mi-Mes T scap						0.10 r	0.10 r	0.10 r		3		0
Chenopodium album Chenopodiaceae a-aut Meg-Alt T scap	0.30 +				0.57 1			0.19 +		10		0
Convolvulvus arvensis Convolvulaceae a SG herb rhiz	1.70 1	1.24 1	0.57 1	0.19 +	0.67 1	1.43 1	0.57 1	0.76 1	0.76 1	78	1.33 1	13
Cynodon dactylon Poaceae a-aut Mes-Mac H caesp										0		0
Euphorbia helioscopia Euphorbiaceae v Mi-Mes T scap								0.38 +	0.19 +	5	0.38 +	4
Euphorbia maculata Euphorbiaceae v-aut Mi-Mes T rept						0.29 +	0.29 +	0.29 +	0.10 r	9	0.48 +	5
Geranium columbinum Geraniaceae v-a Mes T semiros	0.94 1	1.05 1				0.10 r	0.10 r		0.10 r	12		0

Taxon Family Life form	Plot 1 n c	Plot 3 n c	Plot 4 n c	Plot 10 n c	Plot 12 n c	Plot 13 n c	Plot 20 n c	Plot 22 n c	Plot 23 n c	Total No of individuals in all F100 plots	Control n c	Total No of individuals in control
Geranium dissectum Geraniaceae v-a Mes T semiros		1.05 1	1.71 1	4.38 1	3.71 1	4.86 1		0.10 r		158	3.14 1	31
Kickxia spuria Scrophulariaceae a Mes-Meg T scap	0.90 1	1.20 1	3.62 1	0.90 1	0.84 1	1.14 1	4.76 1	12.67 2a	9.71 2a	**357**	2.10 1	21
Lotus corniculatus Fabaceae v-aut Mes H scap									0.10 r	1	0.10 r	1
Mentha longifolia Lamiaceae a Mes-Meg H scap	1.94 1		0.29 +				0.19 +	1.43 1		38		0
Medicago lupulina Fabaceae v Mes-Mac T scap	0.29 +	0.19 +	0.32 +			0.48 +	0.10 r	0.19 +		16	0.19 +	2
Plantago lanceolata Plantaginaceae v-a Mi-Meg H ros								0.10 r	0.10 r	2		0
Poa anua Poaceae n-a Mi-Mes T caesp										0		0
Polygonum aviculare Polygonaceae a-aut Mi-Mes T rept	3.21 2a	1.71 1	1.90 1	1.33 1	1.81 1	3.14 2a	0.57 1	1.71 1	1.24 1	166	0.76 1	8
Portulaca oleraceae Portulacaceae a Mes T scap										0		0
Trifolium repens Fabaceae v-aut Mi-Mes H rept		0.57 1					0.29 +	0.38 +	0.38 +	16		0
Senecio vulgaris Asteraceae n-v Mes-Meg T scap	0.38 +			0.38 +		0.1 r	0.38 +	0.29 +	0.19 +	17	0.19 +	2

Taxon Family Life form	Plot 1 n c	Plot 3 n c	Plot 4 n c	Plot 10 n c	Plot 12 n c	Plot 13 n c	Plot 20 n c	Plot 22 n c	Plot 23 n c	Total No of individuals in all F100 plots	Control n c	Total No of individuals in control
Setaria viridis Poaceae a-aut Mes-Mac T scap								0.19 +	0.76 1	9		0
Solanum nigrum Solanaceae a-aut Mes-Mac T scap							2.95 1	0.48 +	0.95 1	**43**		0
Sonchus asper Asteraceae v-a Mes-Alt T scap							0.76 +	0.19 +	1.24 1	22		0
Sorghum halepense Poaceae a-aut Mes-Meg G rhiz	8.20 2a	9.52 2a	8.57 2a	6.67 2m	8.00 2a	15.9 2a	25.33 2a	20.95 2a	18.57 2a	**1217**	10.48 2a	**105**
Stellaria media Caryophyllaceae n-aut Mi-Meg T rept	0.30 +					0.29 1				6	0.19 +	2
Veronica arvensis Scrophulariaceae v-a Mi-Mes T scap	0.20 +	0.10 r	0.31 +				3.90 1	7.52 2m	10.95 2m	230		0
Veronica persica Scrophulariaceae n-aut Mi-Mes T scap	3.62 1	1.43 1	0.67 +	1.52 1	1.05 1		3.14 1	0.57 1	1.62 1	136	1.90 1	19
Total No of individuals per m^2	24.21	18.5	20.37	16.8	24.2	42.1	53.5	52.3	51.52	∑ 3028	19.33	∑ 370
No taxa	14	11	11	9	9	13	19	22	19		15	
Weed cover (%)	15	12	12	10	15	15	20	20	20		7	
Swiss Chard yield (kg of aboveground part)	10.35	12.45	9.75	8.85	6.75	6.55	8.75	10.25	8.35		4.65	

Table 4. Weed density and weed cover in the plots in the regim F150 (150%). n – average No of individuals per m². c – weed cover in the plot

Taxon Family Life form	Plot 6 n c	Plot 7 n c	Plot 8 n c	Plot 16 n c	Plot 17 n c	Plot 18 n c	Plot 25 n c	Plot 26 n c	Plot 27 n c	Total No of individuals in all F150 plots	Control n c	Total No of individuals in control
Anagalis arvensis Primulaceae v-a Mi-Mes T scap			0.10 r	0.48 +		0.29 +	0.86 1	0.48 +		22	2 1	20
Amaranthus retroflexus Amaranthaceae a-aut Mes-Alt T scap					0.10 r	6.57 2a	1.90 1			86		0
Atriplex patula Chenopodiaceae aut Meg-Alt T scap	2.86 1	2.70 1	0.48 1	0.19 +	0.57 1	3.14 1	0.57 1	2.19 1	14.48 2a	**272**	13.33 2a	**133**
Capsella bursa-pastoris Brassicaceae n-aut Mi-Meg T semiros	0.10 r		0.57 1	0.29 +	0.38 +	0.38 +	0.48 +	1.33 1	0.38 +	39	0.38 +	4
Chaenorrhinum minus Scrophulariaceae v-aut Mi-Mes T scap				0.10 r		0.10 r			0.10 r	3		0
Chenopodium album Chenopodiaceae a-aut Meg-Alt T scap				0.10 r	0.10 r					2		0
Convolvulvus arvensis Convolvulaceae a SG herb rhiz	1.38 1	1.19 1	1.48 1		0.67 1	0.57 1	0.29 +	0.67 1		62	1.33 1	13
Cynodon dactylon Poaceae a-aut Mes-Mac H caesp										0		0
Euphorbia helioscopia Euphorbiaceae v Mi-Mes T scap			0.10 r			0.38 +		0.10 r	0.10 r	7	0.38 +	4
Euphorbia maculata Euphorbiaceae v-aut Mi-Mes T rept	0.10 r	0.10 r		5.67 2m	1.81 1	0.86 1		0.10 r	0.10 r	87	0.48 +	5
Geranium columbinum Geraniaceae v-a Mes T semiros								0.29 +	0.76 1	11		0

Taxon Family Life form	Plot 6 n c	Plot 7 n c	Plot 8 n c	Plot 16 n c	Plot 17 n c	Plot 18 n c	Plot 25 n c	Plot 26 n c	Plot 27 n c	Total No of individuals in all F150 plots	Control n c	Total No of individuals in control
Geranium dissectum Geraniaceae v-a Mes T semiros	3.81 1		3.52 1		0.19 +		0.38 +		0.76 1	114	3.14 1	31
Kickxia spuria Scrophulariaceae a Mes-Meg T scap	1.95 1	3.52 1		1.10 1	2.62 1	5.24 2m	2.48 1	2.86 1		**191**	2.10 1	21
Lotus corniculatus Fabaceae v-aut Mes H scap		1.90 1								0	0.10 r	1
Mentha longifolia Lamiaceae a Mes-Meg H scap	2.43 1						0.38 +			28		0
Medicago lupulina Fabaceae v Mes-Mac T scap	0.67 1		0.48 +	0.57 1	0.29 +	0.38 +				28	0.19 +	2
Plantago lanceolata Plantaginaceae v-a Mi-Meg H ros		0.48 +				0.10 r				1		0
Poa anua Poaceae n-a Mi-Mes T caesp	0.10 r									1		0
Polygonum aviculare Polygonaceae a-aut Mi-Mes T rept	0.52 1		1.30 1	2.29 1	0.62 1	1.33 1	2.57 2a	2.19 1		140	0.76 1	8
Portulaca oleraceae Portulacaceae a Mes T scap		0.48 +		0.48 +	0.29 +			0.38 +	2.67 1	13		0
Trifolium repens Fabaceae v-aut Mi-Mes H rept					0.29 +			0.38 +	0.19 +	7		0
Senecio vulgaris Asteraceae n-v Mes-Meg T scap	0.10 r		0.19 +		0.29 +	0.10 r	0.19 +	0.48 +	0.95 1	23	0.19 +	2

Taxon Family Life form	Plot 6 n c	Plot 7 n c	Plot 8 n c	Plot 16 n c	Plot 17 n c	Plot 18 n c	Plot 25 n c	Plot 26 n c	Plot 27 n c	Total No of individuals in all F150 plots	Control n c	Total No of individuals in control
Setaria viridis Poaceae a-aut Mes-Mac T scap									0.48 +	5		0
Solanum nigrum Solanaceae a-aut Mes-Mac T scap	0.30 +			0.29 +	1.90 1	1.43 1	38.10 2a	3.43 1	20.19 2a	**656**		0
Sonchus asper Asteraceae v-a Mes-Alt T scap					0.10 r	0.19 +	0.10 r	0.76 1	0.48 +	16		0
Sorghum halepense Poaceae a-aut Mes-Meg G rhiz	13.50 2a	14.50 2a	17.30 2a	17.90 2a	17.24 2a	17.33 2a	7.81 2m	23.05 2a	20.48 2a	**1491**	10.48 2a	**105**
Stellaria media Caryophyllaceae n-aut Mi-Meg T rept	0.19 +	0.10 r	0.10 r							4	0.19 +	2
Veronica arvensis Scrophulariaceae v-a Mi-Mes T scap		1.60 1	1.30 1	0.10 r	5.48 2m	5.62 2m	4.29 1	3.81 1	0.10 r	223		0
Veronica persica Scrophulariaceae n-aut Mi-Mes T scap	2.10 1	1.10 1	1.90 1	2.10 1	1.05 1	1.05 1	2.38 1	3.05 1	4.38 1	191	1.90 1	19
Total No of individuals per m^2	30	27.7	28.8	31.6	33.9	45	62.7	45.5	65.8	∑ 3723	19.33	∑ 370
No taxa	15	11	13	14	18	18	15	17	15		15	
Weed cover (%)	12	15	15	12	10	15	30	20	40		7	
Swiss Chard yield (kg of aboveground part)	6.95	10.85	9.45	16.05	15.05	12.55	9.85	10.25	9.75		4.65	

Analysis of the crop yield and weed cover in the plots wih regime of fertilization F50 shown similar results. Among the plots with fertilization regime F50 the highest yield was recorded in the plot 15 (12.05 kg) (table 2), where the content of humus was highest (table 1) and weed cover exceeded the average value for this regime of fertilization (17%). On the other hand, in the plot 11 (table 2) the yield was minimal (4.75 kg) and weed cover was the lowest (10%). Considering regime F100 (table 3), the highest yield was recorded in the plot 3 (12.45 kg), but, unlike other regimes (F150 and F50), the plot with highest yield didn't have highest content of humus in the soil before the treatment (table 1). The highest humus content is reported for plot 10 (table 1), but the plot had lower crop yield (8.85 kg) and the lowest weed cover (10%) (table 3). This can be explained, as mentioned above, especially by the content of available potassium as well as soil EC which frequently positively correlated to crop yield. Thus, Knežević *et al*. (2014) found that the yield of Swiss chard was in positive significant relationship with available soil K. Namely, in soil of the plot 3 the highest content of available K and EC value were measured, but soil of the plot 10 had relatively lower content of K and EC value.

It is known that long term use of fertilizers changes the agroecological conditions for existance of the whole agrophytocoenosis and its separate components (Atanasova *et al*., 2009), thus our future investigations will be focused on this topic.

CONCLUSIONS

Weed sinousia of Swiss chard in Montenegro consists of 29 species, out of 26 genera and 17 families and in taxonomic spectrum it differes from the sinousia of other crops investigated in Montenegro. Dominant families in the specrum are Poaceae, Scrophulariaceae (4 species each) and Fabaceae (3 species). The most abundant species in sinousia are S. halepense, S. nigrum, K. spuria and A. patula. Up to this research A. patula and K. spuria was not consideres as troublesome agricultural weed in Montenegro. In the total weediness dominant weed species participate with 67.3%. Ranking of dominant species differs between the treatments, but athough changes in the relative abundances of weeds caused differences in the community composition between plots without (C) and with different fertilizing regime (F50, F100 and F150), species composition was quite similar.

The analysis of the total biological spectrum indicates the dominance of therophytes with 75.8%, while hemicryptophytes contribute the spectrum with 17.2%. Such spectrum of life forms fits into the general biological spectrum of the flora of Bjelopavlići plain.

Considering the effect of fertilizers on the crop growth and weediness general conclusion is that fertilizers benefit both the crop and weeds. The average crop yield was highest in the regime F150, as well as total weediness F150. Nevertheless our research has shown that increased doses of fertilizers had better effect on the crop yield than on weediness. However, analysing the species

abundance, cover per single plot and crop yield in each regime of fertilization, some deviations are noticed, but in order to find proped explainations additional research are needed.

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IMPLEMENTATION OF PANEL DATA IN MARKETING RESEARCHES IN FORESTRY

SUMMARY

Series of panel data are a combination of comparative data and time series. One of the most significant advantages of using an analysis based on a series of panel data refers to obtaining maximum information from a limited number of observations per unit of observation in a given period, which is very frequent in forestry. The aim of the research is to analyse the individual market factors, examine their interdependence, and establish strength of linkage. In this case study the goal is to examine the influence of independent variables (placement on the domestic market, export and sales prices of non-timber forest products - NTFPs) on the dependent variable - the purchase of raw materials.

Variables that are taken into consideration were: buying, selling on the domestic market, export and selling prices of certain types of NTFPs. In the analysis were used R Program and the special package, which is dealing with panel data. Based on Pooling OLS estimators determined that the variable export and sales on the domestic market are important to purchase of NTFPs. By analyzing the variations between objects of observation (between) it is established, as the previous estimator that the variables export and prices are important to the purchase of NTFPs. According to First difference estimator export and sales price are the factors that most influence the purchase of raw NTFPs. According to all tree estimators: Pooled OLS; between and First differences, it was found that the placement on the domestic market variable which, compared with other, largely determines the purchase of raw NTFPs (in terms of the analyzed data). This is evident based on the greatest values that range in interval 0.80 to 1.04 for the domestic market.

Keywords: panel data, market, forestry, estimator, strategy.

INTRODUCTION

Series of panel data are a combination of comparative data and time series (Jovičić, 2010). As such, they are very suitable for different types of analysis, especially applicable to the analysis of market. These series are mainly oriented to the analysis of heterogeneity among observation units, as well as the analysis of changes in the structure over time (Greene, 2005). Heterogeneity means that the impact of independent variables on the dependent varies from unit to the

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object of observation and / or over time (Dragutinović – Mitrović, 2001, Baltagi, 2008). These models allow examining nature of the variability of parameters that are important for the analysis. In addition, as one of the most significant advantages of using an analysis based on a series of panel data refers to obtaining maximum information from a limited number of observations per unit of observation in a given period (Dragutinović – Mitrović, 2001). Assumptions about the homogeneity of observation units, which are common in classical statistical analysis, often in the economy are not met. In such situations, it is necessary to use models that combine time series and comparative data, because their use does not require the usual assumptions about the constancy of parameters.

Testing of variations through panel data series can be directed in many directions, thus are distinguished:

- Overall (variations in time and within the data to be analysed, namely in the specific case of the types of products),
- Between (variation between species analysed products) (Kennedy, 2008),
- Within variation (among species of analysed products over time) (Račić 2013).

In panel researches are most often used the following regression models (Katchova, 2011):

•model with constant parameters (pooled OLS model) - the assumption is that free members and coefficients of slope are constant in space and in time. A errors in model include individual and the time differences (Gujarati, 2004).

• model with fixed effects (fixed-effects model) - coefficients of slope are constant, but the free members vary according to observation units and / or in time (Gujarati, 2004).

• model with stochastic effects (random-effects model) - free members and the coefficients of slope vary according to observation units and through time.

When testing the variability of regression parameters determines the existence of individual or timed effects, raises the question of the selection of model of panel data. In the case where the data relate to a few geographic regions, industries etc. suggest the fixed effects model. When the units are randomly selected from large basic set, then is used model of stochastic effects. When concluding is conditioned only by the individual characteristics of the sample, than is used model of fixed effects. Before selecting of a linear model in econometric panel analysis, it is necessary to test the heterogeneity of regression parameters, or the significance of individual and time effects. Specifically, the first model is estimated with the restriction (pooling OLS), and then model without limitation (fixed effect, random effect).

The aim of the research is to analyse the individual market factors, examine their interdependence, and establish strength of linkage. In this case study the goal is to examine the influence of independent variables (placement on the domestic market, export and sales prices of non-timber forest products - NTFPs) on the dependent variable - the purchase of raw material.

MATERIAL AND METHODS

The research covered an area of Central Serbia and all related data the purchase, placement on the domestic market, export and selling prices of certain types of NTFPs. Data were collected in the 30 companies that are aimed at marketing of NTFPs, and operate in the specified area. The majority of companies (63) are situated in statistical region of Central Serbia (Keča, 2016). All of the companies are private owned, having more than 10 permanent workers, and conduct placement on domestic and foreign market. The period 2006-2013 was considered.

In this case analysis based on the panel series, compared to other methods has been applied, because of the advantages that are reflected in the fact that it does not require long time series, as is the case for e.g. regression analysis, that the results would be authentic and precise. We did not have the time series longer than 5 years. Therefore, the analysis based panel series is estimated to be adequate in the applied study. On the other hand, this methodological approach is very prominent when, generally, comes to market research (Hsiao, 2003, Wooldridge, 2010).

Variables that are taken into consideration (buying, selling on the domestic market, export and selling prices of certain types of NTFPs) are significant, if we analyze the market of this product category. The starting assumption of the research is that, compared to other, placement sales on the domestic market has the greatest impact on the amount of the purchase of raw materials. This is reflected in the assumption that the increase in placements on the domestic market leads to increased demand for raw materials companies, and therefore a quantitative increase in the purchase of raw materials (in order to ensure higher production and sales at higher market prices).

In the analysis were used R Program (Dessau and Pipper, 2008) and the special package, which is dealing with panel data (Chen, 2013).

Advantages of panel analysis (Klevmarken, 1989, Ripley, 2001) in relation to the others are reflected in the following:

- Panel data contains more data than the corresponding spatial data and time series,
- Dependent variables are changing through the units of observation and time, and obtained estimations derived through panel analysis are more precise,
- Panel data reduced the bias of parameters which occurs due to lack of data,
- Panel data allow defining and testing the complicated econometric models,
- Panel data reduced the problem of multicollinearity (Graham, 2003),
- Allow measuring diversity within the observation unit.

Series of panel data are a combination of comparative data and time series. Each panel has two dimensions structure and time, and where in the econometric analysis of panel data most used are linear models (model of panel data).

For the analysis of panel were identified through variables such as:

• amount of the purchase of raw material per year (t)

• amounts placed on the domestic market (t)

• quantities exported (t)

• the prices at which the products realized on the market (RSD).

Products that have been analyzed for different categories of NTFPs covered the period 2006-2013. Thus, the surveys involve forest fruit (blueberries, juniper, wild rose, raspberry, blackberry and wild strawberries), herbs and mushrooms (boletus and chanterelles). Since the analysis provided data types of products in all units of time, it is a "balanced" or complete panel series. In case the missing data for a variable, it would be the so-called "unbalanced" panel series (Croissant and Millo, 2008).

RESULTS AND DISCUSSION

The first part shows the results of descriptive statistics relating to the dependent variable (the purchase of raw materials) and analyzed independent variables, such as placement on the domestic market, exports and prices at which the products are realized in the market (Table 1). The reason for the separation of purchase as the dependent variable is reflected in the fact that sought to determine the strength of the influence of factors such as market prices, amount of investments into the domestic market and exports (independent variable) on the amount of purchased raw materials in analysed time interval. Raw material is taken as a starting unit of observation, because it preceded, as sales in the domestic market and the export of final products. In subsequent stages, it has been substituted the variables, so that every independent variable would be analyzed as a dependent, with the aim of establishing their interdependence in all relations.

Table 1. Descriptive statistics for analyzed variables

Purchase	Placement	Export	Prices
Min. : 5.0	Min.: 2.0	Min.: 1.0	Min.: 40.0
1st Qu.: 92.5	1st Qu.: 5.0	1st Qu.: 42.5	1st Qu.: 200.0
Median: 200.0	Median : 32.0	Median :141.0	Median: 400.0
Mean: 311.9	Mean: 93.7	Mean:220.5	Mean: 629.9
3rd Qu.: 283.0	3rd Qu.: 99.5	3rd Qu.: 234.5	3rd Qu.: 542.5
Max.: 1200.0	Max.: 600.0	Max.: 858.0	Max.: 2600.0

Results of analysis according to individual estimators are given as follows:

Table 2. Results according to Pooling OLS estimators

Coefficients :	Estimate	Std. Error	t-value	Pr(>|t|)	Sign.
(Intercept)	3.5521434	6.4358014	0.5519	0.5837	
Xplacement	1.0408989	0.0455480	22.8528	<2e-16	***
Xexport	0.9589093	0.0353677	27.1125	<2e-16	***
Xprices	0.0010241	0.0108347	-0.0945	0.9251	
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					
Total Sum of Squares:			6062500		
Residual Sum of Squares:			37042		
R-Squared			0.99389		
Adj. R-Squared :			0.91438		
F-statistic: 2494.23			2494.23		
p-value:			< 2.22e-16		
Residuals :					
Min.	1st Qu.	Median	3rd Qu.	Max.	
-125.00	-3.29	-1.47	4.69	98.40	

Based on Pooling OLS estimators determined that the variable export and sales on the domestic market are important to purchase of NTFPs (***), while the variable price of this estimator, there is no impact on the amount of purchase NTFPs. There was a strong correlation (Adj., R-sqared = 0.914). The coefficient of correlation was statistically significant (F = 2494.2), as well as parameters (p value <2.22e-16) (Table 2).

Table 3. Results according "between" estimators

Coefficients :	Estimate	Std. Error	t-value	Pr(>|t|)	Sign.
(Intercept)	2.02826298	1.80546636	1.1234	0.3042	
Xplacement	1.03013182	0.01545503	66.6535	7.671e-10	***
Xexport	0.96856851	0.01227245	78.9222	2.786e-10	***
Xprices	-0.00038405	0.00357620	-0.1074	0.9180	
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					
Total Sum of Squares:			1190500		
Residual Sum of Squares:			70.337		
R-Squared			0.99994		
Adj. R-Squared :			0.59996		
F-statistic:			33850		
p-value:			4.511e-13		
Residuals :					
Min.	1st Qu.	Median	3rd Qu.	Max.	
-3.030	-1.920	-0.919	1.500	4.540	

By analyzing the variations between objects of observation (between) it is established, as the previous estimator that the variables export and prices are important to the purchase of NTFPs (***), and a variable price, according to the estimator, there is no impact on the amount bathe NTFPs. There was a strong correlation (Adj., R-sqared = 0.999). The coefficient of correlation was

statistically significant (F = 3.3850), as well as parameters (p-value 4.511e-13) (Table 3).

Table 4. Results according to First differences estimators

Coefficients :	Estimate	Std. Error	t-value	Pr(>|t|)	Sign.
(Intercept)	1.874016	5.027649	0.3727	0.71153	
Xplacement	0.804902	0.166348	4.8387	2.457e-05	***
Xexport	0.773047	0.079922	9.6726	1.500e-11	***
Xprices	-0.065969	0.033299	-1.9811	0.05526	
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					
Total Sum of Squares:			129170		
Residual Sum of Squares:			35277		
R-Squared			0.72688		
Adj. R-Squared :			0.65419		
F-statistic: 2494.23			31.9371		
p-value:			3.0077e-10		
Residuals:					
Min.	1st Qu.	Median	3rd Qu.	Max.	
-141.000	-5.810	0.105	7.980	78.800	

According to First difference estimator export and sales price are the factors that most influence the purchase of raw NTFPs (***). Also, the price and in the case of previous estimators indicate that they do not affect the amount of the purchase NTFPs (Table 4).

The relevance based on the F test suggests that in choosing the estimator should take into account the model "fixed effect

Table 5. Results according to F test for individual effects

Products	Year	Byuing	Placement	Export	Prices
Length:50	Min. :2006	Min. : 5.0	Min. : 2.0	Min. : 1.0	Min. : 40.0
Class :character	1st Qu.:2007	1st Qu.: 92.5	1st Qu.: 5.0	1st Qu.: 42.5	1st Qu.: 200.0
Mode :character	Median :2011	Median : 200.0	Median : 32.0	Median :141.0	Median : 400.0
	Mean :2010	Mean : 311.9	Mean : 93.7	Mean :220.5	Mean : 629.9
	3rd Qu.:2012	3rd Qu.: 283.0	3rd Qu.: 99.5	3rd Qu.:234.5	3rd Qu.: 542.5
	Max. :2013	Max. :1200.0	Max. :600.0	Max. :858.0	Max. :2600.0

Byuing of raw NTFPs	Pooled OLS regression	Between	Within or fixed effects	First differences
Placement on domestic market	1.0408989	1.03013182		0.804902
Export	0.9589093	0.96856851		0.773047
Prices	0.0010241	-0.00038405		-0.065969

According estimators: pooled ols; between; first differences, it was found that the placement on the domestic market variable which, compared with other, largely determines the purchase of raw ntfps (in terms of the analyzed data). This is evident based on the greatest values that range in interval 0.80 to 1.04 for the domestic market (table 5) ($f = 126.16$, $df1 = 11$, $df2 = 37$, p-value < 2.2e-16 alternative hypothesis: significant effects). The second by relevance is a variable of export of ntfps, measured by all three estimators. Prices were evaluated as the least important factor as measured by the three estimators.

