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Original scientific paper

CONTENT OF EXCHANGEABLE CATIONS IN ALBIC LUVISOLS IN THE REPUBLIC OF MACEDONIA[#]

Marjan Andreevski[*], Duško Mukaetov

Institute of Agriculture, Ss. Cyril and Methodius University, Skopje, Republic of Macedonia

[*]e-mail: m.andreevski@zeminst.edu.mk

On different locations of the country, 23 soil profiles of Albic Luvisols were excavated and morphologically described on the field. More than half of soil profiles (15) were under forest vegetation, 4 soil profiles were under grass and the other 4 on arable land. The survey showed that the cation exchange capacity (CEC) is higher in the humus accumulative horizon A, but lower in the eluvial horizon. Due to increasing amount of clay in a lower part of a soil profiles, a CEC increases in hor. Bt, BtC and C. The base saturation percentage ranges from 46.93 % to 60.83 %. From these data we can see that the soils are in advanced stage of acidification. In Albic Luvisols, the basic cations (Ca^{++}, Mg^{++}, K^{+}, Na^{+}) are more common in comparison to acid cations (H^{+} + Al^{+++}). Exception is the eluvial horizon, where the content of acid cations higher compared to basic cations. Among the exchangeable basic cations, we can see that the content of Ca^{++} cation has the highest values, Mg^{++} is less represented, while K^{+} and Na^{+} cations have the lowest contents among the exchangeable cations.

Key words: albic luvisols; cation exchange capacity; exchangeable cations

INTRODUCTION

Albic Luvisols are of great importance for the agriculture and forestry in the Republic of Macedonia. Their importance, stems from their moderate production capability and the fact that they are formed and covers big areas in humid regions of the country. One part of these soils are cultivated with different agricultural varieties, while the other parts are under lawns and forest. The content of exchangeable cations is significant indicator for the soil formation conditions. Many processes and characteristics of soil depend of cation exchange capacity (CEC) and its content. The content of the exchangeable ions of Albic Luvisols in the Republic of Macedonia can be found in the previous work of [1–7].

Data regarding the content of the exchangeable cations on Albic Luvisols in the Republic of Macedonia formed under different vegetation can be find in our previous work [8].

MATERIAL AND METHODS

On different locations of the country, 23 soil profiles of Albic Luvisols were excavated and morphologically described on the field. More than half of soil profiles (15) were under forest vegetation, 4 soil profiles were under grass and the other 4 on arable land.

Field examinations have been performed according to accepted methods in Former Yugoslavia [9].

The laboratory analyses have been done according to the standard of adopted methods in Former Yugoslavia and the Republic of Macedonia, as follows:

- Mechanical composition of soil has been determined by the pipette method [10]; the dispersion of the particles has been done with 0.1M Na-pyrophosphate. The separation of the mechanical elements in fractions has been done by the international classification.

[#]Dedicated to academician Gjorgji Filipovski on the occasion of his 100[th] birthday

• pH (reaction) of the soil solution has been determined with glass electrode in water suspension and in MKCI suspension [11].

• Easy available forms of P_2O_5 and K_2O were determinate by Al method [12].

• The content of humus has been determinate at the base of total carbon by the method of Tjurin modified by Simakov [13].

• The total N has been determinate by Kjeldahl micromethod [11].

• Extraction with barium chloride three-ethanolamine in glass colons (Melich method) was used for quantification of acid exchangeable cations (H^+ + Al^{+++}). The extract is titrated with 0.04 M HCl in a presence of mixed indicator [11].

• Extraction with $BaCl_2$ [14] was used for quantification of the exchangeable cations (Ca^{++}, Mg^{++}, K^+, Na^+). The quantity of exchangeable cations was determined by use of atomic absorption spectrometry type "Varian".

• Cation exchange capacity (CEC), sum of basic exchangeable cations, and base saturation percentage (BS) as well as the percentage of particular cations saturation were calculated.

RESULTS AND DISCUSSION

A detailed overview of the mechanical composition and some chemical properties will be given, in order to give a correct interpretation of the results for CEC and a content of exchangeable cations of Albic Luvisols. An average results of 23 soil profiles are presented in Table 1 and 2.

Table 1. Mechanical composition of Albic Luvisol in the Republic of Macedonia (average values)

		in % of fine earth					
	Skeleton	Coarse	Fine	Coarse+fine	Silt	Clay	Silt+
Horizon,		sand	sand	sand			clay
Number of samples	> 2 mm	0.2–2 mm	0.02–0.2 mm	0.02–2 mm	0.002–0.02 mm	< 0.002 mm	< 0.02 mm
A (23)	8.66	10.86	47.13	57.99	24.93	17.08	42.01
E (19)	14.93	11.75	45.62	57.36	26.46	16.18	42.64
Bt (23)	6.78	7.82	35.91	43.73	22.44	33.83	56.27
BtC (9)	5.4	7.62	33.26	40.88	22.27	36.86	59.12
C (15)	6.0	9.88	38.22	48.1	18.53	33.37	51.9

Table 2. Chemical properties of Albic Luvisol in the Republic of Macedonia (average values)

Horizon, Number of samples	Humus	Total	C/N	pH		Easy available mg/100g soil	
	%	N%		H_2O	NKCl	P_2O_5	K_2O
A (23)	5.88	0.27	11.84	5.67	4.70	6.56	28.48
E (19)	2.08	0.12	10.44	5.34	4.14	< 1	13.17
Bt (23)	0.92	0.07	8.01	5.62	4.29	< 1	17.69
BtC (9)	0.69	0.06	6.85	5.78	4.44	< 1	18.44
C (15)	0.46	0.04	6.79	5.92	4.58	< 1	17.59

CATION EXCHANGE CAPACITY

The cation exchange capacity depends on the total amount of clay, the nature of the clay minerals, the content of humus and the reaction of the solution used for its extraction [15]. The data for a cation exchange capacity in Albic Luvisols, are presented in Table 3. Out of the presented data, differences of cation exchange capacity can be detected, between soil horizons. These differences are due to various reasons, among which the most important are: greater accumulation of humus in the humus accumulative horizon, translocation of the clay from the humus accumulative and eluvial horizons to the argilic horizon Bt, stratification of the sediments and the inherited quantities of clay from the previous soil formation stadium.

Cation exchange capacity has its highest values in the humus accumulative horizon with average values of 19.51 cmol(+)kg^{-1} soil. Horizon E shows the lowest values of only 12. 20 cmol(+)kg^{-1} soil (low humus contents and intensive leaching of clay). In the argillic horizon (Bt) and the transitional horizon (BtC), cation exchange capacity increases with average values for hor. Bt of 16.02 cmol(+)kg^{-1} soil, and 16.93 cmol(+)kg^{-1} soil for hor. BtC. The increasing of cation exchange capacity in the lowest parts of the soil profile is due to a larger amounts of clay leached in that part of a soil profiles and probably a higher presence of smectite clay mineral. In the parent material (hor. C), the cation exchange capacity is decreasing and shows average values of 14.46 cmol(+)kg^{-1} soil. The average values of CEC for the Albic Luvisols in Republic of Macedonia reported by Filipovski, [7] are in the ranges of 16.02 for hor. A and 23.55 cmol(+)kg^{-1} soil for hor. Bt. Similar data for the CEC in Albic Luvisols are reported in the previous works of the researchers in the neighboring countries [16–21].

Table 3. Exchangeable cations of Albic Luvisol in the Republic of Macedonia (average values)

Horizon, Number of samples	Exchangeable cations in cmol (+)kg^{-1} soil						cmol (+)kg^{-1} soil	Exchangeable cations in % of CEC					
	Ca^{2+}	Mg^{2+}	K^+	Na^+	S	H^++Al^{3+}	CEC	BSP%	Ca^{2+}	Mg^{2+}	K^+	Na^+	H^++Al^{3+}
A (23)	7.26	2.18	0.47	0.22	10.13	9.38	19.51	54.05	38.83	11.50	2.53	1.21	45.95
E (19)	3.97	1.47	0.17	0.16	5.77	6.43	12.20	46.93	32.12	12.06	1.37	1.33	53.07
Bt(23)	5.69	2.28	0.20	0.25	8.43	7.59	16.02	53.64	36.25	14.44	1.26	1.64	46.36
BtC 9	7.01	2.26	0.18	0.19	9.64	7.29	16.93	58.26	42.24	13.75	1.11	1.16	41.75
C (15)	6.01	1.76	0.17	0.22	8.16	6.31	14.46	60.83	45.19	12.73	1.20	1.70	39.17

SUM OF EXCHANGEABLE BASE CATIONS AND BASE SATURATION PERCENTAGE

The sum of exchangeable base cations depends to the cation exchange capacity and to the base saturation percentage. Out of the data presented, it can be concluded that the sum of an exchangeable base is higher in hor.A, with average value of 10.13 cmol(+)kg^{-1} soil (bioaccumulation). The lowest average values of 5.77 cmol(+)kg^{-1} soil are found in the E horizon. This is due to the intense debasification in this horizon. In the lowest part of the soil profile, the content of basic cations is increasing as a result to the increased values of the cation exchange capacity (more clay) and retention of a part of a leached basic cations from hor. A and E. The average values of exchangeable basic cations for hor. Bt is 8.43 cmol(+)kg^{-1} soil and for the hor. BtC is 9.64 cmol(+)kg^{-1} soil. In the parent material (hor. C), the sum of an exchangeable base shows an average content of 8.16 cmol(+)kg^{-1} soil.

From a data of base saturation percentage we can see that the acidification is in advanced stage. The average value for the base saturation percentage for hor. A is 54.05 %. The lowest percentage of base saturation percentage is in hor. E with 46.93 %. The differences between these two horizons is mostly due to the bioaccumulation of a basic cations in hor. A. As a result to the retention of a leached base ions in the lower part of the soil profile, the values for a base saturation percentage are increasing in hor. Bt with average percentage of 53.64 % in BtC 58.26 % and 60.83 % in C.

This higher saturation with basic cations in the lower parts of the soil profile (horizons BtC and C), leeds to the conclusion that the basic cations are leached deep in the soil profile, underneath the argillic horizon Bt. This increase of the base saturation percentage in the deeper parts of the soil profile is referred by other authors as well [22, 20, 23].

According to Filipovski, [7] the base saturation percentage of cations in Albic Luvisols on the territory of Macedonia in hor. A in average yields 57.37 % while in hor. Bt up to 65.90 %. For comparison, here are cites data for base saturation percentage of cations from other authors in the neighborhood: Škorić, [24, 25] for E horizon reports 30 to 50% of base saturation, and for Bt horizon from 50 to 70 %, Ćirić, [26] from 40 to 70 %, Resulović, [27] 50–70 %, Penkov et al. [28] more than 65 %, Koroxenidis et al., [29] from 43 to 80 %.

THE CONTENT OF EXCHANGEABLE CATIONS

The quantity of a particular adsorbed exchangeable cations in Albic Luvisols depends to the character of the parent material, double layer of a soil profile and the character of the soil forming processes. With the processes of debasification and acidification of the hor. A and E, these horizons are losing the adsorbed basic cations, which are translocated and partially accumulated into hor. Bt, BtC and C. For the soil profiles under nature vegetative cover, actually it's very important the bioaccumulation of a base cations in hor. A. On arable land the content of adsorption cations in hor. Ap is changed due to the mixing during the cultivation of horizons with different content of the adsorbed cations. Out of the data presented in Table 3, we can see that the basic cations in Albic Luvisols, (Ca^{++}, Mg^{++}, K^+, Na^+) are slightly more present than the acid cations ($H^+ + Al^{+++}$). Exception is the eluvial horizon, where acid cations are more present than the basic cations. Among the exchangeable base cations more common cations are Ca^{++}, then Mg^{++}, while K^+ and Na^+ are less represented. The exchangeable Ca^{++} cation is more common in the humus accumulative horizon which is result to its bioaccumulation. An average values of content of exchangeable cation Ca^{++} for hor. A is 7.26 cmol(+)kg^{-1} soil. We can see that in the eluvial horizon has the lowest value of this cation with only 3.97 cmol(+)kg^{-1} soil. The reduction on the content of humus and debasification in the eluvial horizon are the main reasons for this rapid declining of the Ca^{++} content. As a result to the larger clay content and retention of a part of the leached Ca^{++} cations from upper to the lower parts of the soil profile, the contents of this cation increases, hence the content of Ca^{++} in the argillic horizon is 5.69 cmol(+)kg^{-1} soil, in the transitional horizon averages 7.01 cmol(+)kg^{-1} and in a parent material the average contents is 6.01 cmol(+)kg^{-1} soil. In percent's, the average contents of exchangeable Ca^{++} in hor. A is 38. 83 %, in hor. E is 32.12 %, but in the lowest part of a soil profiles increases up to 36.25% in Bt, 42.24 % in BtC and 45.19 % in hor. C. The Mg^{++} has less contents among the basic cations in the adsorption complex of the Albic Luvisols. The content of an exchangeable Mg^{++} in horizon A is 2.18 cmol(+)kg^{-1} soil, or 11.50 % from a cation exchange capacity. The content in the eluvial horizon has the lowest values and in average is 1.47 cmol(+)kg^{-1} soil (12.06 % from CEC). In the lowest part of a soil profile, the exchangeable Mg^{++} is increasing. In Bt horizon, the content of Mg^{++} averages for about 2.28 cmol(+)kg^{-1} soil, or 14.44 % from the cation exchange capacity, while in a transitional BtC horizon 2.26 cmol(+)kg^{-1} or 13.75 % from CEC. In a parent material the content of the exchangeable Mg^{++} is 1.76 cmol(+)kg^{-1} soil or 12.73 % from the cation exchange capacity. Main reason for the lower contents of magnesium compared to the calcium cation in the adsorption complex is result to the fact that magnesium is less contents of this cation in the parent material. On the other side, its exchangeable force is lower, so this cations can be easily leached. In addition, this cation more easily enters the crystal lattice of the clay minerals mining that less quantities stayed available for adsorption. The exchangeable cations K^+ and Na^+ have the lowest content among the exchangeable cations. Potassium is the most common in a humus accumulative horizon, with average quantities of 0.47 cmol(+)kg^{-1} soil or 2.53% from CEC. Main reason for this is its bioaccumulation. The content of this cation decreases with increasing of the depth of a soil profiles. With regards to the adsorbed sodium, we cannot note any meaningfyll rules in its vertical distribution throughout the soil profile. The content of adsorbed sodium ranges from 1.16 % to 1.70 % of CEC. These values of adsorbed sodium can not cause any damages for the plant.

The acid cations ($H^+ + Al^{+++}$) are more common in hor. A, which averages for 9.38 cmol(+)kg^{-1} soil. The E horizon content of acid cations is 6.43 cmol(+)kg^{-1} soil, Bt 7.59 cmol(+)kg^{-1} soil, BtC 7.29 cmol(+) kg^{-1} and in parent material the content of $H^+ + Al^{+++}$ cations in average is 6.31 cmol (+)kg^{-1} soil.

Expressed in percentage from the cation exchange capacity, the exchangeable acid cations ($H^+ + Al^{+++}$) in hor. A yields 45.95%. In hor. E the acid cations contents has a highest values for about 53.07% from CEC, and have higher content than the basic cations. This is due to the intense debasification and acidification, and less intensive bioaccumulation of the basic cations.

In the lower part of the soil profiles, the values of an acid cations declines and are less common than the basic cations. In hor.Bt the content of acid cations is 46.36 %, in BtC 41.75 % while in hor. C they have the lowest values with only 39.17 % from CEC. The decline of the acid cations content in the lower parts of the soil profile is due to the weakening of the process of acidification and retention of the basic cations leached from the upper parts of the soil profile.

CONCLUSION

- The cation exchange capacity is highest in the humus accumulative horizon A (19.51

$cmol(+)kg^{-1}$ soil. The lowest values has the eluvial horizon E (12.20 $cmol(+)kg^{-1}$ soil. – As a result to the increasing of clay content in the lower parts of a soil profile, the cation exchange capacity increases in hor. Bt, BtC and C.

• The sum of the exchangeable base cations is highest in hor. A, while the lowest values are found in hor .E. In fact, the sum of an exchangeable base cations increases in the lowest part of a soil profiles (hor. Bt, BtC and C) as a result to the increasing of the cation exchange capacity and the retention of a part of a basic cations, leached from the upper parts.

• The base saturation percentage ranges from 46.93% to 60.83%. Out of the presented data, it can be concluded that the acidification is in advanced stage.

• In Albic Luvisols, the content of basic cations (Ca^{++}, Mg^{++}, K^{+}, Na^{+}) is more common than acid cations (H^{+} + Al^{+++}). Exception is the eluvial horizon, where acid cations are more common.

• Among the exchangeable base cations, Ca^{++} is the most common cation, then follows Mg^{++}, while K^{+} and Na^{+} are with lower content.

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СОСТАВ НА РАЗМЕНЛИВИТЕ КАТЈОНИ НА ЛЕСИВИРАНИТЕ ПОЧВИ ВО РЕПУБЛИКА МАКЕДОНИЈА

Марјан Андреевски, Душко Мукаетов

Земјоделски институт, Универзитет „Св.Кирил и Методиј", Скопје, Република Македонија

На различити локации на територијата на Република Македонија се ископани и морфолошки проучени 23 основни педолошки профили на лесивирани почви, од кои 15 под шумска вегетација, четири под тревна вегетација и четири на обработливи површини. Резултатите од испитувањата покажаа дека капацитетот на атсорпција е највисок во хумусно акумулативниот хоризонт, а најнизок во елувијалниот хоризонт. Заради зголемување на содржината на глина во долниот дел на профилот, капацитетот на атсорпцијата се зголемува во хор. Bt, BtC и C. Степенот на заситеност со базични катјони се движи од 46.93 % до 60.83 %. Од овие податоци може да се види дека ацидификацијата е напредната. Во лесивираните почви, базичните катјони (Ca^{++}, Mg^{++}, K^{+}, Na^{+}) се малку позастапени од киселинските катјони (H^{+} + Al^{+++}). Исклучок е елувијалниот хоризонт во кој киселинските катјони се малку позастапени од базичните катјони. Од разменливите базични катјони Ca^{++} е најмногу застапен, потоа следува Mg^{++}, додека K^{+} и Na^{+} се многу малку застапени.

Клучни зборови: лесивирани почви; капацитет на атсорпција на катјони; разменливи катјони

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Original scientific paper

CONTENT OF HUMUS AND SOIL pH OF THE SOILS FORMED UPON LIMESTONES AND DOLOMITES[#]

Mile Markoski[1*], Tatjana Mitkova[1], Vjekoslav Tanaskovik[1], Velibor Spalević[2]

[1]Faculty of Agricultural Sciences and Food, University Ss. Cyril and Methodius, Skopje, Republic of Macedonia
[2]Faculty of Philosophy, University of Montenegro, Niksic, Montnegro

[*]e-mail: mmarkoski@zf.ukim.edu.mk

The soils formed on limestones and dolomites have been examined in different locations on the territory of the Republic of Macedonia. The filed researches have been performed in the course of 2010, 2011 and 2012, during which 52 basic pedological profiles were excavated, of which 34 were Rendzic Leptosol, 13 were Chromic Leptic Luvisol on hard limestones and 5 **were** profiles of Rhodic Leptic Luvisol on hard limestones. These soils are characterized with a profile type O-A-R; A-R; A-(B)rz-R. Seventy eight soil samples were taken for laboratory analyses. The field researches carried out in accordance with the accepted methodology in our country.The pH (reaction) of the soil solution was electrometrically determined with a glass electrode in water suspension and in suspension of 1M KCl. The humus content was determined on the basis of the carbon organic C according the Tyurin method, modified by Simakov. Rendzic Leptosols are characterized with the highest content of humus in relation to the other soils formed on limestone and dolomite. The subtype organogenic Rendzic Leptosols has highest mean value (19.47 %). The content of humus in the Amo horizon amounts to 8.50 % on average, and in the cambic horizon (B)rz 5.18 %. In the Rhodic Leptic Luvisol on hard limestones, the average content of humus in the Amo horizon amounts to 5.33 %, and in the cambic horizon B(rz) it amounts to 2.13 %. pH in H_2O in the subtype organogenic Rendzic Leptosols is an average of 6.99, average value of (6.93) belong to the organomineral Rendzic Leptosols. In the Amo horizon with the cambic Rendzic Leptosols pH in H_2O is 6.12 and in the cambic horizon (B)rz, pH is 6.68. In Chromic Leptic Luvisols on hard limestones there is decarbonization and weak acidification, due to which the soil solution is weak acidified and in the Amo horizon and (B)rz the average value of pH in H_2O is 6.63. In the Rhodic Leptic Luvisol on hard limestones the average pH in H_2O in the humus-accumulative Amo horizon is 6.94 and in the cambic horizon (B)rz pH in H_2O is 6.72.

Key words: humus; soil; limestones; dolomites

INTRODUCTION

Humus defines key soil characteristics and its fertility, and it is an indicator of the processes in soil. Therefore, understanding of its content and quality is important for the sustainable management of agricultural land. Although there is a great interest in the role of humus in ecosystem function, there have been few studies providing unequivocal identification and quantification of humus because of the heterogeneous and polydisperse nature of humic substances, and the complexity of the inter- and intra-molecular reactions [1]. The clear objective of this research is to see the difference between the content of humus and soil pH of the soils formed upon limestones and dolomites. The content of humus and pH reaction in different regions, conditions and different types of soils are formed on the same substrate. Humic substances (HS) constitute a major fraction (60–70 %) of soil organic matter and are possibly the most abundant of naturally occurring organic macromolecules on the earth ($2–3 \times 10^{10}$ t), [2].

[#]Dedicated to academician Gjorgji Filipovski on the occasion of his 100th birthday

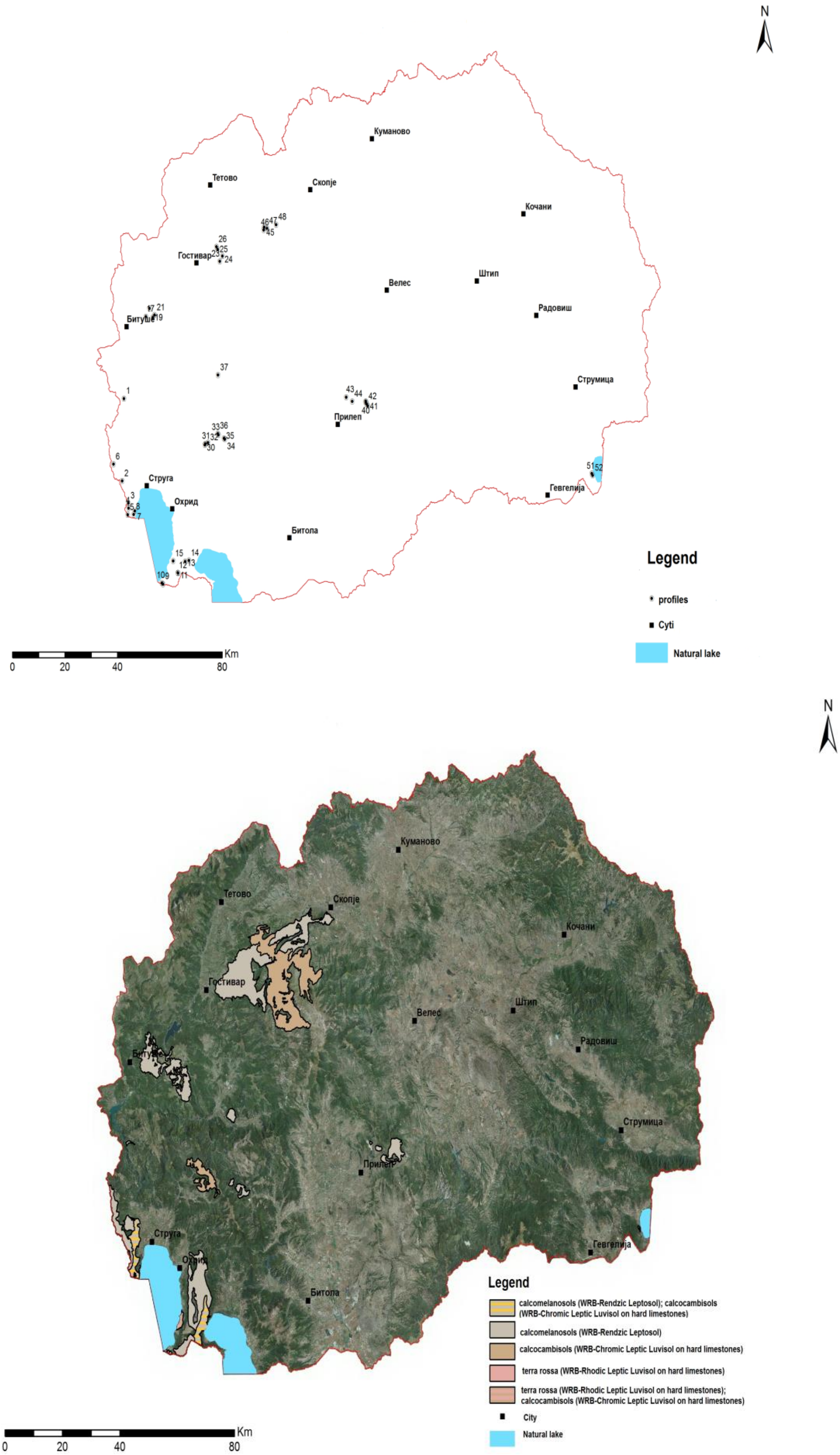

Figure 1. Locations of the soils formed on limestones and dolomites in the Republic of Macedonia

The knowledge of the chemical properties of these soils has a great importance, since these soils are formed only on certain substrates (pure and compact limestones and dolomites), where all physical, physical–mechanical and biological properties greatly depend on the parent material [3]. Data on the humus content, the humus composition, the composition of exchangeable ions, pH - reaction of the soil solution, the sum of exchangeable bases (S), the cation exchange capacity (T), the base saturation percentage (V), and other properties are present in the papers [4–15].

All authors have noted in their research that there is the largest content of humus in the subtype organogenic Rendzic Leptosols [16]. Presented data shows that 38.95 % humus is in the most shallow subtype organogenic Rendzic Leptosols and it reduces to 9.11 % towards Chromic Leptic Luvisols on hard limestones. Also, the content of humus decreases from the Amo horizon to the cambic horizon (B)rz of Chromic Luvic Rendzic Leptosols and Chromic Leptic Luvisols on hard limestones. During the research of the Rendzic Leptosols together with Chromic Leptic Luvisols on hard limestones in Jablanica [17] was found that soils are characterized by acidic and neutral reaction. According to the pH reaction, organogenic and organomineral Rendzic Leptosols fall into three categories (moderately acidic, slightly acidic and neutral). On the Figure 1 can be seen locations of the soils formed on limestones and dolomites in the Republic of Macedonia.

MATERIAL AND METHODS

During 2010, 2011 and 2012, field researches have been carried out on the soils formed on limestones and dolomites, on various locations on the territory of the Republic of Macedonia. The country is divided in 8 climate-vegetation-soil regions, and the studied soils (calcomelanosols) are located in six climate-vegetation belts, in five (calcocambisols) and in two (terra rossa) [18]. The climate of the Sub-Mediterranean modified Mediterranean region differs from the climate of the other regions in terms that the influence of the Mediterranean can be most prominently noticed. The mean annual temperature of this region is 14.2 °C and the average amount of rain is between 611 and 695 mm. According to the Lang's rain factor, the climate is semi-arid, but in four months (from VI until IX) it is arid. The warm-continental region is dominated by the warm continental climate. The mean annual temperature in this region varies from 9.6 up to 11.8 °C (mean 10.9 °C), and the average amount of rain is around 700 mm. The cold continental region covers a narrow belt of 900 to 1100 m above sea level altitude where the cold continental climate is dominant with some influence from the mountain climate, as a result of the larger above sea level altitude. The climate is colder and more humid when compared to the continental region where the mean annual temperature is 8.6-9.6°C or average of 9 °C. The average amount of rain is 800–850 mm. According to the Lang's rain factor, the climate is humid. The Piedmont-continental mountain region covers a vertical belt with an approximate above sea level altitude of 1100 m up to 1300 m. The mean annual temperature is between 7.5 °C and 8.6 °C or an average of around 80 °C, and the average amount of rain is around 900 mm. The number of snowfall is greater when compared to the previous belt, and the duration of the snow cover is longer. According to the Lang's factor, the climate in this belt is humid. The same as in the cold continental region, there is not period of drought. The mountain–continental region covers about 1300 up to 1650 m above sea level. The main difference between this and the previous region arises from the stronger influence of the mountain climate in this area and the mean annual temperature is 1.6 °C lower than the piedmont-continental mountain region and on average it is 6.8 °C, and the average amount of rain is 1044 mm. According to the Lang's rain factor, the climate is humid and approximates to per humid. The sub-alpine mountain region covers a wide belt from 1650 up to 2250 m. The climate in this region differs greatly from all the other regions due to the domination of the mountain climate which causes a significant drop in the average annual temperature, a drop of the average temperatures in all four seasons, but most prominently in the summer and spring months, causing the annual amplitude to drop. There are no hot days, and the number of days with frost increases. The amount of rainfall in this region does not differ drastically from the mountain continental region. The average temperature in this belt is only 4.8 °C. The vegetation season is short, around 100 days. According to the Lang factor, the climate is per humid [19]. The soils formed on limestones and dolomites occupy a large part of the soil cover of Republic of Macedonia. Based on the pedological (soil) map of the Republic of Macedonia in scale 1:200.000 [1], these soils occupy around 12.45 % of the total area of the Republic of Macedonia or 2.571.300ha. In this area, calcomelanosols / Rendzic Leptosol (RL) covers around 220.000ha or 8.55 %, calcocambisols / Chromic Leptic Luvisol on hard limestones (CLL) covers around 100.000ha or 3.88 %, but terra rossa / Rhodic Leptic Luvisol (RLL) on hard limestones rarely form continuous soil cover.

These can be found on really small areas of karst relief, they have concave shape and are characterized with mosaic and fragmented appearance, and cover around 260ha or 1.00 % of the total area. As a result of our field research, the soils formed on limestones and dolomites were found on Jablanica, Galichica, Ilinska Planina, Bistra, Suva Gora, and Suva Planina at the foot of the accumulation Kozjak, Pletvar, Sivec, as well as on the higher parts in Dojran.

Following the field recognition, locations were selected for digging out the basic pedological profiles. A total of 52 basic pedological profiles were dug out, from which 34 are Rendzic Leptosol, 13 Chromic Leptic Luvisol on hard limestones and 5 profiles of Rhodic Leptic Luvisol on hard limestones, [18]. Seventy eight soil samples were taken for laboratory analyses. The field researches were carried out in accordance with the accepted methodology in our country [20]. The pH (reaction) of the soil solution was determined electrometrically with a glass electrode in water suspension [20] and the classification of soils according to the reaction was performed according to the USA classification [21]. The humus content was determined on the basis of the organic carbon C according the Tyurin method, modified by Simakov [22]. The total N was by determination of Kjeldahl- method [21]. For all analysed properties in both horizons, analysis of variance (ANOVA) for samples of different sizes was made. The impact of the substrate, soil type and their interaction, on the variability of all examined properties was determined. The significance of differences between mean values for the analyzed properties per substrate and soil type was determined using the Tukey test, for the level of $p < 0.05$. All statistical analyzes were made with the R software package.

RESULTS AND DISCUSSION

Content humus of the soils formed upon limestones and dolomites varies extensively and depends on the deposition of nearby materials (from the higher places) and on the degree of erosion, altitude, vegetation, relief, evolution and the intensity of the soil forming process [23].

The occurrence of one or other plant communities is closely connected to the heterogenic climate, relief and soil conditions of the environment.

Table 1. Natural conditions of the studied profiles

Prof. Nº	Location	Soil types	Parent material	Geographical position		Altitude (m)	Inclination %	Exposition	Phytocenosi
				N.L	E.L.				
1	Jablanica	Eh	ML	41^{0}12ˋ17 35’’	20^0 34’ 16 83’’	1490	40-50	South	*Ass. Calamintho* **grandiflorae-Fagetum**
2	Jablanica	Eh	ML	41^{0}11ˋ49 89’’	20^0 34’ 34 37’’	1387	50	North-west	*Ass. Calamintho* **grandiflorae-Fagetum**
3	Jablanica	Eh	ML	41^{0}08ˋ06 35’’	20^0 35’ 43 17’’	1494	50-60	East	*Ass. Calamintho* **grandiflorae-Fagetum**
4	Jablanica	Lvd	ML	41^{0}07ˋ23 87’’	20^0 35’ 41 90’’	1440	50-60	East	*Ass. Querno - Quercetum ceries*
5	Jablanica	Eh	ML	41^{0}06ˋ07 06’’	20^0 35’ 49 27’’	1257	70	East	*Ass. Querco – Osttryetum carpinfoliae*
6	Jablanica	Eh	ML	41^{0}14ˋ36 55’’	20^0 32’ 10 30’’	1962	70-80	North	*Ass. Onobrichi – Festucetum cyllenicae*
7	Jablanica	Lf	ML	41^{0}06ˋ14 81’’	20^0 37’ 49 94’’	765	40-50	North	*Ass. Querco Carpinetum orientalis*
8	Jablanica	Eh	ML	41^{0}06ˋ16,66’’	20^0 37’46,10’’	791	40-50	South	*Ass. Querco Carpinetum orientalis*
9	Galičica	Lf	ML	40^0 54, 37’ 90”	20^0 44, 27’ 73˝	740	40-50	South - East	*Ass. Quercetum frainetto – cerris*
10	Galičica	Lf	ML	40^0 54, 38’ 33”	20^0 44, 28’ 83˝	740	40-50	South - East	*Ass. Quercetum frainetto – cerris*
11	Galičica	Eh	ML	40^0 57, 14’ 46”	20^0 48, 45’ 47˝	1650	50	East	*Ass. Seslerietum wettsteinii*
12	Galičica	Eh	ML	40^0 57, 14’ 95”	20^0 48, 45’ 91˝	1650	50	East	*Ass. Seslerietum wettsteinii*
13	Galičica	Eh	ML	40^0 57, 51’ 63”	20^0 48, 48’ 43˝	1460	40-50	West	*Ass. Festuco heterophillae – Fagetum*
14	Galičica	Lvd	ML	40^0 58, 19’ 92”	20^0 48, 32’ 48˝	1320	30-40	West	*Ass. Querco – Osttryetum carpinfoliae*
15	Galičica	Lvd	ML	40^0 58, 06’ 24”	20^0 48, 22’ 27˝	1154	30-40	West	*Ass. Querco – Osttryetum carpinfoliae*
16	Bistra	Eh	DL	41^0 39, 03’ 96”	20^0 42, 48’ 76˝	1706	40-50	North	*Ass. Calumintho gradiflorae – Fagetum*
17	Bistra	Eh	DL	41^0 38, 25’ 68”	20^0 41, 22’ 37˝	1728	40-50	North	*Ass. Bruckenthalietum – Juniperetum*
18	Bistra	Eh	DL	41^0 38, 25’ 68”	20^0 41, 22’ 37˝	1728	40-50	North	*Ass. Bruckenthalietum – Juniperetum*

Table 1 (continuation)

19	Bistra	Eh	DL	41^0 38, 00’ 52”	20^0 42, 44’ 07”	1730	50	North	*Ass. Bruckenthalietum – Juniperetum*
20	Bistra	Lvd	DL	41^0 38, 29’ 88”	20^0 41, 28’ 26”	1720	50	North	*Ass. Bruckenthalietum – Juniperetum*
21	Bistra	Eh	DL	41^0 38, 22’ 98”	20^0 42, 25’ 69”	1750	40-50	North	*Ass. Bruckenthalietum – Juniperetum*
22	Bistra	Lvd	DL	41^0 38, 07’ 51”	20^0 42, 32’ 37”	1730	40-50	North	*Ass. Bruckenthalietum – Juniperetum*
23	Suva Gora	Eh	BM	41^0 48, 17’ 11”	21^0 01, 06’ 12”	1350	40	South - East	*Ass. Onobrichi – Festucelum cyllericae*
24	Suva Gora	Eh	BM	41^0 48, 21’ 62”	21^0 01, 02’ 58”	1370	40	West	*Ass. Onobrichi – Festucelum cyllericae*
25	Suva Gora	Eh	BM	41^0 49, 05’ 77”	21^0 00, 21’ 28”	1270	20	West	*Ass. Onobrichi – Festucelum cyllericae*
26	Suva Gora	Eh	BM	41^0 49, 27’ 97”	20^0 59, 32’ 07”	1060	20-30	North - West	*Ass. Onobrichi – Festucelum carpinfoliae*
27	Suva Gora	Lvd	BM	41^0 49, 27’ 23”	20^0 59, 31’ 12”	1050	20-30	North	*Ass. Querco – Osttryetum carpinfoliae*
28	Suva Gora	Lvd	BM	41^0 49, 44’ 18”	20^0 59, 04’ 78”	938	30-40	North	*Ass. Querco – Osttryetum carpinfoliae*
29	Suva Gora	Lvd	BM	41^0 49, 45’ 47”	20^0 59, 05’ 57”	830	30	North - West	*Ass. Querco – Osttryetum carpinfoliae*
30	Ilinska	Lvd	PL	41^0 17, 32’ 20”	20^0 59, 07’ 21”	1522	30-40	North - East	*Ass. Calumintho gradiflorae – Fagetum*
31	Ilinska	Lvd	PL	41^0 17, 30’ 11”	20^0 59, 11’ 31”	1524	30-40	North - East	*Ass. Calumintho gradiflorae – Fagetum*
32	Ilinska	Lvd	PL	41^0 17, 28’ 49”	20^0 59, 16’ 71”	1570	30	South	*Ass. Calumintho gradiflorae – Fagetum*
33	Ilinska	Eh	PL	41^0 17, 38’ 58”	20^0 59, 02’ 05”	1501	30	North	*Ass. Calumintho gradiflorae – Fagetum*
34	Ilinska	Eh	PL	41^0 17, 39’ 71”	20^0 58, 56’ 62”	1504	30-40	North	*Ass. Calumintho gradiflorae – Fagetum*
35	Ilinska	Eh	PL	41^0 17, 49’ 18”	20^0 58, 48’ 23”	1487	40	South	*Ass. Calumintho gradiflorae – Fagetum*
36	Ilinska	Eh	PL	41^0 18, 25’ 67”	20^0 58, 47’ 92”	1437	30-40	North - East	*Ass. Calumintho gradiflorae – Fagetum*
37	Ilinska	Eh	PL	41^0 18, 27’ 39”	20^0 58, 45’ 73”	1432	30-40	North - West	*Ass. Calumintho gradiflorae – Fagetum*
38	Pletvar	Lvd	DM	41^0 24, 15’ 54”	21^0 40, 28’ 16”	1166	30	West	*Ass. Juniperus communis intermedia*
39	Pletvar	Eh	DM	41^0 24, 15’ 49”	21^0 40, 27’ 57”	1200	20-30	West	*Ass. Juniperus communis intermedia*
40	Pletvar	Eh	DM	41^0 24, 16’ 41”	21^0 40, 27’ 55”	1205	20-30	West	*Ass. Juniperus communis intermedia*
41	Pletvar	Eh	DM	41^0 24, 15’ 05”	21^0 40, 38’ 83”	1174	40-50	North	*Ass. Juniperus communis intermedia*
42	Pletvar	Eh	DM	41^0 24, 14’ 24”	21^0 40, 41’ 61”	1176	40-50	North	*Ass. Juniperus communis intermedia*
43	Pletvar	Eh	LDC	41^0 24, 51’ 88”	21^0 35, 34’ 13”	1035	50-60	North	*Ass. Juniperus communis intermedia*
44	Pletvar	Eh	LDC	41^0 24, 55’ 90”	21^0 35, 37’ 91”	975	50-60	North - West	*Ass. Juniperus communis intermedia*
45	Suva Planina	Eh	LDC	41^0 52, 32’ 87”	21^0 12, 02’ 90”	600	50-60	East	*Ass.Querco - Carpinetum orientalis subass. Buxetosum*
46	Suva Planina	Lvd	LDC	41^0 52, 51’ 52”	21^0 12, 55’ 05”	725	50-60	South	*Ass.Querco - Ostryetum carpinifoliae*
47	Suva Planina	Eh	LDC	41^0 52, 42’ 24”	21^0 12, 54’ 55”	771	50-60	South - East	*Ass.Querco - Carpinetum orientalis.*
48	Suva Planina	Eh	LDC	41^0 53, 24’ 72”	21^0 15, 29’ 90”	945	40-50	East	*Ass.Querco - Ostryetum carpinifoliae*
49	Dojran	Eh	ML	41^0 13, 44’ 54”	22^0 41, 35’ 39”	255	40-50	East	*Ass. Cocciferro – Carpinetum orientalis.*
50	Dojran	Lf	ML	41^0 13, 43’ 68”	22^0 41, 39’ 22”	233	40-50	South - East	*Ass. Cocciferro – Carpinetum orientalis.*
51	Dojran	Lf	ML	41^0 13, 46’ 36”	22^0 41, 39’ 98”	211	40-50	North	*Ass. Cocciferro – Carpinetum orientalis.*
52	Dojran	Eh	ML	41^0 14, 03’ 29”	22^0 41, 26’ 99”	243	40-50	South - East	*Ass. Cocciferro – Carpinetum orientalis.*

Calcomelanosol.- Eh; Calcocambisol - Lvd; Terra rossa – Lf; Massive limestone- ML; Dolomitic limestone – DL; Bituminous marbels – BM; Plate (flat) limestone – PL;Dolomitic marbels – DM; Laminated dolomite and calcite – LDC

Rendzic Leptosols are characterized with the highest humus content compared to other soils formed on limestone and dolomite. There is the highest average value (19.47 %) in the Rendzic Leptosol, subtype organogenic, in organomineral 13.17 %, and with evolution the humus content decreases, especially in the (B)rz horizon. In Rendzic Leptosol, subtype chromic luvic the Amo horizon there is 12.44 % and in the cambic horizon (B)rz there is 6.66 % humus (Tables 2 and 3).

Table 2. Average values for the chemical properties of Amo

Soil type	N	Humus %		Organic C %		Total N %		pH H_2O	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
1	7	19.47c*	2.62	11.29c	1.52	1.14c	0.18	6.99b	0.44
2	22	13.17b	3.93	7.64b	2.28	0.79b	0.24	6.93b	0.58
3	5	12.44b	2.92	7.22b	1.70	0.75b	0.18	6.12a	0.66
4	13	8.50a	1.97	4.93a	1.14	0.69b	0.26	6.63ab	0.66
5	5	5.33a	1.51	3.09a	0.87	0.32a	0.09	6.94b	0.22

*Values in each column marked with the same letter don't differ significantly between themselves;
1. Rendzic Leptosol organogenic; 2. Rendzic Leptosol, organomineral; 3. Rendzic Leptosol, chromic luvic;
4. Chromic Leptic Luvisol on hard limestones; 5. Rhodic Leptic Luvisol on hard limestones

Table 3. Average values for chemical properties of (B)rz

Soil type	N	Humus %		Organic C %		Total N %		pH H_2O	
		$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD	$\bar{x}$	SD
3	5	6.66b	2.02	3.87b	1.17	0.40b	0.12	6.68	0.70
4	14	5.18b	1.48	3.01b	0.87	0.34b	0.09	6.63	0.72
5	7	2.13c*	1.10	1.24a	0.64	0.12a	0.07	6.72	0.33

*Values in each column marked with the same letter don't differ significantly between themselves;
3. Rendzic Leptosol, chromic luvic; 4. Chromic Leptic Luvisol on hard limestones;
5. Rhodic Leptic Luvisol on hard limestones.

Compared to other soils formed on limestone and dolomite, Rendzic Leptosols are formed on higher altitudes so their higher content of humus is due to the lack of moisture and heat in the summer and freezing of the soil mass during the long winter period. The process of mineralization of organic matter during the winter is delayed and slow or entirely prevented (frozen soil), and when there is enough warm periods for decomposition of organic matter, mineralization is slowed down due to lack of soil moisture. Due to the lower amount of rainfall compared to other countries (eg. Montenegro), their altitude, vegetation and conditions under which the soil forming processes (accumulation of humus, decarbonatization and acidification) occur, the limit of 25% humus for the distinction between organogenic and organomineral Rendzic Leptosols is unrealistic for our conditions which was confirmed in the our research. According to [24], a more realistic boundary for distinction of these two subtypes would be where the content of humus is around 15 %. There is less humus in Chromic Leptic Luvisols on hard limestones. They are formed at a lower altitude where the conditions for mineralization are more favourable. The content of humus in the Amo horizon is 8.50 % on average and in the cambic horizon (B)rz it is 5.18 %.

Great decrease in the content of humus is observed in Rhodic Leptic Luvisol on hard limestones as a result of environmental conditions (altitude, type of vegetation), the appearance of the cambic horizon, the manner of utilization (cultivated or uncultivated), erosion and the anthropogenic factor. In addition, the surveyed Rhodic Leptic Luvisols on hard limestones are not in the literal area with a typical Mediterranean climate. The average content of humus in the Amo horizon is 5.33 % and in the cambic horizon (B)rz it is 2.13 %.

Rendzic Leptosols are characterized with the highest content of total nitrogen in relation to the other soils formed on limestone and dolomite. The subtype organogenic Rendzic Leptosols has highest mean value (1.14 %).

The content of total nitrogen (N) in the Amo horizon amounts to 0.69 % on average, and in the cambic horizon (B)rz 0.34 %. In the Rhodic Leptic Luvisol on hard limestones, the average content of total nitrogen (N) in the Amo horizon amounts to

0.32 %, and in the cambic horizon B(rz) it amounts to 0.12 %.

According to [25], Rendzic Leptosols are soils where there is gradual decline in the humus content, while Chromic Leptic Luvisols on hard limestones and Rhodic Leptic Luvisols on hard limestones are soils where there is sharp decline in the humus content. We classified the examined soils according to the content of humus [26], and the results are presented in Table 4.

All organogenic Rendzic Leptosols, belong 100 % to the class of soils with very high content of humus, (Figure 2), 27.27 % organomineral Rendzic Leptosols fall into the class of soils with high content of humus and 72.73 % in the class of soils with very high content of humus. Chromic luvic Rendzic Leptosol, 10.00 % belong to the class of soils with medium content of humus, 60.00 % are soils with high content of humus and 30.00 % are soils with very high content of humus. Chromic Leptic Luvisols on hard limestones, like Chromic luvic Rendzic Leptosol, are classified into three classes as follows: 22.22 % with average humus content, 70.37 % high humus content and 7.41 % belong to the class of soils with very high humus content. In Rhodic Leptic Luvisol on hard limestones there are no samples which fall into the class of soils with high humus content, 41.67 % are soils with low humus contents, there is the same percentage with the soils with medium humus content, and 16.66% are soils with high humus content. The following authors have had similar results on the humus content: [4, 7, 8, 11–14, 16, 17, 23, 27, 28].