CONCLUSIONS

Based on of the research, which referred to NTFPs market, was determined the effectiveness of applying analysis based on panel series, particularly bearing in mind that it was not available long time series. In addition, in this way, we obtained two-dimensional, that is, structural and time comparability among the data within each panel observations. The subject of the analysis were quantitative market factors related to the quantity of raw materials purchase (the dependent variable), placement to the domestic market, exports and resilient price of final products (independent variables). Starting from the purchase of raw materials as the dependent variable, and on the basis of the level of influence of independent variables obtained according to estimators Pooling OLS, Between summary i First differences it was found that placement on the domestic market has the greatest impact on the amount of the purchase of raw NTFPs. Thus is achieved affirmativeness of starting hypothesis that precisely placement on the domestic market largely determines the amount of the purchase of raw materials. The establishment of this hypothetical starting point was that it sought to examine whether increase in placements in the local market causes and increased purchase of raw materials, while the prices and quantities of exports less important factors, if we consider the substantive effect of the independent variable on the dependent one.

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RESPONSE OF SESAME (*Sesamum indicum* L.) TO MULCHING AND INORGANIC FERTILIZER APPLICATION IN A SOUTHERN GUINEA SAVANNAH ZONE OF NIGERIA

SUMMARY

Sesame is best cultivated towards the end of the wet season in the southern guinea savannah zone of Nigeria because when planted early in the season, high moisture regime will predispose the crop to increased vegetative growth at the expense of seed formation, discoloured grains and create an unfavourable environment for harvesting, drying and processing of the seeds. However, growing sesame towards the end of the rainy season may subject it to moisture stress especially with the uncertainty introduced by climate change. This therefore necessitated an investigation into the potentials of incorporating mulch practice to conserve moisture and fertilizer nutrients in sesame production. A field study was conducted in the Teaching and Research Farm of the University of Ilorin, Ilorin- Nigeria during the 2013 and 2014 cropping seasons to evaluate the effects of mulching and NPK fertilizer on the performance of sesame (Ex-Sudan variety). Treatments consisted of three mulch types (no mulch (NM), grass mulch (GM) and wood shavings (WS) mulch applied at the rate of 0.5 t ha^{-1}) and five levels of NPK fertilizer 20:10:10. The experiment was laid out in a 3 x 5 factorial arrangement replicated thrice. Data were collected on soil parameters (some physical and chemical properties), plant growth parameters (plant height, number of leaves and number of branches) and yield parameters (number of capsules per plant, weight of seeds per plant and weight of seeds per hectare). The result of the study indicated that using mulch alone increased the water holding capacity and the organic matter content of the soil. However, using the grass mulch and fertilizer at the rate of 300 $kgha^{-1}$ resulted in an increase in the growth and yield parameters which were significantly different ($P<0.05$) from the other treatments in both years. The average yield of the two years of study

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indicated an overall seventy-one percent increase in seed weight per hectare in the grass mulched plots over the control.

Keywords: sesame, inorganic fertilizer, mulching, water holding capacity, yield

INTRODUCTION

Sesame or beniseed (*Sesamum indicum* L.) is from the Pedaliaceae family is an important oil seed crop which is cultivated in tropical and sub-tropical countries of Asia and Africa for its oil seed. According to Tunde-Akintunde and Akintunde (2007), its oil content is comparable to that of olive tree seed (*Olea europaea*). Young leaves are used as soup ingredients in sub-Saharan Africa. In India and China, the leaves play a role in the treatment of cancer while the seed cake left after oil extraction is an excellent livestock feed, which can also be used as manure, carrier for medicine and perfumes (Vossen and Mkamilo, 2007). It is cultivated in the savannah zones of Nigeria (Alegbejo, 2003) in smallholdings without the use of external inputs, which has adversely affected its yield potentials. The average yield in farmers` field is about 300 $kgha^{-1}$ which is grossly lower than yields elsewhere (Abubakar *et al.*, 1998). The reason for this low yield can be attributed to poor cultural practices (Haruna and Usman 2005).

Usually, sesame is cultivated late in the season in the southern guinea savannah zone of Nigeria during which time there is torrential rainfall, which results in leaching of nutrients accompanied by high temperature that often leads to increased evaporation rate with subsequent low soil moisture content, which invariably affects dissolution of fertilizer. One way of overcoming this problem is by the use of mulch, which is a soil conservation practice that has been used for a long time to increase the organic matter and moisture contents of the soil, reduce soil temperature and prevent leaching of nutrients (Wien *et al*., 1993; Ahamefule and Peter, 2014). Khurshid (2006) stated that mulching has been reported to increase soil moisture content and stability of the topsoil while Anikwe *et al.*(2007) also reported that mulching provides a better soil environment, moderate soil temperature, increase soil porosity and infiltration and Tian (1994) asserted, that mulch materials perform additional function of increasing soil organic matter content, cation exchange capacity (CEC), biological activity, improves soil structure, increases plant nutrients after decomposition and subsequently enhancing plant growth and yield.

The use of mulch will conserve the moisture content in the soil taking into cognizance the time of planting of the crop thereby allowing the full utilization of available moisture and fertilizer nutrients at the root zone of the crop. Mbagwu (1990) reported higher nitrogen use efficiency for maize in mulched soils compared to non-mulched soils while Ahamefule and Peter (2014) similarly reported higher phosphorus use efficiency for cowpea plants. Apart from increasing soil fertility, mulch also serves as soil amendment by adding organic matter to the soil and suppress weed growth (Aguyoh *et al.*,1999). Grass mulch has been found to increase the nutrient content of soil following decomposition

and mineralization; hence can increase the vegetative growth of plants, which ultimately results in high yield (Singh *et al*., 2007; Ahamefule and Peter, 2014) attributable to the reduction in soil temperature and improved moisture holding capacity of the soil (Lal, 1974).

Mineral fertilizer on the other hand, has been used to improve the yield of crops for many years especially in the southern guinea savannah zone of Nigeria, where it is a sine – qua-non in fertility management because of the inherently low organic matter content of the soils in the region. The fertility status has been further depleted as a result of constant bush fire and the reduction in the fallow periods due to increased population and infrastructural development. Benett and Wood (1995) had earlier reported that fertilizer rate depends on soil fertility, soil type and the previous crop. Rao *et al*. (1994) reported increase in sesame yield from the application of nitrogen fertilizer and Schilling and Cattan (1991) reported similar increase in sesame yields from the application of fertilizer containing nitrogen, phosphorus and sulphur.

Complementary use of NPK fertilizer and mulch will prove a sound fertility management practice in soils of the southern guinea savannah zone of Nigeria, because the nutrients N, P and K, are among the most limiting in the zone. This practice will enhance increased crop productivity as mulch will reduce the rate of leaching of fertilizer nutrients and significantly increase the available phosphorus and potassium contents in the soil (Cadavid *et al*., 1998). Liasu and Achakzai (2007) had reported that combination of mulch and inorganic fertilizer improved the growth and yield attributes of tomatoes.

Although, sesame can be cultivated in semi-arid regions because of its tolerance to drought, nevertheless in a world confronted with global warming and hence unpredictable rainfall, using mulch and fertilizer may bridge the yield differential between the guinea savannah zone of Nigeria and other sesame growing areas of the world. The objective of this study was therefore to evaluate the effects of mulching and NPK fertilizer on the growth and yield of sesame.

MATERIAL AND METHODS

The Experimental Site

The experiment was carried out during the wet seasons of 2013 and 2014 at the Teaching and Research Farm of the University of Ilorin, Ilorin (9^o 29' N, 4^o 35' E and 307 m above sea level) in a southern guinea savannah zone of Nigeria. The area is characterized by a mean annual rainfall of 1186 mm, mean annual temperature of 29 ºC while the average annual relative humidity is about 85%. The site was a two-year fallow land, which had previously been cropped, to maize and cassava prior to the establishment of the experiment.

Field layout

The land was ploughed and harrowed and raised seedbeds were made before marking out into plots. The size of each plot was 2 m × 5 m with a 0.50 m avenue between the plots. Sowing was done by drilling the sesame seeds in shallow furrows and then covered lightly with soil. This was done in 2013 and

2014.The plants were thinned down to two plants per stand at a spacing of 90 × 30cm between and within rows. Fertilizer (NPK 20:10:10) was applied three weeks after planting and weed control was done manually.

Soil samples collected from 0 - 30 cm depth (fifteen locations) prior to planting within the experimental plots were bulked and a sub-sample was taken for physical and chemical characterization.

Experimental Design

The experiment was laid out in a 3 × 5 factorial arrangement in randomized complete block design (RCBD). The factors were mulching (no mulching (NM), mulching with grass (GM) and mulching with wood shavings (WS)) and NPK fertilizer. These gave 15 treatment combinations which were replicated thrice.

Grass mulch from a nearby lawn containing pure stand of grasses and wood shavings from a nearby sawmill were collected and applied (0.5 t/ha) as mulch yearly.

Data Collection

Data were collected on vegetative (plant height, number of leaves, number of branches) and yield traits number of capsules, seed weight per plant).

Vegetative Traits

The plant height was assessed by measuring from the soil level to the crown of the plant in five randomly selected tagged plants using a measuring tape at 4, 6 and 8 weeks after planting. The number of leaves was assessed by visual count of the green leaves from the five-tagged plants at 4, 6 and 8 weeks after planting and the number of branches was assessed by visual count of the number of primary and secondary branches at 4, 6 and 8 weeks after planting.

Yield and Yield components

The number of capsules per pant was assessed by visual count of the number of capsules, the seed weight per plant was assessed by taking the weight of the five-tagged plants after drying and threshing using a sensitive balance. The seeds of the five-tagged plants were weighed and recorded and extrapolated to get the yield per hectare (kg ha^{-1}). The combined yields for the two years were summed up and the mean recorded for the calculation of the two years yield.

Laboratory analysis

Soil pH was measured (soil: water ratio, 1:2) using a glass electrode; total N content was determined by micro - Kjeldahl method (Bremner, 1965); available phosphorus was determined following Bray No 1 (1N NH4F + 0.5N) HCl extractant by vanadomolybdophosphoric acid method (Kuo, 1996), organic carbon was determined by using the modified Walkley–Black method (Nelson

and Sommers, 1996) and extraction of exchangeable bases was done by using 1N ammonium acetate, exchangeable K and Na were determined using flame photometry while calcium and magnesium were analyzed by atomic absorption spectrophotometry. Total porosity was determined according to Ahamefule *et al*. (2014) and soil moisture contents at a depth of 15 cm was calculated as weight of wet soil (g) - weight of dried soil/ weight of dried soil multiplied by 100 using standard procedure.

Data analysis

Data collected were subjected to analysis of variance (ANOVA) using Genstat software package 12th Edition and where significant treatment effects were detected, the least significant difference (LSD) was used to compare means at 5% level of probability.

RESULTS AND DISCUSSION

Soil Physico-chemical Properties

Pre and post treatment soil investigations indicated that the soil had a moderately acidic reaction, the organic matter content was very low to moderately low, the nitrogen content was very low to moderately low, the phosphorus, potassium, calcium, and magnesium contents were low to moderately low (Table 1).

Table 1. Soil properties of the experimental site before and after cropping in 2013 and 2014.

Year	Soil pH	Org C	Org. Matter	Total N	P	K	Ca	Mg
		%			Ppm	cmol/kg		
2013 a	5.5	0.33	0.57	0.05	0.37	0.97	5	0.14
2013 b	5.7	1.41	2.44	0.14	0.81	0.59	10	2.1
2014	5.4	0.59	1.02	0.06	3.01	0.18	6	0.69

*a. Before initial cropping, b. End of first year cropping

Meteorological Data

The data on rainfall, temperature and relative humidity are presented (Table 2). The data on rainfall indicated that the month of September in the two years of study experienced the highest rainfall. This rainfall comes in torrent but sometimes for a short duration; the temperatures and relative humidity were high. The meteorological data for the two years (Table 2) clearly indicated that the

highest monthly rainfall and maximum temperature was experienced during the cultivating seasons for the two years (August – October).

Table 2. Meteorological Data of the experimental site in 2013 and 2014

Months	Rainfall (mm)		Temperature ^{o}C (2013)		Temperature ^{o}C (2014)		Relative Humidity (%)	
	2013	2014	Min.	Max	Min.	Max	2013	2014
January	0.5	6.3	19.4	34.2	20.6	34.5	81	81
February	39.2	34.2	22.7	34.8	20.7	35.3	81	82
March	39.	71.0	24.2	35.6	23.8	34.8	81	81
April	181.8	321.4	23.6	32.3	22.5	32.7	81	81
May	81.8	163.8	22.7	31.5	22.7	39.6	81	81
June	132.9	154.4	20.9	34.2	21.9	30.4	80	81
July	107.3	82.1	21.8	28.0	21.9	29.6	80	81
August	17.7	94.9	21.4	27.8	21.3	27.5	80	80
September	202.5	391.6	21.5	29.2	21.2	28.5	80	80
October	154.3	259.4	21.7	31.0	21.7	31.6	80	81
November	0.0	0.0	23.4	31.5	22.7	32.5	83	81
December	11.4	0.0	19.4	33.5	19.4	33.2	82	82
Mean	969.0	1579.4	21.9	32.0	21.7	32.5	81	81

Soil Porosity and Water holding capacity

The data on porosity is presented in (Figure 1). The data showed that the mulched plots had significantly higher total porosity compared to control whereas plots under grass mulch had a higher total porosity relative to those under wood shavings. It was however observed, contrary to expectations that total porosity fell by at least 3% in the second year regardless of mulch applied. Similar trend was observed for the water holding capacity (Figure 2) of treated soil but at least 0.61% reduction in the second year of the experiment. Varying the amount of mineral fertilizer applied to the soil did not affect the total porosity and water holding capacity of the soil. There were no significant interaction effects between the treatments on total porosity and water holding capacity.

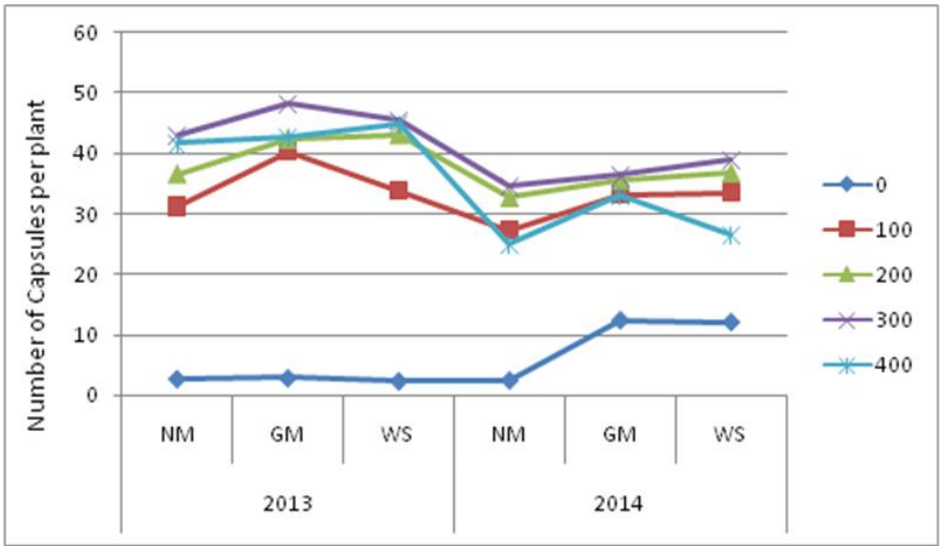

Fig 1. Effects of mulching and NPK fertilizer on the number of capsules per plant of sesame in 2013 and 2014

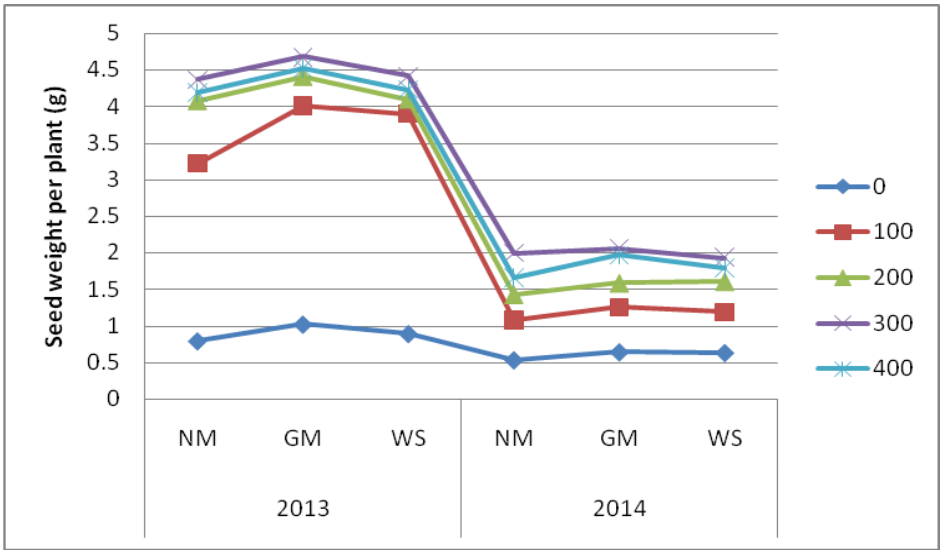

Fig 2. Effects of mulching and NPK fertilizer on the seed yield per plant (g) of sesame in 2013 and 2014

Vegetative Traits of Sesame in 2013 and 2014

Growth parameters of Sesame as influenced by mulching and Inorganic fertilizer rate in 2014.

The data on plant height of sesame at 4, 6 and 8 WAP are presented in Table 4. The data indicated that there was a trend in the performance of sesame across the three sampling periods. Increasing fertilizer rates resulted in an

increase in the plant height of sesame up to the highest rate of 400 kgha-1 but no significant difference was observed except at 8WAP when significant difference ($P<0.05$) was observed between the treated plots and the control. Using grass mulch produced the tallest plants, although there was no significant difference between the three mulch types. In addition, there was no significant interaction between mulch types and fertilizer rates across the three sampling periods. In 2014, a similar trend of what happened in 2013 occurred but significant ($P<0.05$) differences across the three sampling periods using inorganic fertilizer was observed.

Table 4: Effects of mulching and NPK fertilizer on plant height (cm) of sesame at 4, 6 and 8 WAP in 2013and 2014

		4 weeks after planting				6 weeks after planting				8 weeks after planting			
	Fertilizer Rate kg/ha	No mulching	Grass Mulch	Wood shavings	Mean	No mulching	Grass mulch	Wood shavings	Mean	No mulching	Grass mulching	Wood shavings	Mean
	0	10.83	11.90	10.43	11.06	24.30	21.47	20.93	22.23	40.8	47.50	40.6	43.0
	100	15.37	15.67	16.53	15.86	49.87	45.33	43.87	46.36	83.4	95.5	82.3	87.1
2013	200	18.90	20.40	21.07	20.12	63.90	71.87	63.50	66.42	96.5	113.6	109.5	106.5
	300	23.17	25.80	25.87	24.94	72.00	87.70	71.20	76.97	113.1	125.3	126.4	121.6
	400	26.50	31.87	29.63	29.33	79.15	99.17	85.90	88.07	128.6	134.9	135.3	132.9
	Mean	18.90	21.13	20.71		57.84	65.11	57.08		92.5	103.3	98.8	
	LSD-0.05	Mulching (M) ns				ns				ns			
		Fertilizer (F) ns				ns				13.32			
		Interaction (M x F) ns				ns				ns			
	0	22.37	21.20	17.50	20.36	35.3	45.0	53.3	44.6	66.27	71.50	69.63	69.13
	100	19.93	27.00	22.33	23.42	75.0	75.7	79.0	76.6	103.40	105.93	102.07	103.13
2014	200	23.90	31.87	27.00	27.59	88.7	84.0	96.3	89.7	134.07	139.10	131.27	134.31
	300	36.83	35.33	29.70	23.96	100.0	104.3	107.3	103.9	142.30	144.93	149.90	145.71
	400	30.07	37.47	32.47	33.37	128.7	137.0	123.7	129.8	145.13	145.53	151.20	147.79
	Mean	26.62	30.57	26.00		85.5	89.2	91.9		117.83	121.40	120.81	
	LSD-0.05	Mulch (M) ns				ns				ns			
		Fertilizer (F) 5.713				11.28				5.016			
		Interaction (M x F) ns				ns				ns			

Number of leaves at 4, 6 and 8 are in Table 5. The data at 4 and 6WAP showed that, there was a trend as the level of fertilizer increased. The highest number of leaves was produced at the 400 kgha-1 treated plots which was at par with the 300 kgha-1 but significantly different ($P<0.05$) from the other treatments. The highest number of leaves was produced by the grass mulched

plots which was significantly different (P<0.05) from the other mulch types. However, no significant mulch and fertilizer interaction was observed. At 8 WAP, the data showed that increasing fertilizer rates also resulted in an increase in the number of leaves. The highest number of leaves was attained at the 400 kgha-1rate of application, which was significantly different (P<0.05) from the control. Although the grass mulched treated plots produced the highest number of leaves, there was no significant difference between the mulch types. However, no significant mulching and fertilizer rate interaction effect was observed.

Table 5: Effects of mulching and NPK fertilizer on the number of leaves per plant of sesame at 4, 6 and 8 WAP in 2013 and 2014

		4 weeks after planting				6 weeks after planting				8 weeks after planting			
	Fertilizer Rate kg/ha	No mulching	Grass Mulch	Wood shavings	Mean	No mulching	Grass Mulch	Wood shavings	Mean	No mulching	Grass mulching	Wood shavings	Mean
	0	9.07	9.83	9.80	9.57	17.90	21.51	21.40	20.90	36.60	44.77	44.60	41.99
	100	10.00	10.90	10.27	10.39	24.37	26.40	26.23	25.67	65.93	72.90	71.23	70.02
2013	200	10.17	11.30	11.07	10.84	25.53	28.47	28.13	27.38	68.93	80.27	67.13	72.11
	300	10.63	11.40	11.27	11.10	26.83	34.63	32.47	31.31	67.67	77.23	75.17	73.23
	400	10.90	11.87	11.40	11.39	30.47	35.45	34.67	33.53	70.03	81.17	70.47	73.89
	Mean	10.15	11.06	10.76		25.02	29.31	28.58		61.83	71.27	65.72	
	LSD(0.05)	Mulching (M) 0.314				1.183				ns			
		Fertilizer (F) 0.405				1.528				6.036			
		Interaction (M x F) ns				ns				ns			
	0	8.86	9.66	9.81	9.44	29.51	31.92	30.51	30.65	40.57	44.50	43.88	42.98
	100	15.59	17.62	17.44	16.88	28.09	42.24	39.14	36.49	61.27	80.77	77.92	73.32
2014	200	17.84	19.36	18.72	18.64	49.09	49.23	48.42	48.25	76.97	78.73	89.52	81.74
	300	20.54	24.16	23.54	22.75	50.10	57.30	56.65	54.35	85.78	95.15	91.78	90.91
	400	22.26	24.79	24.75	23.93	51.96	58.08	57.73	55.83	94.53	97.65	94.78	95.66
	Mean	17.02	19.12	18.85		41.35	47.76	46.23		71.82	79.36	79.58	
	LSD(0.05)	Mulching (M) 0.347				ns				ns			
		Fertilizer (F) 0.449				5.61				5.93			
		Interaction (M x F) 0.778				ns				ns			

Data on the number of leaves in 2014 indicated that at 4 WAP, significant differences (P<0.05) were observed by using mulch, fertilizer and there was mulching and fertilizer interaction effects. At 6 and 8 WAP, using fertilizer increased the number of leaves up to the highest rate of application and significant differences (P<0.05) was observed between the treated plots and the control but no significant mulching effect. Nevertheless, no significant mulching and fertilizer interaction effect was observed.

The number of branches of sesame at 4.6 and 8 WAP are in Table 6. The data on the number of branches showed that using mulch produced the highest number of branches significantly (P<0.05) across the three sampling periods but no significant fertilizer, mulching and fertilizer interaction was observed. In 2014, the data on the number of branches showed that significant effects (P<0.05) were observed by using mulch and fertilizer but no significant mulching and fertilizer interaction across the three sampling periods.

Table 6: Effects of mulching and NPK fertilizer on the number of branches per plant of sesame at 4, 6 and 8 WAP in 2013 and 2014

		4 weeks after planting				6 weeks after planting				8 weeks after planting			
	Fertilizer Rate kg/ha	No mulching	Grass Mulch	Wood shavings	Mean	No Mulching	Grass mulch	Wood shavings	Mean	No mulching	Grass mulching	Wood shavings	Mean
	0	0.15	0.10	0.04	0.09	0.20	0.15	0.08	0.14	0.26	0.18	0.12	0.19
	100	0.24	1.20	0.48	0.64	0.56	1.56	1.11	1.08	0.97	1.89	1.76	1.54
2013	200	0.41	1.27	0.75	0.81	0.76	1.81	1.23	1.27	1.14	2.12	1.92	1.73
	300	0.48	1.53	0.96	0.99	1.30	1.92	1.29	1.48	1.59	2.01	2.01	1.87
	400	0.64	1.58	1.20	1.14	1.40	1.99	1.53	1.64	1.66	2.00	2.00	1.89
	Mean	0.38	1.18	0.69		0.85	1.49	1.05		1.13	1.56	1.56	
	LSD-0.05	Mulching (M) 0.237				0.362				0.235			
		Fertilizer (F) ns				ns				ns			
		Interaction (M x F) ns				ns				ns			
	0	0.14	0.14	0.80	0.36	0.72	0.95	1.29	0.99	1.40	1.60	1.80	1.60
	100	0.28	0.29	0.87	0.48	0.85	1.09	1.01	0.99	1.64	2.14	2.10	1.96
2014	200	0.37	0.43	0.91	0.57	0.94	1.30	1.25	1.16	1.98	2.40	2.20	2.20
	300	0.58	0.54	0.96	0.69	1.07	1.52	1.35	1.31	2.21	2.82	2.53	2.52
	400	0.64	0.68	0.98	0.77	1.14	1.63	1.54	1.43	2.41	2.96	2.93	2.77
	Mean	0.40	0.42	0.90		0.94	1.30	1.29		1.93	2.38	2.31	
	LSD-0.05	Mulching (M) 0.031				0.142				0.107			
		Fertilizer (F) .040				0.184				0.138			
		Interaction (M x F) 0.069				ns				ns			

Yield parameters of sesame in 2013 and 2014

The data on the number of capsules per plant of sesame in 2013 and 2014 are presented in (Figure 1).The data indicated that the grass-mulched plots produced the highest number of capsules per plant but no significant difference was observed. There was a trend when different rates of fertilizer was used. Increasing the rate of fertilizer resulted in an increase in the number of capsules per plant up to 300 kgha-1 and declined at the 400kgha-1 rate of application which was significantly different ($P<0.05$) from the other treatments. However, no significant mulching and fertilizer interaction was observed across the two years.

The seed weight per plant of sesame for the two years of study as affected by mulching and fertilizer rates presented in (Figure 2) showed that using grass mulch produced the highest seed weight per plant and outpaced other mulch types significantly ($P<0.05$); also there was a trend, as the level of fertilizer application increased up to 300 kgha-1 rate of application and declined at the 400 kgha-1 , there was an increase in the seed weight of sesame which was significantly different ($P<0.05$) from the control and there was mulch x fertilizer interaction effects ($P<0.05$) in the 2013 but no interaction effects in 2014.

Data on the seed weight per hectare as influenced by mulching and NPK fertilizer application in 2013 and 2014 presented in (Figure 3). The data indicated that using grass mulch produced the highest seed weight per hectare which significantly (P<0.05) differed from other mulch types. Using NPK fertilizer at the rate of 300 kgha-1 produced the highest seed weight per hectare which was significantly different (P<0.05) from the control and there was mulch x fertilizer interaction effects (P<0.05) in the 2013. However, no significant mulch x fertilizer interaction effects was observed in 2014.

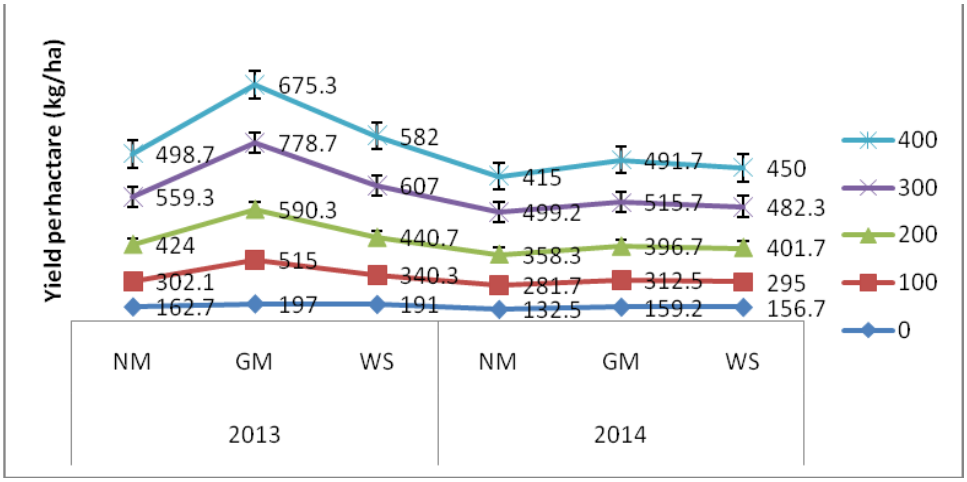

Fig. 3 Effects of mulching and NPK fertilizer on the yield per hectare (kg) of sesame in 2013 and 2014

Combined Yield of sesame (2013 and 2014)

The result of the effect of mulching and fertilizer rates on the combined yield (2013 and 2013) of sesame presented in (Fig 4). The result showed that the grass mulched plots out-yielded the no mulch and wood shaving mulched plots significantly (P<0.05); using NPK fertilizer at 300 kgha-1 produced the highest yield per hectare which was significantly (P<0.05) different from the other treatments but no significant mulch and fertilizer interaction effects was observed.

The moderately low fertility status of the experimental site could be attributable to increased cropping intensity. Eifediyi et *al.* (2013) had earlier reported that soils of the study area are deficient in nutrients especially N, P and K. Gbedegasin and Akinbolu (1995) reported that soils of the southern guinea savannah zone of Nigeria are low in organic carbon, total nitrogen and available phosphorus. The poor nutrient status of the soil could also be adduced to constant bush fire, which deprives the soil of much needed organic matter thereby making it bare and prone to the vagaries of weather, which often results in soil erosion. These poor soil conditions coupled with the comparatively higher rainfall and maximum temperature experienced during the cultivating season therefore affirms the need for mulching as a soil conservation approach on this soil for

sustainable sesame production. Ansary and Roy (2005) had earlier posited that mulching brings about moisture conservation at the root zone and hence prevent leaching of fertilizer nutrients thereby promoting growth and yield parameters in tomatoes. The mulch was able to absorb some of the radiation energy thus functioning as insulators. The soil under a mulch cover therefore receives less heat during the day and less heat means less evaporation and hence increased soil moisture content (Muller – Samann and Kotschi, 1994).

The significantly higher porosity in unmulched plots may be attributable to improved organic matter content whereas differences between mulched types may be due to their C/N ratio which will determine the extent of their microbial activity and hence the speed of decomposition and subsequent mineralization. Grass mulch is thought to have lower C/N ratio compared to wood shavings and hence will therefore support relatively higher microbial activity which will invariably culminate in higher porosity, infiltration rate and water holding capacity (Martens and Frankenberger, 1992). The positive effect of organic mulches in increasing the available plant nutrient content in the soil is well documented (Saroa and Lal, 2004).

The increase in growth parameters of sesame in mulched plots can be adduced to sufficient soil moisture near the root zone of the crop that minimized evaporation loss due to mulching. Mulch is a source of organic matter to the soil and when it decomposes with subsequent mineralization releases nutrients that are useful to plants; hence increased growth attributes of sesame. Liasu and Achakzai (2007) had earlier reported that mulch and fertilizer when combined improved the growth attributes of tomatoes than when fertilizer or mulch is used alone. Malik *et al.* (2003), Olowe and Busari (2000) also had reported significant increase in plant growth attributes of sesame when NPK fertilizer was applied. The increase in growth attributes can be adduced to a combination of factors such as increased water holding capacity, increased porosity and reduction in the leaching of nutrients. Lal *et al.* (1980) and Manrique (1995) earlier posited that mulches enhance crop productivity by improving water conservation, soil physical and chemical properties.

From the results obtained, it is clear that yield and yield attributes of sesame were significantly increased by the application of fertilizer. Both yield and yield attributes of sesame were optimum at 300 $kgha^{-1}$ of fertilizer application because the fertilizer used during the experiment has higher nutrient content compared with mulch. The effects of mulching on the number of capsules per plant were significantly higher than the control. Similar responses had earlier been reported by Farios- Larios and Orozos- Santos (1997) who suggested that length of fruit of watermelon was greatly enhanced by the application of mulch compared to the control. The unmulched plots may have experienced increased evaporation from soil, increased leaching of nutrients and competition from weeds.

The significant effects produced by the 400 $kgha^{-1}$ over the 300 $kgha^{-1}$ in terms of growth parameters could not be translated into yield components as the

yield of sesame was significantly increased by the application of 300 $kgha^{-1}$ of NPK. This may be due to antagonistic behavior of one nutrient over the other, which may have promoted vegetative growth at the expense of seed formation. Arancibia and Motsenbocker (2008), Farios-Larios and Orozco–Santos (1997) had reported that marketable yield from mulched treatments were higher than those produced on unmulched plots in watermelon. Aruna et al. (2007) also reported increase in tomato in mulched plots and Angrej-Ali and Gaur (2007) in strawberry and Ibarra *et al.* (2001) in muskmelon.

In addition, the increase in yield of sesame could be attributed to soil conservation (Bhella, 1988) and reduced weed infestation in plots that were treated with mulches. Weed infestations were reduced in plots irrespective of the mulch type. Mulches, particularly grass mulch gave higher yields and affected other yield factors when compared to the wood shavings and non- mulch applications. This can be attributed to the fact that mulches reduce leaching, modify soil nutrient status and hence increase fertilizer use efficiency (Mbagwu, 1990). The significant interactions between mulching and NPK fertilizer application on the seed yield of sesame is an affirmation of the fact that combined applications of both mulch which is a form of organic manure and inorganic manure are essential for increased yield. This finding is in conformity with the findings of Olowe and Busari (2000) who asserted that inorganic and organic fertilizer increased the growth and yield of sesame.

The average yield of the two years of study indicated an overall seventy-one percent increase in seed weight per hectare in the grass-mulched plot over the control. Several workers had earlier reported yield increases by applying mulch. Abak *et al.* (1991) observed an increase in total yield (62%) and yield of early melon covered by mulch while Lal (1978) reported an increase of 38, 10 and 22 consecutive years for maize under mulch.

The differences in yield in the two years of study could be attributed to weather variability bearing in mind that the same variety and cultural practices were carried out during the period. Semenov and Porter (1995) had earlier reported that rainfall and temperature are major determinants of crop growth and yields especially in rain fed agriculture; rainfall and temperature from season to season greatly affects water availability to crops and thus pose crop production risks.

CONCLUSIONS

The higher yields of sesame were obtained in the mulched plots because of better water holding capacity and nutrient conservation compared to the unmulched plots. Mulch types had effect on the cultivation of sesame. The best agronomic and yield traits was observed from the plots mulched with grass. Grasses are suitable as mulch materials in the savanna zone as they are available all the time and its use should be encouraged and recommended for effective production of sesame for optimum yield especially when rainfall is erratic or too much.

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CONTENT OF VITAMIN C IN THE FRUITS OF JUJUBE (*ZIZIPHUS JUJUBA MILL.*) VARIETIES AND THEIR PROGENIES

SUMMARY

We have investigated the content of very important bioactive compound such as vitamin C in fruits of six varieties of Chinese jujube progenies: Zu tao czao, Da bai czao, Kitaiski 2A, Wild midleasian jujube type, Ja czao and Vahshski 45-2. It has been studied the inheritance of the vitamin C content in the fruits of 131 types in progenies, which were obtained from crossing of those six Chinese jujube varieties by open pollination, considering of the high jujube variety self-incompatibility. Studies were done on the plants that were 7-9 years old, or in full fertility.

Fruits from variety Da bai czao have the highest content of vitamin C (370.7 mg/100 g fruit). Compare with the other varieties, the fruit from variety Kitaiski 2A is characterized with the lowest content of vitamin C (185.2 mg/100 g fruit), and it's progeny, on the contrary, is characterized with the highest content of vitamin C in average (290.8 mg/100 g fruit), compare with the other investigated progenies. The largest number of the types in progeny with content of vitamin C higher than 300 mg/100 g fruit were characterized the varieties Zu tao czao and Da bai czao.

Keywords: Fruit, Chinese jujube, open pollination, type,variety, vitamin C

INTRODUCTION

The jujube fruits are rich with bioactive compounds which are very important for human health. The laboratory investigations show that the jujube fruits contain 20-28% total sugars, 0.3-2.5% total acids, 2.9% proteins, 500-600 mg of the vitamin C (in 100 g fruit pulp), vitamins from the B complex, and also they are rich source for minerals (Azam-Ali S. et al., 2006). According to literature data (Mratinic E., 2012) for other fruit kinds, the jujube fruits are in the very top by the content of vitamin C. The vitamin C is the most investigated and described between the vitamins, with a powerful influence on many biological processes. Such as strong antioxidant, vitamin C, participate in collagen and carnitine synthesis and in metabolism of the fatty acids. L-Ascorbic acid, the

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active biological form of vitamin C, is mostly presented in plants. The primates, including humans, cannot make vitamin C in their bodies and the primary source of the vitamin C are fruits, vegetables and fresh meat, among them the fruits are the richest sources for this vitamin (Hernández Y., et al, 2006). The ascorbic acid in jujube fruits is concentrated in the deep layers of the mesocarp, around the seed, and not below the peel, which is the case with other fruit kinds.

It is quite stable in jujube fruits during thermal processing of the fruits (the compotes and jams from jujube fruits contain usually 150-200 mg vitamin C) (Surhaev G., 2006). The vitamin C content in the jujube fruits decrease 8-10 times, 20 days after the harvest. It's enough to take only one fresh jujube fruit per day to reach the adult needs for the vitamin C and vitamin B complex as "recommended daily allowance (RDA)" by FAO (Azam-Ali et al, 2006). The highest content of the vitamin C is found in the time before fruits ripening (Markovski et al., 2009). This vitamin content rise from fruit's stone to peel and from fruit's remains of style to pedicel according to Bowe C. (Azam-Ali et al., 2006). The high content of vitamin C quantitatively inherited into some fruits as in fruits of the tomato varieties. Also, according to Stevens et al., (2007) the vitamin C content in tomato fruits is quantitatively inherited, that is shown with "quantitative trait locus (QTL) mapping" for ascorbic acid. Our investigations are focused on content of the vitamin C in jujube varieties and their progenies fruits, with aim to examine the inheritance of this characteristic. Using the results from the investigation, it is expecting to know which of the jujube varieties and types can be used as parents for obtaining the hybrids with higher content of the vitamin C in further breeding process (Yoo et al, 2012).

MATERIAL AND METHODS

The three year investigations in the selection orchards of Agricultural Institute in Skopje, Macedonia were performed with planting distance of 4 x 2 m, planted in 1997 with two years old seedlings of Chinese jujube varieties. During the investigations plants were 7-9 years old, apropos that were in the beginning of full fruitfulness (Markovski A. et al, 2015). The plants were grown without intervened pruning, left to develop naturally shaped crown, specific for each genotype. About 130 genotypes in the progenies which were obtained by open pollination between six Chinese jujube introduced varieties were examined. 35 seedlings of each variety-mother were planted in (with 38% surviving loses in total). As a control were used grafted trees of six varieties-mothers obtained with grafted technique whip grafting, in three repetitions, performed in 1998 (Markovski, A. Petkovski, D. 2012).

Determination of the content of vitamin C was performed by "volumetric method by titration with DCPIP" (dichlorophenol indophenol) (Papuc C. et al, 2001).

The examined characteristic was variational statistically analysed and tested by "Fisher LSD test" at two levels of probability (0.05 and 0.01).

The genetic parameters was estimated according to Wolie A. et al. (2013) biometrical methods and formulas to finding: Genotypic co-efficient of variation (GCV) = (σ_g / x) x 100, Phenotypic co-efficient of variation (PCV) = $(\sigma_p / \tilde{x})$ x 100, Heritability (H^2) = $(\sigma^2_g / \sigma^2_p)$ x 100, and Genetic advance (GA) = k H^2 σ_p, where, σ_p = phenotypic standard deviation, σ_g = genotypic standard deviation, $\tilde{x}$ = grand mean for the characteristic, σ^2_g = genotypic variance, σ^2_p = phenotypic variance, and k = selection differential (at 5% selection intensity).

RESULTS AND DISCUSSION

The investigations show high content of vitamin C in the Chinese jujube varieties and progenies fruits. From the mother varieties with the highest content of the vitamin C in the fruits is characterized the variety Da bai czao (370.7 mg/100 g fruit), while with the lowest, almost double less content of vitamin C is characterized the variety Kitaiski 2A (185.2 mg/100 g fruit). It is found that the size of the varieties fruits has no influence on content of the vitamin C. For example, the variety Vahshski 45/2 which is characterized with large fruits and the variety Da bai czao with the smallest fruits from all investigated varieties have high content of the vitamin C in the fruits. The content of the vitamin C in fruits of the Chinese jujube is very high, even higher than any other species from Ziziphus genus such a ber-Indian jujube (Ziziphus mauritiana L). It's proposed for plant breeders to planning the interspecies breeding programs for obtaining the hybrids with maximum fruit size of the Indian jujube and content of vitamin C of the Chinese jujube (Azam-Ali S. et al., 2006).

The coefficient of variation of the vitamin C content in Chinese jujube types fruits from progenies of the Chinese jujube varieties ranges between 18-22% (Table 1).

A difference in the average content of the vitamin C among the types from the progenies is three times lesser (52.9 mg/100 g fruit) than the differences in the average content of the vitamin C among the mother varieties (185.5 mg/100 g fruit).

The significant statistical difference in the average fruit vitamin C content between the Chinese jujube varieties and types in the progenies is determined, with exception of the difference in the fruits vitamin C content between the varieties Zu tao czao and Da bai czao and their progenies. Very significant statistical difference in vitamin C content is determined between the some types in the progenies of the varieties Zu tao czao (7 types) and Kitaiski 2A (10 types) compared with their mother varieties. In the progenies of the other mother varieties the types with the highest vitamin C content in the fruits statistically significant does not differ from the mother varieties. The ascorbic acid content is depends from the period of fruit ripening, or late ripening Chinese jujube varieties have the highest content of vitamin C, while the content of catechin decreased up to 20 times below of the level at the beginning of the fruit ripening period. Between the content of this two compounds a high correlation coefficient is determined during the fruit ripening period (Thayer, J., 2012).

Table 1. Content of the vitamin C (mg/100 g fruit) in fruits of the jujube progenies.

	Zu-tao czao		Da bai czao		Ja czao		Wild midleas.		Vahshski 45/2		Kitaiski 2A	
	Type	mg/ 100 g	Type	mg/ 100 g	Type	mg/ 100 g	Type	mg/ 100 g	Type	mg/ 100 g	Type	mg/ 100 g
P	13/1	428.6	30/2	419.1	12/3	346.0	17/4	400.1	26/5	328.6	13/6	411.2
	25/1	385.2	15/2	360.8	19/3	315.2	16/4	374.9	15/5	321.0	33/6	388.7
	20/1	349.2	22/2	359.9	10/3	303.8	28/4	333.6	16/5	316.4	22/6	338.2
R	10/1	325.8	35/2	353.7	22/3	301.0	14/4	333.4	7/5	285.5	37/6	300.6
	17/1	324.7	13/2	338.7	14/3	283.6	31/4	307.7	27/5	279.1	40/6	285.5
	33/1	324.2	31/2	336.1	38/3	264.8	21/4	290.8	32/5	262.4	9/6	271.6
O	29/1	310.2	11/2	333.0	13/3	264.7	22/4	274.1	23/5	256.3	24/6	270.4
	8/1	291.1	7/2	325.0	24/3	257.8	35/4	267.1	35/5	251.7	14/6	255.4
	16/1	290.4	25/2	310.0	23/3	237.9	29/4	264.5	17/5	239.7	21/6	250.8
G	21/1	290.0	21/2	306.6	26/3	237.2	23/4	260.0	25/5	222.8	31/6	227.5
	34/1	289.6	23/2	299.4	15/3	236.2	26/4	258.0	38/5	212.5	23/6	198.7
	15/1	284.0	14/2	283.6	21/3	231.6	13/4	256.4	31/5	210.3		
G	22/1	281.8	37/2	278.6	33/3	213.1	24/4	227.4	18/5	203.0		
	27/1	272.4	32/2	269.6	32/3	212.6	34/4	220.2	33/5	196.7		
	11/1	272.1	33/2	268.5	16/3	210.9	25/4	204.4	6/5	189.7		
N	35/1	268.4	3/2	260.0	17/3	206.2	27/4	195.8	19/5	131.8		
	37/1	261.7	8/2	252.8	31/3	180.4	18/4	189.1	24/5	123.9		
	26/1	254.2	29/2	242.9	30/3	177.8	19/4	171.5				
I	28/1	250.1	26/2	240.4	25/3	175.2	32/4	163.0				
	32/1	236.6	36/2	238.0	20/3	174.6						
	18/1	231.7	28/2	234.8	37/3	168.9						
E	7/1	230.9	19/2	217.1	28/3	160.5						
	6/1	226.3	18/2	217.0								
	23/1	224.7	17/2	211.7								
S			9/2	177.1								
			6/2	173.8								
	CV %	18		22		22		18		22		22
	$Lsd_{0.05}$	55.7		100.9		59.2		72.2		50.1		38.7
	$Lsd_{0.01}$	74.1		134.2		78.7		96.0		66.7		51.5

The Kitaiski 2A progeny is characterized with the highest content of the vitamin C among the progenies (290.8 mg/100 g fruit), while the Ja czao progeny has the lowest content of the vitamin C (235.4 mg/100 g fruit). The types from the Kitaiski 2A progeny are clearly separated from types from others progenies, especially above the range of 260 mg vitamin C/100 g fruit. Otherwise, below that range (260 mg/100 g fruit) are stand out the most of the types from the Vahshski 45/2 and Ja czao progenies.The mother varieties which have in average

the lowest content of vitamin C (Kitaiski 2A and Zu tao czao) gives progeny with the highest content of vitamin C in average (Table 1).

The type 13/1 from the progeny of the mother variety Zu tao czao is characterized with the highest content of vitamin C (428.6 mg/100 g fruit).

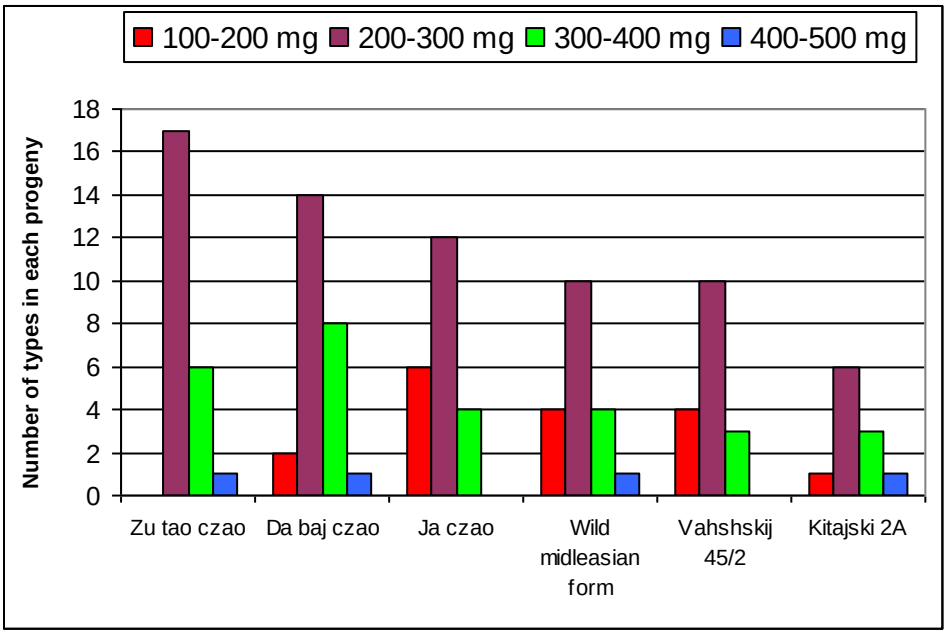

Figure 1. Four group of distribution of the types in progenies by content of the vitamin C (mg/100 g fruit).

Actually, only four types (13/1, 30/2, 13/6 and 17/4) are characterized with higher content of vitamin c over 400 mg/100 g fruit, also only few types (13/1, 30/2, 13/6, 17/4, 25/1, 16/4 AND 33/6) from the progenies have higher content of vitamin c than the mother variety with the highest content of vitamin c, da bai czao (370.7 mg/100 g fruit).

The presence of vitamin c in the types from the progenies is the best expressed in the figure 1. Especially is important the types number in the progenies which belong to the second (200-300 mg/100 g fruit) and third group (300-400 mg/100 g fruit), by the content of vitamin c.

These two groups are presented mostly in the progenies of the varieties zu tao czao and da bai czao, or 17 types in the second and 6 types in the third group from progeny of the zu tao czao and respectively 14 types in the second and 8 types in the third group in the progeny of da bai czao.

Can be noted that the variety kitaiski 2a despite that has in average the highest content of vitamin c in the progeny, also has considerably the lower number of types with high content of the vitamin c in total, than the varieties zu tao czao and da bai czao. Ascorbic acid content in varieties da bai czao and nikitskii 17 researching in yalta, crimea (russia) shown differ results than ours, or

almost double content of the vitamin c (774 mg/100 g fruit) and also high pectin content in some chinese jujube varieties (up to 1%). It indicates that in the northern countries fruits of the same chinese jujube varieties have greater content of vitamin c. Among 25 chinese jujube varieties are made the different lists of the best varieties depending of the used postharvest fresh handling and processing technologies, according to sinko l.t., (Surhaev g., 2006).

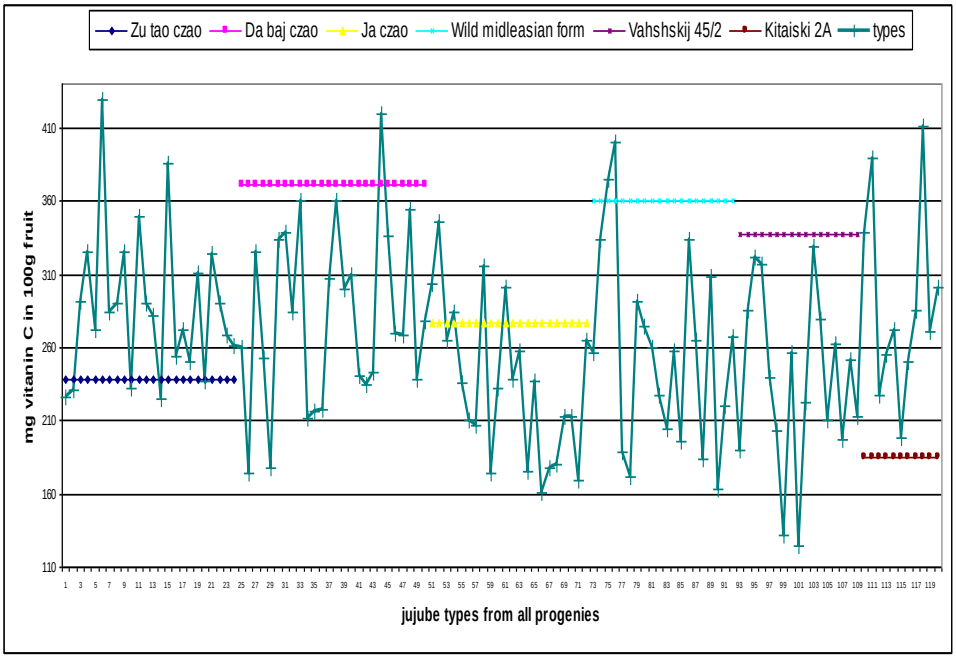

Figure 2. Comparison of average content of the vitamin C, between mother (varieties horizontal lines) with types in progeny (zig-zag lines).

The variety da bai czao produce the progeny with the greatest number of types (8) which belong to second group (300-400 mg/100 g fruit) by content of vitamin c. The progeny of the variety ja czao has the greatest number of the types with low content of vitamin c in the fruits (100-200 mg/100 g fruit). The evaluation of many chinese jujube varieties in south-west turkmenistan, shown that with the best bioactive compounds content is characterized the variety ja czao according to lomakina m.i., (azam-ali s et al., 2006).

Among the varieties da bai czao, ja czao, wild midleasian and vahshski 45/2 is recorded weak influence of variety to content of vitamin c in fruits of the types from their progenies, where the mothers have the higher content of vitamin c than the most of the types in their progenies. The exceptions are the varieties zu tao czao and kitaiski 2a where the most of the types have higher content of vitamin c in the fruits than their mothers (Figure 2.).

The determination of the genetic parameters clarify the investigated characteristic variation in progenies (Table 2).