Table 4. Number of soil samples according to soil type and subtype allocated into groups with different content of humus

Soil type and subtype	**Humus %**			
	1 – 3	**3 – 5**	**5 – 10**	**> 10**
Organogenic Rendzic Leptosol	0.00	0.00	0.00	100
Organomineral Rendzic Leptosol	0.00	0.00	27.27	72.73
Chromic luvic Rendzic Leptosol	0.00	10.00	60.00	30.00
Chromic Leptic Luvisol on hard limestones	0.00	22.22	70.37	7.41
Rhodic Leptic Luvisol on hard limestones	41.67	41.67	16.66	0.00

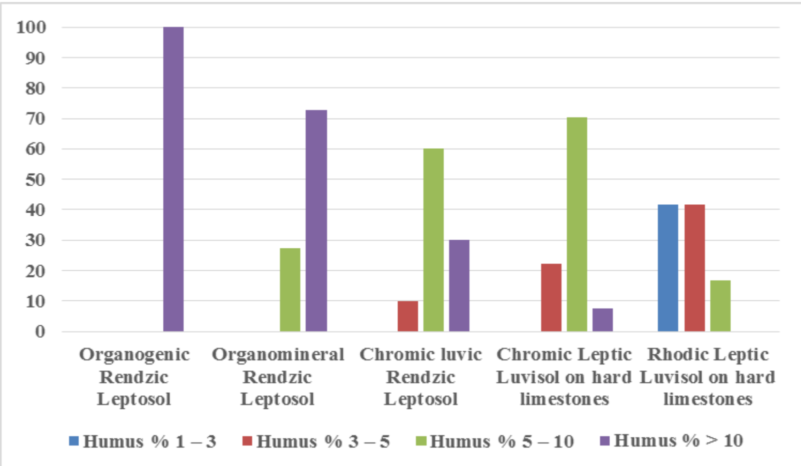

Figure 2. Soil samples according to soil type and subtype allocated into groups with different content of humus in procent

Analysis of the variance (Table 5) showed that in both horizons, the soil type has a significant impact on the variability of the humus content. The parent material, similar as the soil type has significant influence over the content of humus in the Amo horizon.

The soils do not contain carbonates. The reaction of the soil solution varies widely, depending on the developmental stage of the soil type, altitude, vegetation, duration of acidification, erosion, and the manner of use. The brownification that begins to occur in the Chromic luvic Rendzic Leptosols subtype and later the appearance of illimerization in some Rhodic Leptic Luvisol on hard limestones contributes to the gradual change of the reaction of the soil solution in soils.

In order to provide better overview of the heterogeneity of the reaction in these soils, Figure 3 presents data on the reaction in H_2O according to horizons.

Table 5. Analysis of variances of chemical properties of the humus accumulative Amo and cambic horizon (B)rz

Hor.	Factors	Mean Sq					
		Df	**Humus**	**Total C %**	**Total N %**	**pH H₂O**	**pH KCl**
Amo	**Soil type**	4	200.82***	67.50***	0.51***	0.78**	1.05**
	Parent material	5	30.26**	10.19**	0.22***	1.84***	2.75***
	Type x substrate	9	12.83*	4.23*	0.03	0.05	0.16
	Error	33	5.73	1.93	0.02	0.17	0.22
(B)rz	**Soil type**	2	34.42***	11.59***	0.13***	0.01	0.11
	Parent material	5	1.16	0.38	0.00	1.28***	1.95***
	Type x substrate	2	6.15	2.09	0.02	0.07	0.04
	Error	16	2.11	0.72	0.00	0.17	0.29

* Significant at the level of 0.05; ** significant at the level of 0.01; *** significant at the level of 0.001.

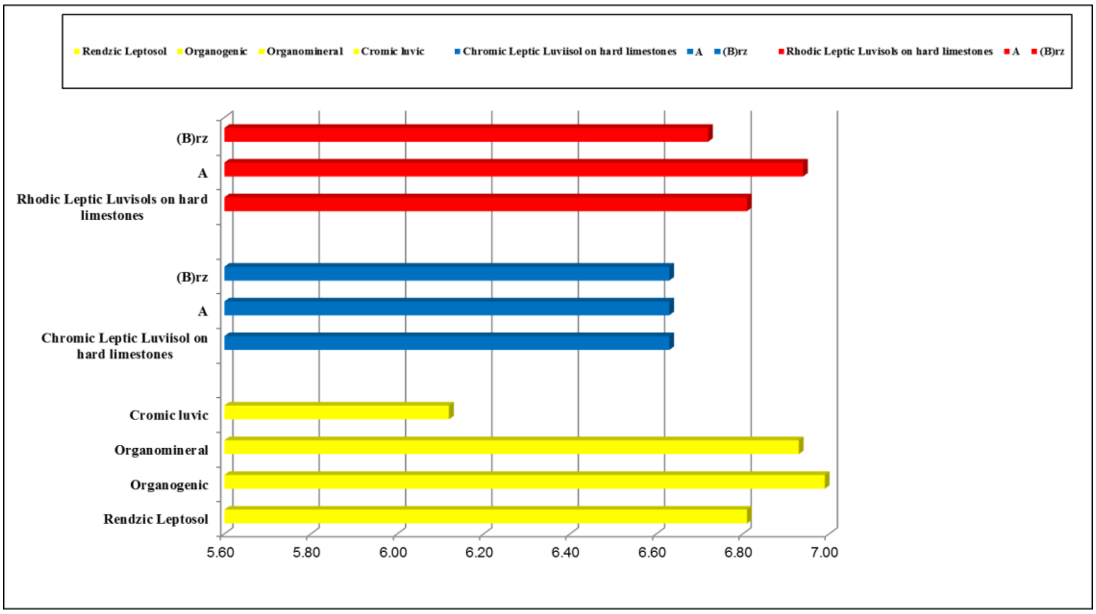

Figure 3. Overview of the pH reaction in H_2O in the soils formed on limestone and dolomites

The high heterogeneousness of the reaction of the soil solution can also be seen from the results in Tables 1 and 2. The average value of pH in H_2O at the subtype organogenic Rendzic Leptosols is 6.99. Organomineral Rendzic Leptosols have slightly lower value (6.93). In the Amo horizon, at the Chromic Luvic Rendzic Leptosols, the average value is 6.12 (statistically lowest value for this soil type) and in cambic horizon (B)rz, the average value is 6.68. With the evolution of the Rendzic Leptosols into Chromic Leptic Luvisols on hard limestones, it comes to a debazification and acidification, due to which the soil solution becomes slightly acidic and the average pH value in H_2O in the horizon Amo and (B)rz is 6.63. In Rhodic Leptic Luvisol on hard limestones, the average pH in H_2O in the Amo horizon is 6.94, while in the cambic horizon (B)rz it is 6.72. Difference was not found between the reactions of the soil solution in Rhodic Leptic Luvisol on hard limestones at various locations and altitudes. We noticed higher values of the reaction of the soil solution at the soils formed on limestone and dolomites in comparison to the same soil types in other countries (Montenegro, Serbia, Bosnia and Herzegovina, Croatia, Slovenia, Albania, Greece, Spain and other) [23].This is a result of a lower quantity of rainfalls and the previously mentioned conditions. Similar values for the reaction of the soil solution for the soils formed on limestone and dolomite, can be found in the studies of: [15, 17, 29, 30]. On the basis of the American classification (Soil Survey Manual, 1951) cit. [20], the reaction of the soils formed on limestone and dolomites ranges from slightly acidic to neutral. At the Rendzic Leptosols, it ranges from slightly acidic to neutral, whereby the subtypes organogenic and organomineral Rendzic Leptosols belong to the class of neutral soils, and the Chromic Luvic Rendzic Leptosols belong to the slightly acidic class. Chromic Leptic Luvisols on hard limestones and Rhodic Leptic Luvisol on hard limestones belong to the classes of neutral soils. The variance analysis has shown that the soil type has strong influence in the Amo horizon over the variability of the pH reaction, while in the cambic horizon (B)rz, the soil type has no influence over the reaction of the soil solution. Unlike the soil type, the parent material has significant influence on the pH-reaction in H_2O in the both horizons Amo and (B)rz, (Tables 1 and 2), whereby the statistically lowest average pH value in H_2O (5.76) can be found in the soils formed on limestone slabs, and the highest value (7.40) can be found at the soils formed over slabs of dolomite and calcite. There is no significant deviation in influence of the other substrates from the highest average value.

CONCLUSION

Rendzic Leptosols are characterized with the highest content of humus when compared to the other soils formed on limestone and dolomite. Chromic Leptic Luvisols on hard limestones have lower content of humus. They are formed on a lower altitude where the conditions for mineralization are favorable. The mean content of humus in the Amo horizon is 8.50 %, while in the cambic horizon (brz) it is 5.18 %. A large decline in the content of humus was noticed in Rhodic Leptic Luvisol on hard limestones, as a result of the environmental conditions (altitude, the type of vegetation), the occurrence of cambic horizon (B)rz, the manner of utilization (cultivated or non-cultivated), the erosion and the anthropogenic factor. In addition, the examined Rhodic Leptic Luvisol on hard limestones are not located in the literal zone with typical Mediterranean climate. The average content of humus in the Amo horizon is amounting to 5.33 %, while in the cambic horizon (B)rz it is amounting to 2.13 %. The information from the variance analysis indicate that in both horizons, the soil type has a significant influence on the variability of the content of humus, and the parent material, similar to the soil type, has significant influence on the content of humus in the Amo horizon.

The soils do not contain carbonates. Based on the American classification, the reaction of the soils formed on limestone and dolomites ranges from strongly acidic to neutral. At the Rendzic Leptosols, it ranges from extremely acidic to neutral, whereby the subtypes organogenic and organomineral Rendzic Leptosols belong to the class of poorly acidic soils, while the Chromic Luvic Rendzic Leptosols belongs to the class of moderate acidic soils. Chromic Leptic Luvisols on hard limestones belong to the class of extremely acidic to neutral soils, and the Rhodic Leptic Luvisol on hard limestones belongs to the class of strongly acidic to slightly acidic soils.

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СОДРЖИНА НА ХУМУС И pH РЕАКЦИЈА КАЈ ПОЧВИТЕ ОБРАЗУВАНИ ВРЗ ВАРОВНИЦИ И ДОЛОМИТИ

Миле Маркоски[1], Татјана Миткова[1], Вјекослав Танасковиќ[1], Велибор Спаљевиќ[2]

[1]Факултет за земјоделски науки и храна, Универзитет Св. Кирил и Методиј, Скопје, Република Македонија

[2] Факултет за филозофија, Универзитет во Црна Гора, Никшиќ, Црна Гора

Испитувани се почвите образувани врз варовници и доломити на различни локации на територијата на Република Македонија. Теренските истражувања се извршени во текот на 2010, 2011 и 2012 година, при што беа ископани 52 основни педолошки профили од кои 34 се калкомеланосоли, 13 калкокамбисоли и 5 профили на црвеница. На овие почви оишани се нивната генеза, еволуција, класификација и хемиските својства. Овие почви се карактеризираат со тип на профил О-А-R; А-R; А-(B)rz-R. Калкомеланосолите се карактеризираат со најголема содржина на хумус во однос на останатите почви образувани на варовник и доломит. Најголема средна вредност (19,47 %) има во поттипот органогена В.Д.Ц. Во калкокамбисолите има помалку хумус. Содржината на хумус во хоризонт Аmo средно изнесува 8,50 %, а во камбичниот хоризонт (B)rz 5,18 %. Кај црвеницата просечната содржина на хумус во хоризонтот Аmo изнесува 5,33 %, а во камбичниот хоризонт (B)rz 2,13%. pH во H_2O кај поттипот органогена В.Д.Ц просечно изнесува 6,99, просечна вредност (6,93) имаат органоминералните В.Д.Ц. Во хоризонтот Аmo кај браунизираната В.Д.Ц изнесува 6,12 а во кабичниот хоризонт (B)rz, средно 6,68. Во калкокамбисоли доаѓа до дебазификација и ацидификација, заради што почвениот раствор се закиселува и во хоризонтот Аmo и (B)rz просечната вредност на pH во H2O изнесува 6,63. Во црвениците просечната pH во H_2O во хумусно-акумулативниот хоризонт Аmo изнесува 6,94 а во камбичниот хоризонт (B)rz 6,72.

Клучни зборови: хумус; почва; варовници; доломити

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Original scientific paper

RISK ASSESSMENT RESULTING FROM RADIONUCLIDES IN SOILS OF THE REPUBLIC OF MACEDONIA[#]

Zdenka Stojanovska[1], Blažo Boev[2], Mimoza Ristova[3], Ivan Boev[2], Sorsa Ajka[4], Zora S. Žunić[5], Kremena Ivanova[6]

[1]Faculty of Medical Sciences, Goce Delcev University, Stip, Republic of Macedonia
[2]Faculty of Natural and Technical Sciences, Goce Delcev University, Stip, Republic of Macedonia
[3] Institute of Physics, Faculty of Natural Sciences and Mathematic, Ss Cyril and Methodius University, Skopje, Republic of Macedonia
[4]Croatian Geological Survey, Zagreb, Croatia
[5]Vinča Institute of Nuclear Sciences, University of Belgrade, Belgrade, Serbia
[6]National Center of Radiobiology and Radiation Protection, Sofia, Bulgaria

[*]e-mail: blazo.boev@ugd.edu.mk

In the last decade, many studies have been made for measuring natural radioactivity in regions of the Republic of Macedonia. However, the information on terrestrial radiation exposure, and, consequently, risk assessment were not available. In this study, a risk assessment was done considering a specific activity concentration of ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs in the topsoil. The results indicate that the external gamma doses due to natural radionuclides in soils are higher than those of ^{137}Cs. The absorbed dose rate in air varies in intervals: from 4.3 to 57 nGy/h (due to ^{226}Ra); from 3.9 to 88 nGy/h (due to ^{232}Th); from 3.3 to 58 nGy/h (due to ^{40}K); and from 0.01 to 5.3 nGy/h (due to ^{137}Cs). In addition, the mean annual effective doses due to natural radionuclides and ^{137}Cs in the soils of the Republic of Macedonia are estimated to be 78 μSv and 1.01 μSv, respectively. As well as, the values of external hazard index H_{ex} indicate in general low gamma radiation risk for populations living in the Republic of Macedonia.

Key words: outdoor; gamma dose; external hazard index

INTRODUCTION

The study of different radioactive sources in the environment has led to extensive surveys in many countries. Cosmic and terrestrial radiation as well as the associated public exposure due to it, depend primarily on the geological and geographical conditions and seem at completely different levels of every region within the world [1, 2].

Public exposure due to cosmic radiation varies with altitude and solar activity while exposure from the terrestrial materials depends on its geological origin. Terrestrial radioactivity arises mainly from natural radionuclides, like ^{40}K, also the radionuclides from ^{232}Th- and ^{238}U-chains [2]. These radionuclides, which are formed as a result of the primary processes during the formation of the Earth, are named "primordial radionuclides". Because of their very long half-lives, they still exist within the Earth crust up to nowadays. In addition to primordial radionuclides in the soil, the artificial ^{137}Cs occurs mostly as a result of the Nuclear weapon tests and Chernobyl reactor accident [3] within the last century. The radionuclides presence in the Earth surface leads to external exposure of the people, proportionally to their concentration in soil. The natural radionuclides concentrations in soil depend on the radioactivity of the rock from which the soil is formed. Furthermore, many processes, such as sorption, deposition, and washout of radionuclides upon the influence of natural waters in the soil, caused changes in the radionuclides concentrations.

[#]Dedicated to academician Gjorgji Filipovski on the occasion of his 100th birthday

In specific, radon decay products and gamma radiation emitted by terrestrial natural radionuclides arise in the soil and in building materials are the main sources of the internal and external exposures of the peoples. Studying of the radionuclides content in the soils provides essential radiological information about the background level and the possibility for population risk assessment considering its spatial variation.

The quantities commonly used for population risk assessment due to external exposure on terrestrial radioactivity are external absorbed dose rate in air at 1 m above the ground level and the annual effective dose. In many studies, for the population radiological risk evaluation due to natural radionuclides in the soil the external hazard index is engaged as well [4–6]. Even, it has been developed for building materials [7], its application for soils was widely used because of the proportionality between the outdoor, and the indoor gamma doses.

Concerning the great benefits from the studies of radioactivity in the environment, extensive surveys in the Republic of Macedonia were conducted within the last decade. Beside several investigations of radon and thoron [8–18] and radioactivity in some types of cement [19] investigations of the radionuclides in the surface soils across the country were conducted as well [20–23]. In this work, estimation of the external radiation risk was done using the results of ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs specific activities measured in the 213 soil samples, sampled over the entire territory of the country.

MATERIAL AND METHODS

The Republic of Macedonia is located within the central part of the Balkan Peninsula, occupying an area of 25 713 km^2. The area is characterized by diverse relief and complex geology. Over 50 % of the total area is mountain massive. The territory is organized into 8 statistical regions: Polog (POL); Southwest (SW); Pelagonija (PEL); Skopje (SKO); Vardar (VAR); Southeast (SE); East (EAST); Northeast (NE).

Soil samples and measurements

The topsoil (0–20cm) was sampled throughout the period of 2007–2010 from 213 locations. The sampling locations were on the uncultivated fields in/or close to inhabited areas. Their geographical positions across the eight statistical regions of the country are shown in Figure 1. In the laboratory, the samples were crushed, dried at 105 °C, sieved and then transferred in 500 ml Marinelli beakers. Furthermore, each sealed Marinelli was kept aside for about one month to ensure secular equilibrium between ^{226}Ra and its decay products prior to gamma spectrometry measurements.

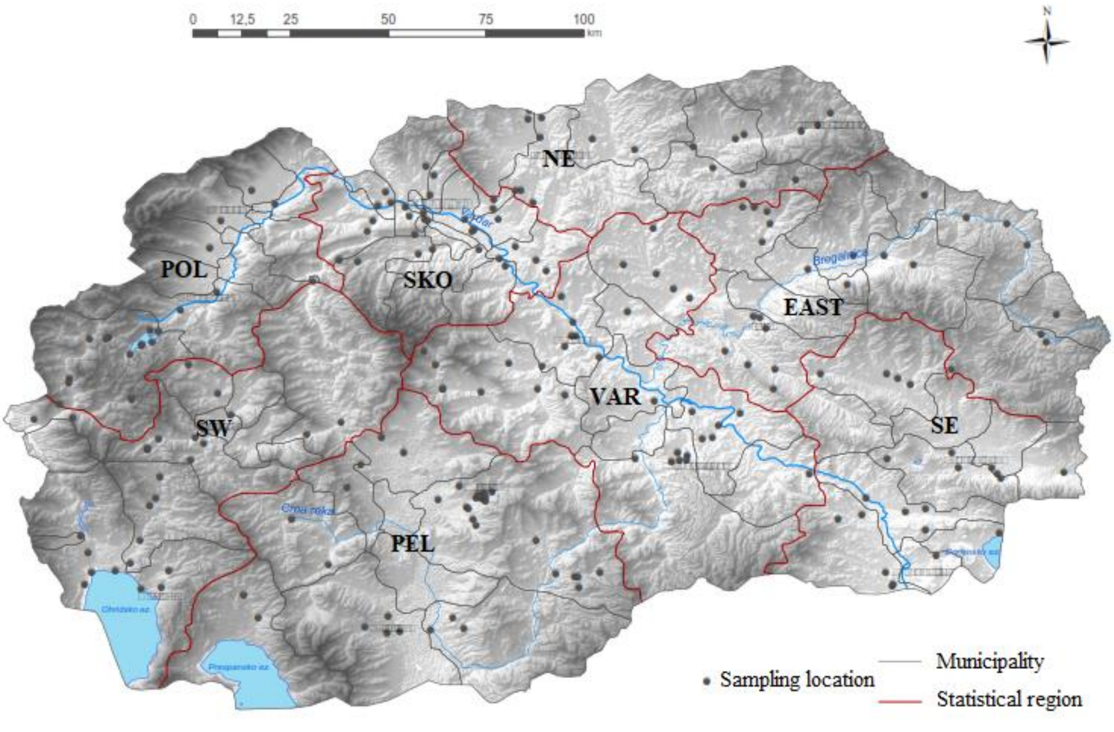

Figure 1. Soil sampling locations across the territory of the Republic of Macedonia

The measurements were carried out with a p-type HPGe detector (Canberra Inc.; 25 % relative efficiency, resolution of 1.79 keV at 1.33 MeV, 8192 ch. digital analyzer), and with software GENIE 2000 system for spectrum evaluation. The methodology of measurements is already explained in our previous published paper [20].

External effective dose due to natural and artificial radioactivity in soil

The external gamma dose rate in the air at 1 m above ground level was determined from measured specific activities considering factors of 0.462, 0.604, 0.042 and 0.030 for converting the activities of ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs to absorbed dose [1–3], as given below:

$$D = 0.462 A_{Ra} + 0.604 A_{Th} + 0.042 A_{K} + 0.030 A_{Cs} \quad (1)$$

Where D is the dose rate in nGy/h and A_{Ra}, A_{Th}, A_{K} and A_{Cs} are the specific activities in Bq/kg of ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs, respectively.

Furthermore using the estimated absorbed dose rate D, the annual effective dose is determined by [1, 2]:

$$D_{E} = D \cdot 0.7 \cdot 8760 \cdot 0.2 \quad (2)$$

where to determine the biological hazard to which a person is exposed, Gy is converted to Sv considering the conversion factor of 0.7, and the occupancy factor of 0.2 specifies the proportion of the total time (8760 h/y) spent outdoors.

External hazard index due to natural radioactivity in soil

The external hazard index is a useful coefficient for comparing the activities of materials that contain ^{226}Ra, ^{232}Th, ^{40}K considering the radiation effect associated with them. The external hazard index [7] is defined as follows:

$$H_{ex} = \frac{A_{Ra}}{370} + \frac{A_{Th}}{259} + \frac{A_{K}}{4810} \quad (3)$$

where, A_{Ra}, A_{Th}, A_{K} and A_{Cs} are the specific activities (Bq/kg) of ^{226}Ra, ^{232}Th and ^{40}K, respectively. If $H_{ex}<1$, the radiation hazard is insignificant that corresponds to the dose limit of 1 mSv for the population [24].

RESULTS

Descriptive statistics of the total gamma dose rates in the air at 1 m above ground level as well separately due to ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs in soil collected at 213 different locations across the entire territory of the Republic of Macedonia is presented in Table 1.

Table 1. Descriptive statistic of the gamma dose rates in the air at 1 m above ground level from 213 different locations across the whole country territory

Statistic	^{40}K		^{226}Ra		^{232}Th		^{137}Cs		D(tot) nGy/h
	D nGy/h	%	D nGy/h	%	D nGy/h	%	D nGy/h	%	
Min.	3.34	14	4.34	17	3.93	18	0.01	0	13
Max.	57.96	58	57.03	76	87.73	84	5.34	7.61	176
AM	24.41	36	18.76	27	24.91	36	1.15	1.80	69
SD	7.99	7	8.26	6	10.88	7	0.93	1.46	24
GM	22.92	35	17.17	26	22.78	35	0.82	1.27	65
GSD	1.47	1.24	1.53	1.22	1.55	1.19	2.58	2.62	1.43

The values of the gamma doses rates due to both natural and artificial isotopes D(tot) varies between 13 nGy/h and 176 nGy/h with geometric mean (GM) of 65 nGy/h. In general, higher mean gamma doses rates that originate from the natural radionuclides were found, compared to doses from the artificial ^{137}Cs. On the other hand, the gamma doses rate due to exposure to ^{232}Th and ^{40}K are higher compared to doses from ^{226}Ra (Kruskal-Wallis test, error probability $p < 0.0001$). The dispersions of the results, expressed through the standard deviation and the geometric standard deviation, are smaller for natural radionuclides than for the ^{137}Cs. All data fitted well with log-normal distribution (Kolmogorov-Smirnov test, $p > 0.05$).

Descriptive statistics of the gamma dose rate due to natural radionuclides and ^{137}Cs in the soil, sampled in the 8 statistical regions of the Republic of Macedonia is given in Table 2.

Table 2. Descriptive statistic of external gamma dose rate due to Natural and artificial radionuclides in the soils of the 8 statistical regions of the Republic of Macedonia.