Table 2. Genetic parameters for vitamin C content of the jujube genotype fruits

Variety progenies	Range			Genetic parameters			
	Min. mg /100g	Max. mg /100g	Mean mg /100g	GCV (%)	PCV (%)	Heritability (%)	GA (%)
Zu tao czao	224.7	428.6	287.7	9.7	18.2	49.8	8.8
Da bai czao	173.8	419.1	281.1	22.7	33.4	21.6	7.1
Ja czao	160.5	346.0	234.5	13.6	25.5	47.6	11.8
Wild	163.0	400.1	258.8	13.6	27.0	50.4	13.2
Vahshski 45/2	123.9	328.6	237.2	8.6	20.9	57.6	11.7
Kitaiski 2A	198.7	411.2	290.8	4.3	15.0	62.9	9.2
All progenies	160.5	428.6	287.6	34.3	46.2	45.1	19.7

The genotypic coefficient of variation (gcv) indicate for much higher variability of all genotypes in progenies than in the frame of the separate single mother-variety progeny. Considering that, the bigger are the values of gcv, heritability and genetic advance (ga) for some characteristic, same characteristic can be more improved through the selection process (islam m.n. et al., 2010), therefore at chinese jujube for the same purpose is recommended using of a larger number of parents in the hybridizations. The difference between gcv and phenotypic coefficient of variation (pcv) suggested for some environment influence which is not too much expressed. Heritability is an important genetic parameter that measures the relative degree to which a character is transmitted from parent to progeny (fotrić akšić m, et al, 2011). The percent of heritability is higher in the progeny of the mother variety kitaiski 2a (62.9%), which is mean that in this variety is much easier the obtaining of inheritance with high content of the vitamin c in fruits, through individual selection. However, moderate to low heritability and genetic advance suggested for polygenic influence and quite weak additional gene effect (kitaiski 2a) in creation of the characteristic for content of vitamin c in the chinese jujube fruits.

On basis of the obtained results, with aim to perform individual selection of types by production of vitamin c, are investigated the dependents on it with total yield of fruits, the size of the fruits, and also the volume of the tree crown of the types (markovski, petkovski, 2012) (table 3). By the evaluation of the yield (kg per tree) it is found that the type 17/4 is characterized with the highest production of vitamin c in fruit (44 g vitamin c per tree). If we considered the vigour of the type or yield kg/m3, then with the highest production is characterized the type 15/2 (12.3 g vitamin c per m3 volume of the crown). The variety zu tao czao participate with the greatest number of types among the first four with the highest production of vitamin c per tree and also per volume of the crown (Table 3).

Table 3. Top ten types with the highest production of vitamin C in the fruit, from all obtained jujube progenies.

Type	Vitamin C mg/100 g fruit	Mass of fruits g	Yield kg/tree	Yield per crown kg/m^3	Vitamin C production	
					g/m^3 crown	g/tree
17/4	400.1	9.4	11.0	0.1	0.5	44.0
20/1	349.2	12.3	11.4	1.2	4.1	39.8
25/1	385.2	9.0	5.6	0.2	0.8	21.6
37/6	411.2	3.9	5.2	0.9	3.7	21.4
15/2	360.8	3.1	4.1	3.4	12.3	14.8
16/4	374.9	1.8	3.9	0.3	1.3	14.6
22/2	359.9	4.7	3.9	0.7	2.3	14.0
12/3	346.0	7.9	3.3	0.1	0.4	11.4
13/6	388.7	3.4	1.0	1.4	5.6	3.9
13/1	428.6	4.1	0.8	1.3	5.7	3.4

CONCLUSIONS

The investigation in general shown that the content of the vitamin C in Chinese jujube varieties progenies inherited intermediate, but in some progenies such as the progeny of Kitaiski 2A and Zu tao czao the inheritance is partial dominantly, compared with the mother variety. The varieties Zu tao czao and Da bai czao produced the largest number of types with high content of the vitamin C, and is recommended using of these two varieties in further breeding programs.

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AGRARIAN LANDSCAPE BETWEEN TRANSITION AND SUSTAINABILITY - GRAČANICA AREA CASE STUDY

ABSTRACT

During the past two decades, the area of Kosovo and Metohija has been subjected to extensive changes which can be studied through various layers of complex transition process and its outcomes. The emergence of new zones of rural settlements accounts for one of the major spatial results of the overall societal transition. In every of these administrative units – Štrpce, Ranilug, Gračanica and others, thorough spatial transformations continuously appear since the beginning of the transition process, to arrive to the point where sustainability of agrarian landscape is brought into question.

By focusing the research on causal relations and on specific case example – the area of Gračanica, this paper studies the evolution of transition and concurrently investigates and defines its impacts on agrarian landscape. Multi-layered space-time analysis has shown that the agrarian landscape of Gračanica currently rests on the borderline between sustainability and unsustainability. Since the beginning of the transition process, Gračanica landscape has been exposed to different physical, ecological, economic and socio-cultural pressures which contravene its value and qualities. The main current threats to the sustainability are found in development plans, erratic constructional and agricultural practice, environmental conditions, overall development, and inflow of capital which stimulates non-agricultural development and simultaneously causes multilayered loss of value of the most fertile agricultural land. Agrarian landscape of Gračanica area, as an absolute natural and cultural heritage, needs systemic preservation measures.

Keywords: agricultural land, Gračanica rural area, spatial transformations, sustainability versus transition, preservation, visual landscape

INTRODUCTION

In rural areas, the land has ultimate ecological, social and cultural value. In agrarian landscape, the need to study nature and culture together is evident (Palang *et al.* 2006). "The patterns humans impose on the earth, purposefully as well as inadvertently, through land-use change are fundamental determinants of

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

local, regional, and global ecological processes that ultimately influence the sustainability of both biological and cultural landscapes, and thus human quality of life." (Redman, 2008: 3) The changes in agrarian landscape, that are provoked by major shift in overall circumstances (such as the large-scale social conflicts), at beginning represent an informal response to newly emerged conditions. The first phase of post-conflict spatial transformations is usually followed by unorganised, uncontrolled development, illegal occupation of free agricultural land and its random utilization for construction. In later phases, spatial changes gradually become organised and planned, even though the alterations that were previously introduced often remain forever present, dictating the course of further development and impacting further transformation of the agrarian landscape in physical, ecological, socio-cultural and economic terms.

Over the past two decades, predominantly rural landscape of Kosovo and Metohija underwent significant spatial transformations because of the migratory flows, population displacement, damaged built stock, defeated multiculturalism and numerous economic implications. Major ethnic division caused spatial rebordering and reordering; in overall terms, this referred, inter alia, to the formation of enclaves. Every of these newly emerged rural entities nowadays is faced with various challenges from inside and outside that impact the achievement of sustainability and resilience, with particular regard to agricultural landscape loss, fragmentation, transformation, isolation, vulnerability and pollution.

In the transition process initiated by 'geography of division' and 'demographic shifts' (Murtagh, 1998) and later followed by (sub)urbanisation, the agrarian landscape of Gračanica area in the central part of Kosovo and Metohija has been subjected to different rapid changes and their consequences. By focusing the research on causal relations, this paper studies physical, ecological, economic and socio-cultural impacts of transition on Gračanica agrarian landscape and concurrently evaluates the sustainability of subject rural area.

MATERIALS AND METHODS

Study area

Until the end of the last century, Gračanica was a single village located 8 km away from the town of Priština, separated from the urban tissue by a sloppy void. In social terms, the village was a city satellite; in administrative, a unit under the governance of the Municipality of Priština; in spatial and agricultural terms, Gračanica was a rural settlement with households active in keeping the livestock or, more often, growing the crops. Because of the arrival of a part of population from Priština and surrounding rural settlements, the relocation of existing public institutions, as well as the establishment of seats of international peacekeeping and non-governmental organisations in nearby Priština, the population of Gračanica started to increase since 1999. During the following years, the settlement has been transformed gradually into the centre for basic need satisfaction (health and administrative service provision, education, culture,

shopping, and others). At the same time, spatial conditions in Gračanica surroundings also were changing, to finally get to the present point of deep spatial interfering and blurred edge determinants.

With the administrative reorganisation, Gračanica area became in 2009 the separate municipality, and Gračanica village became centre to 16 cadastre zones: Badovac, Batuse, Čaglavica, Dobrotin, Gračanica, Donja Gušterica, Gornja Gušterica, Laplje Selo, Lepina, Livađe, Preoce, Skulanevo, Sušica, Suvi Do, Radevo i Ugljare. The Municipality encompasses the territory of 122.25 km^2 with about 25,000 inhabitants, and borders with the Municipality of Priština on the north, the Municipality of Lipljan on the south, and with the Municipality of Kosovo Polje on the west. (Figure 1)

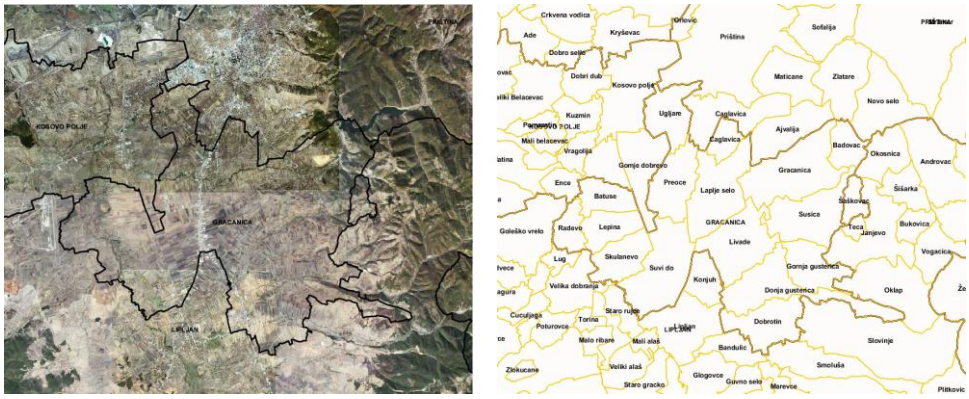


Figure 1. Gračanica Municipality: orthophoto image (left) and belonging cadastre zones (right) (Image source: geoportal.rks-gov.net)

The settlements of Gračanica area are rural, with predominant irregular layout and compact form which was kept from the period of their foundation (Urošević, 1965). The largest number of settlements is set on plain terrain, while few are developed on the undulating terrain on the edge of Kosovo Valley. Terrain morphology has allowed for the formation of the typical rectangular shape of agricultural parcels and the typical spatial organisation of the settlements, with the residential part in the centre and the agricultural - production part around it (Figures 2a, 2b and 2c).

Gračanica area has favourable climatic conditions and high-quality cultivable soil of the first or second category. The land is mainly used for cultivating crops - wheat, barley, maize and sunflower. Vegetable gardens and orchards are commonly found within residential plots. Livestock breeding is sporadically present, as well as the beekeeping, mushroom farming, and production of spices and herbs.

BADOVAC

First mention: *demography tracks from XIX century (Urošević, 1965);* ***Pattern****: isolated households (previous village immersed in 1991);* ***Form:*** */;* ***Layout:*** *irregular;* ***Terrain****: slope;* ***Development stage:*** *rural;* ***Cadastre zone:*** *Badovac;* ***Households****: 3;* ***Agrarian landscape****: forest and deforestation/ lake*

BATUSE

First mention*: demography tracks from the beginning of XIX century (Urošević, 1965);* ***Pattern****: village;* ***Form****: cluster;* ***Layout****: irregular;* ***Development stage****: rural dispersion;* ***Terrain****: plain;* ***Cadastre zone:*** *Batuse;* ***Households****: 41;* ***Agrarian landscape****: crop fields, meadows*

ČAGLAVICA

First mention*: demography tracks from XVIII century (Urošević, 1965);* ***Pattern****: settlement;* ***Form****: mixed;* ***Layout****: overall irregular;* ***Development stage****: suburbanization / urbanization;* ***Terrain****: plain to slightly undulating;* ***Cadastre zone****: Čaglavica;* ***Households****: 571;* ***Agrarian landscape****: crop fields*

DOBROTIN

First known mention*: XVIII century;* ***Pattern****: village;* ***Form****: elongated cluster;* ***Layout****: irregular;* ***Development stage****: rural;* ***Terrain****: plain;* ***Cadastre zone****: Dobrotin;* ***Households****: 257;* ***Agrarian landscape****: crop fields*

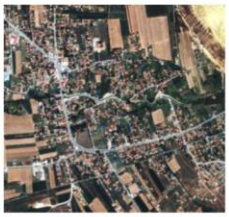
DONJA GUŠTERICA

First mention*: XVIII century (Urošević, 1965);* ***Pattern****: village;* ***Form****: elongated cluster;* ***Layout****: irregular;* ***Development stage****: rural;* ***Terrain****: plain;* ***Cadastre zone****: Donja Gušterica;* ***Households****: 280;* ***Agrarian landscape****: crop fields*

GORNJA GUŠTERICA

First mention*: Middle Ages;* ***Pattern****: village;* ***Form****: mixed radial and linear;* ***Layout****: irregular;* ***Development stage****: rural;* ***Terrain****: plain, at the edge of Kosovo valley;* ***Cadastre zone****: Gornja Gušterica;* ***Households****: 133;* ***Agrarian landscape****: crop fields, meadows, forest*

Figure 2a. The characteristics of Gračanica settlements: Badovac, Batuse, Čaglavica, Dobrotin, Gornja Gušterica and Donja Gušterica



GRAČANICA

***First mention**: 1321; **Pattern**: central settlement; **Form**: cluster with linear branch expansion; **Layout**: irregular; **Development stage**: suburbanization / urbanization; **Terrain**: plain; **Cadastre zone**: Gračanica; **Households**: 915; **Agrarian landscape**: crop fields*

LAPLJE SELO

***First mention**: VIX century (Urošević, 2009); **Pattern**: village; **Form**: cluster; **Layout**: irregular; **Development stage**: rural + suburbanization; **Terrain**: plain; **Cadastre zone**: Laplje Selo; **Households**: 343; **Agrarian landscape**: crop fields*

LEPINA

***First mention**: demography track from Middle Ages (Urošević, 1965); **Pattern**: village; **Form**: cluster type; **Layout**: irregular; **Development stage**: rural; **Terrain**: plain; **Cadastre zone**: Lepina; **Households**: 83; **Agrarian landscape**: crop fields*

LIVAĐE

***First mention**: Middle Ages (Лиѵађие) (Urošević, 2009); **Pattern**: village; **Form:** cluster; **Layout**: regular organization along irregular branched road matrix; **Development stage**: rural; **Terrain**: plain; **Cadastre zone**: Livađe; **Households**: 159; **Agrarian landscape**: crop fields, meadows*

MARIGONA RESIDENCE

***First mention**: New development (undergoing finalization); **Pattern**: settlement; **Form**: elongated grid; **Layout**: regular; **Development stage**: urbanization; **Terrain**: plain; **Cadastre zone**: Preoce; **Households**: n/a; **Agrarian landscape**: -*

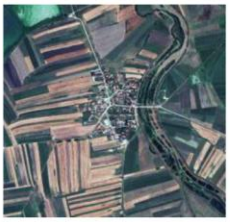
NOVI BADOVAC

***First mention:** 1991 (formed after immersion of village Badovac); **Pattern**: hamlet; **Form**: cluster; **Layout**: regular; **Development stage**: suburbanization; **Terrain**: slightly undulating; **Cadastre zone**: Gračanica/Sušica; **Households**: n/a; **Agrarian landscape**: -*

Figure 2b. The characteristics of Gračanica settlements: Gračanica, Laplje Selo, Lepina, Livađe, Marigona Residence and Novi Badovac

PREOCE

***First mention**: demography tracks from XVIII century (Urošević, 1965); **Pattern**: village; **Form**: cluster; **Layout**: regular; **Development stage**: rural + suburbanization; **Terrain:** plain; **Cadastre zone**: Preoce; **Households**: 168; **Agrarian landscape**: crop fields*



RADEVO

***First mention**: n/a; **Pattern**: village; **Form**: cluster; **Layout**: irregular; **Development stage**: rural; **Terrain**: plain; **Cadastre zone**: Radevo; **Households**: 52; **Agrarian landscape**: crop fields; meadows; fish ponds*

SKULANEVO

***First mention**: XIV century; **Pattern**: village; **Form**: cluster with roadside construction; **Layout**: irregular; **Development stage**: rural; **Terrain**: plain; **Cadastre zone**: Skulanevo; **Households**: 73; **Agrarian landscape**: mainly crop fields, meadows*

SUŠICA

***First mention**: 1321; **Pattern**: village; **Form**: cluster with branched hamlets; **Layout**: irregular; **Development stage**: rural; **Terrain**: undulating; **Cadastre zone**: Sušica; **Households**: 160; **Agrarian landscape**: crop fields, forest / deforestation*

SUVI DO

***First mention**: 1321; **Pattern**: village with two hamlets (Ašani and Novo Naselje); **Form**: gridded clusters; **Layout**: regular; **Development stage**: rural + suburbanization on the boundary with Lipljan Municipality; **Terrain**: plain; **Cadastre zone**: Suvi Do; **Households**: 176; **Agrarian landscape**: crop fields, meadows*

UGLJARE

***First mention**: Middle Ages (Locus dictus Uliare); **Pattern**: village with hamlet Emšir; **Form**: clusters with roadside expansion; **Layout**: irregular; **Development stage**: rural dispersion and suburbanization; **Terrain**: plain; **Cadastre zone**: Ugljare; **Households**: 353; **Agrarian landscape**: crop fields*

Figure 2c. The characteristics of Gračanica settlements: Preoce, Radevo, Skulanevo, Sušica, Suvi Do and Ugljare

The area of Gračanica contains abundant built heritage, displaying different layers of history and carrying significant cultural value (Figure 3):

- archaeological site of Roman cultural and administrative centre Municipum Ulpiana Splendissima, i.e. the renewed Byzantine town Iustiniana Secunda, from the 6th century AD;
- Gračanica Monastery, built in 1321 on foundations of the Byzantine basilica from the 6th century AD as the last of 42 endowments of King Milutin. Ever since, the monastery represented an important educational, religious and cultural centre in the region and kept this role until the present day. The monastery church and the overall complex, with its paramount architectural value, are inscribed in UNESCO List of World Heritage in Danger in 2006;
- sacral architectural objects: Church of Sveti Dimitrije in Dobrotin (1855); Church of Sveta Nedelja in Batuse (1864); Church of Knez Lazar in Donja Gušterica (1905); Church of Sveti Grigorije Bogoslov in Livađe (1935); Church of Sveta Petka in Laplje Selo (1938), and others;
- traditional housing units from the 19th and the early 20th centuries;
- monuments and memorial houses from the First (WWI) and the Second World War (WWII);
- newly built monuments, churches and art installations with cultural value.

Figure 3. Cultural heritage of Gračanica area: Ulpiana archeological site (top left); Gračanica Monastery (top right); Sveta Nedelja Church in Batuse (bottom left); Memorial house from the WWII in Suvi Do (bottom right)

The agrarian landscape of Gračanica, distinguishing by its visual uninterruptedness and spatial continuity, represents the outmost natural and cultural value of the area. (Figure 4)

Figure 4. Agrarian landscape of Gračanica: Suvi Do Cadastre zone (top left); Laplje Selo cadastre zone (top right); Batuse cadastre zone (bottom left); Gračanica cadastre zone (bottom right)

Research methodology

Redman (2008: 15) wrote that, in order "to understand the richness, diversity, and complexity of agrarian landscapes and their transformations, we must monitor them at varying spatial and temporal scales, and place them in a context of former cycles of change, human perception of the land and lifeways, and the emergence of institutions associated with natural resources."

The first step of the research - identification of research problem, subject and study area is based on in-situ detection of a significant spatial phenomenon that previously hasn't been studied scientifically (to-date, Vöckler [2008] remains as the only author who profoundly investigated transition-related spatial transformations in the region, although from the urban growth aspect). Detailed description of the case study area, necessary for the understanding of its traditional characteristics, value, current undergoing changes and their reflective implications, is the result of the analysis of all cadastre zones and included settlements. The settlements in Gračanica area (Figures 2a, 2b and 2c) were investigated based on their pattern, form, layout, development stage, location in space, and agrarian landscape (Fikfak, Popović and Kosanović, 2015). The core research work relates to the study of space-time transformations of Gračanica agrarian landscape, by implementing the following research methods:

- analysis, comparison and interpretation of recent historical and actual remote sensed data (satellite imagery, aerial photography and orthophoto);
- field visits and photographing - sensing in-situ, with the legitimate use of "holistic ability of human perception to recognize patterns" (Antrop and Van Eetvelde, 2000: 47) and of "linguistic models for interpreting landscapes" (Claval, 2005: 9);
-overview and analysis of statistical data, regulatory/development plans and policies, ecological studies and impacts, and relevant international published literature.

RESULTS AND DISCUSSION

The study has shown that transition process had and still has significant impacts on the agricultural landscape of Gračanica area. According to the sustainability aspects, these impacts can be classified as: physical, ecological, economic and socio-cultural.

Physical impact

The analysis of spatial transformations occuring since the beggining of the present century shows that there is a growing pressure on Gračanica spatial boundaries and its belonging agricultural land, because of the expansion of surrounding urban and suburban areas of Priština, Kosovo Polje and partially Lipljan municipalities.

Cadastre zones Gračanica (Figure 5), Čaglavica (Figure 5) and Ugljare (Figure 6) to-date have been significantly affected by urban growth and formation of urban-rural fringe.

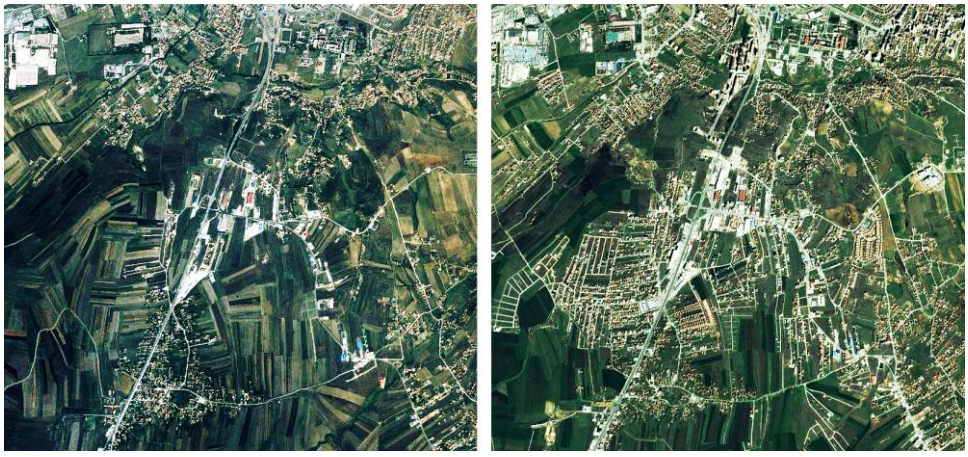


Figure 5. Spatial transformations on triple boundary between Priština, Gračanica and Čaglavica – state on 09.05.2003. (left) and 22.03.2014. (right) (Image source: Google Earth)

On the other hand, the conversion of agricultural land into residential or business purposes also occurs in the inner part of Gračanica zone (Figures 7 and 8). These new spatial formations (such as Marigona Residence development, Figures 7 and 8) - isolated urbanised areas developed in the middle of agrarian landscape, can be classified as a less frequent type of remote urban-rural fringe sections lying in deeper rural hinterlands of Gračanica zone and actually belonging to the central city of Priština.

Traffic infrastructure development (highway, bypasses and other roads) in past years has attracted new content, again on account of agricultural land. With the new road net, the fragmentation of rural landscape deepens (Figures 6 and 7), ecological flows are disrupted, transport journeys maximise and car dependence enlarges, all of which, as Raco (2007) notes, represent the features of unsustainable communities.

Figure 6. Spatial transformations on the boundary between Kosovo Polje and Ugljare – state on 09.05.2003. (left) and 30.01.2016. (right) (Image source: Google Earth)

Figure 7. Boundary between Laplje Selo and Preoce cadastre zones on 09.05.2003. (left), 19.08.2010. (middle) and 30.01.2016. (right), with the emergence of Marigona Residence development (Image source: Google Earth)

Figure 8. The examples of agricultural land conversion: Suvi Do zone (top left); triple boundary between Suvi Do, Livađe and Laplje Selo (top right); Preoce zone - Marigona (bottom left); Ugljare zone (bottom right)

Ecological impact

Almost two decades long transition neglected environmental quality; instead, the priority has been given to the conversion of agricultural land into developed purposes, urbanisation and suburbanisation, all of which are ecologically aggressive processes. The inability to support growing demands with the municipal systems, inadequate wastewater and solid waste management, the lack of public transportation, as well as the uncontrolled use of the pesticides in agricultural production resulted with the pollution and degradation of agricultural land. Wastewater generated throughout the Municipality ends either in permeable septic tanks and further in underground water flows, or in streams and rivers, without any prior filtering or purification. Inadequate (and mainly illegal) municipal landfills for unseparated waste disposal and the two tailing ponds of the mines that worked in the past (in Gračanica and Badovac cadastre zones) pollute in sequence all three natural elements – water, air and soil. Even though the area of Gračanica has abundant water resources, their pollution is high; rivers Gračanka, Sitnica, Prištevka, Janjevka and Žegovka with their seriously compromised ecological quality carry high risks to surrounding agricultural soil pollution, especially in the periods of abundant rainfall leading to agricultural land flooding.

Economic impact

Agricultural soil pollution and loss, outdated methods of crops growing and aggravated access to agricultural products market (Rikalo, 2011) lead to the decrease in production competitiveness and, taking into consideration long-

lasting presence of restricted factors, carry the risk to agricultural production abandonment.

Socio-cultural impact

Imposed changes bring to the forefront the question on how people perceive Gračanica agrarian landscape in transition. With the insertion of new materialised artefacts, such as buildings, developments, roads, and other (Figures 5-8), the wholeness of the visual landscape has been disrupted and new scenery with universal visual patterns has been introduced (such as ribbon-building along the roads, industrial corridors, inner and outer urban-rural fringe and other), causing the difficulties in landscape reading and the loss of its authentic value - continual plain crop fields and compact villages with low rise unobtrusive structures. Gračanica's cultural heritage is fused with the agrarian landscape; because of the introduced spatial changes, its significance is impacted.

Disscussion

Redman (2008) conceptualised the cycle of agrarian transformation affecting landscapes, of altered landscapes affecting ecological processes, of both influencing the ways in which humans monitor and respond to their surroundings, and of human responses endangering further cycles of change. For Antrop and Van Eetvelde (2000: 45), landscape is dynamic and in continuous transition, not only by natural processes, but also by changing economical needs and cultural values. However, major social conflict and its outcome have led to the emergence of the 'surprise' (Redman, 2008) in the cycle of agrarian transformation, i.e. to the initiation of accelerated shifts in Gračanica agrarian landscape.