		Gamma dose rate (*D*) in the air at 1 m above ground							
	Statistical regions	SKO	VAR	SE	EAST	NE	PEL	SW	POL
Natural radionuclides	No. of observations	33	31	20	24	14	38	34	19
	Minimum (nGy/h)	32	39	13	43	36	56	51	38
	Maximum(nGy/h)	71	134	138	108	108	175	103	79
	AM (nGy/h)	50	70	57	69	63	86	75	61
	SD (nGy/h)	11	25	33	18	22	22	16	13
	GM (nGy/h)	48	66	48	67	60	84	73	59
	GSD	1.27	1.39	1.87	1.29	1.39	1.26	1.25	1.25
Artifitial radionuclides	Minimum (nGy/h)	0.15	0.20	0.07	0.15	0.01	0.01	0.36	0.03
	Maximum (nGy/h)	2.68	3.75	1.98	3.19	4.11	5.19	5.34	4.50
	AM (nGy/h)	1.08	1.08	0.73	1.15	0.85	1.15	1.46	1.56
	SD (nGy/h)	0.69	0.89	0.51	0.81	1.07	0.88	1.05	1.36
	GM (nGy/h)	0.83	0.81	0.57	0.87	0.43	0.86	1.19	0.87
	GSD	2.26	2.12	2.17	2.28	4.37	2.70	1.86	4.01

The geometric mean (GM) values of the gamma dose rate due to natural radionuclides and artificial (^{137}Cs) in soils of the statistical regions were in intervals from 48 nGy/h to 84 nGy/h and from 0.43 nGy/h to 1.19 nGy/h, respectively. Geometric standard deviations (GSD) of the doses were in intervals from 1.25 to 1.87 and from 1.86 to 4.37 for natural and artificial radionuclides, respectively. In all regions, the contribution of ^{226}Ra to the dose is lower compared to those of ^{232}Th and ^{40}K (Figure 2).

The descriptive statistic and interpolated maps of the annual effective doses estimated due to the natural radionuclides and ^{137}Cs activities in the soil are shown in Table 3 and Figure 3.

In the present work, the annual effective dose due to the natural radionuclides ranges between 15 μSv and 215 μSv with the geometric mean value of 78 μSv; the annual effective dose due to the ^{137}Cs ranges between 0.01 μSv and 6.55 μSv with the geometric mean value of 1.01 μSv (Table 3).

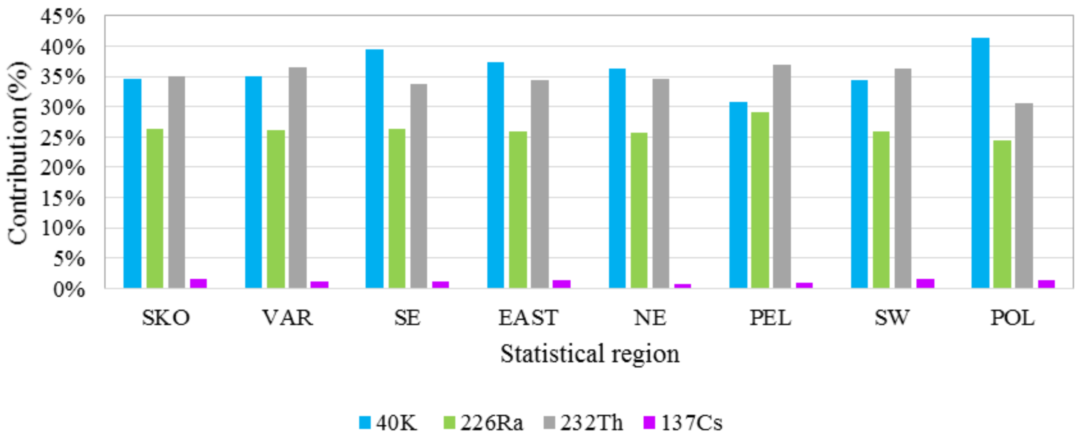

Figure 2. Different contributions of the ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs into total absorbed dose rate through 8 statistical regions

Table 3. Descriptive statistic of the annual effective doses due to Natural and artificial radionuclides in soil

	Annual effective dose D_E	
	Natural radionuclides	^{137}Cs
No. of observations	213	213
Minimum (μSv/y)	15	0.01
Maximum (μSv/y)	215	6.55
Arithmetic mean (μSv/y)	83	1.42
Standard deviation (μSv/y)	29	1.14
Geometric mean (μSv/y)	78	1.01
Geometric standard deviation	1.44	2.58

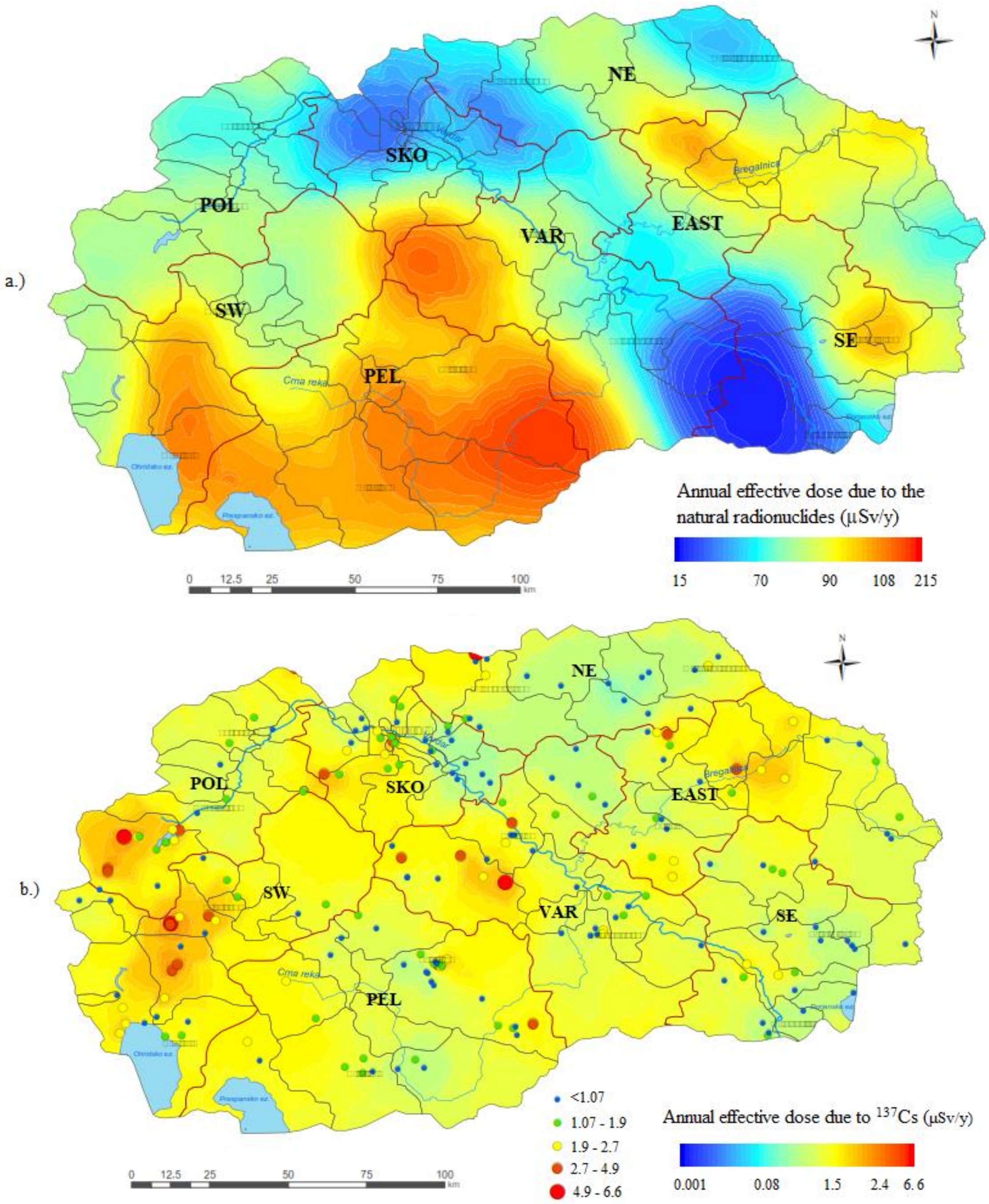

Figure 3. Interpolated maps of the annual effective doses due to the natural radionuclides (a) and ^{137}Cs (b) specific activities in the soil

As can be seen from Table 3 and Figure 3, the annual effective dose received due to the exposure to natural radionuclides is much higher than the annual effective dose due to ^{137}Cs, i.e., the contribution of ^{137}Cs in the total external exposure of the population is negligible.

On the map in Figure 4, the values of the external hazard index for each sampling location are shown. The estimated hazard index values (H_{ex}) ranged from 0.07 to 1.05. The value of the external hazard index exceeded 1 only at one location.

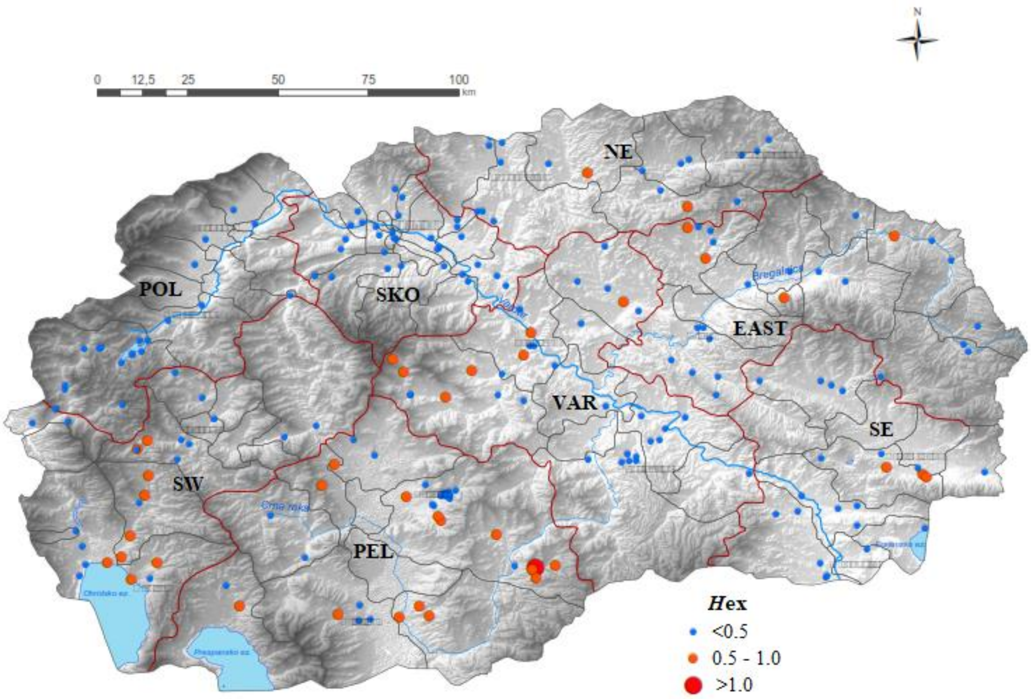

Figure 4. External hazard index (H_{ex}) values through the territory of the Republic of Macedonia

DISCUSSION

External exposure due to gamma radiation emitted by ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs in the soils appear at different levels across to the country territory. In general, the contribution of natural radionuclides (^{226}Ra, ^{232}Th, ^{40}K) and ^{137}Cs to the total gamma dose rate are different (Table 1). Specifically, the mean contributions of ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs into the total gamma dose rate are 26%, 35%, 35% and 1.27% respectively.

The results show a negligible contribution of the ^{137}Cs in the total external gamma exposure of the population in the Republic of Macedonia (Table 2 and Table 3). As mention previously, the ^{137}Cs in soils are different in origin: nuclear weapon tests and Chernobyl accident. Considering that no published results related to ^{137}Cs activity in the country before the Chernobyl accident makes the evaluation of the origin's relevant contributions demanding. Overall, people living in the western part of the country receive a higher dose compared to those living in the east (Figure 3b). It could be a result of the complex dispersion pattern of cesium in the environment but also linked to higher levels of precipitation in the western part of the country.

Otherwise, the specific activities of ^{226}Ra, ^{232}Th and ^{40}K in the soils are associated to the formation of each lithological area, particularly to its content in the rock from which the soils originate [20]. Commonly, the geology of the Republic of Macedonia comprises a variety of complex of sedimentary, metamorphic and volcanic rocks with different age and mineral content. The highest gamma dose rate was related to the soil sample from Mariovo that belongs to the Pelagonia region (Figure 3a). Higher gamma dose rates are found in South West, as well, compared to other statistical regions (Table 2). The South West statistical region appertains into the Western Macedonian geotectonic zone while as the Pelagonija statistical region belongs to the Western Macedonian zone and Pelagonian Massif. Both geotectonic zones comprised rocks of volcanic origin [25]. The contribution of the natural radionuclides into doses varied within regions as well as. For example, maximum contributions due to such radionuclide into a total absorbed dose were found to be in PEL (29%) due to ^{226}Ra; VAR (37%) and PEL (37%) due to ^{232}Th and POL (41%) due to ^{40}K.

The mean gamma dose rate for the whole country of 65 nGy/h was similar to that of 62.8 nGy/h reported for the neighboring country Serbia [26] but higher than the dose of 54 nGy/h obtained from survey in Kosovo and Metohija [27] and dose of 46.2 nGy/h assessed for population in the Thessaloniki city in Greece [28].

The mean annual effective dose value of 78 μSv/y due to natural radionuclides in the soils of the Republic of Macedonia is slightly higher than the worldwide value of 70 μSv/y reported in UNSCEAR reports [1–3].

CONCLUSION

The gamma exposure of the population due to ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs contained in the soils of the Republic of Macedonia have been analyzed. The gamma doses emitted from ^{137}Cs was negligible compared to the dose originated from natural radionuclides. The contribution of the natural ^{226}Ra, ^{232}Th and ^{40}K in the total dose of the population in the Republic of Macedonia were 26%, 35% and 35% respectively. In general, in the regions where bedrocks are from the volcanic origin the exposure due to ^{226}Ra, ^{232}Th, ^{40}K in soil is higher in comparison with other parts of the country. The obtained results are comparable with the results reported in the literature from neighboring countries.

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ПРОЦЕНКА НА РИЗИКОТ ОД РАДИОНУКЛИДИТЕ ВО ПОЧИТЕ НА РЕПУБЛИКА МАКЕДОНИЈА

Зденка Стојановска[1], Блажо Боев[2], Мимоза Ристова[3], Иван Боев[2], Сорса Ајка[4], Зора С. Жуниќ[5], Кремена Иванова[6]

[1]Факултет за медицински науки, Универзитет „Гоце Делчев“, Штип, Република Македонија
[2]Факултет за природни и технички науки, Универзитет „Гоце Делчев“, Штип, Република Македонија
[3] Институт за физика, Факултет за природни науки и математика,
Универзитет „Св. Кирил и Методиј“, Скопје, Република Македонија
[4]Хрватски геолошки завод, Загреб, Хрватска
[5]Винча институт за нуклеарни науки, Универзитет во Белград, Србија
[6]Национален центар за радиобиологија и заштита од радијација, Софија, Бугарија

Во последната деценија се направени многу студии за мерење природна радиоактивност во региони на Република Македонија. Сепак, информациите за експозицијата на терестријалните зрачења и следствено процената на ризикот од нив не беа достапни. Во оваа студија, процената на ризикот беше направена врз основа на специфичните активности на ^{226}Ra, ^{232}Th, ^{40}K и ^{137}Cs во површинската почва. Резултатите укажуваат на тоа дека надворешните гама-дози што потекнуваат од природните радионуклиди во почвите се повисоки во однос на оние од ^{137}Cs. Брзината на апсорбираната доза во воздухот варира во интервали: од 4,3 до 57 nGy / h (од ^{226}Ra); од 3,9 до 88 nGy / h (од ^{232}Th); од 3,3 до 58 nGy / h (од ^{40}K); од 0.01 до 5.3 nGy / h (од ^{137}Cs). Проценетите средни годишни ефективни дози што потекнуваат од природните радионуклиди и ^{137}Cs во почвите на Република Македонија се 78 μSv и 1.01 μSv, соодветно. Вредностите на индексот на надворешна опасност Hex општо укажуваат на низок ризик од гама-зрачење за населението кое живее во Република Македонија

Клучни зборови: надворешна гама доза; индекс на надворешна опасност

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Original scientific paper

DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR DETERMINATION OF SOME PESTICIDE RESIDUES IN WATER SAMPLES[#]

Lenche Velkoska-Markovska[*], Biljana Petanovska-Ilievska

Faculty of Agricultural Sciences and Food, Ss. Cyril and Methodius University,
Skopje, Republic of Macedonia

[*]e-mail: lencevm@fznh.ukim.edu.mk

The development of a new reversed-phase high-performance liquid chromatography method (RP-HPLC) with ultraviolet-diode array detection (UV-DAD) for simultaneous determination of 2,4-D, atrazine, malathion, fenitrothion and parathion residues in different water samples are described in this paper. The developed method has been validated according to European Commission guidelines for pesticide residue analytical methods, and all performance characteristics were found within acceptance criteria. The best separation and quantitative determination of the analytes were achieved using a LiChrospher 60 RP-select B (250 × 4 mm, 5 µm) analytical column, under the isocratic elution with mobile phase consisting of acetonitrile/water (60/40, *V/V*), flow rate of 1 ml/min, constant column temperature at 25 °C and UV-detection at 220 nm and 270 nm. The run time of analysis under the stipulated chromatographic conditions was about 10 min.

Key words: RP-HPLC; UV-DAD; method validation; pesticide residues; water samples

INTRODUCTION

As it is well-known, pesticides are natural or synthetic chemical compounds destined to destroy or prevent the growth of any pest (insects, weeds, diseases, fungi, etc.) that threatens the production of agricultural crops [1]. Farmers are extensively applying pesticides to increase yields while saving time and money [2]. However, only a small part of the applied pesticides reach the target plants, and the remainder remains in the air, soil and water. Excessive use of pesticides leads to pollution of water, soil and air, as well as, causes their accumulation in agricultural crops [3]. Water is the most important and crucial for life, and its pollution is a major problem nowadays. Due to the solubility of pesticides in water, they can cause serious environmental pollution (soil, water and air) and human health disorders [4]. Through primary agricultural products, they can be found in processed products for human consumption.

2,4-D and malathion are among the most used pesticides in R. Macedonia, and until several years ago, fenitrothion, parathion and atrazine were also widely used chemical plant protection products. Although atrazine, fenitrothion and parathion are forbidden for use in the EU, they are still allowed in the United States (except for parathion) and in some third countries. In addition, as a result of the unauthorized use of these pesticides, they can be found in environmental samples (water, soil, air) and food.

Herbicides from the chlorophenoxy carboxylic acids group, such as 2,4-D (Figure 1a) are characterized by relative stability and photostability in the natural waters because they are considered as persistent organic pollutants and pose a serious ecological problem [5].

Triazines, especially atrazine (Figure 1b) [6], are among the most commonly used herbicides in the world. Their use causes great concern because of their mobility and high solubility in water that allows them to pass into underground and surface waters [7]. Chemical pollution of surface waters is a threat to the aquatic environment causing negative effects such as acute and chronic toxicity to aquatic

[#]Dedicated to academician Gjorgji Filipovski on the occasion of his 100th birthday

organisms, accumulation in the ecosystem, loss of biodiversity, and a threat to human health. Atrazine represents a significant risk to the aquatic environment and it is one of the 45 priority harmful substances according to Directive 2013/39/EU of the European Parliament and the Council of 2013 [8].

Organophosphate pesticides, such as malathion (Figure 1c), fenitrothion (Figure 1d) and parathion (Figure 1e) are toxic for both humans and animals, and they are also quite stable under natural environmental conditions [9]. Their improper use can cause their presence in agricultural products and the environment.

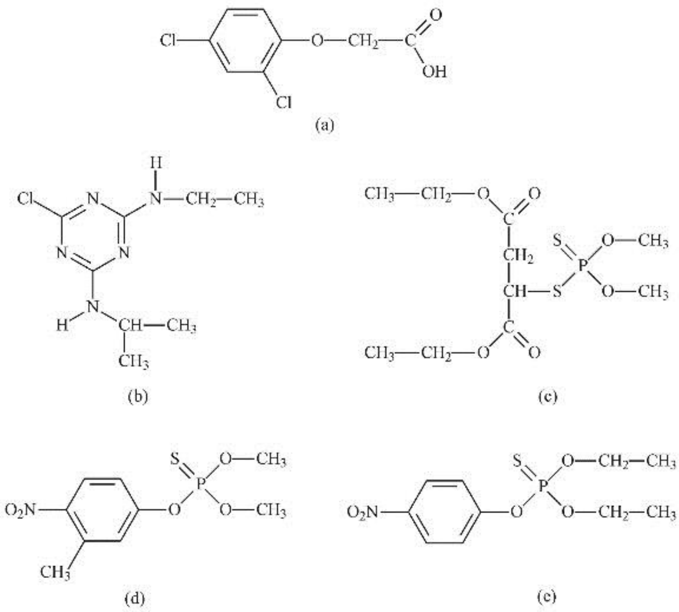

Figure 1. Structural formulas of 2,4-D (a), atrazine (b), malathion (c), fenitrothion (d) and parathion (e)

In order to protect the consumers' health from possible adverse effects, controlling the content of pesticides and their residues in environmental and food samples is necessary. In order to avoid any negative impact on human health, as well as to manage good agricultural practices, maximum residue levels of pesticides (MRLs) in food and water have been stipulated in most countries. The MRLs of pesticides in waters of class I and II, including drinking water, mineral waters and some surface waters are regulated by Directive 98/83/EC in the EU [10] and by the Water Safety Rule [11] in R. Macedonia, and they are fixed at 0.1 μg/l individually for each pesticide or 0.5 μg/l for the total quantity of all pesticides.

Of particular importance is using the highly sensitive and selective analytical methods, as well as their continuous improvement for the monitoring of pesticide residues in food and water samples. The most widely used analytical techniques are gas chromatography [12] with different detectors, such as: mass spectrometry (MS) [13, 14], flame photometric detector (FPD) [15], nitrogen phosphorous detector (NPD) [16], electron capture detection (ECD) [17], and also liquid chromatography with tandem mass spectrometry (MS/MS) [18, 19] and fluorescence detector [15]. Despite that they are less sensitive, HPLC (High Performance Liquid Chromatography) methods with diode array detection (DAD) are still used [20].

Gas and liquid chromatography are very powerful techniques for analyzing pesticides in different samples, but sample preparation, such as the extraction or concentration of the analytes before their chromatographic determination, is usually required. Several extraction techniques are known that can be used to extract pesticides from different matrices, especially from water samples [21] such as liquid-liquid extraction (LLE) [22], liquid-phase microextraction (LLME) [23], solid-phase extraction (SPE) [18], solid-phase microextraction (SPME) [24], and recently used, a quick, easy, cheap, effective, rugged and safe (QuEChERS) method [25]. However, classical LLE and SPE are the most commonly used techniques for concentrating pesticides from different matrices [18]. One of the more commonly used adsorbents for solid-phase extraction of pesticides, including the investigated pesticides is C18 [26–28].

In a previous study, HPLC method was developed for the determination of 2,4-D, atrazine, malathion, fenitrothion and parathion residues in water samples, using LiChrospher 60 RP-select B (125 × 4 mm, 5 μm) analytical column and mobile phase consisted of acetonitrile and water [26]. The purpose of this study was to investigate the other possibilities for the determination of 2,4-D, atrazine, malathion, fenitrothion and parathion residues in water samples by reversed-phase high-performance liquid chromatography (RP-HPLC) method and ultraviolet diode array detection (UV-DAD) using different analytical column and mobile phases.

EXPERIMENTAL

Reagents and Chemicals

In the development of the method, the Pestanal analytical standards of 2,4-D (98.6 % purity), atrazine (98.8 % purity), malathion (97.2 % purity), fenitrothion (95.2 % purity) and parathion (98.8 % purity) were purchased by Sigma-Aldrich (Germany).

For the preparation of mobile phases, HPLC-grade acetonitrile, methanol, water, as well as buffer solutions were used. The buffer solutions were made using phosphoric acid (H_3PO_4), potassium hydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), acetic acid (CH_3COOH) and sodium acetate (CH_3COONa) produced by Sigma Aldrich (Germany). Samples for the analysis of target pesticide residues were taken from tap water, bottled non-carbonated water, purchased from local supermarket and water from the Vardar River.

Instrumentation

The analyses were carried out using an Agilent 1260 Infinity Rapid Resolution Liquid Chromatography (RRLC) system equipped with: vacuum degasser (G1322A), binary pump (G1312B), autosampler (G1329B), a thermostatted column compartment (G1316A), UV-VIS diode array detector (G1316B) and ChemStation software. An ultrasonic bath "Elma" was used for preparing the stock solutions. The separation and determination of analytes were performed on a LiChrospher 60 RP-select B (250 × 4 mm, 5 μm, Merck) analytical column. A vacuum manifold Visiprep (Supelco) was used for the SPE and for vortexing of samples was used IKA Vortex Genius 3 (Germany).

Preparation of Standard Solutions

Stock solutions of 2,4-D, atrazine, malathion, fenitrothion and parathion were prepared by dissolving 0.0253 g, 0.0113 g, 0.0330 g, 0.0225 g and 0.0188 g of the pure analytical standards with acetonitrile in a 25 ml volumetric flask. To better dissolve the analytical standards, the prepared standard solutions were ultrasonified in an ultrasonic bath for a period of 15 minutes. According to the principles of SOP's (Standard Operating Procedure) [29], the standard solutions were stored in a refrigerator at a temperature of 4 °C. Under these conditions, the stability of the analytical standards was greater than one month. The stock solutions were used to prepare standard working solutions and standard mixtures of all examined pesticides with different pesticide concentrations (2.56 – 616.24 ng/mL for 2,4-D, 1.42 – 170.25 ng/mL for atrazine, 22.23 – 2672.5 ng/ml for malathion, 16.36 – 1967.0 ng/ml for fenitrothion and 20.90 – 2513.26 ng/ml for parathion) in 10 ml volumetric flasks by dilution with the acetonitrile/water mixture (50/50, *V/V*), as well as to enrich the water samples for method validation.

Sample preparation

The samples from the Vardar River were taken in brown glass bottles of 2.5 L, and immediately upon arrival in the laboratory, the samples were filtered through a 0.45 μm nitrocellulose membrane filter (Millipore, Ireland). Subsequently, the samples were subjected to solid-phase extraction and HPLC analysis, and each sample was injected with 5 μl.

Method Validation

Specificity, selectivity, linearity, precision, recovery and limit of quantification (LOQ) were tested for the method validation.

The calibration curves for determining the linearity of the method for determination of pesticide residues in water were obtained by threefold injection of samples of distilled water enriched with the investigated pesticides in 3 concentration levels (0.1, 0.2 and 0.5 μg/l for each pesticide analyzed) after the solid-phase extraction through Supelclean ENVI-18 columns. Each solution was injected with 5 μl.

The recovery was determined by adding a precisely determined volume of a standard solution (at three concentration levels) from each analyzed pesticide to 1 L of distilled water, as follows: 0.1, 0.2 and 0.5 μg/l. Samples that have not been added pesticides were used as blank samples. For each concentration level, 4 samples were prepared (n = 4). Subsequently, the samples were subjected to solid-phase extraction and HPLC analysis, and each sample was injected with a volume of 5 μl.

Solid-phase extraction (SPE)

The solid-phase extraction was performed using columns of the type Supelclean ENVI-18 (Supelco, Sigma-Aldrich), with a volume of 6 ml and a mass of the adsorbent of 0.5 g.

The solid-phase extraction procedure consists of several steps. SPE columns conditioning was performed by passing 5 ml of acetonitrile and then 5 ml water at a flow rate of 2 ml/min. Throughout the conditioned columns, the samples (1 L water previously filtered through a nitrocellulose membrane filter with a pore size of 0.45 μm) were passed through at a flow rate of 8–10 ml/min. The retained compounds of interest and the impurities on the SPE packing were rinsed through with wash solutions (5 ml of distilled water), and then the columns were dried under vacuum for 20 min. The elution of the selected components was carried out in two portions of 2 ml of acetonitrile. The eluates were evaporated to dryness under the gentle stream of nitrogen at a temperature of 40 °C and then the dry residue was dissolved with 1 ml of acetonitrile and water mixture (50/50, *V/V*) using Vortex for 1 min. Before performing the HPLC analysis, the final extract was filtered through an Iso-Disc PTFE syringe filter (Supelco, Sigma-Aldrich) with a pore size of 0.45 μm and transferred to vials for analysis. Each sample was injected with a volume of 5 μl.

RESULTS AND DISCUSSION

The first step in the method development was the selection of the wavelength at which the chromatographic processes will be monitored.

Based on the UV spectra of the components of interest recorded in a solution of acetonitrile and water, with a volume ratio of 50/50 (Figure 2), the wavelength at which the chromatographic analysis was performed was selected. As can be seen from Figure 2a, two maxima were observed in the UV spectrum of component 2,4-D, one at about 230 nm and the other significantly less intensive at about 285 nm. In a solution of acetonitrile and water (50/50, *V/V*), atrazine exhibits maximum absorption around 220 nm (Figure 2b). In the recorded wavelength range (Figure 2c) under these conditions, maximum absorption of malathion cannot be observed, but it was noticeable that the absorption increases with decreasing wavelength. Fenitrothion shows a maximum UV absorption at about 270 nm (Figure 2d), and parathion at about 280 nm (Figure 2e). In the spectrum of the latter two compounds, absorption at a wavelength of 220 nm was observed.

For these reasons, the HPLC analysis for the simultaneous determination of 2,4-D, atrazine, malathion, fenithrothion and parathion was carried out at a wavelength of 220 nm. Additionally, the chromatographic process was followed at 270 nm, because at this wavelength fenithrothion and parathion exhibit maximum absorption, which means that the intensity of their chromatographic peaks at this wavelength was higher.

In order to develop a simple HPLC method for separation and determination of the investigated components in water samples, the chromatographic process was conducted using isocratic elution, *i.e*, the use of a constant composition of the mobile phase.

Chromatographic analysis was performed using the LiChrospher 60 RP-select B (250 × 4 mm; 5 μm) analytical column, which is characterized by a higher number of theoretical plates (55 000 plates/m) [30], and hence with higher efficiency compared to the shorter column with the same C-8 stationary phase, LiChrospher 60 RP-select B (125 × 4 mm; 5 μm), in which the number of theoretical plates is 44 000 plates/m, used in a previous study [26].

To obtain optimal conditions for separating analytes with satisfactory purity index values, a series of preliminary experiments were accomplished by changing the composition of the mobile phase. Namely, acetonitrile, methanol and water, as well as, 0.1 % acetic acid, phosphate buffer and ammonium acetate buffer were used for the preparation of mobile phases. The following mobile phases were used: acetonitrile/water (45–80 % acetonitrile), methanol/water (60–80 % methanol), acetonitrile/0.1 % acetic acid, methanol/0.1 % acetic acid, as well as methanol/phosphate buffer (pH = 2.5, 3.5 and 4.5) and methanol/ammonium acetate buffer (pH = 4.5, 5.5 and 6.0) (Figure 3). The performed investigations showed that when using methanol as a constituent of the mobile phase, longer retention times for analytes and a noisy baseline were obtained.

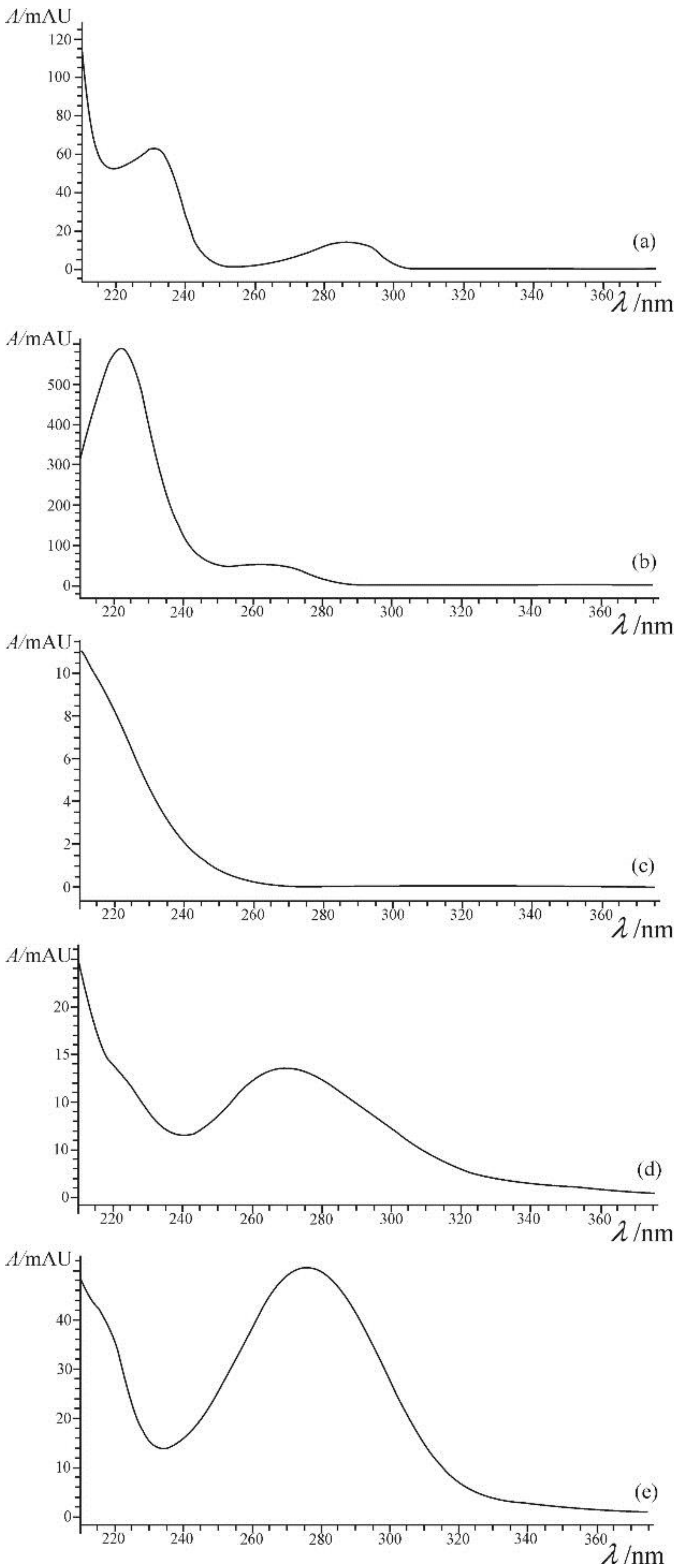

Figure 2. The UV spectra of pure analytical standards of 2,4-D (a), atrazine (b), malathion (c), fenitrothion (d) and parathion (e) in acetonitrile/water (50/50, *V/V*)

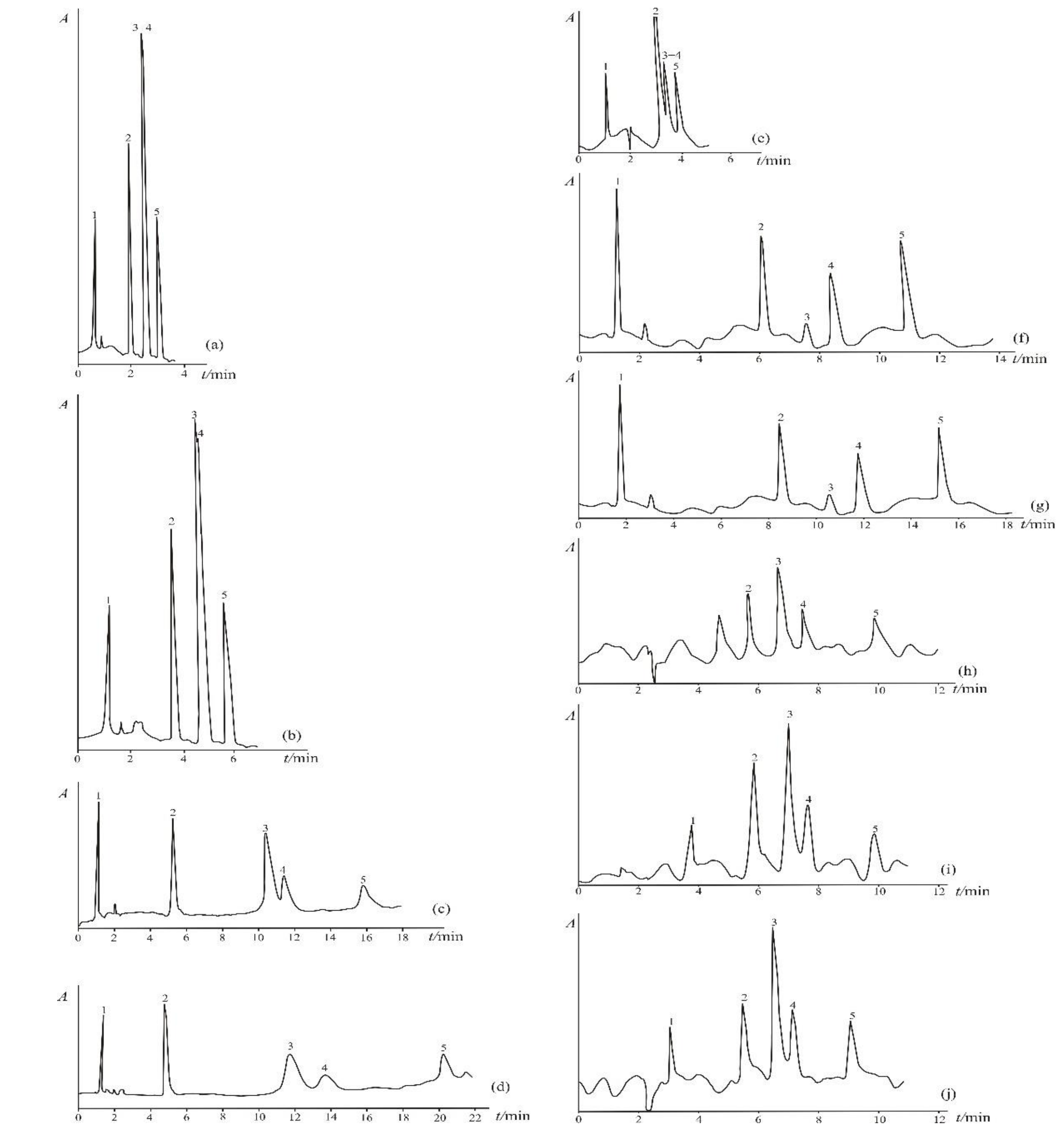

Figure 3. Chromatograms obtained from standard mixture of 2,4-D (1), atrazine (2), malathion (3), fenitrothion (4) and parathion (5) at 220 nm on LiChrospher 60 RP-select B (250 x 4 mm; 5 μm) column with mobile phase consisted of acetonitrile/water (80/20, *V/V* (a), 70/30, *V/V* (b), 50/50, *V/V* (c), 45/55, *V/V* (d)), methanol/water (80/20, *V/V* (e), 70/30, *V/V* (f), 60/40, *V/V* (g)), methanol/0.1 % acetic acid (70/30, *V/V* (h)), methanol/phosphate buffer (70/30, *V/V*, pH = 3.5 (i)) and methanol/ammonium acetate buffer (70/30, *V/V*, pH = 6.0 (j))

Using a mobile phase consisting of acetonitrile and water with a volume ratio (60/40, *V/V*), a flow rate of 1 ml/min, a constant column temperature at 25 °C and UV detection at 220 nm and 270 nm were shown to be the optimum separation conditions of the tested components with symmetrical peak shapes and satisfactory resolution purity index (Figure 4). Table 1 shows the obtained values for the column dead time (t_0), the retention times (t_R) of the analytes, their retention factors (k'), the separation factors (α) and the resolution (R_S) of the adjacent peaks. According to these data, the calculated values for the retention factors (k') were less than 10, the separation factor (α) of two adjacent chromatographic peaks was greater than 1, and the resolution (R_S) at the adjacent peaks was higher than 1.5. Consequently, it can be concluded that the proposed method allows optimal conditions for separation of analytes [31] for a total run time of 10 min.

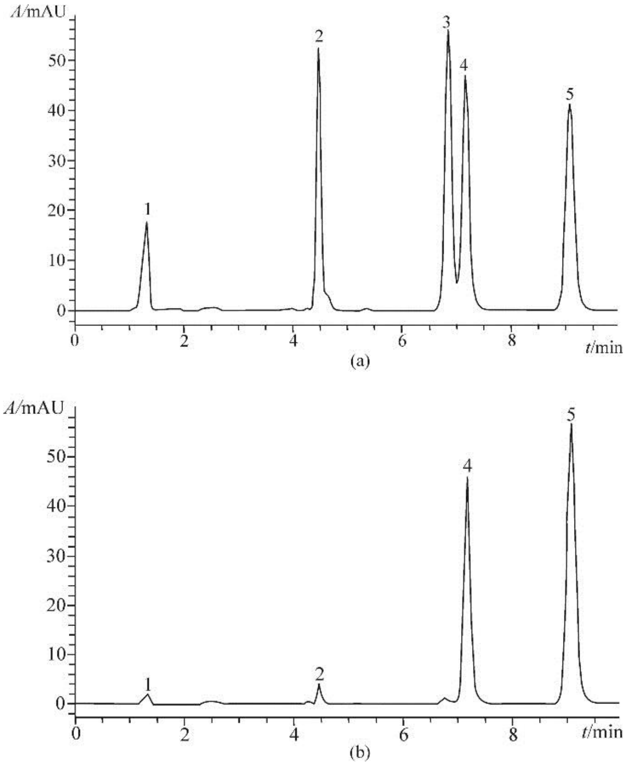

Figure 4. Chromatograms obtained from standard mixtures of 36.48 ng 2,4-D (1), 12.48 ng atrazine (2), 935.00 ng malathion (3), 223.68 ng fenitrothion (4) and 257.02 ng parathion (5) at 220 nm (a) and 270 nm (b) with developed method

Table 1. Data for retention times (t_R), retention factors (*k'*), separation factors (α) and resolution (*Rs*) for the investigated pesticides

Compound	t_R (min)	*k'*	α	*Rs*
dead time	1.09	–	–	–
2,4-D	1.39	0.27	12.92	29.68
atrazine	4.49	3.49	1.54	19.02
malathion	6.89	5.32	1.05	2.18
fenitrothion	7.20	5.60	1.32	11.54
parathion	9.17	7.41	–	–

In order to be able to perform qualitative and quantitative analysis of the investigated pesticide residues in water samples, their prior concentration was necessary. This occurs as a result of the fact that the calculated values for LOD and LOQ, from the analysis carried out with the standard mixture of the tested pesticides in the lowest concentration area, without concentrating the analytes were greater than 0.1 μg/L, which is equal to MRLs of pesticide residues in water, prescribed by the law in the Republic of Macedonia [11] and with the European Regulation [10].

Before the HPLC analysis, the concentration of the analytes and sample clean-up were carried out by solid-phase extraction using Supelclean ENVI-18 columns.

The development and validation of an analytical method for simultaneous determination of 2,4-D, atrazine, malathion, fenitrothion and parathion residues in water samples were performed according to the Guidance document on pesticide residue analytical methods [32]. Consequently, specificity, selectivity, linearity, precision expressed as repeatability of retention time and peak area, recovery and limit of quantification (LOQ) for all analytes were tested.

Specificity and selectivity. UV-diode array detection was applied to check the peak purity and analyte peak identity, in order to prove the specificity of the developed method. The purity indexes for all analytes were not less than 999 (the maximum value for the peak purity index (PPI) should be 1000), meaning that no other component influenced the chromatographic peaks of the analytes. Furthermore, the identification of the components of interest was accomplished by comparing the retention times of the analytical standards with those of the same components in the water samples. Additionally, the values of the match factors obtained by overlapping the UV spectra of the pure analytical standard and the absorption spectrum of the same analyte present in water samples were used. Moreover, in accordance with the EU criteria [32], to demonstrate the selectivity of the method, chromatograms of a standard mixture of investigated pesticides with a concentration corresponding to MRL (a), a matrix blank (distilled water) (b) and a sample of distilled water spiked with pesticides with a concentration equal to the MRL for each analyte (c) are presented in Figure 5. It can be seen that by applying the proposed method, the examined components can be determined in water samples after solid-phase extraction.

Linearity. The linearity of the method was determined by the construction of calibration curves which represented the dependence of the concentration of analytes and the obtained response as peak area or peak height.

As can be seen from Table 2, the proposed method was linear for all components of interest (R^2 > 0.99) using the peak areas and peak heights. The calculation of the results was done using the peak areas for each analyte.

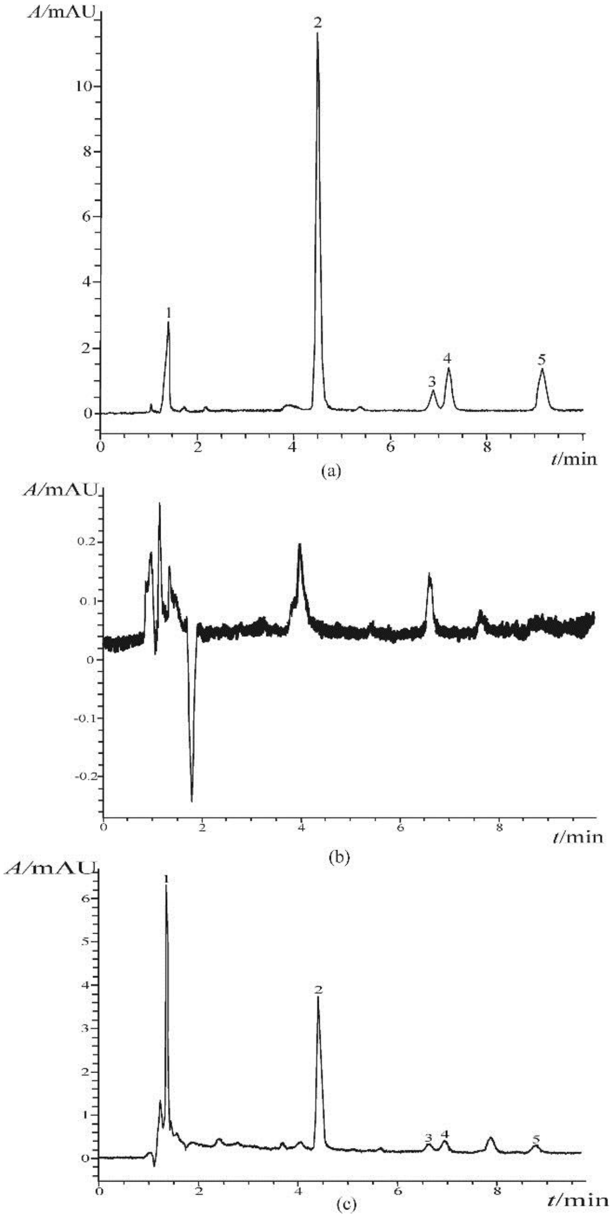

Figure 5. Chromatograms from standard mixture of 2,4-D (1), atrazine (2), malathion (3), fenitrothion (4) and parathion (5) at the concentrations which correspond to MRLs (a), matrix blank (b) and samples of distilled water fortified at the concentration equal to MRL for each analyte (c).

Table 2. Statistical data for linearity of the method

Compound	Linearity range (μg/L)	Regression equation	R^2
2,4-D	0.1 – 0.5	[1]y = 59.1x + 9.542 [2]y = 8.3008x + 6.0438	0.9975 0.9919
atrazine	0.1 – 0.5	[1]y = 83.824x + 14.765 [2]y = 12.901x + 2.2897	0.9992 0.9998
malathion	0.1 – 0.5	[1]y = 2.4476x + 1.1549 [2]y = 0.4031x + 0.1056	0.9990 0.9978
fenitrothion	0.1 – 0.5	[1]y = 14.877x + 0.614 [2]y = 1.6061x + 0.0819	0.9974 0.9981
parathion	0.1 – 0.5	[1]y = 17.767x + 0.7515 [2]y = 1.5217x + 0.0836	0.9992 0.9992

[1]y = peak area, [2]y = peak height

Limit of quantification (LOQ). The signal-to-noise ratio (S/N) at the lowest concentration level for each compound was found to be ≥ 10 for all investigated pesticides. Hence, the LOQ was estimated to be 0.1 μg/L for all examined pesticides. These obtained values for LOQs are acceptable for determining the pesticide residues in water samples according to the rules of the European Commission Guidance document on pesticide residue analytical methods [32].