The study has shown that the threats to sustainability of agricultural landscape of Gračanica area are laid in development plans, erratic construction and agricultural practice, weak environmental conditions, poor overall development, and capital inflows, which stimulates non-agricultural development and simultaneously causes multilayered loss of value of the most fertile agricultural land.

According to the adopted development plan for the period 2014-2029, Gračanica Municipality is located in the 'Blue Zone' intended for economic growth of Priština. The plan foresees significant future transformation, i.e. the urbanization of Gračanica rural zone on account of valuable and high-quality agricultural land, which is primarily reflected through the development of new transportation networks/corridors and splitting of agricultural entirety into smaller segments by inserting business and industrial intermediate zones, to the point where edge cadastre zones - Suvi Do, Skulanevo, Lepina, Batuse and Radevo are under the risk of being cut-off from the rest of Gračanica agricultural entirety. It is very likely that these changes will trigger new ecosystems disruption and boost the pollution brought by urbanization. The Development Plan for Gračanica (2013) carries the risk of future agricultural land fragmentation also because it foresees the development for larger than realistic population size. At the same time, this document omits smart land use and

precious agricultural land preservation, even that it offers a series of measures for agricultural production enhancement.

Although Priština tends to extend, it does not tend to explode, even if the two phenomena, as Dickinson (1967) explained, go together in different geographical settings. Hence, justification of the penetration of the city into agriculture land is weak, which is adding credibility to agricultural land preservation. The formation of the so-called 'urban-rural fringe' (Pryor, 1969) on the boundaries between Gračanica and adjacent municipalities will likely lead to new spatial transformations, as "an important issue in the urban-rural fringe is the rapid pace of change" (Weaver and Lawton, 2001: 440). "The landscapes created by the transformation of the countryside around urban centres // are considered highly dynamic, but remain poorly understood." (Antrop and Van Eetvelde, 2000: 45) Despite the scenario of centric growth of existing settlements in Gračanica area, foreseen by the Development plan of Gračanica Municipality 2014-2029, it is also possible, according to present trends, that easily accessible greenfield areas will become locations for housing construction and for people in active liberal professions, and others, so that "distinctly urban programmes and forms of built patterns also move to rural areas" (Fikfak, Popović and Kosanović, 2015: 320).

Any future development without adequate infrastructural support and environmental protection measures implementation unavoidably will raise new threats to agricultural land sustainability.

Murtagh (1998) noted that the size of the community is vital in its ability to sustain itself in highly contested space. To preserve the agricultural landscape of Gračanica area means to secure the survival of its inhabitants, which, for the region with high unemployment rate and low income, accounts for the highest priority. "...A sustainable place is one in which employment, mixed housing and social facilities are co-present and available to a range of socio-economic groups. It is populated by sustainable citizens who are politically, socially and economically active and self-reliant. They are 'nondependent' on the state, and provide for themselves through private-sector (market) provision." (Raco, 2007: 306-307) "...Under the precarious conditions that characterise rural survival in many low income countries, diversification has positive attributes for livehood security that outweigh negative connotations it may possess. Policy should facilitate rather than inhibit diversity. Diverse rural livelihoods are less vulnerable than undiversified ones." (Ellis, 2000: 289) In wider sense, "the linkages between community relations, conflict resolution and area planning is a major agenda for exploration in the formulation of relevant and genuinely sustainable local development strategies" (Murtagh, 1998: 230).

The preservation of rural landscape "fits in the framework of the protection of cultural and natural heritage" (Antrop, 2006: 188). Palang et al. observe landscape is an instrument, "because even today people have more of an emotional attachment to it than they have to a formal region or to nature as such" (Palang *et al.* 2006: 354). The sense of self, created by adopting ready-made

identities through historical association and tradition (Saar and Palang, 2009), is impacted with altered narratives of the place. When the bond between the individual and the place in transformed cultural circumstances is weakened, it potentially impacts identity-feeling and attachment to the place, and puts into question social sustainability of the area, especially when taking into account the fact that Gračanica's spatial changes, for the most part, occur without the involvement of local population. The struggle between a landscape that signifies possibilities for better economic wealth for the insiders, and a landscape that provides possibilities for amenities and power play for the outsiders, described by Palang et al. (2006), thus is valid for Gračanica area as well.

Future research

Research results pave the road to future detailed investigation of the impact of transition process on the most jeopardized cadastre zones of the Municipality of Gračanica, in terms of agrarian landscape loss, fragmentation, pollution and degradation and the transformation of its meaning, as well as of the possibilities for agricultural landscape preservation from the current standpoint.

CONCLUSIONS

The research has shown that the transition process caused major changes in the agrarian landscape of Gračanica area by impacting all dimensions of its sustainability - physical, ecological, economical and socio-cultural. The analyses of transition trends and of current state indicate that the changes in agrarian landscape of Gračanica are still taking effects, and that their outcome is uncertain to the point of doubt that Gračanica area is capable to sustain itself as autonomous rural unit. Clearly, the agrarian landscape of Gračanica area, as an absolute natural and cultural heritage, needs systemic preservation measures.

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MONITORING OF MULTI–CLASS PESTICIDES IN HONEY SAMPLES FROM MACEDONIA BY ULTRA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY – TANDEM QUADRUPOLE MASS SPECTROMETRY

ABSTRACT

The possible presence of pesticide residues in honey has impelled the need for setting up monitoring programs to determine the proper assessment of human exposure to pesticides. This paper describes an effective multi-class method using a modified QuEChERS sample preparation for detection and quantification of 18 pesticides with Ultra-high-performance liquid chromatography (UHPLC) – tandem quadrupole mass spectrometry. The method was validated according to the requirements laid down in DG SANCO 12571/2013 document. Levels of detection and quantification were lower than the established MRLs, the obtained precision was better than 20 %, and the recovery values were between 74.4 and 104.1 %. Fifty honey samples within the national monitoring program were collected from August to November, 2014 and tested for presence of carbaryl, carbofuran, fenvalerate, cypermethrin, deltamethrin, permethrin, bifenthrin, amitraz, coumaphos, bromopropylate, dichlorvos, diazinone, malathion, parathion, dimethoate, omethoate, methomyl and thiametoxam. Trace levels of methomyl, diazinone, bromopropylate and fenvalerate were detected somewhat above the reporting level for these pesticides (10 μg/kg). This contamination indicates on existing of possible moderate cross-contamination during pollen and nectar collection by bees, and residues of substances used in beekeeping. Although traces of pesticides were found in 16 of the samples tested (32 %), the levels found did not pose increased toxicological risk for the population.

Keywords: pesticides, honey, multi class method, liquid chromatography, tandem quadrupole, QuEChERS.

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INTRODUCTION

Honey is a valuable commodity that can be used as a final product or food ingredient. It is a highly-energetic natural carbohydrate product containing significant number of compounds with high benefit for the humans (Eissa et al. 2014). The quality of honey is mainly determined by its sensorial, physicochemical and microbiological characteristics. The honey quality criteria are well defined by the EC Directive 2001/110 (EC, 2001). Agricultural contamination of food with pesticides is a challenging problem that could not be neglected. In recent years, analysis of pesticide residues in food becomes a substantial requirement for consumers, producers and food safety authorities (Eissa et al. 2014). Pesticide residues might cause genetic mutations and cellular degradation, thus presenting a potential risk for the human health, mainly due to their cumulative properties (Blasco et al. 2003).

The analysis of pesticides residues in honey is significant analytical challenge, due to the complex and variable honey composition dependable on the type of plant where bees collect nectar (Tomasini et al. 2012). It is considered that there are two forms of contamination of honey: during pollen and nectar collection of bees, indirect contamination and through treatment of hives, in which pesticides can migrate in honey, i.e. direct contamination (Orso et al. 2014). The evidence for the presence of pesticide residues in honey (Blasco et al. 2003, Orso et al. 2014) has impelled the need for setting up monitoring programs for obtaining a proper assessment of human exposure to pesticides through honey consumption (Wallner, 1999). Different national regulations have established maximum permitted concentrations of pesticides (MRLs) in honey, but the lack of harmonized regulative cause problems in international trade (Blasco et al. 2003).

Many publications have reported various extraction methods for determination of pesticide residues in honey, employing solid phase-extraction (SPE), matrix solid-phase dispersion (MSPD), supercritical fluid extraction (SFE) and accelerated solvent extraction (ASE) (Amendola et al. 2011). The pesticide determination is usually performed by GC or LC coupled with various detectors. Only few of these methods allow multi-class detection of pesticide residues, as well as their quantification at very low concentration levels. In the recent years MSPD sample preparation has been identified as powerful tool for multi-residual analysis of pesticides in honey samples due to its compatibility both with GC and LC chromatographic systems and mass spectrometric detection systems (Tomasini et al. 2012; Orso et al. 2014, Rodríguez-López et al. 2014; Fidente et al. 2005; Tanner and Czerwenka, 2011).

The lack of information about pesticide residues in honey samples from Macedonia implies the necessity to determinate the pollution of this bee product.

Accordingly, the aim of this study was to identify the current honey contamination with 18 pesticides applying validated multi-class ultra high-performance liquid chromatography (UHPLC) – tandem mass spectrometry method. On basis of pesticides found in a representative number of 50 honey samples, we could conclude whether the honey samples were exposed to direct or indirect contamination, as well as their safety from the toxicological point of view.

MATERIAL AND METHODS

Chemicals and materials

As an analytical standards carbaryl, carbofuran, fenvalerate, cypermethrin, deltamethrin, permethrin, bifenthrin, amitraz, coumaphos, bromopropylate, dichlorvos, diazinone, malathion, parathion, dimethoate, omethoate, methomyl and thiametoxam were purchased from Fluka (Pestanal quality). Acetonitrile was with HPLC grade, while methanol and water were with LC/MS grade, all supplied from Carlo Erba. Formic acid (Suprapur grade) and ammonium formate were products of Sigma-Aldrich. The sorbents used for MSPD were magnesium sulfate anhydrous (Merck), sodium chloride (Carlo Erba), primary-secondary amine - PSA and C-18 (Supelco).

Sample collection

A total of 50 multifloral honey samples were collected at individual beekeepers from different geographical locations in Macedonia in the period August-November, 2014. Upon collection, all honey samples were placed into clean glass jars, labeled and placed on +4 oC, transferred to the laboratory and kept at 4 oC until analysis. The sample size needed for obtaining the required sample homogeneity was at least 500 g.

Sample preparation: modified QuEChERS procedure

The extraction method used was based on the original QuEChERS method developed by Anastassiades et al. (2003). Briefly, for the extraction, 5.0 g of honey was weighed in a 50 mL polypropylene centrifuge tube and 10 mL of water was added. The mixture was vortexed for 1 min. Afterwards 10 mL acetonitrile was added and the sample was homogenized. After adding 4 g magnesium sulfate and 1 g sodium chloride, the sample was vortexed again and centrifuged at 8000 rpm for 10 min. Six mL of the upper layer was placed into 15 mL polypropylene tube containing 900 mg magnesium sulfate, 150 mg PSA and 150 mg C-18 sorbent. After 1 min homogenization the tube was centrifuged at 3000 rpm for 5 min. Four mL of the solution were evaporated to dryness, re-dissolved in 2 mL mobile phase, filtered through 0.45 μm filter and placed into autosampler vial and further analyzed with UHPLC-TQD system.

Instrument and experimental conditions

UHPLC with mass spectrometric detection was performed on Acquity – TQD Waters system (Waters, Milford, MA, USA), utilizing Ascentis® Express of 2.7 µm particle size, 50 mm length and 2.1 mm. i.d. (Supelco, Bellefonte, PA, USA). Source temperature was 150 oC, desolvation temperature 450 oC. Detection by MS/MS was performed using electrospray ionization (ESI) operating in positive mode. For each compound the optimum collision energies with aim of getting two multiple reaction monitoring (MRM) transitions with the best signal intensity were selected.

The mobile phase components for the UHPLC system were methanol (A) and water (B), both modified with 0.1 % formic acid and 5 mM ammonium formate, applying suitable gradient program. The flow rate was 0.6 mL/min and the total run time 23.10 min.

Method validation

The optimized UHPLC-MS/MS method employing modified QuEChERS sample preparation was validated regarding the requirements laid down in SANCO Guidance document on analytical quality control and validation procedures for pesticide residue analysis in food and feed (SANCO, 2013). Accordingly, the method validation includes the following parameters: limits of detection and quantification (LODs and LOQs), linearity, precision and recovery. The range of determination was 5-250 ng/mL, the precision and accuracy was estimated at reporting level 10 µg/kg and five times reporting level i.e. 50 µg/kg.

RESULTS AND DISCUSSION

Optimization of the LC-ESI-MS-MS parameters

The chromatograms were recorded in MRM mode with ESI interface in positive ionization mode. The MRM analysis introduces high method specificity based on the detection of both parent ion and one of its known fragments (Sampaio et al. 2012). For each compound, the in-house optimized collision energies were selected with the aim of getting two characteristic MRM transitions with the best intensity (Table 1).

The main advantage of the method was the application of MS/MS since it provides high confidence level for identification of the target pesticides. The monitoring of the second fragmentation products allows more efficient discrimination of the interfering matrix, than the products of the primary fragmentation (Sampaio et al. 2012). The first MRM transitions exhibited higher signals and accordingly higher sensitivity, thus, they were used for quantification of the compounds examined. The second MRM transitions are so-called target ions and are mandatory for confirmatory purposes.

Table 1. MRM conditions for the simultaneous determination of pesticides with electrospray ionization in positive mode (dwell time 0.025 s)

Pesticide	Parent ion/(*m/z*)	Product ion/(*m/z*)	CV[a]/(V)	CE[b]/(eV)	RT[c]/(min)
Carbaryl	202.0	202>127.1 202>145.1	22 22	15 10	5.87
Carbofuran	222.1	222.1>123.1 222.1>165.1	80 80	20 5	5.47
Fenvalerate	420.4	420.4>145.1 420.4>219.2	12 12	32 10	5.72
Cypermethrin	165.1	165.1>55.0 165.1>72.0	46 46	18 30	5.38
Deltamethrin	506.9	506.2>281.0 506.2>92.9	38 38	14 62	1.87
Permethrin	183.0	183.0>109.0 183.0>155.0	40 40	18 8	1.14
Bifenthrin	440.4	440.4>181.1 440.4>165.9	18 18	16 54	2.15
Amitraz	294.0	294.0>181.1 294.0>211.0	28 28	24 12	2.06
Coumaphos	363.2	363.2>227.0 363.2>307.1	46 46	26 18	7.42
Bromopropylate	341.4	341.4>57.0 341.4>71.0	40 40	24 30	7.52
Dichlorvos	221.0	221.0>79.0 221.0>109.0	36 36	32 18	4.96
Diazinone	305.2	305.2>153.1 305.2>169.1	44 44	20 22	7.89
Malathion	331.2	331.2>99.0 331.2>127.0	28 28	24 12	9.07
Parathion	292.1	292.1>181.0 292.1>221.0	26 26	12 10	2.05
Dimethoate	230.0	230.0>125.0 230.0>199.0	24 24	22 10	3.05
Omethoate	214.0	214.0>155.0 214.0>183.0	26 26	16 12	1.08
Thiametoxam	292.2	292.2>181.1 292.2>211.0	26 26	24 12	2.08
Methomyl	163.1	163.1>104.6 163.1>122.6	36 36	24 16	3.78

[a]CV- cone voltage
[b]CE – collision energy
[c]RT – retention time

Method validation

To ensure that the optimized procedure was suitable for the application in routine analysis, the analytical performance parameters were determined and assessed. The obtained linearity (R2) in the range 5-250 ng/mL for all compounds was better than 0.99. The determined LODs and LOQ were lower than the reporting level 10 µg/kg, except in the case of coumaphos and thiametoxam where obtained LOQ was 11.4 and 12.2 µg/kg, respectively (Table

2). Furthermore, the obtained LOQ values were substantially lower than the established MRLs for pesticides in honey (EU Pesticide database; EC, 2010).

The recovery experiments exhibited values within the required range 70-120 %, laid down as acceptable in the SANCO document (2013), except for omethoate, whereas the obtained recovery was 152.7 and 181.2 % for 10 and 50 µg/kg, respectively. According to some previous investigations (Tomasini et al. 2012), a recovery value over 150 % is considered as high matrix effect. Such effect occurs when molecules co-eluting with the compounds of interest alter the ionization efficiency in reference to conditions in absence of matrix. Practically, it means that for accurate omethoate quantification we shall use matrix-matched calibration curve that will compensate the matrix effect. Optimized method exhibited satisfactory precision; the obtained RSD, for all compounds investigated, for both validation levels, was lower than 20 % (Table 2).

Table 2. Validation parameters for the pesticide compounds according to the SANCO requirements [9]

Pesticide	LOD/ µg/kg	LOQ/ µg/kg	Recovery at 10 µg/kg (RSD/%)	Recovery at 50 µg/kg (RSD/%)
Carbaryl	1.8	5.8	80.3 (8.5)	81.7 (8.4)
Carbofuran	2.5	8.1	79.7 (12.6)	89.4 (6.2)
Fenvalerate	1.4	4.7	91.1 (9.4)	84.6 (16.2)
Cypermethrin	1.9	6.2	81.3 (13.5)	91.8 (13.0)
Deltamethrin	0.5	1.7	86.9 (9.8)	77.6 (7.4)
Permethrin	1.6	5.2	87.0 (10.6)	85.0 (14.8)
Bifenthrin	2.3	7.6	76.9 (17.0)	75.0 (19.3)
Amitraz	1.8	6.0	91.7 (10.1)	80.7 (11.3)
Coumaphos	3.4	11.4	93.7 (13.2)	83.2 (7.3)
Bromopropylate	2.8	9.2	96.3 (12.4)	92.7 (7.6)
Dichlorvos	0.5	1.6	73.8 (9.5)	74.4 (6.9)
Diazinone	1.2	4.0	92.1 (13.7)	82.2 (5.8)
Malathion	0.9	3.9	97.1 (12.9)	104.1 (12.1)
Parathion	1.3	3.3	82.5 (8.8)	79.6 (5.7)
Dimethoate	2.4	7.8	84.1 (14.5)	81.0 (7.7)
Omethoate	3.0	9.7	152.7 (11.3)	181.2 (9.6)
Thiametoxam	3.7	12.2	100.2 (15.9)	102.4 (9.7)
Methomyl	2.0	6.6	75.7 (8.6)	83.4 (7.7)

The recovery experiments revealed values within the required range 70-120 %, laid down in the SANCO document (2013), except for omethoate, whereas the obtained recovery was 152.7 and 181.2 % for 10 and 50 µg/kg, respectively. According to some previous investigations (Tomasini et al. 2012), a recovery value of over 150 % is considered as high matrix effect. Such effect occurs when molecules co-eluting with the compounds of interest alter the ionization efficiency in reference to conditions in absence of matrix. Practically, it means that for accurate omethoate quantification we shall use matrix-matched calibration curve that will compensate the matrix effect.

Honey samples analysis

When analyzing real honey samples, internal quality control was implemented to provide the correct working of the method and the UHPLC-MS/MS system. A duplicate analysis of honey samples was accompanied with a recovery experiment within each batch, to check the extraction efficiency. Positive samples were quantified with reference to the four - level calibration curves obtained for each batch of samples. Furthermore, it enables avoiding adverse effects impact on the quantification process coming from the instrument. Each positive result (over the analyte reporting level) was corrected for the recovery rate obtained for each batch of samples.

The method was applied to 50 multifloral honey samples collected at individual beekeepers from different geographical locations in Macedonia. In these samples five different pesticides, namely bromopropylate, methomyl, diazinone, fenvalerate, dichlorvos, cypermethrin and omethoate were detected in concentration range between 6.9 and 26.5 μg/kg in 16 honey samples (32 %) (Table 3). The positive findings were confirmed by acquiring two transitions for quantification and identification in compliance with the EU guidelines (SANCO, 2013). The findings demonstrated that the method could be useful for analyzing pesticides in real samples of honey. Carbaryl, carbofuran, fenvalerate, deltamethrin, permethrin, bifenthrin, amitraz, coumaphos, malathion, parathion, dimethoate and thiametoxam were present in concentrations lower than the LOQ (Table 3).

Table 3. Positive findings of pesticides in 50 honey samples, using the proposed method.

Pesticide	Min (μg/kg)	Max (μg/kg)	Mean[a] (μg/kg)	MRL (μg/kg)
Cypermethrin	<6.2	24.6	16.5 (2)[b]	50
Bromopropylate	<9.2	21.5	15.8 (3)[b]	10
Dichlorvos	<1.6	11.5	9.2 (2)[b]	10
Diazinone	<4.0	26.5	24.0 (3)[b]	10
Fenvalerate	<7.8	10.6	-- (1)[b]	10
Omethoate	<9.7	16.3	14.0 (2)[b]	10
Methomyl	<6.6	24.1	18.5 (3)[b]	10

[a]mean value of positive findings
[b]number of positive findings

Bromopropylate, diazinone and methomyl were detected and confirmed over the limits of quantification at three samples (6 % positivity), cypermethrin, dichlorvos and omethoate at two samples (4 % positivity) and fenvalerate at one honey sample (2 % positivity). The determined concentrations for all positive findings for bromopropylate, diazinone, omethoate and methomyl (Table 3), exceeded the required reporting concentration level according to EU pesticide database, being 10 μg/kg. For dichlovos the respective level (10 μg/kg) was exceeded only at one sample, while for cypermethrin, the detected concentration levels for both positive samples were below the level of interest (50 μg/kg).

The presence of methomyl, fenvalerate and omethoate in the analyzed honey samples can be attributed to environment contamination since there are no known beekeeping practices using these pesticides (Bogdanov, 2006). On the other hand, bromopropylate and diazinone are known as used against parasites and diseases in the conventional beekeeping practice (Bogdanov, 2006; Fernandez-Garsia et al. 1994).

Likewise, other researchers reported no significant residues of insecticides in honey (Bogdanov, 2006; Naccari et al. 2014). Several investigations conducted on different types of honey and applying various analytical methods showed, instead, the presence of pesticides in significant extent (Rodríguez-López et al. 2014; Herrera et al. 2005; Mukherjee, 2009). In another study of honey with origin from Spain and Portugal (Blasco et al. 2003) most of the compounds found were chlorinated pesticides.

According to some previous publications (Bogdanov, 2006) the relatively low concentration of pesticides in honey seems to be due to a filtering effect of bees. Initially high pesticides concentrations in nectars are decreased approximately by factor of 1000. In addition, many of the modern pesticides used today are unstable and disintegrate quickly after use. Considering the fact that pesticide residues concentrations found in honey are comparable to the ones found in other foodstuffs (Kuchen et al. 1998) and the low honey inclusion in the diet, is can be concluded that pesticide residues found in honey within this study, are safe from toxicological point of view.

CONCLUSION

The QuEChERS in combination with UHPL-MS/MS method provides high-quality results, minimizes the number of analytical steps, and uses reagents in small quantities. The method showed good performance, which was verified through the method validation procedure. The achieved recoveries and precision were acceptable, and in line with the legislation requirements. Matrix-matched calibration curves should be used for accurate quantification of the positive findings. The data for pesticide findings from this study and the frequency of pesticide detection are an indication for the presence of both direct and indirect honey contamination. However, no significant amounts of investigated pesticides are detected, that could pose health risk to the consumers. This research is the first insight on the monitoring of residues of pesticides in honey samples from different geographical parts of Macedonia.

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Teerawong LAOSUWAN , Yannawut UTTARUK[1]

ESTIMATING ABOVE GROUND CARBON CAPTURE USING REMOTE SENSING TECHNOLOGY IN SMALL SCALE AGROFORESTRY AREAS

SUMMARY

The purpose of this study was to estimate the above ground carbon capture in small scale agroforestry areas of Inpang Community Forest Network, Sakon Nakhon Province, Thailand using remote sensing technology. This procedure was conducted from the satellite imagery Landsat 7 ETM+ information, which would be modified by atmospheric correction to verify it and generate Modified Soil Adjusted Vegetation Index 2 (MSAVI-2) along with model of determining Fractional Cover (FC) in order to determine the energy reflectance of vegetation index and fractional cover. The data obtained from the satellite through the above process were brought to determine the correlation with data from field surveys with statistical methods. The resultant equation was the Y = 0.0259e0.067X. Such equation could be used to calculate the above ground carbon capture in the areas of interest. The results of estimating carbon content and carbon equivalent of small scale agroforestry areas with total space of 749.71 rai represented above ground carbon capture volume of 6,171.99 tons per rai or 22,630.63 tons of carbon dioxide equivalents (Ton CO2 Equivalent).

Keywords: Above ground carbon capture, remote sensing technology, small scale agroforestry.

INTRODUCTION

Carbon dioxide is the most primary greenhouse gas in the atmosphere. From the estimated increase of carbon dioxide finding, it is found that Carbon dioxide (CO2) has tendency to rise to about 440-660 ppm by the year of 2050. The change in the carbon cycle in forests is the most important factor affecting the changes in greenhouse gas concentrations of the atmosphere in the atmospheric release and absorption of carbon dioxide (Wasun et al., 2010). The major reasons that cause climate change is modification of patterns of land use. In particular, the destruction of forest resources by 20 percent results in the loss of carbon storage in xylems (Office of Environmental Policy and Planning, 2000). Forests have ability that can absorb and store CO2, accumulating carbon in biomass of different parts. The parts of trees include the stem, branches,

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

leaves, and roots, which potentially accumulate CO2; it is depended on ecological system including wild species, the density of the forest, topography and environmental factors (Ogawa et al., 1965; Senpaseuth et al., 2009). To estimate the above ground carbon capture of forests is regarded as extremely important. It can be noted from the Kyoto protocol that has recognized the issues of Land Use, Land - Use Change and Forestry (LULUCF) as part of the reduction of greenhouse gas (IPCC, 2007; UNFCCC, 2010a; UNFCCC, 2010b; Chicago Climate Exchange, 2010; Laosuwan et al., 2011).

Remote sensing technology is considered as an important factor because the data from the satellite has evolved rapidly. In terms of record keeping and data analysis methods, The record system (Sensor) that has developed in terms of spatial resolution and spectral resolution causes a wide range of applications in various fields increases (Laosuwan, 2010; 2013). In addition, data from satellites today is well established that can be used in monitoring the dynamics of the natural and caused by human action in a timely manner. It can also be used in conjunction with Geographic Information System (GIS) effectively (Gomasathit et al., 2011; Laosuwan et al., 2011). Presently, remote sensing technology assists in the assessment of above the ground carbon capture. Satellite data can also be recorded because of the reflection of electromagnetic waves and simultaneous several wavelengths (Multi-spectral) that can take the properties of the wave, various electromagnetic. The satellite record estimates above-ground carbon capture in forest areas quickly (Lu et al., 2002; Schlerf et al., 2005; Patel et al.,2007; Samaniego et al.,2009; Kamusoko et al.,2009;Wang et al., 2011)]. This study aimed to estimate above ground carbon capture in small scale agroforestry areas of Inpang Community Network, Sakon Nakhon Province, Thailand using remote sensing technology.

MATERIAL AND METHODS

The setting and data collection

Scope of Setting

This study selected the small scale agroforestry areas of the farmers in Inpang Community Network, Sakon Nakhon Province, Thailand (Figure 1). As the pilot project in the province, the area was allocated in the boundary of 11 sub districts. There were 50 family farmers joining the program. The total small scale agroforestry area was 749.71 rai (1 hectare = 6.25 rai) or 1,199,536 m2.

Satellite Data

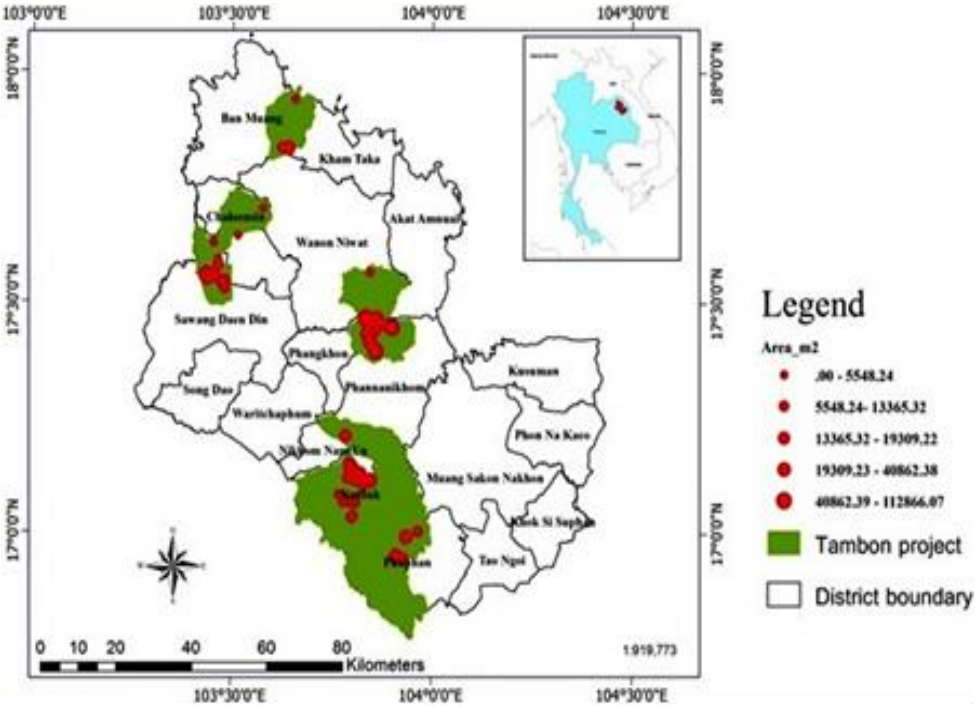
This study used data from the Landsat-7 satellite in the Enhanced Thematic Mapper Plus (ETM +) 2013 by The US Geological Survey. Data can be downloaded from the website http://glovis.usgs.gov/.

Data Analysis

The study was divided into two main steps below:

1) Analysis of satellite data from Landsat-7 ETM +.

2) Exploration and field data to determine the amount of carbon in the plot area.



The final step will be to determine statistical correlation based on satellite Landsat-7 ETM + data and field survey data to calculate the carbon content per area.

Figure 1.Inpang Community Network, Sakon Nakhon Province, Thailand

Analysis of Satellite Data from Landsat-7 ETM +

(1) The satellite data from Landsat-7 ETM+ data downloaded from the USGS had errors caused by failed sensors on satellites. The crash of such information was called "Scan Line Corrector (SLC) failed". Thus, it is necessary to make adjustments to the data using the Gap-filling. In this study, the researcher recreated a tool to fix Gap-filling on the program ERDAS (Spatial Modeler Language: SML).

(2) Adjustments of Atmospheric Correction ensured the accurateness of the information conducted furnished by the act in two processes: 1) The process of making Convert Digital Number to Radiance Values and 2) the process of making Convert Radiance to TOA Reflectance. The study was modeled in two

steps above mentioned on the program ERDAS (SML) by creating such models based on Equation 1 and Equation 2 below.

$$L_\lambda = Grescale * Qcal + Brescale \quad (1)$$

Where;

$$Grescale = \frac{LMAX_\lambda - LMIN_\lambda}{Qcalmax - Qcalmin}$$

$$Brescale = LMIN_\lambda - [LMAX_\lambda - LMIN_\lambda / Qcalmax - Qcalmin] \times Qcalmin$$

Where;

L_λ = Spectral radiance at the sensor's aperture [W/(m^2 sr µm)]

$Qcal$ = Quantized calibrated pixel value [DN]

$Qcalmin$ = Minimum quantized calibrated pixel value corresponding $LMIN_\lambda$

$Qcalmax$ = Maximum quantized calibrated pixel value corresponding $LMAX_\lambda$

$LMIN_\lambda$ = Spectral at sensor radiance that is scaled to $Qcalmin$ [W/(m^{2}sr µm)]

$LMAX_\lambda$ = Spectral at sensor radiance that is scaled to $Qcalmax$ [W/(m^{2}sr µm)]

$Grescale$ = Band specific rescaling gain factor [(W/(m^2 sr µm))/DN]

$Brescale$ = Band specific rescaling bias factor [W/(m^2 sr µm)]

$$\rho_\lambda = \frac{\pi \times L_\lambda \times d^2}{E_{SUN_\lambda} \times \cos\theta_s} \quad (2)$$

Where;

ρ_λ = Unitless planetary reflectance

π = 3.14

L_λ = Spectral radiance at sensor's aperture (Wm^{-2} sr^{-1}µm^{-1})

d = Earth-sun distance in astronomical units

E_{SUN_λ} = Mean solar exoatmospheric irradiances

θ_s = Solar zenith angle

(3) The results of the data analysis in (2) were used for determining the energy reflectance of vegetation index with MSAVI-2, setting up the pixel of satellite data from Landsat-7 ETM +. If greater than 0 to 1, it would be the vegetation. If the value was equal to or less than 0 and 0, it was non-vegetation. Moreover, the finding of FC was to set up pixels to classify plants that were from 0 to 100. Also, such model was based on Equation 3 and Equation 4 below.

$$MSAVI-2 = \frac{(2NIR+1) - \sqrt{(2NIR+1)^2 - 8(NIR - \mathrm{Re}\,d)}}{2} \quad (3)$$

Where;
MSAVI-2 = Vegetation index
NIR = Near infrared band reflectance (0.76 – 0.90 µm)
RED = Red band reflectance (0.60 – 0.70 µm)

$$FC = \frac{(VI - VI_{open})}{\left(VI_{canopy} - VI_{open}\right)} \times 100 \quad (4)$$

Where;
FC = Tree canopy fractional cover
VI = Vegetation index
VI_{open} = Vegetation index of open areas
VI_{canopy} = Vegetation index of tree canop

Field Survey to Determine the Carbon Content in the Plot Areas

(1) Selection and creation of permanent plot

- Stratified method was used for areas selection, the knowledge from farmers who were members of the community, and then stratified random method was used to be a group study case. Then randomly select permanent plot according to the species or group of species cultivated plants or by age of transplant or agroforestry management system whose property is similar.

- The researchers made 20m x 25m of permanent sample plot in 50 plots in small scale agroforestry areas in order to assign 50 permanent plots as representatives of the study area of 749.71 rai. Later, the researcher collected data on types and amounts of large plants (wood with Diameter at Breast Height (DBH) ranging from 4.5 cm or more) as well as measured the height of plants by using clinometers. Data obtained from the survey would be recorded in a record form.

(2) Estimation of Carbon Capture

The results of the data obtained from (1) were used to calculate the biomass of permanent plots using allometric equation for agroforestry plants in Thailand (Usa et al., 2011). Such method was used to estimate the above ground carbon capture as shown in the equation 5.

$$\begin{aligned} Ws &= 0.0389\left(D^2H\right)^{(0.9417)} \quad r^2 = 0.9106 \\ Wb &= 0.0678\left(D^2H\right)^{(0.6618)} \quad r^2 = 0.8347 \\ Wl &= 0.0084\left(D^2H\right)^{(0.7660)} \quad r^2 = 0.9109 \end{aligned}$$

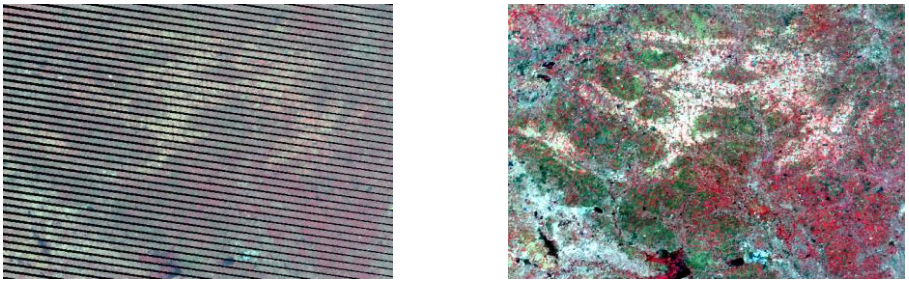
(5)

Where;
Ws = Stem; Wb = Branch; Wl = Leaf

RESULTS AND DISCUSSION

Analysis of Satellite Landsat-7 ETM +

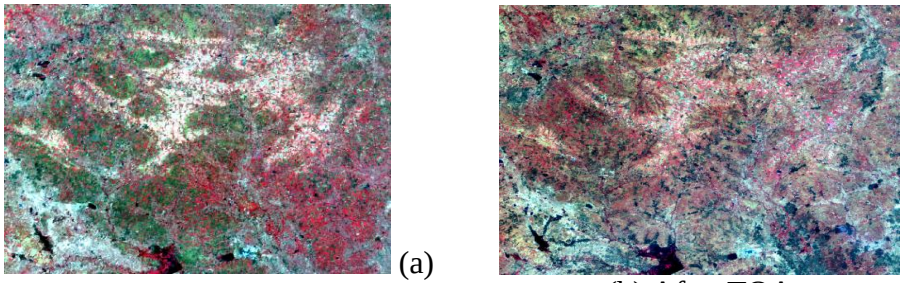
(1) Results from the gap-filling to fix the failure of the scan line corrector (SLC) resulted in visual continuity of satellite data. The results of the gap-filling were shown in Figure 2.



(a) Before Gab-filling (b) After Gab-filling

Figure 2. Gap-filling method

(2) Developing a model of Atmospheric Correction to ensure the accuracy of the data was shown in Figure 3.



(a) Before TOA (b) After TOA

Figure 3. Atmospheric Correction (TOA)

(3) The results of determining the vegetation index with MSAVI-2 model and results of adjustment with the FC model were shown in Figure 4.

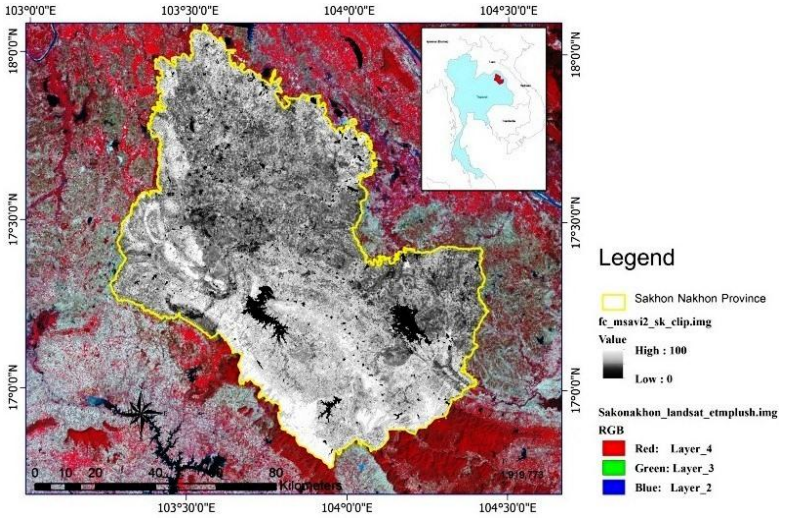


Figure 4. FC model

Results of the field survey

For 50 permanent plots in the study area, the researcher measured the height of trees with 4.5 cm or above and DBH of trees at a height of 130 cm. Then the researcher recorded species of plants and heights of trees. Later, the above ground biomass was analyzed. However, the relationship between the FC and the carbon content could be shown in Figure 5. From the figure, it was found that when the number of FC was high, the carbon content increased as well. This has led equation $Y = 0.0259e^{0.067X}$ to represent the model. The result was shown in Figure 6, in which red color indicated the low volume of carbon capture and green color indicated the high volume of carbon capture respectively.

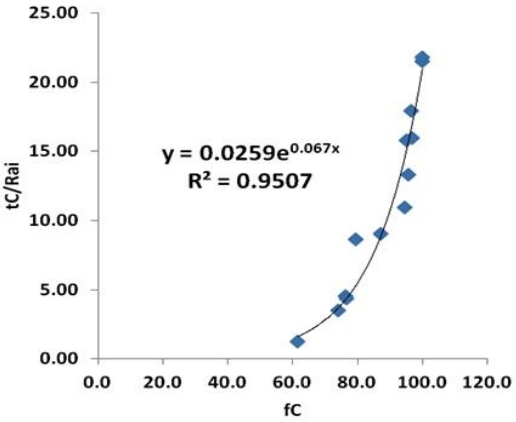


Figure 5. Relationship between the FC and the carbon content

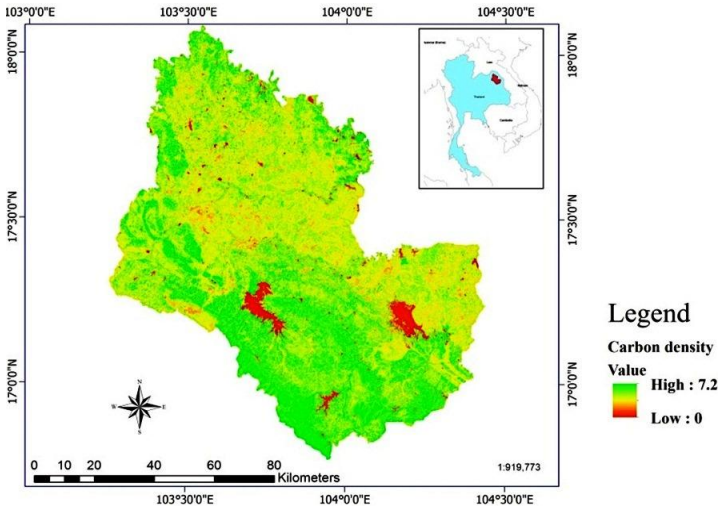


Figure 6. Carbon density

When the equation $Y = 0.0259e^{0.067X}$ was used to calculate the amount of above ground carbon capture per area (ton per rai) using the FC value from satellite Landsat 7 ETM +. The total size of small scale agroforestry areas of 749.71 rai found that the area with lowest density contained carbon capture at 1.00 ton per rai; the area with highest density contained carbon capture at 21.04 ton per rai. The mean was 8.87 ton per rais. The accumulation of carbon content was equivalent to 6,171.99 ton per rai and calculated carbon content was equal to 22,630.63 ton of carbon dioxide equivalents (Ton CO2 Equivalent). The plot with the lowest above ground carbon capture had 1.11 ton or 4.06 ton of carbon dioxide equivalents. The area with the highest above ground carbon capture had 547.76 ton per rai or 2,008.44 ton of carbon dioxide equivalents. The average above ground carbon capture was 123.44 ton or 452.61 ton of carbon dioxide equivalents.

In addition, the researcher has concluded results of the amount of carbon that could be analyzed with data from the satellite Landsat 7 ETM +, compared with data from field surveys, data from a total of 50 plots with Paired Sample t-test. The results showed a correlation of 0.926, with statistically significance indifference of 0.01. This indicated that the application of the data from the satellite Landsat 7 ETM + to estimate above ground carbon capture in the areas of Inpang Community Network, Sakon Nakhon Province, Thailand was very reliable.

CONCLUSIONS

The study on estimating above ground carbon capture in the area of small scale agroforestry of Inpang Community Network, Sakon Nakhon Province, Thailand by using the remote sensing technology can be concluded as follows. The total small scale agroforestry areas used was 749.71 rai, which enabled them

to capture carbon content of 6,171.99 ton per rai or 22,630.63 ton of carbon dioxide equivalents. The small scale agroforestry areas are considered as the good reservoirs of carbon content because there are a few large-sized trees. This means that the small-sized to medium sized trees in the small scale agroforestry areas can grow further if local people in the areas help enrich them. The small-sized to medium sized trees in these areas have potential to accumulate the carbon content than large-sized trees because they have lower rate of growth. Additionally, some related organization and interested people can use the results of this study to be applied on the estimation of above ground carbon capture in the other areas of small scale agroforestry in Thailand.

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STATISTICAL ASSESSMENT OF THE IMPACT OF NANO-CHELATED ELEMENTS AND SULFUR ON CHICKPEA PRODUCTION UNDER SUPPLEMENTAL IRRIGATION

SUMMARY

Chickpea is an important source of plant protein source and has a major role at people nutrition in semi-arid regions. Soils of these regions have high pH and low organic matter, which reduce the availability of most micronutrients. In order to investigate the effects of application of sulfur (0, 15, 30 kg ha^{-1}) and three nano-chelated micronutrients (nano-Zn, nano-Fe and nano-Mn) on yield and some morphological traits of chickpea, a field experiment was conducted. Day to maturity (DM), first pod height (FPH), primary branch per plants (PBP), secondary branch per plant (SBP), number of pods per plant (NPP), number of empty pod per plant (EPP), number of seeds per plant (NSP), seed yield (SY), straw yield (ST), biological yield (BY), harvest index (HI), and 1000 seed weight (TSW) were measured. Results showed that the first two principal components (PC1 and PC2) were used to create a two-dimensional treatment by trait (TT) biplot that accounted percentages of 53% and 26% respectively of total variation. The vertex treatments in polygon of TT biplot were S1-Nano1, S1-Nano2, S1-Nano3, S2-Nano1, and S3-Nano1 which S3-Nano1 treatment combination indicated high performance in DM, FPH, PBP, SBP, NPP, NSP, SY, ST, BY and TSW. According to ideal treatment biplot, the S3-Nano1 (30 kg ha^{-1} sulfur plus nano-chelated zinc) might be used in selecting superior traits and it can be considered as the candidate treatment for chickpea production. Treatment combinations which are suitable for obtaining of high seed yield performance were identified in the vector-view biplot and showed S3-Nano1 as the best treatment suitable for obtaining of high seed yield. In conclusion, application of nano-fertilizer could increase crop yield and improve the fertilizer efficiency.

Keywords: Nano-manganese, Nano-iron, Nano-zinc, micronutrients.

INTRODUCTION

Chickpea is a food legume, which ranks third after beans and field peas among the world's pulse crops and is grown in a wide range of environments in many countries around the world because it is a rich source of quality protein for a majority of the population (FAO, 2014). It is cultivated on an area of about 12

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million hectares with an annual production of 9.5 million tones and an average productivity of 880 kg ha1 while in Iran, it is cultivated on 550 thousand hectares annually producing 300 thousand tons with an average productivity of 540 kg ha-1 (FAO, 2014). The country ranks sixth after major chickpea producing countries include India, Australia, Pakistan, Turkey and Myanmar. One of the main reasons for chickpea low productivity in Iran is cultural management such as application of fertilizers (Johnson et al. 2005). Micronutrients are needed in very small quantities for optimum crop growth and play a vital role in human health while the lack of trace elements or imbalance among them may result in growth suppression or even inhibition of crop production (Farooq et al. 2012). Fertilizers have a fundamental role in improving the productivity of crops but the nutrient use efficiencies of conventional fertilizers is relatively low and relatively few investigations have analyzed the application of the new micronutrient fertilizers on chickpea and agronomic managements for improving crop productivity (Subramanian et al. 2015).

Among different micronutrients, the deficiencies of zinc (Zn), iron (Fe) and manganese (Mn) are the most important yield-limiting factors in semi-arid regions because the availability of most of micronutrients is greatly depends on soil pH because high pH cause to unavailability of trace elements such as iron, manganese, zinc and sulfur application can be suitable option for solution of this problem (Plaster, 2013). Some studies showed that the soils of rainfed areas in arid and semi-arid environments are deficient in sulfur (Khalid et al. 2011; Islam, 2012). Also, information regarding effects of sulfur on micronutrients especially Zn, Fe and Mn availability and uptake by crop plants is very scarce. Zinc deficiency is common throughout the world and lack of Zn can limit the growth and productivity of a wide range of crops (Harris et al. 2008). Fertilizers containing Zn are commonly added to soils where necessary and it can also be effective to use foliar sprays. Mn deficiency is common in some soils and chickpea is the most sensitive on Mn deficiency and chloroplast ranks first among the organelles to Mn deficiency, which this deficiency symptoms was inversely related to root length while it is associated to the 100-grain weight (Bozoglu et al. 2008). The role of Fe as an essential nutrient and its function in metabolism have been investigated in detail by some investigations (Marschner, 1995; Fox et al. 1998), however, it is abundant in most soils especially under alkaline or calcareous conditions. Therefore, Zn, Fe and Mn micronutrients and sulfur are essential elements for crop growth because it can affect the crop development and plays some critical rules in crops and given the importance of chickpea, it seems that one way of improving the its low productivity can be application of new nano-fertilizers micronutrients.

Nanoparticles are one potential output that could be a major innovation for agriculture (Derosa et al. 2010). Nano-fertilizers could be more soluble or more reactive than bulk fertilizers and they can exactly release their active ingredients in responding to environmental triggers (Mastronardi et al. 2015). Nano-sized nutrients could be perhaps predicted to have a considerable effect on fertilizer

efficiency and crop productivity. However, beneficial aspects of nano-particles have now been explored by researchers in different fields (Nietzold and Lisdat, 2012). The importance of nano-fertilizers for improving saffron yield was reported also by Amirnia et al. (2014), who found that application of iron nano-particles enhanced chlorophyll, carbohydrate, essential oil, fresh weight and dry weight, and foliar spray treatment was found to be more effective than soil addition of nano-fertilizer. According to Soliman et al. (2015), iron nano-particles applied in the form of spray on soybean increased dry weight. The present field survey was conducted to investigate the effect of sulfur fertilizer and three nano-form of zinc, iron and manganese on yield and some other traits of chickpea crop in rainfed condition of semi-arid area under supplemental irrigation.

MATERIAL AND METHODS

A field experiment was conducted using cultivar Kakaie of chickpea at Takab district (47°70' E; 36°23' N), Northwest of Iran with an average annual rainfall of 340 mm and mean annual temperature of 12.3 °C. The trial was performed in a split-plot experiment according to randomized complete block design with three replication (plot size of 2 ×2 m^2) keeping sulfur in main plots and nano-chelated micronutrient in sub plots. The soil texture was sandy loam, with 7.8 pH, with0.44% organic carbon, 0.044% N, 4.34 mg. kg^{-1} P, and 227 mg. kg^{-1} K and the recommended NPK fertilizer was 30 kg N and 75 kg P_2O_5 ha^{-1}. Sulfur fertilizer was applied in three levels (S1: no application, S2:15 Kg ha^{-1}, S3:30 Kg ha^{-1}) were mixed with top soil and nano-chelated micronutrients were including N1: nano-chelated zinc, N2: nano-chelated iron, and N3: nano-chelated manganese. Nano-chelated fertilizers were applied at rate of 1 kg ha^{-1} through fertigation 30 and 60 days after sowing date. Nano chelate fertilizers were obtained from the Sepeher Parmis Company, Iran, which contained zinc oxide, ferric oxide and manganese (II) oxide nanoparticles with morphological properties which were characterized by scanning electron microscope (Figure 1).

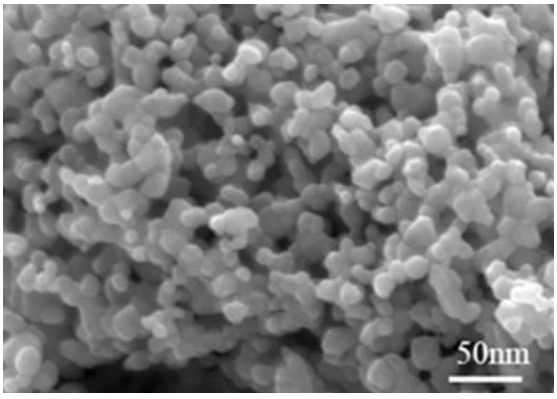

Figure 1. Scanning Electron Microscope (SEM) image of synthesized nanoparticles of zinc oxide utilized for nano-chelated fertilizer.

Chickpea was sown manually in the third week of April in 10 rows, at 20 cm row-to-row spacing and 8 cm plant-to-plant spacing. Two supplemental irrigations applied during flowering and grain filling stages. The amount of irrigation water was calculated to restore water content in the root zone to field capacity. Weeds were controlled by frequent hand weeding and. Traits day to maturity (DM) was recorded for each experimental plot. Plants were harvested by hand at June and some agronomic traits including first pod height (FPH), primary branch per plants (PBP), secondary branch per plant (SBP), number of pods per plant (NPP), number of empty pod per plant (EPP), and number of seeds per plant (NSP) were recorded on 10 randomly selected plants in each plot. Seed yield (SY), straw yield (ST) and biological yield (BY) were determined by harvesting the middle three rows of each plot after avoiding border effects and harvest index (HI) of each plot was calculated according to the ratio of seed yield to biological yield. The 1000 seed weight (TSW) was measured after harvesting and drying from three random sample of each plot. The two-way layout of treatment × trait (TT) biplot model is used according to (Yan and Rajcan, 2002) via GGEbiplot software (Yan, 2001).