Precision. To determine the precision of the developed method, five consecutive injections (5 μl) of a distilled water sample fortified with the investigated pesticides at the MRL level (0.1 μg/L) were made. Table 3 shows the precision of the method expressed as the repeatability of the results obtained for the retention time and the peak area for each analyte. From the calculated values for RSD of retention times (0.12–0.25 %) and the peak areas of the analytes (0.61–5.85 %), it is evident that the method was characterized by a satisfactory precision for quantitative determination of the analyzed pesticide residues in water.

Recovery. The obtained results of the recovery of the developed method, as well as the relative standard deviation (RSD) of the recovery for each concentration level, are shown in Table 4. The calculation of the recovery results was done using the peak areas for each of the components.

Table 3. Statistical data for Intra-day precision of retention time and peak area (n = 5)

Compound	t_R (min) ± SD	RSD (%)	peak area ± SD	RSD (%)
2,4-D	1.34 ± 0.002	0.12	15.95 ± 0.40	2.53
atrazine	4.40 ± 0.007	0.17	22.77 ± 0.14	0.61
malathion	6.61 ± 0.011	0.17	1.40 ± 0.02	1.83
fenitrothion	6.92 ± 0.017	0.25	2.27 ± 0.08	3.43
parathion	8.75 ± 0.019	0.22	2.65 ± 0.15	5.85

Table 4. Results from recovery experiments (n = 4)

Compound	Fortification level (μg/L)	Total analyte found (μg/L ± SD)	Recovery (%)	RSD (%)
2,4-D	0.1	0.108 ± 0.007	108.51	6.29
	0.2	0.186 ± 0.013	92.83	7.29
	0.5	0.464 ± 0.065	92.81	13.94
atrazine	0.1	0.096 ± 0.002	95.51	1.75
	0.2	0.202 ± 0.007	101.16	3.72
	0.5	0.498 ± 0.001	99.61	0.22
malathion	0.1	0.100 ± 0.010	100.41	10.44
	0.2	0.201 ± 0.013	100.77	6.32
	0.5	0.505 ± 0.014	101.08	2.79
fenitrothion	0.1	0.112 ± 0.005	111.67	4.71
	0.2	0.185 ± 0.009	92.53	4.76
	0.5	0.505 ± 0.003	100.92	0.63
parathion	0.1	0.107 ± 0.009	107.15	8.16
	0.2	0.190 ± 0.010	94.99	5.47
	0.5	0.506 ± 0.010	101.15	2.04

The recovery values for each concentration level (92.53–111.67 %) and the relative standard deviation (RSD ≤ 13.94 %) were within the acceptable values for these parameters according to the EU criteria [32]. They confirm that the method was precise and accurate enough for determining analyzed pesticide residues in water samples.

The developed reversed-phase high-performance liquid chromatography method based on solid-phase extraction was applied for the determination of 2,4-D, atrazine, malathion, fenitrothion and parathion residues in different water samples (tap water, non-carbonated water and water from Vardar River). Typical chromatograms of the tested water samples are presented in Figure 6. As can be seen from Figure 6, the analyzed samples did not found residues of the investigated pesticides at a concentration corresponding to the MRL or higher.

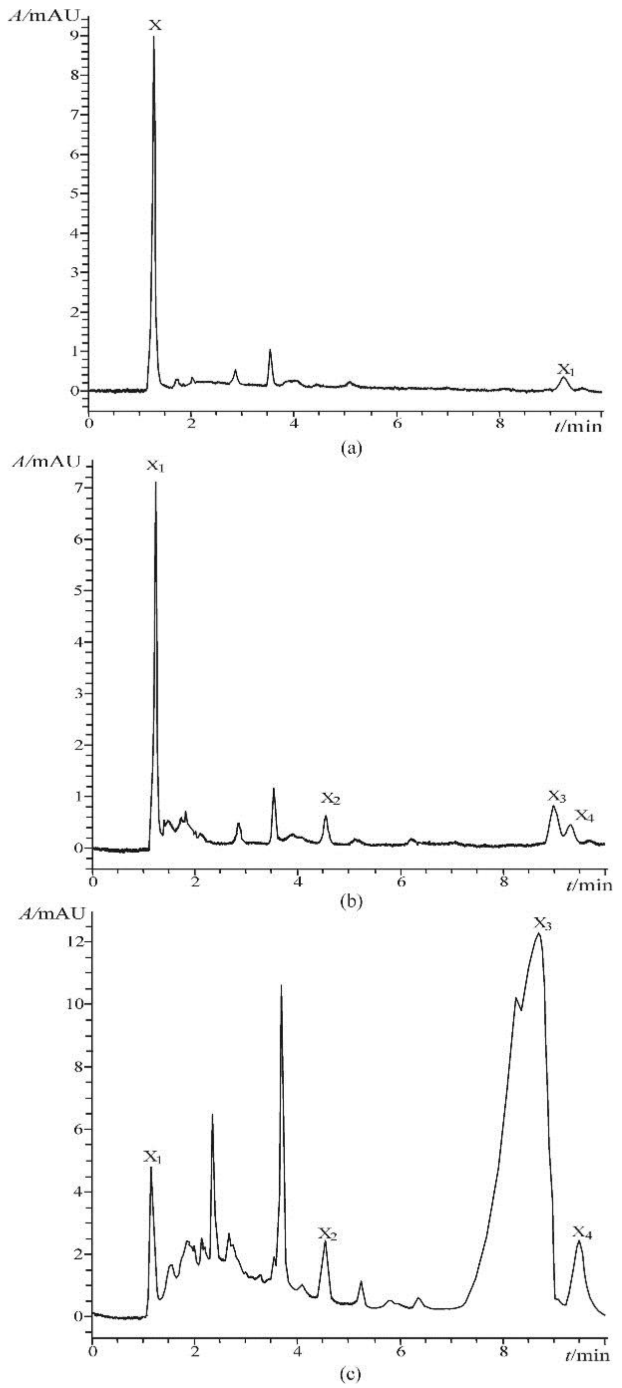

Figure 6. Typical chromatograms of water samples obtained from tap water (a), non-carbonated water purchased at the local market (b) and water from the Vardar River (c) at 220 nm.

On the chromatogram of tap water (Figure 6a) chromatographic peaks with a similar retention time of 2.4-D (1.25 min (X_1)) and parathion (9.27 min (X_2)) can be seen. Chromatographic peaks with retention time similar to the peaks of 2,4-D, atrazine and parathion (1.23 min (X_1), 4.55 min (X_2), 9.01 min (X_3), and 9.33 min (X_4)) also occur in the sample of purchased non-carbonated water (Fig. 6b). In the sample of the Vardar River (Fig. 6c) there are peaks at 1.16 min (X_1), 4.56 min (X_2) and 9.5 min (X_4). Also, on this chromatogram, a broad irregularly formed a chromatographic peak with two peaks (X_3), which starts at about 7.5 min and ends at 9 min, can be observed. This high-intensity peak did not overlap the peaks of malathion, fenitrothion, and parathion. Comparing the UV-spectra of the unknown substances to those of the analytical standards confirms that no residues of pesticides of interest were found in the analyzed water samples.

This paper describes a new possibility for successful determination of 2,4-D, atrazine, malathion, fenitrothion and parathion residues in water samples using reversed-phase high-performance liquid chromatography (RP-HPLC) method and ultraviolet - diode array detection (UV-DAD). Prior to HPLC analysis, a solid-phase extraction (SPE) was used for analytes concentration and sample clean-up. Specificity, selectivity, linearity, precision, recovery and limit of quantification (LOQ) were examined to assess the validity of the developed method. The method had satisfactory values for all correlation coefficients for calibration curves ($R^2 > 0.99$) and excellent precision for the retention times and peak areas for all examined pesticides. Under the established condition, the recovery of analytes was 92.53–111.67 %, with relative standard deviations below 13.94 %.

The developed method was successfully applied for the determination of selected pesticide residues in tap water, non-carbonated water and water from Vardar River. The obtained results showed that analyzed water samples did not contain detectable residues of investigated pesticides above 0.1 μg/L.

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РАЗРАБОТКА И ВАЛИДАЦИЈА НА RP-HPLC МЕТОД ЗА ОПРЕДЕЛУВАЊЕ НА НЕКОИ ОСТАТОЦИ ОД ПЕСТИЦИДИ ВО ВОДНИ ПРИМЕРОЦИ

Ленче Велкоска-Марковска, Биљана Петановска-Илиевска

Факултет за земјоделски науки и храна, Универзитет „Св. Кирил и Методиј“,
Скопје, Република Македонија

Во овој труд е опишана разработката на нов метод со реверзно-фазна високоефикасна течна хроматографија (RP-HPLC) и ултравиолетов детектор со низа од диоди (UV-DAD) за истовремено определување на остатоци од 2,4-Д, атразин, малатион, фенитротион и паратион во различни водни примероци. Разработениот метод е валидиран во согласност со насоките на Европската комисија за аналитички методи за остатоци од пестициди и добиените резултати за сите тестирани параметри се во границите на прифатливи вредности. Најдобро раздвојување и квантитативно определување на аналитите се постигнати со помош на аналитичката колона LiChrospher 60 RP-select B (250 × 4 mm, 5 μm), при изократско елуирање со мобилна фаза составена од ацетонитрил/вода (60/40, *V/V*), проток од 1 ml/min, константна температура на колоната од 25 °C и UV-детекција на 220 nm и 270 nm. Времето на спроведување на анализата под пропишаните хроматографски услови е околу 10 min.

Клучни зборови: RP-HPLC; UV-DAD; валидација на методот; остатоци од пестициди; водни примероци

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Original scientific paper

SEDIMENTATION RATES AND LIFESPAN ANALYSES IN THE RESERVOIR "KALIMANCI"[#]

Ivan Mincev[*], Ivan Blinkov, Aleksandar Trendafilov

[1]Faculty of Forestry, University Ss. Cyril and Methodius, Skopje, Republic of Macedonia

[*]e-mail: i_mincev@sf.ukim.edu.mk

Erosion and filling of reservoirs with sediment is one of the main problems leading to reduced lifespan of the reservoir with large environmental and economic implications. Reservoir sedimentation also provides valuable information on erosion intensity and sediment transport within a drainage basin and it can serve as the outlet of a giant erosion plot. With the invention of the global navigation satellite system (GNSS), the bathymetric measurements of the sediment in the reservoirs became much easier. Measurements performed with GNSS-supported echosounder are completed much faster and with considerable accuracy. The subject of this study is the Kalimanci reservoir. The reservoir was built in 1969 and until the present there have been 13 measurements, and gathered data is of great service for examination of the rates of deposition. It can be inferred that two mayor deposition periods exist, with braking point in the mid-80s. In the first period, the average annual deposition in the reservoir was 493,055 m^3 / year, and in the second period the average annual deposition in the reservoir was 214.325 m^3 / year, i.e. the deposition rate has reduced more than double between the two periods.

Key words: water reservoir; sediment transport; bathymetry; soil erosion; lifespan

INTRODUCTION

Reservoirs are designed to operate for a limited amount of time, but often their lifespans are reduced by sedimentation. Despite the designed life, reservoirs realistically have a project life defined as the "period during which the reservoir can reliably serve the purposes it was originally constructed for". Reaching of the project life, the failure to meet designed needs occurs typically before half of the storage volume of the reservoir is reduced from sedimentation (Morris *et al.* [12]; Dendy *et al.* [4]; Murthy [13]). The storage capacity, or reservoir yield, is expressed "as a function of available storage volume in the conservation pool" Nikitina *et al.* [14].

Reservoir sedimentation is a serious consequence of soil erosion with large environmental and economic implications. On the other hand, reservoir sedimentation also provides valuable information on erosion problems and sediment transport within a drainage basin. *A reservoir can be considered as a large-scale experiment, as the outlet of a giant erosion plot* (de Vente *et al.* [7].

Reservoirs are a unique category of objects because their lifespan is little dependent on the constructive elements of the dam itself, but are largely dependent on erosion and sedimentation processes. If the sediment regime is properly managed, then the reservoirs can last much longer than their projected lifespan. Schnitter (1994) in his work emphasized the presence of 12 reservoirs which lasted over 2000 years. Four of these dams/reservoirs are still functional (Morris *et al.* [12]).

The primary purpose of a reservoir sedimentation survey is to determine the volume and weight of sediment accumulated between surveys, or during the recorded period of storage. Information obtained from reservoir sedimentation surveys may be used to: estimate sediment yield for a given watershed, evaluate sediment damages, provide basic data for

[#]Dedicated to academician Gjorgji Filipovski on the occasion of his 100[th] birthday

planning and designing reservoirs, evaluate the effects of watershed protection measures, determine the distribution of sediment in a reservoir, and/or predict a reservoir's sediment storage life expectancy, or period of useful operation (Hall [5]).

It is estimated that more than 0.5 percent of the total reservoir storage volume in the world is lost annually as a result of sedimentation (White [18]). In comparison, for Republic of Macedonia, the reservoir "Tikveš" was built in 1968 with total storage of 475 10^6 m^3. The last measurements of the sediments in 1991 show accumulation of 29.3 10^6 m^3 of deposed sediment, or, an average annual deposition of 1.27 10^6 m^3, which is 0.26 percent of the total storage, little above the European average value (Trendafilov [16]). All previous measurements were done by the Water Development Institute of RM, using echosounder without GNSS (Global navigation satellite systems) support, with established geodetic polygonal network on already established measuring profiles. This approach included: setting up a polygonal network on already established measuring profiles, after which a boat with fitted echosounding equipment moved over established profiles and its position was determined by a geodetic instrument (distomat). This approach was very labor intensive and included mobilization of a lot of manpower.

With the invention of GNNS, bathymetric measurements of the sediment in reservoirs became much easier. The latest equipment for bathymetric measurements includes echosounder with spatial support of GNSS (mainly GPS). Measurements taken with GNSS-supported echosounder are completed much faster with considerable accuracy. Because the time needed for the measurements is much shorter, additional measurements can be taken, other than measurements of the established profiles. Thus, this new method allows for total measurement of the reservoirs on profiles and contour measurement.

The aim of this study was to define the regime of sedimentation within the reservoir Kalimanci and to calculate its lifespan.

The objectives of this study were:

– collecting previous data about erosion intensity, reservoir siltation, other related data;

– modelling erosion processes and transported sediments in the current state;

– bathymetric measuring;

– calculation of deposed sediment;

– defining sedimentation regime over time;

– defining reservoir lifespan.

MATERIAL AND METHODS

Study area

The catchment area of the reservoir is situated in the eastern part of the country and covers the upper part of Bregalnica river, in the area of combined valley-hilly and mountain region that belong to old Balkan relief structures.

The watershed of Bregalnica is located in the eastern part of the country, draining surface waters from an area of 4307 km^2 or 16.7% of the country. The upper part of the Bregalnica watershed extends upstream of the dam od Kalimanci (in the most eastern part of the country), from 22°27’44” to 23°02’03” East longitude, and from 41°35’09” to 42°09’16” North latitude. It covers an area of 1124.7 km^2 which is 26.1 % of total area, or 4.4 % of the country. The climate in the watershed is conditioned by the geographical location of the area and its topographic features. Although Malesh and Pijanec are two adjacent areas, they differ significantly. Berovo Valley, which is on higher altitude, has a climate formed under the influence of eastern continental and mountain climate of the neighbouring mountains, and the climate is considered to be moderate-cold-continental. The Delchevo Valley, which is at lower altitude and open through Istibanjska Gorge, has a climate type determined as moderately-warm-continental. The highest parts of the basin are considered to be with typical mountain climate. The basin has 2 meteorological stations (Berovo and Delchevo) and 14 pluviometric stations. For the zone above altitude of 1200 m, there are no measurements. Lithology of the research area is represented by various metamorphic (gneiss, mica-schist’s, schist’s), magmatic (granitites, gabro) and clastic sedimentary rocks, with variable erodibility. It is important that huge areas in the central part of the catchment (up to 1200 m of altitude), are composed by easily erodible Pliocene sands and sandstones – deposits from the Neogene lacoustrine phase in the Malesh and Pijanec basins. Soil pattern consist of various soil types, fluvisols, colluvial, various cambisol, regosol, lithosol, ranker etc. Generally, land cover in the upstream part of the basin is not favourable to develop high-intensity and widespread erosion processes. Unfavourable conditions on some location even cause extreme erosion processes. On the other hand, in the part closest to the reservoir Kalimanci, as a result of mass deforestation in the distant past, erosion processes are considered as highest in the country (Kojcevska [8]).

The catchment area is characterized with high-intensity erosion processes and the mean erosion coefficient (Z) is higher than average value in the country.

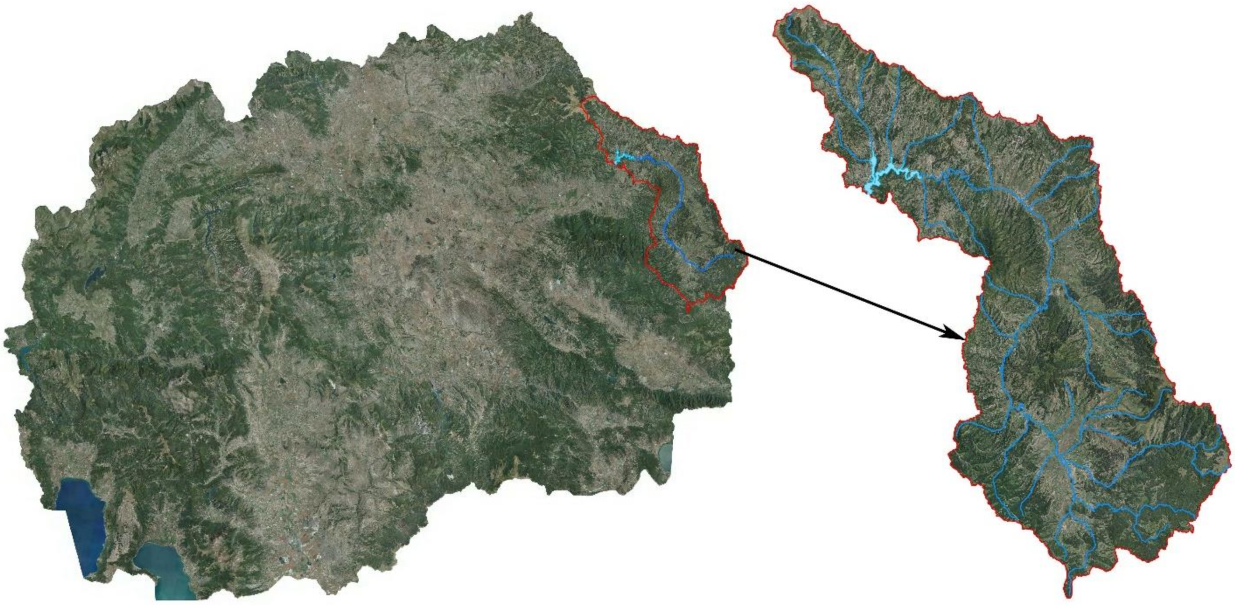

Figure 1. Location of the catchment of the reservoir Kalimanci

Collecting previous data about erosion intensity, reservoir siltation, other related data

Various data was collected from the previous related research as follow: Erosion Map of the Republic of Macedonia (Water Development Institute – 1993), data in Blinkov [1], Trendafilov and Blinkov 2002 [17], Milevski [9], Kojcevska [8], Mincev [10, 11], mainly data about the previous state of the reservoir and catchment, bathymetric data, statistical data, climatic data.

Modeling erosion intensity and sediment transport

For this purpose, we employed the Erosion Potential Method by Gavrilovic. According to several authors, EPM is the most appropriate method for estimation of off-site damage, especially total annual transported material to any reservoir and the most appropriate for hilly-mountainous and mountainous regions (Blinkov and Kostadinov [3], Trendafilov [16], Spalevic *et al.* [15], Mincev [10, 11]).

The old erosion map was created through direct on-field mapping and later calculation of the produced sediments using EPM, produced mostly in the 1980's and beginning of 1990's. Later, digital data were developed based on available maps, as follows: soil map, geological map, map of visible erosion processes, map of land cover/use, precipitation map, temperature map. Special attention was stressed on the development of the "Xa" factor, where as a base was used Land cover data from topographic maps 1:25.000. Further on, the land cover map was fine-tuned for forests and transitional woodland classes using manual photointerpretation of aerial photo images. This fine-tuning proved to be a game changer since it significantly improved the results of the erosion map. Beside this step also was added a layer with gullies as an extreme form of erosion. All these analyses were done in GIS environment.

$$G = T\ H\ p * Z^{1.5}\ Rn \quad [\ m^3/km^2/year]$$

$$Z = \gamma * Xa * (\varphi + J_{sr}^{0.5}) \quad (1)$$

where G – quantity of transported sediment, Z – erosion coefficient

Bathymetry

Bathymetry is the measurement of the depth of water in water bodies. Acoustic echo-sounding relies on accurate measurement of time and voltage. A sound pulse of known frequency and duration is transmitted into the water, and the time required for the pulse to travel to and from a target (e.g., a submerged object or the bottom of a water body) is measured.

The distance between sensor and target can be calculated using the following equation:

$$D = (S \times T) \quad (2)$$

where D = distance between sensor and target, S = speed of sound in water, and T = round-trip time.

To acquire information about the nature of the target, intensity and characteristics of the received signal are also measured. The echo-sounder has four major components: transducer, which transmits and receives the acoustic signal; signal generation computer, which creates the electrical pulse; the global positioning system, which provides precise latitude/longitude coordinates; and the control and logging computer. Prior to conducting a bathymetric survey, geospatial data (including geo-referenced aerial photography) of the target lake are acquired, and the lake boundary is digitized as a polygon shape file. Transect lines are predetermined based on project needs and reservoir size. Immediately before or after the bathymetric survey, elevation of the lake surface is determined [6].

For the purpose of this study, it was done an additional measurement of the sediment in order to estimate the rate of filling the reservoir with sediment. The measurements were done with Color GPS chartplotter and sounder GPSMAP 521s. This was the main echo-sounder used for the lake bathymetry. This is not a professional echosounder and it has poor output capabilities. It was checked for accuracy on the field and it showed good results. This model has the capability of dual frequency measurements for the measuring the bottom level and the compaction of the sediment.

The bathymetry of Kalimanci reservoir was done in executed in June 2013. Before measurement we selected the most appropriate approach, dependent on previously available data.

For the measurement of reservoir Kalimanci performed by Water Development Institute of the Republic of Macedonia (WDI) in the past, polygonal net was established and fixed cross profiles. Geodetic data for the profiles and “0-measuring” of the bottom before closing of the dam were obtained.

First, the maps and profiles were scanned and moderated or georeferenced. The profiles, further, in the appropriate GIS software were digitized and the surface of the transverse profile in the "zero state" was determined. On the other hand, from data of previous measurements of the sediment, the total quantities of deformed deposits were taken over time periods.

The recording of the reservoirs was carried out on the already established profiles by WDI in order to enable the temporal continuity of the recording and to have comparability of the data.

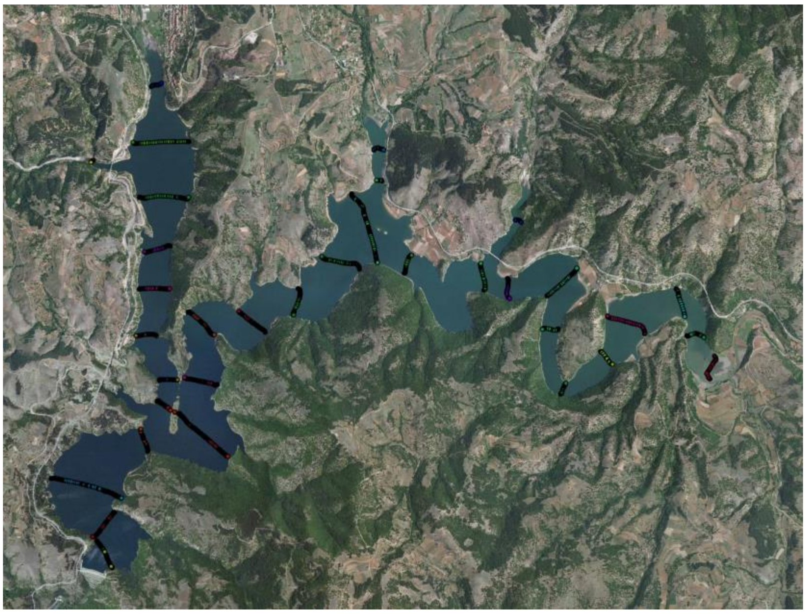

Figure 2. Position of measuring transect profiles on the reservoir "Kalimanci"

Calculation of deposed sediment

For further calculation we used the geodetic volumetric method using cross profiles. The volume of the total deposit is obtained as the product of the distances between the adjacent cross sections and the arithmetic mean of the surface of the coarser layer between two adjacent profiles). In the end, all these products are summarized and the total volume of settled deposits in one section of the reservoir is obtained. The same procedure is repeated for all tributaries of the accumulation and is summarized at the level of the reservoir.

Sedimentation Regime of the reservoir and lifespan analyses

For defining siltation regime in the case of Kalimanci reservoir, we used data from previous measurements and used an empirical curve that expresses the trend of siltation. To define variation of the siltation in various periods we analysed the following factors:

– change of climate parameters;

– migration processes and decrease of rural population;

– change of land use;

– the effects of erosion control;

– consolidation of the sediment (known phenomenon but is not researched in this study because of lack of equipment).

RESULTS AND DISCUSION

Erosion and sediment transport

The current erosion map was developed by modelling using the EPM method and the results were validated with bathymetric measurements of the reservoir from 2013. The Erosion map was developed during a period of 12–15 years with on-field mapping and the final map was published in 1993 but the data for the map show the state of erosion in the first half of the 1980’s.

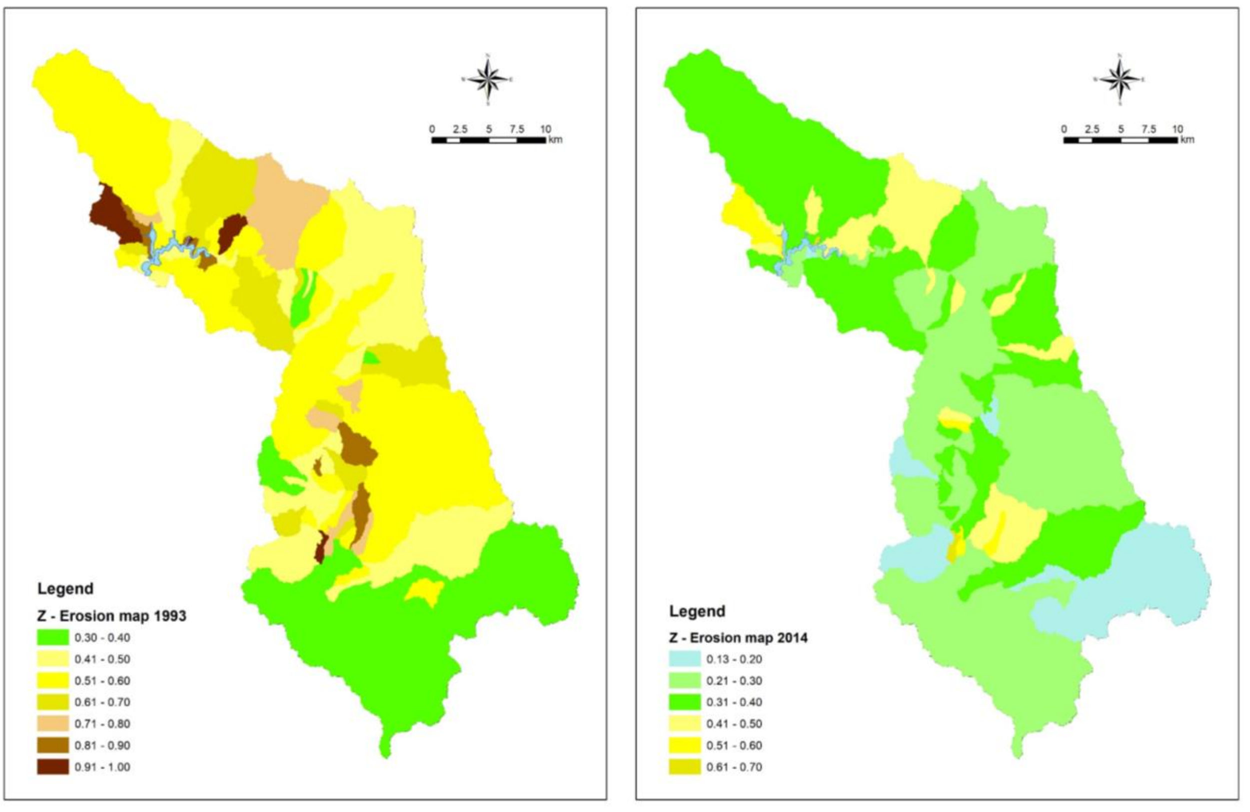

Figure 3. Change of erosion coefficient (1993 erosion map - 2014 erosion modeling)

The calculations for the coefficient "Z" were made by applying the previously calculated input coefficients at the level of the sub-catchment. Values at the level of sub-catchment for the reservoir "Kalimanci" range from 0.13 to 0.69 or averaged 0.36. For the catchment of the river Bregalnica "Z" is 0.33. This suggests that the river basin Bregalnica is less erosive than the other immediate tributaries of the Kalimanci reservoir (mean "Z" = 0.44). The average erosion coefficient in the erosion map of 1993 is 0.54 compared to the current state of 0.36.

The amount of transported sediment to the Kalimanci dam is 277.393 m^3 / year. It is estimated that 53% of the deposit or 146.404 m^3 / year is transported to the measured profile Ochipale on the river Bregalnica. This means that the remaining 47% of the total deposit comes from the immediate catchment of the reservoir or 130,989 m^3 / year [10].

Measured deposed sediment in the reservoir

The reservoir "Kalimanci" is designed at a total volume of 127 mil. m^3. Measurements in 2013 showed that the reservoir has 13.89 mil. m^3 deposits, or on average annually 315,682 m^3, or specific 278.13 m^3 / km^2 / year are deposited.

If Table 1 is analysed, it can be concluded that in the last measurement period 1997–2013 the annual deposited material is 136,319 m^3 / year, which is a considerable difference from the previous measurements.

Table 1. Volume of deposited sediment in the Kalimanci reservoir

No.	Year	Between two measurements	Cumulative	Mean Annually
		m^3	m^3	m^3/year
0	1969	/	/	/
1	1971	1,661,225	1,661,225	830,612.5
2	1972	258,075	1,919,300	258,075.0
3	1973	147,625	2,066,925	147,625.0
4	1975	2,210,590	4,129,890	1,105,295.0
5	1977	1,046,775	5,176,565	523,387.5
6	1978	323,225	5,499,790	323,225.0
7	1980	1,233,190	6,732,908	616,595.0
8	1984	749,9	7,482,880	187,475.0
9	1985	406,01	7,888,890	406,010.0
10	1991	1,514,690	9,403,580	252,448.3
11	1997	2,305,308	11,708,888	384,218.0
12	2013	2,181,112	13,890,000	136.319,5

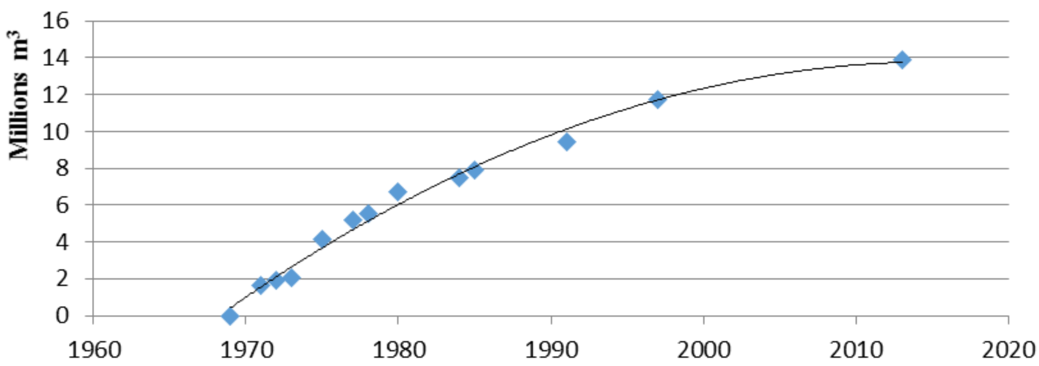

Figure 4. Rate of deposition of the Kalimanci reservoir (1969–2013)

Two periods can be deduced from the graph – 1969–1985 and 1986–2013. In the period 1969–1985, the average annual deposition in the reservoir is 493.055 m^3 / year, and in the period 1986–2013 the average annual deposition in the reservoir is 214.325 m^3 / year. In the first period the filling is more than double than in the second period.

When comparing the modelled erosion intensity from the Erosion map (mapped in the mid of 1980's) and mapped/modelled in 2013 show significant decrease of erosion intensity.

In the erosion map Z = 0.54, while current value is Z = 0.36.

What are reasons for this difference?

Several factors affect this phenomenon.

Change of climate parameters

From table 2, a decrease of annual rainfall by 2–12% and increase in temperatures by 0.5 to 1 °C is easily noticeable.

Table 2 Difference in precipitation and temperature for the periods 1971-1985 and 1986-2010

	Berovo	**Delchevo**	**Kochani**
	Precipitation		
1971-1985	638.1	580.8	546.3
1986-2010	603.3	563.8	478.5
decrease%	5.45	2.93	12.41
	Temperature		
1971-1985	8.4	10.1	13.0
1986-2010	9.0	10.0	14.0

Source: State hydro meteorological service

Migration processes

Migration processes and the decrement of the rural population during time (change of ratio of rural vs urban population from 76:24 in 1970, to 35:65 in 2010 [22, 23], also diminish the rural practices of the population such as deforestation, animal husbandry, agriculture.

Change of land use

Since the population in the rural area is missing, also the nature is going back to the abandoned agriculture land and pastures. So according to statistical data there is decrease of 3.056 ha arable land, decrease of bare land, and increase of forest for 14.250 ha and pastures for 9.856 ha; From this figures it can be seen that the pressure of the nature is diminished and all of the not used parcels are a new front for expanding natural areas [19–21].

Effects of erosion control measures

In the period after the building of the dam, according to Blinkov [1], in the catchment of the reservoir there are several bio-ameliorative erosion control measures undertaken. There are 1.544 cross objects built: 100 check dams, 64 thresholds, 1.308 stone rustically walls and other. Also, there are afforested 5.710 ha for the purpose of combating erosion.

All of these works had an immense effect of retention of erosive material in the upper catchments and after check dams and according to Trendafilov and Blinkov [2] approximately 1.5 million m^3 sediments were deposed behind check dams in Kamenicka River catchment. Over time, because of the diminished pressure of the local population they became even more effective, because after the afforestation there should be a period in which the forests will grow and forest cover will be closed.

Sediment consolidation and compaction in the reservoir

As mentioned in the beginning, the consolidation of the sediment has a big role in diminishing the volume of the deposited sediment and therefore it reduces the actual numbers of the bathymetry. Almost every time the modelled sediment transport has slightly larger numbers comparing with the actual measurements.

Lifespan analyses of the "Kalimanci" reservoir

The lifespan of any reservoir depends on: the bulk density of the deposited sediment, storage capacity, average annual suspended sediment load and the trapping efficiency. Trapping efficiency could be estimated using the Brune's Curve that depends on a mechanical composition of the sediment (colloidal, fine grained sediments, primary highly flocculated and coarse-grained sediments) (Morris *et al.* [12]). In this study we used a modified approach because some of the data were missing.

Blinkov [1] presents high correlation between annual precipitation and high intensity precipitation with water discharge and with the sediment discharge.

Lifespan of the reservoir "Kalimanci" was calculated in 2 ways:

1. Using the current value of mean annual deposed sediment (Vann) for the period 1985–2013 corrected with the ratio (R) of mean annual sum of precipitation (P) and the precipitation for the period 1985–2013 (Pn).

$$R = P/Pn = 624/\ 614 = 1.016287 \quad (3);$$

$$Vann = Vn * R = 214{,}000 * 1.016287 = 217{,}485\ m^3/ann \quad (4)$$

Up to 2013 from the total storage of 127 000 000 m^3, 13 890 000 m^3 are sediment i.e. free storage (Vf) is 113,110,000 m^3.

N = Vf / Vann = 113,110,000 / 217,485 = 520 years up to filling the reservoir storage (5)

2. Using precipitation as variable and creation correlation using cumulative values

The following equation was used:

$$Y = 355.01*x + 5,000,000 \quad (6)$$

where

Y – total deposed sediment into the reservoir – m^3

X – cumulative precipitation on meteorological station Berovo – mm

$$X = (113.110.000 – 5.000.000)/ 355 \quad (7)$$

i.e. X = 304.535 mm until full siltation

N – years until fulfilling the reservoir storage

$$N = X / P = 304,535 / 624 = 488 \text{ years} \quad (8)$$

Both approaches result in similar values (years until fulfilling the reservoir storage) 520 vs 488 years and that is much different than the first calculation based on the first erosion map and first measuring.

Although the lifespan of the reservoir is long there are 2 noticeable facts:

- Storage of the reservoirs is divided in "dead" storage (for Kalimanci reservoir it is 7 million m^3) and useful storage (120 million m^3). According to Trendafilov and Blinkov [17], there is a negative phenomenon in sedimentation regime i.e. 72.5% of the sediments are deposed in the useful storage.

- According to the engineering practice when the reservoir storage is 50% filled, then operation of the reservoirs is not effective.

It means that, in case of no significant changes of precipitation regime or land-use, practically the operation of this reservoir would be efficient for a maximum of 250 years.

But scenarios for climate changes are not favorable and continuation of erosion control works are recommended and the activities in agriculture and forestry sector should be sustainable with the aim of prolonging the efficient operation of the reservoir.

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ЗАПОЛНУВАЊЕ СО НАНОСЕН МАТЕРИЈАЛ И АНАЛИЗИ НА ЖИВОТНИОТ ВЕК НА АКУМУЛАЦИЈАТА „КАЛИМАНЦИ"

Иван Минчев, Иван Блинков, Александар Трендафилов

Шумарски факултет, Универзитет „Св. Кирил и Методиј", Скопје, Република Македонија

Ерозијата и заполнување на акумулациите со наносен материјал е еден од главните проблеми за намалување на животниот век на акумулацијата со големи еколошки и економски импликации. Заполнувањето на акумулацијата, исто така, обезбедува информации за интензитетот на ерозија и транспортот на нанос во рамките на сливот и може да послужи како контрола за ерозивни модели. Со појавата на глобалниот навигациски сателитски систем (ГНСС), батиметриското мерење на наносот во акумулациите стана многу полесно. Мерењата направени со ехосондер со ГНСС-поддршка се прават многу побрзо со зголемена точност. Интересот на оваа студија е акумулацијата „Калиманци". Изграден е во 1969 година и досега има 13 батиметриски мерења и затоа е идеален за испитување на интензитетот на таложење. Може да се издвојат два периода на таложење, со гранична точка во средината на 1980-тите години. Во првиот период, просечниот годишен транспорт на нанос во акумулацијата е 493.055 m3/г., а во вториот период просечниот годишен транспорт на нанос во акумулацијата е 214.325 m3/г. Стапката на таложење е намалена повеќе од двојно.

Клучни зборови: водна акумулација; транспорт на нанос; батиметрија; почвена ерозија; животен век

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Original scientific paper

EVALUATION OF FIELD RESPONSE TO YELLOW RUST AND SEPTORIA LEAF BLOTCH OF MACEDONIAN BREAD WHEAT GENOTYPES[#]

Biljana Kuzmanovska[1*], Mirjana Jankulovska[1], Ljupcho Jankuloski[2], Sonja Ivanovska[1], Rade Rusevski[1], Dane Boshev[1]

[1]Faculty of Agricultural Sciences and Food, Ss. Cyril and Methodius University, Skopje, Republic of Macedonia
[2]Plant Breeding and Genetics Section, International Atomic Energy Agency, Vienna, Austria

[*]e-mail: bkuzmanovska@fznh.ukim.edu.mk

Our study had several goals: assessment of field response of 20 Macedonian bread wheat genotypes to yellow rust and Septoria leaf blotch; identification of resistant and moderately resistant genotypes; and identification of potential sources of resistance which may be used for breeding programs in the future. Field experiments were conducted during two consecutive growing seasons in two regions in the Republic of Macedonia. Examined genotypes showed different genetic response to *Zymoseptoria tritici* and *Puccinia striiformis* f. sp. *tritici*, in both regions and in the separate years of investigation. The genotype MAC10 was immune to both wheat pathogens and MAC16 was identified as immune to yellow rust and moderately resistant to Septoria leaf blotch in both regions. These genotypes will be recommended to local wheat producers, and will be used as genetic source for resistance in national wheat breeding programs.

Key words: wheat genotypes; response; yellow rust; Septoria leaf blotch

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is the main cereal crop in the Republic of Macedonia, cultivated on approximately 80,000 ha. Over the past several years, wheat production in Macedonia faced serious damages and yield losses as a result of foliar diseases, such as yellow rust and Septoria leaf blotch, mainly due to favorable conditions for their development. Yellow rust (*Puccinia striiformis* f. sp. *tritici*), is one of the most important wheat diseases worldwide, which causes yield losses from 10–70 % and reduces the quality of grain and forage [1, 2], especially in regions with cool climate [3]. However, in recent years, this disease has become a rising problem in warmer areas, which were previously considered as unfavorable for yellow rust epidemics [4–6], such as Macedonia. Besides environmental conditions, virulence of the pathogen and host resistance are also the key factors for disease severity [2, 7]. Septoria leaf blotch, caused by the *Zymoseptoria tritici* (syn. *Septoria tritici*; teleomorph *Mycosphaerella graminicola*) is one of the top two or three diseases in most wheat-growing areas around the world, including Europe, USA, South America and Australia [8]. In areas with conditions favorable for its development (high rainfall), yields can be reduced over 50 % in highly susceptible genotypes [9]. Effective control of foliar diseases in wheat can be achieved by cultural practices, use of resistant genotypes and application of fungicides [2, 6, 10–13]. In order to protect the wheat from yellow rust and Septoria leaf blotch, at least two fungicidal treatments are applied during the growing season [6, 14, 15], which significantly increases the production costs and contributes to environmental pollution [13, 16]. Moreover, fungal pathogens are able to develop resistance to commonly used fungicides, which is another disadvantage of chemical control [17, 18]. Growing re-

[#]Dedicated to academician Gjorgji Filipovski on the occasion of his 100[th] birthday

sistant genotypes is the most economically efficient and environmentally friendly approach to control foliar diseases in wheat [10, 18, 19]. Therefore, this study was carried out (1) to evaluate the field response of Macedonian bread wheat genotypes to yellow rust and Septoria leaf blotch; (2) to determine resistant and moderately resistant genotypes, which would be recommended to local wheat producers, and (3) to identify potential sources of resistance which may be used for breeding programs in the future.

MATERIAL AND METHODS

Field experiments were conducted during two consecutive growing seasons (2014/2015 and 2015/2016) in two regions in the Republic of Macedonia (Skopje and Gradsko). A total of 20 bread wheat genotypes (MAC1 to MAC20, consecutively) were evaluated for their response to yellow rust and Septoria leaf blotch in the field. The experiment was set as randomized block design with two replications. Each plot consisted of 5 rows, with 1 m length and between-row distance of 20 cm. Trials were conducted based on natural infections by yellow rust and Septoria leaf blotch pathogens. During each growing season, standard agrotechnical practices for wheat were applied. In each plot, 50 plants were randomly selected for evaluation of the severity of Septoria leaf blotch (at GS 55 – heading) and yellow rust (at GS 73 – early milk development, according to Zadoks' scale) [20]. The severity of Septoria leaf blotch was evaluated using the modified Saari and Prescott's double-digit scale (00–99). The first digit refers to vertical disease progress of the plant (1–9) and the second digit indicates severity measured as diseased leaf area (1–9; where 1 = 10 %; and so forth, consecutively up to 9 = 90 %) [8]. After evaluation, genotypes were classified in 7 categories: immune (00), highly resistant (11–14), resistant (15–34), moderately resistant (35–44), moderately susceptible (45–64), susceptible (65–84) and highly susceptible (85–99) [8]. Severity of yellow rust was evaluated by using the modified Cobb scale (1–100 %), along with the variety response [21]. Response refers to the infection type and is classified according to the following scale: 0 – no visible infection; R – resistant; necrotic areas with or without small pustules; MR – moderately resistant; small pustules surrounded by necrotic areas; M – intermediate; pustules of variable size; some necrosis and/or chlorosis; MS – moderately susceptible; medium-sized pustules; no necrosis, but some chlorosis possible and S – susceptible; large pustules, no necrosis or chlorosis. These scores were then converted to coefficient of infection (CI) by multiplying severity by an assigned constant value for the variety response, where R = 0.2, MR = 0.4, M = 0.6, MS = 0.8, and S = 1 [22]. The genotypes were then classified into: immune (0); highly resistant (1–5); resistant (6–10); moderately resistant (11–20); moderately susceptible (21–30) and susceptible (31–100).

RESULTS AND DISCUSSION

Examined genotypes showed different genetic response to *Zymoseptoria tritici* and *Puccinia striiformis* f. sp. *tritici*, in two consecutive growing seasons. In 2015, disease pressure by Septoria leaf blotch was higher in comparison with yellow rust, while in 2016, climate conditions were particularly favorable for development of P. *striiformis f. sp. tritici*, which resulted in severe yellow rust infections.

In 2015, in both regions, 4 wheat genotypes (MAC2, MAC10, MAC12 and MAC 15) were evaluated as immune and three genotypes (MAC3, MAC11 and MAC18) were found to be highly resistant to Septoria leaf blotch infections. Resistance was observed in genotypes MAC4, MAC8 and MAC17, while MAC16 and MAC19 expressed moderate resistance. Genotypes MAC9 and MAC13 were moderately resistant to moderately sensitive, MAC1 and MAC14 were moderately sensitive, MAC6 was marked as sensitive and MAC20 as moderately sensitive to sensitive. The response of other 2 genotypes (MAC5 and MAC7) significantly differed between two regions (sensitive to highly resistant and moderately sensitive to resistant, respectively). In 2016, in most of the genotypes leaf area was almost fully covered with yellow rust pustules, which obstructed infections by *Zymoseptoria tritici*. Namely, out of 20 genotypes, 15 were determined as immune to Septoria leaf blotch infections, one genotype was highly resistant (MAC3), one was resistant (MAC11) and one was moderately resistant (MAC16). Genotype MAC13 was moderately sensitive to moderately resistant, while the genotype MAC9 was determined as sensitive to *Z. tritici* infection. However, it is important to point out that genotypes MAC2, MAC10, MAC12, MAC15, MAC3 and MAC16 displayed uniform host response regarding the region and growth seasons. Field response of other 14 investigated genotypes significantly differed depending on the region and the growing season.