RESULTS AND DISCUSSION

The analysis of treatment × trait interaction through biplot analysis indicated that the first and second principal components (PC1 and PC2) analysis together could explain 79% of the total variation. The polygon-view biplot (Figure 2), indicated the nine treatment combinations (3 sulfur levels × 3 nano-chelate fertilizer levels) fell into three sections of five possible sections and the measured traits could be grouped into three groups, suggesting that number of empty pod per plant (EPP) and harvest index (HI) could be identified as different traits from the other remained traits. The vertex treatment combinations were S3-Nano1, S1-Nano2, S1-Nano3, S1-Nano1, and S2-Nano1 which S1-Nano3 treatment had better performance in EPP; S1-Nano2 treatment had better performance in HI. Also, S3-Nano1 treatment indicated high performance in day to maturity (DM), first pod height (FPH), primary branch per plants (PBP), secondary branch per plant (SBP), number of pods per plant (NPP), number of seeds per plant (NSP), seed yield (SY), biological yield (BY), and 1000 seed weight (TSW) traits (Figure 2). Chickpea responded positively to the Zn application (Roy et al. 2006) and Brennan et al. (2001) reported the relative response of chickpea to Zn application is greater than that of other crops. As with other pluses, nano-Zn application resulted in more vegetative growth (Singh et al. 1992), leading to higher dry matter production and greater seed yield. Also, Zn application increased significantly chickpea growth and development (Khan et al. 2000) and thus at maturity plants fertilized with nano-Zn had a greater total dry weight.

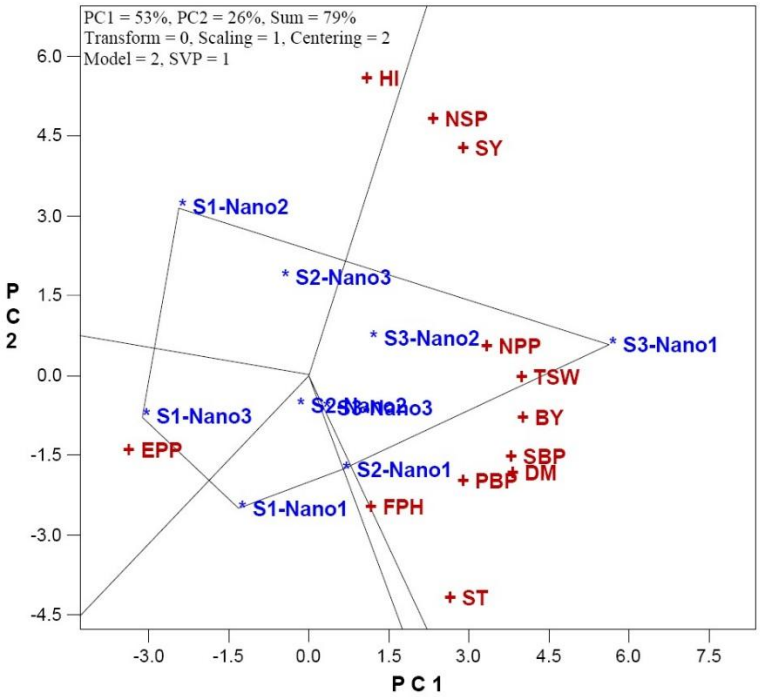

Figure 2. Polygon view of TT biplot showing which fertilizer treatment combination had the highest values for which traits

The vector-view biplot indicates the relationships among traits by the angles between the vectors of traits, which are the lines that connect each trait point with the origin point of the biplot and the cosine of the angle between vectors approximately represents the correlation between two traits, and an acute angle indicates a positive correlation, otherwise a negative correlation between the two traits (Yan and Tinker, 2006). The NPP, TSW, BY, SBP, DM and PBP traits are positively correlated because of the acute angles among their vectors (Figure 3). Also, ST with FPH, and NSP, SY and HI were positively correlated due to their acute angles. A near zero correlation between ST and FPH with EPP, between NSP, SY and HI with HI, between EPP, and between NSP, SY and HI with NPP, TSW, BY, SBP, DM and PBP as indicated by the near perpendicular vectors (Figure 3). Also, a negative association between NSP, SY and HI with ST and FPH and between EPP with NPP, TSW, BY, SBP, DM and PBP as indicated by the large obtuse angles (Figure 3). These results are in good accordance with those reported by Noor et al. (2003). According to Yucel et al. (2006) the chickpea yield showed positive correlation with first pod height, number of secondary branch, number of total full pods, number of full pods per plant, and number of seeds per plant. The main purpose of farmers is to achieve high yield in chickpea which its components are multi-genic traits, and are influenced by the environmental factors such as fertilizer application. The TT biplot analysis of

chickpea's dataset shows visual interrelationships among the traits, which provides more information in comparison to correlation coefficients that only describe the relationships between two traits (Janmohammadi et al. 2015). Most of the above results can be grasped from the simple correlation coefficients (Table 1), but some others are not consistent with them and such discrepancies are observed because the TT biplot explained lower than 100% (in present study, 79%) of the variation

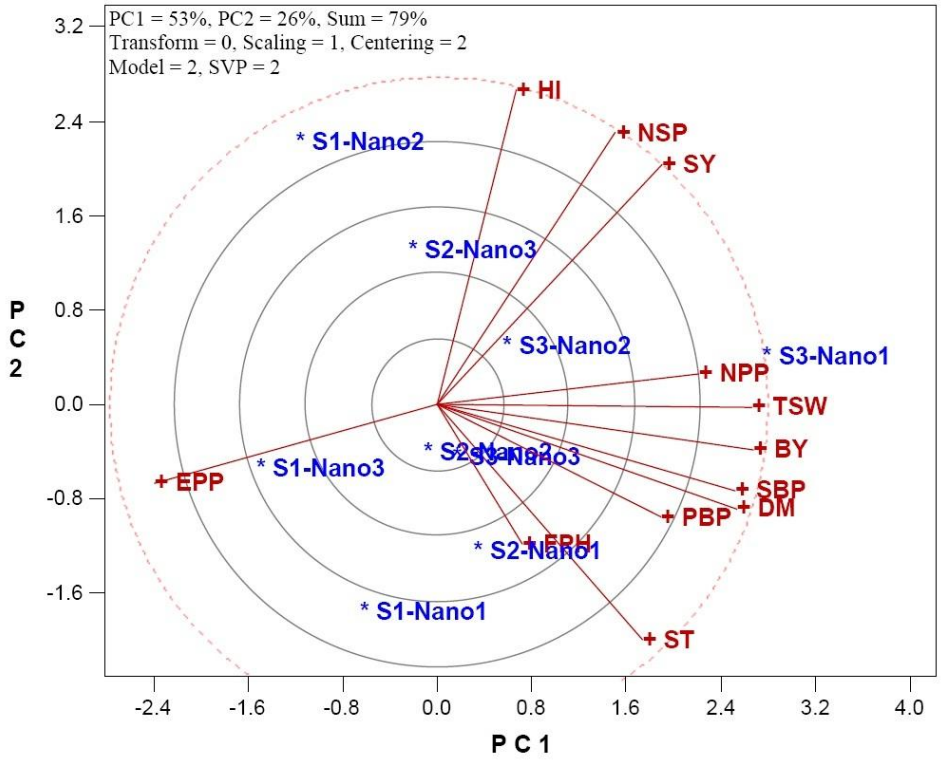

Figure 3. Vector view of TT biplot showing the interrelationship among measured traits under different fertilizer treatment combinations.

The ideal trait is a tester that is most discriminating and representative among all traits (Yan and Tinker, 2006) and the center of concentric circles on the average tester coordinate indicates the ideal trait (Figure 4). The distance from the ideal trait to the biplot origin is equal to the longest vector of all traits and so TSW might be used in selecting superior treatments, which it could be useful in achieving high performance via application of different fertilizer treatments. In other word, TSW is good indicator for discriminant among different sulfur and nano-chelated fertilizer treatments. The ranking of the other traits based on ideal trait were: NPP > BY > SBP > DM > PBP. The concept of ideal treatment is the entry that is most favorable treatment among all treatments (Yan and Tinker, 2006) and it has been shown that the distance between one

treatment and the ideal treatment is a more repeatable parameter to evaluate the treatments performance.

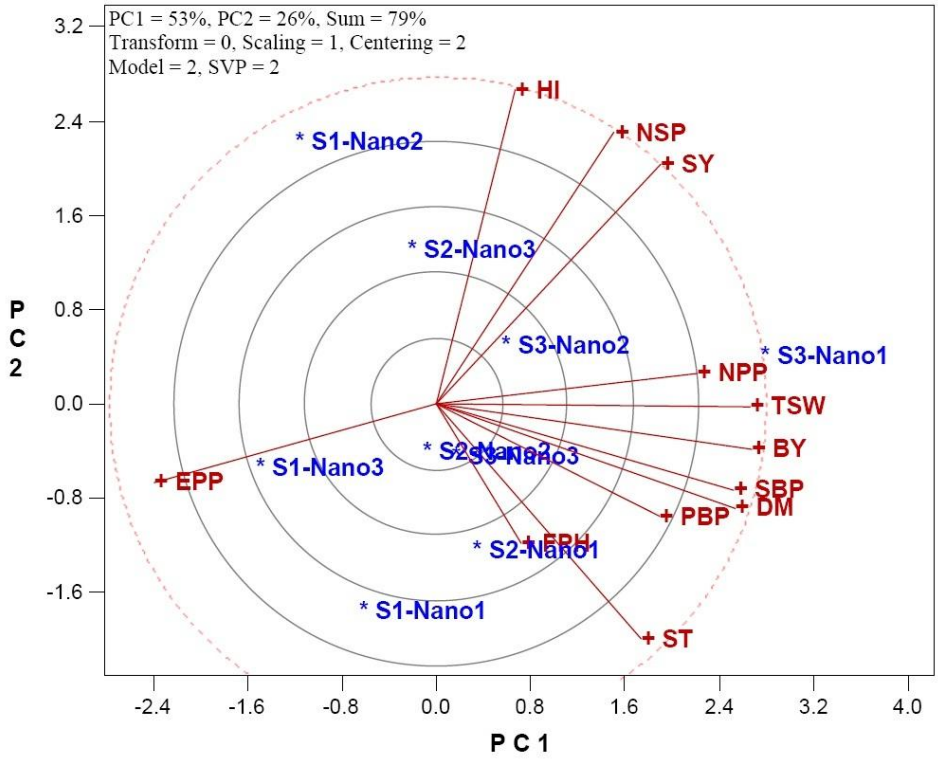

Figure 4. Ideal entry view of TT biplot, showing the relationships of different fertilizer treatment combinations with ideal entry.

In a TT biplot, the center of the concentric circles on the average tester coordinate indicates the ideal treatment (Figure 5), which is equal to the length of treatment vector with the highest performance and so the distance between the ideal treatment and the biplot origin is equal to the longest vector among all treatments. Thus, the S3-Nano1 (30 kg ha-1 sulfur plus nano-chelated zinc) might be used in selecting superior traits and it can be considered as the candidate treatment. The performance of measured traits of chickpea under application of S2-Nano1 (15 kg ha-1 sulfur plus nano-chelated zinc), S3-Nano2 (30 kg ha-1 sulfur plus nano-chelated iron) and S3-Nano3 (30 kg ha-1 sulfur plus nano-chelated manganese) treatment combinations were observed above average while the other treatments (S1-Nano1, S1-Nano2, S1-Nano3, S2-Nano2 and S2-Nano3) were below average (Figure 5).

Suitable treatment combinations for obtaining of high seed yield (SY) of chickpea could be identified in the biplot of Figure 6 which is a vector-view function of TT biplot model and shows treatments that have close association with a target trait among other traits. Based on this type of biplot, S3-Nano1 (30

kg ha-1 sulfur plus nano-chelated zinc) treatment combination was identified as optimal fertilizer treatment suitable for obtaining high seed yield.

Table 1. Pearson's simple correlation coefficients among chickpea traits.

	FPH	PBP	SBP	DM	NPP	EPP	NSP	TSW	SY	BY	ST
FPH											
PBP	0.17										
SBP	0.40	0.82									
DM	0.34	0.79	0.86								
NPP	0.24	0.32	0.49	0.74							
EPP	-0.18	-0.29	-0.66	-0.63	-0.82						
NSP	-0.17	0.09	0.29	0.17	0.46	-0.66					
TSW	0.11	0.64	0.82	0.84	0.65	-0.68	0.48				
SY	-0.10	0.23	0.45	0.38	0.52	-0.68	0.93	0.66			
BY	0.19	0.57	0.86	0.83	0.67	-0.78	0.40	0.95	0.55		
ST	0.31	0.51	0.68	0.70	0.41	-0.41	-0.23	0.63	-0.10	0.77	
HI	-0.24	-0.08	0.02	-0.04	0.22	-0.35	0.87	0.22	0.87	0.06	-0.59

Critical values of correlation $P<0.05$ and $P<0.01$ (degrees of freedom = 7) are 0.67 and 0.75, respectively.

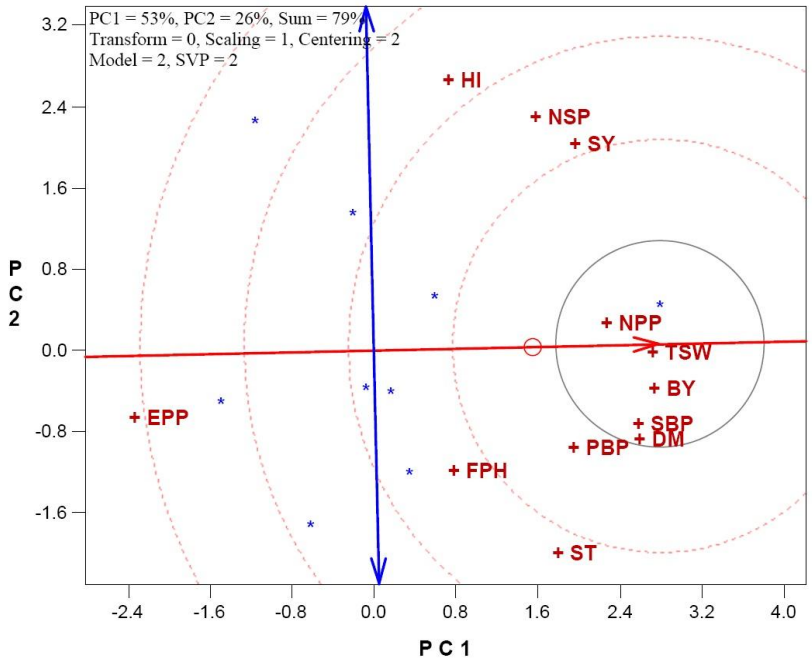

Figure 5. Ideal tester view of TT biplot, showing the relationships of different traits with ideal tester.

Traits were: day to maturity (DM), first pod height (FPH), primary branch per plants (PBP), secondary branch per plant (SBP), number of pods per plant (NPP), number of empty pod per plant (EPP), number of seeds per plant (NSP), seed yield (SY), straw yield (ST), biological yield (BY), harvest index (HI) and 1000 seed weight (TSW).

Thus, application this treatment is expected to lead to improved seed yield under supplemental irrigation conditions in semiarid environment. The ranking of the treatment combinations based on high performance of SY were: S3-Nano2 > S2-Nano3 > S1-Nano2. The best treatments combinations for obtaining of high TSW of chickpea could be determined from the vector-view biplot of Figures 3 and 6, and it indicates treatments that have close association with a target trait among other traits. According to biplot of Figures 3 and 6, S3-Nano1 (30 kg ha-1 sulfur plus nano-chelated zinc) treatment combination was identified as optimal fertilizer treatment suitable for obtaining high TSW and the ranking of the other treatment combinations based on high performance of TSW were: S3-Nano2 > S2-Nano1 > S2-Nano2 > S3-Nano3. This suggests that using nano-sized micronutrient fertilizer will not only result in the development of high seed yield but also cause to achieving desirable TSW trait as well as the other yield components that enhance wide use of such treatment.

Bala et al. (2014) have observed beneficial role of nano-fertilizer application in chickpea crop growth due to increase in activity of growth hormone gibberellins. Liu et al. (2010) found that nano-particles were safe for seed germination and growth of wheat seedlings and also conclude that use of nano-sized fertilizers is useful in crop production. Kharol et al. (2014) showed that application of sulfur and zinc increased the yield performance of chickpea and application of sulfur recorded fifty percent higher in seed yield over control treatment. Due to widespread deficiency of sulfur and Zn in the semiarid regions because of poor (Srinivasarao et al. 2006) and depletion under continuous cropping without application of these nutrients (Rego et al. 2007), their application caused to significant increase in most traits of chickpea. Our results clearly demonstrated significant seed yield responses of different rainfed crops due to application of Zn and sulfur. The deficiencies of Zn and sulfur nutrients assume critical importance for increasing and sustaining chickpea crop productivity of rainfed conditions.

It could be concluded that the TT biplot analysis is excellent graphical tool for visual data analysis and similar reports demonstrated that its efficiency for visualizing treatment-by-trait data and revealing the interrelationships among traits (Peterson et al. 2005). The TT biplot provided a proper tool for visual comparison among treatment combinations on the basis of multiple traits and it effectively revealed the interrelationships among the treatment combinations (sulfur levels in nano-fertilizers). Yan and Kang (2003) suggested that, if there are no clear cut tester by entry pattern, a TT biplot based on values across all treatments should be suffice and if there are clear groups of treatments, TT biplot should be constructed and studied for each groups of treatments.

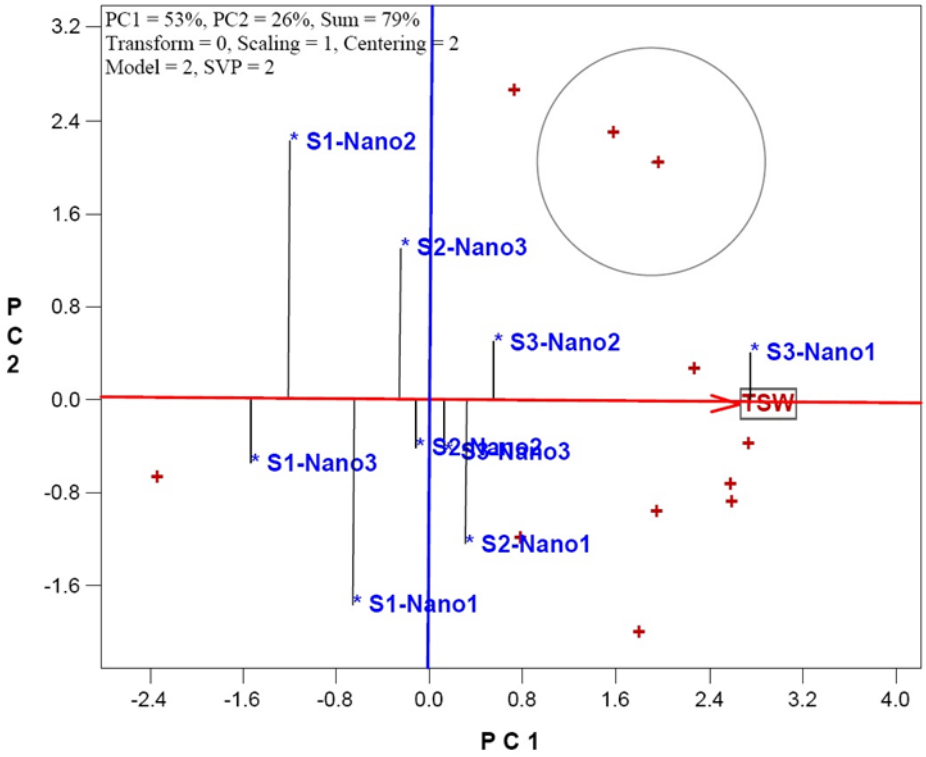

Figure 6. Vector view of TT biplot, showing the relationships of different fertilizer treatment combustions with target trait (TSW, thousand seed weight).

CONCLUSIONS

This research indicated that nano-chelated Zn and sulfur application increase seed yield, primarily due to an increase in the number of seeds per plant, secondary due to an increase in the harvest index, tertiary due to an increase in the number of pod per plant. High levels of sulfur fertilizer (30 kg ha-1) and nano-Zn can cause a significant increase in seed yield and 1000 seed weight. Nano-Zn and sulfur application can increase the yield of chickpea cultivated in semiarid soils with supplemental irrigation.

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IMPACT OF CLIMATE FACTORS ON YIELD AND QUALITY OF VINE VARIETY CABERNET SAUVIGNON IN PODGORICA WINE GROWING REGION

SUMMARY

The influence of climatic factors on grape yield, grape cluster weight, sugar and acidity content in stum in variety Cabernet Sauvignon was measured in period from 2011 to 2013 at the experimental field of Biotechnical Faculty in Podgorica. The study was conducted in the vineyard of the Biotechnical Faculty in Podgorica in the stage of full grape maturity.

The highest grape yield as well as the cluster weight were measured in 2012 (1.2 kg/m^2 and 125 g respectively), while the lowest values were measured in 2013 - 0.88 kg/m^2 and 92 g. Highest sugar content in stum was measured in 2011, and lowest in 2013. The highest acid content was measured in 2013 (6.50 g/l) as a result of heavy rainfall during the growing season, especially in August and September. Differences between the studied parameters were statistically significant.

The results showed that the yield and quality of grapes were in direct relation with the weather conditions in certain years of experiments.

Keywords: Cabernet Sauvignon, climatic factors, yield, quality of grapes.

INTRODUCTION

Due to favourable soil and climatic conditions Podgorica wine growing region was always considered an area very suitable for vine growing. However, the climatic parameters are significantly fluctuating leading to deterioration of usual weather conditions in recent years. There are many indicators confirming that the climate in this wine growing region is changing rapidly: the number of days with tropical temperature is rising, periods of drought are longer and more frequent especially during the summer months, the number of days without rain is increasing, but also the intensity of precipitation. Precipitation is particularly intense during the winter months and often has flood character (Micev, 2014).

Climate change is taking place in every part of the globe in a greater or lesser extent - from the equator to the poles (Gearheard et al., 2010). The consequences of climate change are evident in many viticulture areas of the

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world and predominantly manifested in the changes occurring in the phenophases of vines, as well as the harvest date (Jones et al., 2007). Although Montenegro, as a small geographical area, does not affect the global climate change, it does increasingly suffer from its negative impact, and consequently vine production as well.

Numerous studies worldwide predict that extreme future weather conditions will be more frequent, and negative impact on agricultural production will be more noticeable. All listed above impose a constant need for research of impact of changing climate on agro-technological and economical characteristics of the dominantly cultivated varieties of vine in Montenegro, and therefore the variety Cabernet Sauvignon as well, which is one of the leading vine varieties for the production of high quality red wines. However, it should always be considered that in addition to agro ecological conditions (relief, exposure, temperature sums, radiation, physical and mineral properties of soil), agro biological, economical and technological properties of grape vines are significantly affected by the level of applied ampelotechnics, the type of rootstock, growth form, the pruning technique and many others. (Brighenti et al., 2010; Van Leeuwen and Seguin, 1994).

The aim of this study is to evaluate the impact of major climatic parameters on the yield and quality of grape variety Cabernet Sauvignon, especially during the vegetation season in Podgorica wine growing region.

MATERIAL AND METHODS

The study of the influence of climatic factors on the yield and quality of grape variety Cabernet Sauvignon was conducted in 2011, 2012 and 2013. The study was performed at the experimental field of the Biotechnical Faculty in Podgorica. Experimental vineyard was planted in 2005 with the planting distance of 2.5 m between rows and 1 m within rows. Growing form of vine is double horizontal cordon with trunk height of about 80 cm. Mixed pruning was applied. Tests were carried out on 30 vines, that is, three repetitions with 10 vines.

During the three-year study grape yield was examined (kg/m2), cluster weight (g), sugar (%) and acid content (g/l) in stum. Yield was obtained by measuring the harvested grapes and calculating grape weight per m2, cluster weight was determined from the ratio of the yield of ten vines and number of grape clusters. The sugar content in the grape juice was determined with areometrically (Oeschle must balance), and proportion of the total acid in the grape juice by neutralization of acids and their salts with n/10 NaOH solution using bromothymol blue indicator.

In the analysis of climate in Lješkopolje, data from weather stations in Podgorica were used. Statistical analysis of the results was performed using analysis of variance and LSD test.

RESULTS AND DISCUSSION

Yield quantity and quality is heavily influenced by climate and the prevailing meteorological conditions in production regions (Mirošević and Karoglan - Kontić, 2008). Air temperature exerts dominant influence on vines apropos vine phenological dynamics, and in current climate changing condition, heat regime changes are the most pronounced.

Tab.1. Mean monthly, annual and air temperature in vegetation period (^{o}c)

Year	Month												Mean annual	Mean veg.	Veg. sum
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII			
2011	5.8	7.8	1 .7	16.8	20.3	25.6	27.2	29.3	26.3	16.3	9.9	8.0	17.0	23.1	4947.1
2012	4.9	3	13.1	14.5	19.9	27.3	30.4	29.7	23.9	18.2	13.3	5.6	16.2	23.4	5007.6
2013	6.7	7.7	10.8	17.1	20.1	24.5	28.5	28.8	21.3	16.7	12.5	6.6	16.8	22.4	4793.6
2011-2013	5.8	6.2	8.5	16.1	20.1	25.8	28.7	29.3	23.8	17.0	11.9	6.7	16.5	23.0	4916.1
1985-2010	6.2	7.4	10.5	15.8	20.5	24.4	28.0	27.6	21.6	16.2	10.8	7.3	16.3	22.0	4715.3

Table 1 clearly shows that air temperature in Podgorica wine growing region is in constant growth, especially in the summer period. If we analyze climate anomalies, expressed through the differences in the values of climate parameters between the multi-year average (1985-2010) and the period from 2011 to 2013, we come to the conclusion that the climate in this wine-growing region is rapidly changing. Compared to long-term averages, average annual air temperature in 2011 was 0.7°C higher than the long term average, in 2013 0.5°C, while in 2012 was at the level of long-term averages.

The mean temperature in vegetation period in the three-year average was 23°C which is 1°C more than in the multi-year average. In the second research year, medium temperature in vegetation period was 23.4°C, and was slightly higher than in the first year (23.1°C) and significantly higher than in the third year in which the mean temperature during the vegetation period was 22.4°C.

Tab. 2. Absolute maximum air temperatures

Year	Months												Year max
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
2011	16.6	21.3	23.7	27.6	31.5	37.6	39.9	41.2	37.8	31.0	21.9	16.8	41.2
2012	15.7	19.3	26.3	31.1	33.5	38.3	40.7	44.0	36.1	33.6	22.6	15.4	44.0
2013	16.7	17.2	20.3	32.6	34.1	38.4	39.4	41.3	31.9	29.2	24.8	17.8	41.3

The average sum of active temperatures during the vegetation period for the period 1985-2010 was 4715.3°C, while in the studied period was significantly higher - 4916.1°C. In the studied period, the highest temperature sum in vegetation period was in 2012 (5007.6°C). Table 2 shows that in the period May - Aug - July - August in 2012 was measured significantly higher maximum air

temperature compared to the same period in 2011 and 2013. In other months, the maximum air temperatures were at the same level. In 2012, in the period May - September, was also measured the highest minimum air temperature (tab. 3).

Tab. 3. Absolute minimum air temperatures

Year	Months												Year min
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
2011	-3.0	-3.1	-2.3	4.7	9.9	16.7	14.9	16.9	17.5	2.9	0.3	-2.6	-3.1
2012	-5.5	-5.7	2.5	0.4	10.7	14.9	19.6	17.7	10.9	7.2	4	-5.5	-5.7
2013	-2.3	-2.3	-0.6	8.6	10.0	11.3	16.9	18.5	11.1	1.7	-1	-3.3	-3.3

Jovović et al. (2015) state that the number of tropical days and duration of the warm tropical waves have significantly increased in Podgorica. They further alleged that 59% of June, 87% of July and 86% of August days had a tropical character, and that 15% of the days during June, 40% of the days in July and 45% of the days during August had high tropical temperatures (maximum temperature reaches and passes 35°C). Also, the average number of summer days (maximum temperature during the day reaches and exceeds 25°C), has increased from 129 to 145 days.

Tab. 4. The monthly, annual and precipitations during vegetation period (mm)

Year	Months												Annual sum	Veg. sum
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII		
2011	79.5	113.8	100.5	44.1	89.5	25.8	31.4	2.1	43.3	73.6	37.1	254.2	894.9	309.8
2012	60.3	200.9	0.0	351.2	132.0	33.7	11.3	0.5	86.6	296.3	285.8	305.0	1763.6	911.6
2013	324.3	246.8	518.6	101.2	211.2	51.0	10.3	123.1	178.4	239.3	341	45.8	2391.0	914.5
2011-2013	219.8	203.3	220.5	165.5	144.2	36.8	17.6	41.9	102.7	203.0	309.0	231.0	1683.1	711.8
1985-2010	195.1	190	210.9	103.9	68.5	102.1	21.3	56.6	115.4	181.6	289.3	242.8	1777.5	649.5

The results given in Table 4 show that in Podgorica wine growing region mean annual rainfall for the period 1985-2010 was 1777.5 mm, while in the period studied (2011-2013) was lower and was 1683.1 mm. These data show that in precipitation amounts between the observed periods there were no significant changes, but the changes were significant in the precipitation regime - pronounced rainy and drought periods during the year. In 2011, the annual amount of rainfall was only 894.9 l/m^2, or 882.6 l/m^2 less than long-term averages. In the second year, the annual amount of precipitation (1763.6 mm) was at the level of multi-year average, while in the third year it was significantly higher and was 2,391 l/m^2.

When analysing rainfall in the vegetation period (tab. 4) it is clear that in 2011 precipitation level of only 309.8 l/ m2 was significantly lower than in 2012 and 2013 in which during the vegetation period was measured 911.6 l/ m2 and 914.5 l/ m2, respectively. It is interesting to note that in 2013, for the first three

months, it was measured record amount of rain - 1090 l/ m2. Most of the rainfall in this year fell in March - 518.6 mm; three and a half times more than the usual rainfall for this month. Since the measuring of rainfall exists in Montenegro (for 64 years) it has not happened that this much rain falls in March (Micev, 2015).

The average amount of precipitation during the vegetation period from 2011 to 2013 was 711.8 l/m2. Although in the studied period, compared to the multi-year average (649.5 l/ m2), was registered more rainfall, this does not mean that the vines in these years were sufficiently provided with water. Namely, in 2011 and 2012, the distribution of rainfall was quite unfavourable, with a strong deficit in the warmest period of the year.

Grape yield per area unit, as an absolute indicator of productivity of varieties, is conditioned by a number of factors. Among the more important are biological characteristic of the variety and environmental conditions in the studied years. The results in Table 5 show that the yield of grapes was in accordance with the weather conditions in studied years. The lowest yield of grapes (0.83 kg/m2) was measured in 2013, with highest precipitation, both on annual basis, and during the vegetation period. This year there was a very high intensity of plant diseases, which, along with noticeable reduction in yield and increased use of chemicals, significantly increased the production costs. The highest yield was measured in 2012 (1.20 kg/m2), while in 2011 it was 0.96 kg/ m2. Statistical analysis of data showed that the yield of grapes in 2012 was significantly higher compared to 2013, and less significant compared to 2011. The difference in the yield of grapes between the first and the third year of study did not have the statistical significance for any level of probability. Yields in all years of study have been at the level for the variety Cabernet Sauvignon cited by other authors (Pejović, 1996; Burić, 1995; Cindrić, 2000; Mirošević, 2008 etc.).

Tab. 5. The yield and quality of grape variety Cabernet Sauvignon

Year	(kg/m^2)	Grape cluster weight (g)	Sugar content (%)	Acid content (g/l)
2011	0.96	104	24.10	5.9
2012	1.20	125	23.00	6.0
2013	0.83	89	21.0	6.5
Average	1.00	106	22.7	6.1

	LSD 0.05	LSD 0.01
Grape yield	0.18	0.27
Grape cluster weight	15.3	22.2
Sugar content in stum	0.81	1.18
Acid content in stum	0.39	0.58

The results of the study of average cluster weight of variety Cabernet Sauvignon are given in Table 5. Analyzing the average values in years of study it can be noted that in 2012 cluster weight was significantly higher than in 2013

and less significant in comparison to 2011. The higher cluster weight in first two years of the experiment (125 and 104 g respectively) was due to significantly more favourable environmental conditions in those years, particularly temperature. During these years, the average air temperatures in the vegetation period were significantly higher compared to 2013. The results are consistent with the results reported by Popović et al, 2013, which stated that the cluster weight of variety Vranac in Podgorica wine growing region was higher in climatically favourable years. The average weight of clusters in this research was at the level of values that the variety Cabernet Sauvignon has in the same agro-ecological conditions as stated by Pejović et al., 1996 and Pajović et al., 2009. According to Božinović (2010) the average cluster weight of variety Cabernet Sauvignon is ranging from 90 to 120 g, which also supports the results obtained.

Trough influence on physiological processes of vine, ecological potential of wine growing regions significantly affects the quality of grapes, primarily the content of sugars and acids. The amount of sugar in grapes, apart from the variety, greatly depends on the degree of maturity and health status of grapes, as well as of the climatic conditions in the ripening stage of grapes (Ranković - Vasić, 2011).

Based on the results shown in Table 5, the highest sugar content of the grapes was measured in 2011 (24,10%) and the lowest in 2013 (21.00%). This is the direct consequence of the variation in weather conditions that prevailed in years of performing experiments. In 2013, with the lowest mean air temperature in vegetation period and the highest amount of precipitation in the vegetation period, the sugar content in stum was lowest. Similar results were reported by Vukosavljević et al. (2011) who measured higher sugar content in years with higher mean temperatures in vegetation period. The importance and impact of growing conditions on the yield and quality of grapes indicate Santalucia et al. (2007) and Mota et al. (2008) as well.

Acids in stum are an important indicator of the quality of grapes, as taste and harmony of the wine produced depends on their presence. The results of three-year research showed that the average content of acids in stum was satisfying and typical for the variety Cabernet Sauvignon grown in the agro ecological conditions of Podgorica wine growing region. The highest content of acids was measured in 2013 - 6.50 g/l, as a result of higher precipitation during the vegetation period, especially in August and September (123.1 and 178.4 l/m^2 respectively). Such conditions have significantly affected the quality of the grapes, primarily the acid content. Statistical analysis of data revealed that in 2013 the acid content was significantly higher compared to the other years studied. These results are consistent with the results of Pejović et al., (1996) and Pajović et al., (2014). However, Mirošević et al., (2008) reported higher values for the content of acids in grape variety Cabernet Sauvignon (7-9 g/l), which was probably a consequence of different agro-ecological conditions in which they conducted their research.

CONCLUSIONS

Based on the research done, it can be concluded:

The average yield of grapes in the three-year period was 10 t/ha, sugar content 22.7%, and the acid content 6.10 g/l. Yield and cluster weight were lowest in 2013, which is explained by the large amount of rainfall during the vine vegetation period.

Very high sugar content was measured in 2011 and 2012 (24.1 and 23.0% respectively) as a result of higher average air temperature in the vine vegetation period.

The highest acid content was measured in 2013 as a result of heavy rainfall in August and September.

The results of these studies indicate significant impact of climatic factors on the amount and quality of yields of this variety. However, despite the increasing influence of climate change, there are still very favourable agro-ecological conditions for growing Cabernet Sauvignon in Podgorica wine growing region.

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Dusko VUJACIC and Velibor SPALEVIC [1]

ASSESSMENT OF RUNOFF AND SOIL EROSION IN THE RADULICKA RIJEKA WATERSHED, POLIMLJE, MONTENEGRO

SUMMARY

Soil erosion is a natural process that is causing environmental concerns such as land degradation, soil loss, water pollution and ecosystem alteration. The Erosion Potential Method (EPM) is extensively used for identifying watersheds with such problems. One of 57 studied river basins of the Polimlje Region was Radulicka Rijeka Watershed where we studied soil erosion processes using the analytical and computer-graphic method IntErO, based on the EPM method. Calculated peak discharge from the river basin was 185 m^3s^{-1} for the incidence of 100 years and the net soil loss was 13157 m^3 yr^{-1}, specific 327 m^3 km^{-2} yr^{-1}. The use of EPM method and the IntErO model to predict sediment yield, according to our experiences, is recommended for this Region and may also be suitable for making management decisions. At the same time, further research is needed to address model limitations regarding the further development in relation to the GIS adaptations.

Keywords: Erosion, Soil erosion assessment, watershed, Land use, IntErO model.

INTRODUCTION

Sediments play an important role in elemental cycling in the aquatic environment as they 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). Soil degradation caused by erosion, together with rapid population increase, are ranked as the most important environmental problems in the world (Stoffel and Huggel, 2012), where the erosion is a key driver of land degradation heavily affecting sustainable land management in various environments worldwide (Ballesteros-Cánovas *et al*, 2015; Al-Turki *et al*, 2015; Behzadfar et al, 2015; Stoffel *et al*, 2013; Khaledi Darvishan *et al*, 2012).

The South and Southeast regions of Europe are significantly prone to water erosion. In Montenegro water erosion is the most important erosion type (Spalevic, 1999). The main forces of water erosion are precipitations and

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consecutive runoff; but not less important is fluvial erosion in water streams (Kostadinov *et al*. 2006).

Water erosion has affected 95% of Montenegro. Alluvial accumulation characterises the remaining area, where the deposition of sediments is also affecting agricultural land. Erosion caused by water is dominant in terrain with high slopes due to complex physical and geographical conditions paired with reckless logging (Spalevic *et al*, 2015a; Gazdic et al, 2015; Spalevic *et al*, 2014a; Spalevic *et al*, 2013a; Spalevic *et al*, 2013b; Fustic and Spalevic, 2000).

According to Blinkov (2015) the most erosive countries in Europe are the Balkan countries Albania and Montenegro, where the mean annual intensity of erosion is more than 10 t ha^{-1}. This is confirmed by Spalevic *et al* (2012) for the Mediterranean watersheds of Montenegro: 1900 $m^3km^{-2}yr^{-1}$ for the Zeljeznica river basin of the Adriatic Watershed. On the other hand, for the Polimlje (North of Montenegro, the Black Sea Watershed), the calculated soil losses per km^2 for the 57 river basins were in average 331 $m^3km^{-2}yr^{-1}$ (Spalevic *et al*, 2015b; Spalevic *et al*, 2015c; Spalevic *et al*, 2014b; Spalevic *et al*, 2014c; Spalevic *et al*, 2013c; Spalevic *et al*, 2000).

All stated make clear why erosion risk assessment and its quantification is an important question for this Region.

The idea of this research was the assessment of runoff and soil erosion in the Radulicka Rijeka Watershed, Polimlje, Montenegro, studying spatial characteristics of the various natural phenomena; geological, soil, land use and climate characteristics.

MATERIAL AND METHODS

We studied soil erosion processes in the Radulicka Rijeka Watershed (40 km^2), a right-hand tributary of the river Lim, located in the mountainous area of the Polimlje Region, north of Montenegro (Figure 1).

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 data of geological and pedological research from Zivaljevic (1989), Fustic and Djuretic (2000), who analysed all geological formations and soils of Montenegro including the studied area of the Radulicka Rijeka.

Climatological data were received from the Institute of Hydrometeorology and Seismology of Montenegro (for the period 1948-2015). We analysed torrential rains, annual air temperatures, and average annual precipitations.

Many researchers have attempted to elucidate the interaction between land cover pattern and ecological processes (Fu *et al*., 2011); and this become the focus of the landscape ecology studies in the field (Casermeiro *et al*., 2004; Bautista *et al*., 2007; Fridley *et al*., 2007; Claessens *et al*., 2009; Bisigato *et al*., 2009).

Data in relation to the land use and vegetation cover we received from the Institute of Forestry of Montenegro (IoFoM), Statistical Office of Montenegro (MONSTAT), Google Maps and our own research.

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

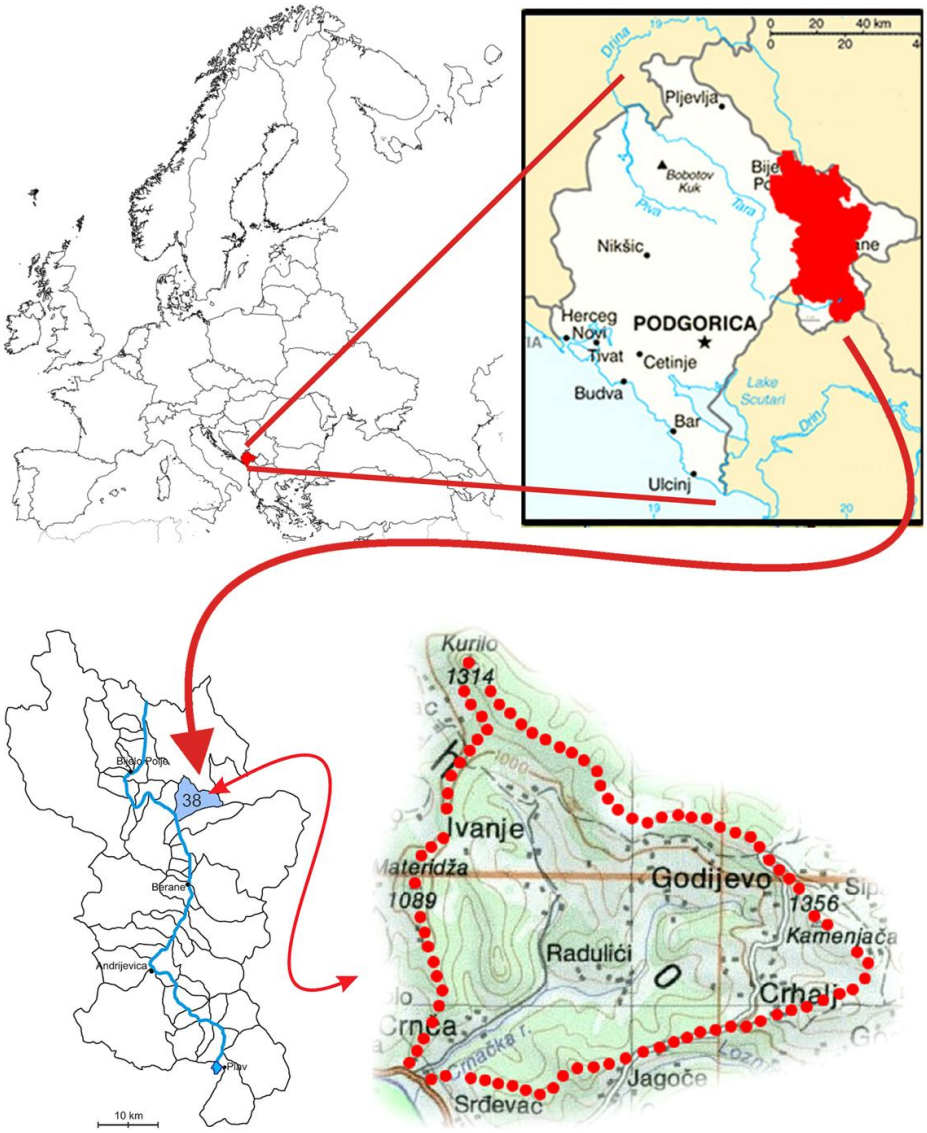

Figure 1. Study area of the Radulicka Rijeka Watershed, Polimlje, Montenegro (H_{min}: 42°57'49.0"N 19°50'29.6"E)

We applied the IntErO model (Spalevic, 2011) [2] for the assessment of runoff and soil erosion. The analytical equation is the following:

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

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

The actual sediment yield was calculated as follows:

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

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

Climate. The climate in the studied area of the Radulicka Rijeka is continental, with the absolute maximum air temperature of 35°C and negative of 29.8°C. The location of the studied river basin is classified as Dfb by Köppen and Geiger.

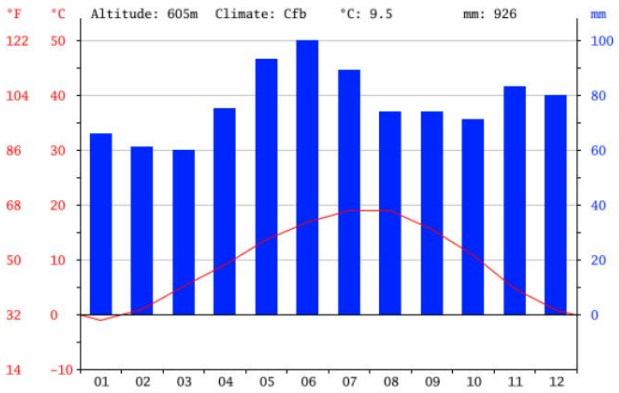

Figure 2. Climate graph (Bijelo Polje)

[2] The IntErO software available on: www.agricultforest.ac.me/Spalevic/IntErO

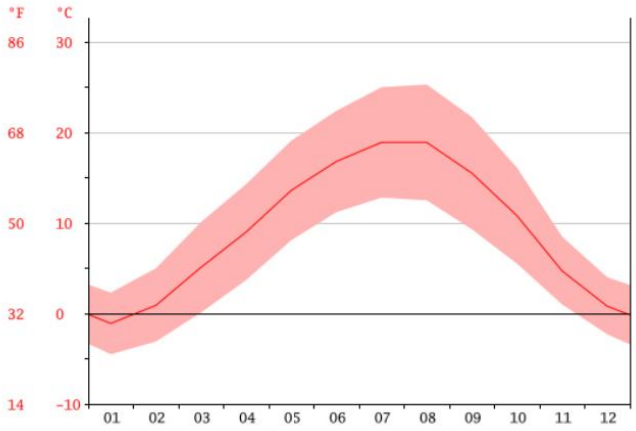

Figure 3. Temperature graph (Bijelo Polje)

The amount of torrential rain, hb, is calculated on 157.6 mm. The average annual air temperature, t0, is 8.9 °C. The average annual precipitation, Hyr, is 983.7 mm. The temperature coefficient of the region, T, is calculated to be 0.99.

Geology and soils. The study area belongs to the Durmitor geotectonic unit of the inner Dinarides of Northern and North - eastern Montenegro. The geological structure of that part consists mainly of Paleozoic clastic, carbonate and silicate volcanic rocks and sediments of the Triassic, Jurassic, Cretaceous – Paleo-gene and Neogene sediments (Frankl *et al*., 2015).

The coefficient of the region's permeability, S1, according to the analysis of geological substrate is calculated on 0.9.

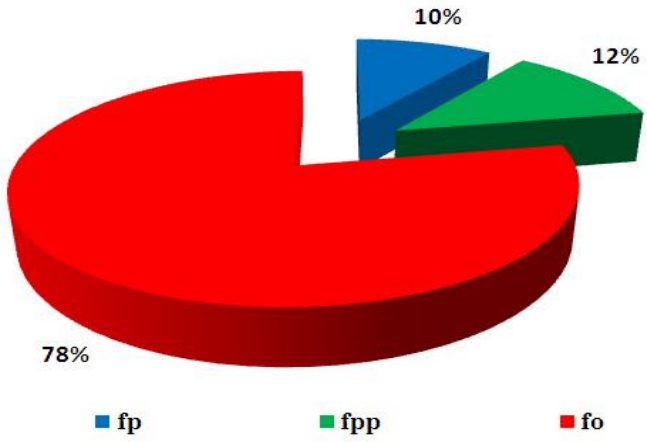

Figure 4: The structure of the river basin, according to bedrock permeability (fp: very permeable; fpp: medium; fo: low permeability).

Based on the results of pedological research (Fustic & Djuretic, 2000; Spalevic, 2011), and our own research, the most common soil types in the watershed are: *Dystric Cambisols* (36.8 km^2 of the studied river basin), *Eutric Cambisols* (1.2 km^2), *Colluvial Fluvisols* (2 km^2).

The structure of the river basin, according to the soil types is presented at the Figure 5.

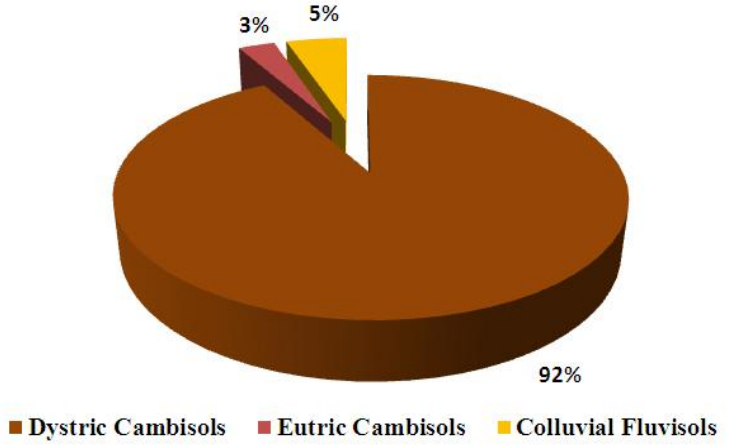

Figure 5: The structure of the river basin, according to the soil types

Land use. The Radulicka Rijeka drainage basin is mainly covered by diverse forms of forests, covering the area of about 50% of the studied river basin. Meadows, pastures and Orchards are covering the area of about 35%. Arable land covers a total surface area of 15%, concentrated in the areas with low slope gradients.

The detailed structure of the river basin, according to the land use is presented at the Figure 6.

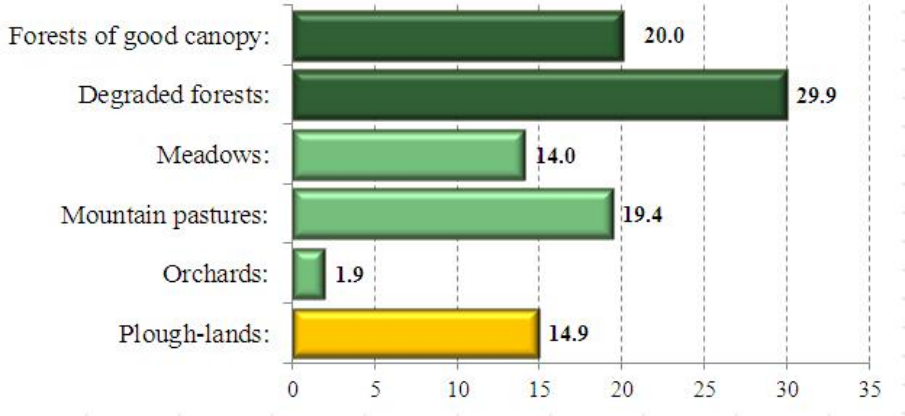

Figure 6: The land use structure of the studied river basin

Figure 7. Details from the studied Radulicka River Basin

Soil erosion characteristics. The dominant erosion form in the studied river basin is sheet erosion. Some problems of overgrazing and livestock traces are recorded also, in the areas close to the village Godijevo and Radulici.

The assessment of runoff and soil erosion in the Radulicka Rijeka Watershedis presented in the Table 1.

Table 1. Part of the IntErO report for the Radulicka Rijeka Watershed

Inputs			
River basin area	F	40.23	km²
The length of the watershed	O	29.88	km
Natural length of the main watercourse	Lv	8.47	km
The shortest distance (fountainhead and mouth)	Lm	7.08	km
River basin length measured by a series of parallel lines	Lb	12.37	km
The area of the bigger river basin part	Fv	12.17	km²
The area of the smaller river basin part	Fm	27.78	km²
Altitude of the first contour line	h0	700	m
The lowest river basin elevation	Hmin	619	m
The highest river basin elevation	Hmax	1356	m
The volume of the torrent rain	hb	157.6	mm
Average annual air temperature	t0	8.9	°C
Average annual precipitation	Hyr	983.7	mm
Types of soil products and related types	Y	1.1	
Coefficient of the river basin planning	Xa	0.51	
Numeral equivalents of visible erosion process	φ	0.31	

Results

Coefficient of the river basin form	A	0.69	
Coefficient of the watershed development	m	0.38	
Average river basin width	B	3.31	km
(A)symmetry of the river basin	a	0.76	
Density of the river network of the basin	G	0.31	
Coefficient of the river basin tortuousness	K	1.2	
Average river basin altitude	Hsr	881.74	m
Average elevation difference of the river basin	D	262.74	m
Average river basin decline	Isr	29.46	%
The height of the local erosion base of the river basin	Hleb	737	m
Coefficient of the erosion energy of the basin's relief	Er	93.15	
Coefficient of the region's permeability	S1	0.9	
Coefficient of the vegetation cover	S2	0.73	
Analytical presentation of the water retention in inflow	W	1.68	m
Energetic potential of water flow during torrent rains	2gDF^½	455.4	m km s
Temperature coefficient of the region	T	0.99	
Coefficient of the river basin erosion	Z	0.497	
Production of erosion material in the river basin	Wyr	43364	$m^3\ yr^{-1}$
Coefficient of the deposit retention	Ru	0.303	
Real soil losses	Gyr	13157	$m^3\ yr^{-1}$
Real soil losses per km^2	Gyr (km^2)	327	$m^3km^{-2}yr^{-1}$

CONCLUSIONS

(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 0.31, indicates there is low density of the hydrographic network. The value of 29.46% indicates that in the river basin prevail steep slopes.

Calculated peak discharge from the river basin was 185 m^3s^{-1} for the incidence of 100 years.

The value of Z coefficient of 0.497 indicates that the river basin belongs to III destruction category; according to the erosion type, it is surface erosion.

The net soil loss was 13157 m^3km^{-2}, specific 327 m^3km^{-2} per year, what indicates, according to Gavrilovic, that the river basin belongs to V category; region of very weak erosion.

The use of EPM method and the IntErO model to predict sediment yields, according to our experiences, is recommended for this Region and may also be suitable for making management decisions. At the same time, further research is needed to address model limitations regarding the further development in relation to the GIS adaptations.

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