Table 1. Severity of Septoria leaf blotch infection and wheat genotype response during 2015 and 2016 growing seasons in Skopje and Gradsko

	2015				2016			
	Skopje		Gradsko		Skopje		Gradsko	
Genotype	Double digit score	Response	Double digit score	Response	Double digit score	Response	Double digit score	Response
MAC1	45	Moderately sensitive	46	Moderately sensitive	00	Immune	00	Immune
MAC2	00	Immune	00	Immune	00	Immune	00	Immune
MAC3	12	Highly resistant	11	Highly Resistant	11	Highly resistant	13	Highly resistant
MAC4	34	Resistant	33	Resistant	00	Immune	00	Immune
MAC5	75	Sensitive	13	Highly resistant	00	Immune	00	Immune
MAC6	75	Sensitive	73	Sensitive	00	Immune	00	Immune
MAC7	45	Moderately sensitive	34	Resistant	00	Immune	00	Immune
MAC8	26	Resistant	23	Resistant	00	Immune	00	Immune
MAC9	43	Moderately resistant	33	Resistant	67	Sensitive	82	Sensitive
MAC10	00	Immune	00	Immune	00	Immune	00	Immune
MAC11	13	Highly resistant	11	Highly Resistant	33	Resistant	24	Resistant
MAC12	00	Immune	00	Immune	00	Immune	00	Immune
MAC13	45	Moderately sensitive	41	Moderately resistant	46	Moderately sensitive	37	Moderately resistant
MAC14	64	Moderately sensitive	52	Moderately sensitive	00	Immune	00	Immune
MAC15	00	Immune	00	Immune	00	Immune	00	Immune
MAC16	35	Moderately resistant	35	Moderately resistant	42	Moderately resistant	43	Moderately resistant
MAC17	21	Resistant	23	Resistant	00	Immune	00	Immune
MAC18	11	Highly resistant	12	Highly Resistant	00	Immune	00	Immune
MAC19	44	Moderately resistant	36	Moderately resistant	00	Immune	00	Immune
MAC20	76	Sensitive	46	Moderately sensitive	00	Immune	00	Immune

As previously mentioned, climate conditions during 2016 were favorable for yellow rust development, which lead to severe infections. As a result, disease severity ranged from 15 to 100 % in both investigated regions (Table 3). The highest disease severity was observed in genotypes MAC1, MAC5 and MAC15 in both regions. On the contrary, in 2015, disease severity was significantly lower and ranged between 10 in the region of Gradsko and 45 % in the region of Skopje (Table 2). Out of 20 examined genotypes, 3 genotypes (MAC9, MAC10 and MAC16) were found to be immune, while 2 genotypes (MAC11 and MAC12) were identified as resistant in both regions, during the two consecutive years. Other 15 genotypes (MAC1, MAC2, MAC3, MAC4, MAC5, MAC6, MAC7, MAC8, MAC13, MAC14, MAC15, MAC17, MAC18, MAC19 and MAC20) exhibited inconsistent response to yellow rust in terms of region and growing season. Differences in the genotypes' response to yellow rust (*Puccinia striiformis f.sp. tritici*) and Septoria leaf blotch (*Zymoseptoria tritici*) are in agreement with many previous studies [18, 23–26]. However, in those studies only resistant and moderately resistant genotypes were identified, while immune genotypes to yellow rust and Septoria leaf blotch were not observed.

Table 2. Severity and coefficient of infection (CI) of yellow rust and wheat genotype response during 2015 growing season in Skopje and Gradsko

Genotype	Skopje			Gradsko		
	Severity and response	CI	Reaction type	Severity and response	CI	Reaction type
MAC1	25 MR	10	R	10 R	2	HR
MAC2	0	0	I	0	0	I
MAC3	0	0	I	0	0	I
MAC4	10 R	2	HR	0	0	I
MAC5	40 MS	32	S	0	0	I
MAC6	45 S	45	S	0	0	I
MAC7	40 MS	32	S	0	0	I
MAC8	20 MR	8	R	0	0	I
MAC9	0	0	I	0	0	I
MAC10	0	0	I	0	0	I
MAC11	20 MR	8	R	20 R	8	R
MAC12	20 MR	8	R	15 MR	6	R
MAC13	0	0	I	0	0	I
MAC14	0	0	I	0	0	I
MAC15	0	0	I	0	0	I
MAC16	0	0	I	0	0	I
MAC17	0	0	I	0	0	I
MAC18	0	0	I	0	0	I
MAC19	0	0	I	0	0	I
MAC20	0	0	I	20 MR	8	R

Table 3. Severity and coefficient of infection (CI) of yellow rust and wheat genotype reaction during 2016 growing season in Skopje and Gradsko

Genotype	Skopje			Gradsko		
	Severity and response	CI	Reaction type	Severity and response	CI	Reaction type
MAC1	95 S	95	S	100 S	100	S
MAC2	70 S	70	S	40 S	40	S
MAC3	50 S	50	S	45 S	45	S
MAC4	60 S	60	S	40 S	40	S
MAC5	100 S	100	S	75 S	75	S
MAC6	75 S	75	S	60 S	60	S
MAC7	75 S	75	S	50 S	50	S
MAC8	80 S	80	S	85 S	85	S
MAC9	0	0	I	0	0	I
MAC10	0	0	I	0	0	I
MAC11	25 MR	10	R	15 MR	6	R
MAC12	20 MR	8	R	15 MR	6	R
MAC13	20 MR	8	R	30 MS	24	MS
MAC14	80 S	80	S	100 S	100	S
MAC15	90 S	90	S	95 S	95	S
MAC16	0	0	I	0	0	I
MAC17	70 S	70	S	60 S	60	S
MAC18	80 S	80	S	95 S	95	S
MAC19	25 MS	20	R	40 S	40	S
MAC20	50 S	50	S	65 S	65	S

If we compare the results for genotypes response to *Puccinia striiformis f.sp. tritici* and *Zymoseptoria tritici*, during the two years in two examined regions, it is noticeable that the genotype MAC10 was immune to both wheat pathogens. Moreover, the genotype MAC16 was identified as immune to yellow rust and moderately resistant to Septoria leaf blotch in two consecutive years, in both regions. These two genotypes can be recommended to local wheat producers, and in the future they should be used as genetic source for resistance in national wheat breeding programs.

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ЕВАЛУАЦИЈА НА РЕАКЦИЈАТА НА МАКЕДОНСКИ ГЕНОТИПОВИ ПЧЕНИЦА КОН ЖОЛТАТА ’РЃА И СЕПТОРИОЗАТА ВО ПОЛСКИ УСЛОВИ

Билјана Кузмановска[1*], Мирјана Јанкуловска[1], Љупчо Јанкулоски[2], Соња Ивановска[1], Раде Русевски[1], Дане Бошев[1]

[1]Факултет за земјоделски науки и храна, Универзитет „Св. Кирил и Методиј“, Скопје, Република Македонија
[2]Оддел за генетика и селекција на растенија, Меѓународна агенција за атомска енергија, Виена, Австрија

Испитувањата беа спроведени со неколку цели: да се евалуира реакцијата кон жолтата ’рѓа и септориозата на 20 македонски генотипови пченица во полски услови, да се идентификуваат отпорни и умерено отпорни генотипови и да се идентификуваат потенцијалните извори на отпорност што би се вклучиле во идните селекциски програми. Полските опити беа спроведени во текот на две последователни вегетациски сезони, во два региона во Република Македонија. Испитуваните генотипови во двете вегетациски сезони покажаа различна генетска реакција кон двата патогена, *Zymoseptoria tritici* и *Puccinia striiformis* f. sp. *tritici*, во двата региона. Генотипот MAC10 покажа имуна реакција кон двата патогена, додека генотипот MAC16 покажа имуна реакција кон жолтата ’рѓа и умерена отпорност кон септориозата, во двата испитувани региона. Овие генотипови ќе се препорачаат на локалните производители на пченица за одгледување, а воедно ќе се користат и во националните селекциски програми како извор на гени за отпорност кон овие два патогена.

Клучни зборови: генотипови пченица; реакција; жолта ’рѓа; септориоза на листот

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Original scientific paper

GENOTYPE AND LOCATION INTERACTION FOR YIELD AND MORPHOLOGICAL TRAITS IN RICE (*Oryza sativa* L.)[#]

Emilija Simeonovska[*], Danica Andreevska, Dobre Andov, Gordana Glatkova, Trajche Dimitrovski

Institute of Agriculture, Ss. Cyril and Methodius University, Skopje, Republic of Macedonia

[*]e-mail: e.simeonovska@zeminst.edu.mk

The objectives of this study were to determine the genotype and location interaction for paddy yield and morphological traits. Two field trials were conducted in a randomized block design with 3 repetitions in the Kocani rice producing region in two locations in 2014. The Turkish varieties, Halilbey, Gala and Gönen and standard San Andrea were used in the experiment. The combined variance analysis for paddy rice yield, biological yield, harvest index (HI), plant height, panicle length and the number of panicles per m^2 has been performed. Highly significant differences among examined genotypes and locations were found for almost all the traits. Genotype × location interaction was significant only for the biological yield and number of panicles per m^2. The highest means for most of yield traits were observed for Gala and Halilbey varieties. Due to their performances, Halilbey, Gala and Gönen can be recommended for the rice production in the Republic of Macedonia.

Key words: rice variety, genotype × location interaction, yield trait, morphological trait

INTRODUCTION

In order to promote Macedonian rice production, one of the measures is the introduction of new rice varieties. The main criteria for their selection are high yielding and quality performances, but also, adaptability to local environmental conditions and growing practices.

Beside the Macedonian registered rice varieties (No. 51, No. 69, Osogovka, Kochanski, Biser 2, Ranka, Nada 115, Prima riska and Montesa), in the entire production, the Italian rice varieties dominate. The oldest introduced and registered varieties are Monticelli, R-76/6, Drago and Baldo (prevalent during the nineties), then the last fifteen years San Andrea was the most widespread. Within 2009–2013, the Italian varieties: Galileo, Bianca, Brio, Ellebi, Opale, Arpa and Onice entered in the domestic rice production.

In 2013, the introduction of fourteen Turkish rice varieties (TR-2121, TR-2024, TR-1981, Paşalı, Çakmak, Kızıltan, Gönen, Kırkpınar, Tunca, Halilbey, Durağan, Gala, Hamzadere and Efe) started through a joint Turkish-Macedonian project "Development of production and quality of rice in the Republic of Macedonia 2013-2014" [1]. Three of these varieties (Gönen, Halilbey and Gala) were registered in the Macedonian National list of Varieties in 2016.

In all these stages, the new varieties were the results of many fields and laboratory investigations, with special attention to their yield performances and morphological traits [2–9].

Paddy yield was always in the focus of all these studies, since it considered as one of the most important traits for the rice growers, besides the quality traits. In some trials, several introduced varieties (Brio, Ellebi, Opale, Arpa and Onice) were lower yielding, compared to control varieties Prima riska – Macedonian variety and R-76/6, Italian domesticated variety [6, 7]. In other research, where San Andrea was used as a control variety, the Turkish rice varieties Kızıltan, Gala, Halilbey, Gönen and Paşalı achieved better paddy yield than control [9].

Plant height of the new rice varieties was also of particular interest. Over the years, in Macedonian

[#]Dedicated to academician Gjorgji Filipovski on the occasion of his 100^{th} birthday rice growing, taller varieties dominated [5, 6, 8]. Beside their yielding ability, they were not very suitable for intensive production systems mostly because of their susceptibility to lodging, especially in case of high doses of inorganic fertilizers. Therefore, during the recent variety selection special attention was done to semi-dwarfs, high-yielding and good quality rice varieties.

Although biological yield, panicle length and the number of productive panicles per m^2 were regularly explored at the national level [5, 6, 8] - up to now, harvest index of rice has not been the subject of research under the conditions of the Republic of Macedonia.

For grain crops, harvest index (HI) is the ratio of harvested grain to total dry matter, and this can be used as a measure of reproductive efficiency. In agronomic terms, HI is calculated as the ratio of harvested product to total aboveground biological yield, which is an economic HI [10]. Harvest index of rice is the result of various integrated processes with involvement of the number of panicles per unit area, the number of spikelets per panicle, the percentage of fully ripened grains, and 1,000 grain weight [11]. HI is one of the yield determining factors. The partitioning of dry matter to grain and straw varied among the genotypes [12]. The HI of crop plants has increased over time due to breeding for higher yield and more recently, specifically for HI. Shorter-statured, modern crop cultivars have higher harvest indices than their taller forebears, although total dry matter production is most often very similar [10]. However, the genetic control of the harvest index plays an important role in crop production [13].

The performance of the cultivar is mostly influenced by genotype, growth environment and the response of genotype to the present environment [14]. The study of genotype × environment interaction is critical for accurate cultivar evaluation in large multi-environmental trials. Cultivars that exhibit high levels of mean performance and stability across a wide range of environmental conditions are desirable for rice production [15].

A genotype × environment interaction may be defined as a change in the relative performance of a “character” of two or more genotypes measured in two or more environments. Interactions may therefore involve changes in rank order between environments and changes in the absolute and relative magnitude of the genetic, environmental and phenotypic variances between environments. These changes in rank order and in variances are found separately and together [16].

Regarding the rice grain yield, analysis of variance showed highly significant differences among genotypes (G), environments (E) and G × E interaction in multi-location trials with several rice genotypes [17]. The significant G × E interaction effect demonstrated that the genotypes responded differently to the variation in the environmental condition of the testing sites and grain yield fluctuated accordingly. This indicates the necessity of multi-location testing of genotypes before recommending for general cultivation.

The aims of this study were to determine the influence of the genotype, location and their interaction on several yields and morphological traits on the rice varieties Halilbey, Gala, Gönen and San Andrea.

MATERIALS AND METHODS

Three newly registered Turkish rice varieties: Halilbey, Gala and Gönen were included in the experiment, together with the standard variety San Andrea (Italian rice variety, widely used and domesticated in Macedonian rice production). During 2014, two identical field trials were set up on two locations within the entire Kocani rice-producing region. Location 1 was in Cesinovo village, on a private rice plot, while location 2 in Kocani, on the field of the Rice experimental Station Kocani, unit of the Institute of Agriculture, Skopje. The distance between locations is 15 km and both of them have continental sub-Mediterranean climate characteristics [18]. Thus, they don’t differ significantly regarding the climatic conditions and the data presented in Table 1 for Kocani cite are considered as representative for both locations as well as for the entire rice producing region in the country.

The soil characteristics of the experiment locations were presented in Table 2. As it is seen in the table, they have different soil contents.

The experiment was designed as a randomized blocks system with 3 repetitions and $5m^2$ plot size. The seeding was manual - wet seeds were broadcasted into standing water. The seed rate was 500 seeds per m^2. Standard production technology was applied during the rice cultivation period. The data were recorded for plant height, panicle length, biological yield, harvest index, the number of panicle per m^2 and paddy yield.

Using IBM SPSS Statistics 23 statistical program, the variance analysis was conducted to determine the differences among the varieties for the examined traits and interaction between location and genotype. The differences among mean values were tested by using LSD test.

Table 1. The average monthly temperatures and monthly sums of rainfalls during the rice vegetation period in Kocani in 2014

Year	Months							Average	
	IV	V	VI	VII	VIII	IX	X	per year	per vegetation
Average monthly temperature [C°]									
2014	12.4	16.8	20.8	23.2	23.8	18.3	13.8	13.8	18.4
Average 1998/2012	13.8	18.6	22.9	25.6	25.1	20.0	14.7	14.1	20.1
Average monthly maximum temperature [C°]									
2014	18.0	23.0	28.1	30.9	31.8	25.0	20.7	20.1	25.4
Average 1998/2012	19.2	23.9	28.7	31.6	31.5	26.2	20.2	19.4	25.9
Average monthly minimum temperature [C°]									
2014	7.4	10.8	14.0	16.5	16.6	13.4	8.7	8.5	12.5
Average 1998/2012	5.7	10.1	13.3	15.1	15.0	10.8	6.8	6.3	11.1
Monthly sum of rainfalls [mm]									
2014	121.0	92.0	116.0	65.0	31.0	89.0	37.0	794.0	551.0
Average 1998/2012	39.7	49.4	54.5	27.6	34.5	42.7	60.4	489.5	308.9

Table 2. Chemical properties of soils from Cesinovo and Kocani

Location	Depth [cm]	pH		$CaCO_3$	Easily available mg/100g of soil	
		H_2O	nKCl	%	P_2O_5	K_2O
Cesinovo	0–20	7.9	6.8	1.24	26.51	24.86
	20–40	7.6	6.6	1.44	22.72	19.74
Kocani	0–20	5.9	5.1	0	16.88	14.03
	20–40	6.2	5.6	0	12.66	11.15

RESULTS AND DISCUSSION

The variance analysis results (Combined ANOVA) showed highly significant differences ($P \leq 0.01$) among investigated genotypes for all the traits, except for the harvest index where the differences among genotypes were significant ($P \leq 0.05$) (Table 3). The differences between locations were not significant only for panicle length, while for all the other characteristics were highly significant. Genotype × location interaction was highly significant ($P \leq 0.01$) only for biological yield and significant ($P \leq 0.05$) for a number of panicles per m^2.

The non-significant G × L interaction for grain yield in this research contradicted the findings of Tariku et al. [19], Upreti et al. [17], Bose et al. [20], Sürek et al. [21] and Blanche et al. [15]. In their studies, they all revealed highly significant genotype × location interaction for rice grain yield where the genotypes interacted considerably with environmental conditions. Also, the non-significant G × L interaction for plant height (Table 4) in this study differs from the results of Tariku et al [19], who reported highly significant G × E interaction for this trait on the tested lowland rice varieties.

In this study, the paddy rice yield varied between 6.10 t/ha (San Andrea, Cesinovo) and 12.27 t/ha (Gala, Kocani), as presented in Table 4. The highest average paddy yield was obtained with Gala (9.96 t/ha) and the lowest with San Andrea (7.42 t/ha). The other Turkish varieties Halilbey and Gönen also achieved higher mean paddy yield than standard San Andrea, but not significantly. The location of Kocani provided better environmental conditions for grain yield to all the examined varieties than the location Cesinovo, as it is obvious also from Figure 1. The reported potential for paddy rice yield of the related varieties from the Trakya Agricultural Research Institute [22] was: 9–10,000 kg/ha for Gala, 11.000 kg/ha for Halilbey and 8.500 kg/ha for Gönen. Akay et al. [14] recorded similar results for Gala rice variety, as one of the best yielding cultivars with 6.623 kg/ha.

Table 3. Combined ANOVA for investigated traits of rice varieties

Sources of variation	DF	Paddy rice yield	Biological Yield	Harvest index	Plant height	Panicle length	Number of panicles per m^2
		Mean Squares					
Replication	2	0.267	1.5100	0.001	61.976	1.018	424.5
Location	1	72.84 **	2836.461 **	0.031 **	1985.62 **	0.027	273920.67 **
Genotype	3	7.332 **	29.071 **	0.005 *	369.13 **	12.181 **	3818.56 **
Genotype × location	3	1.053	22.515 **	0.001	2.429	0.314	1117.889 *
Error	14	0.593	2.6250	0.001	13.344	0.749	317.357
Total	24	–	–	–	–	–	–

* significant at level P ≤ 0.05, ** significant at level P ≤ 0.01

Table 4. Mean values for examined traits of rice varieties

Genotypes (Varieties)	Locations	Paddy rice yield [t/ha]	Biological Yield [t/ha]	Harvest index	Plant height [cm]	Panicle length [cm]	Number of panicles per m^2
	Cesinovo	6.10	18.67	0.32	108.10	16.70	287.00
San Andrea	Kocani	8.73	35.77	0.25	125.13	16.10	483.33
	Mean	**7.42 b**	**27.22 ab**	**0.29 a**	**116.62 a**	**16.40 ab**	**385.17 b**
	Cesinovo	7.39	16.53	0.33	93.40	15.23	232.67
Halilbey	Kocani	10.58	41.82	0.30	112.33	15.67	486.00
	Mean	**8.98 ab**	**29.18 a**	**0.31 a**	**102.87 b**	**15.45 ab**	**359.33 c**
	Cesinovo	7.65	18.18	0.41	92.37	14.33	321.33
Gala	Kocani	12.27	42.75	0.30	109.57	14.50	516.33
	Mean	**9.96 a**	**30.47 a**	**0.35 a**	**100.97 b**	**14.42 b**	**418.83 a**
	Cesinovo	6.33	15.45	0.36	104.20	17.90	269.67
Gönen	Kocani	9.82	35.46	0.29	123.80	17.63	479.67
	Mean	**8.08 b**	**25.46 b**	**0.33 a**	**114.00 a**	**17.77 a**	**374.67 b**
	LSD (0.05)	*1.99*	*3.60*	*0.38*	*7.08*	*2.72*	*13.32*

Distinct letters in the row indicate significant differences according to LSD test (P ≤ 0.05)

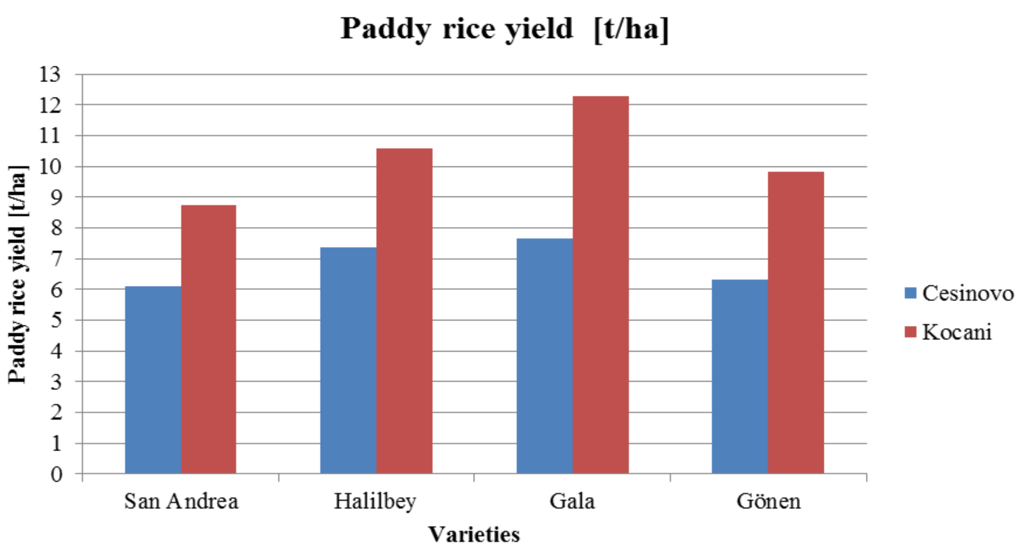

Figure 1. Paddy rice yield of the examined rice varieties

The mean biological yield of the examined varieties (Table 4) ranged from 15.45 t/ha (Gönen, Cesinovo) to 42.75 t/ha (Gala, Kocani). Gala achieved the highest mean biological yield (30.47 t/ha), significantly higher only from the lowest mean found in Gönen (25.46 t/ha). The control variety San Andrea did not differ significantly from Turkish rice varieties, regarding the mean value of this trait. Biological yield values were strongly affected not only by genotype but mostly by the environmental conditions (location) and it was the only trait with highly significant genotype × location interaction (Table 3). In general, higher total biomass in all cultivars was obtained in the Kocani location compared to Cesinovo (Figure 2). In Kocani, the highest value was observed in Gala (42.75 t/ha), while in Cesinovo, the most abundant biomass was produced by San Andrea (18.67 t/ha). Gönen was the variety with the lowest biological yield in both locations (15.45 t/ha in Cesinovo and 35.46 t/ha in Kocani).

Since biological yield was significantly affected by location and genotype × location interaction (Table 3), it suggests genotypic sensitivity to differences in environmental conditions at the two examined locations [13]. In some other trials conducted in the Kocani region during 2013 and 2014 [9], in both years of research, San Andrea produced significantly higher biological yield than Halilbey, Gala and Gönen. Entirely, Unkovich et al. [10] reported maximum values for rice biological yield (DM) of 31.6 t/ha for dryland conditions of Australia.

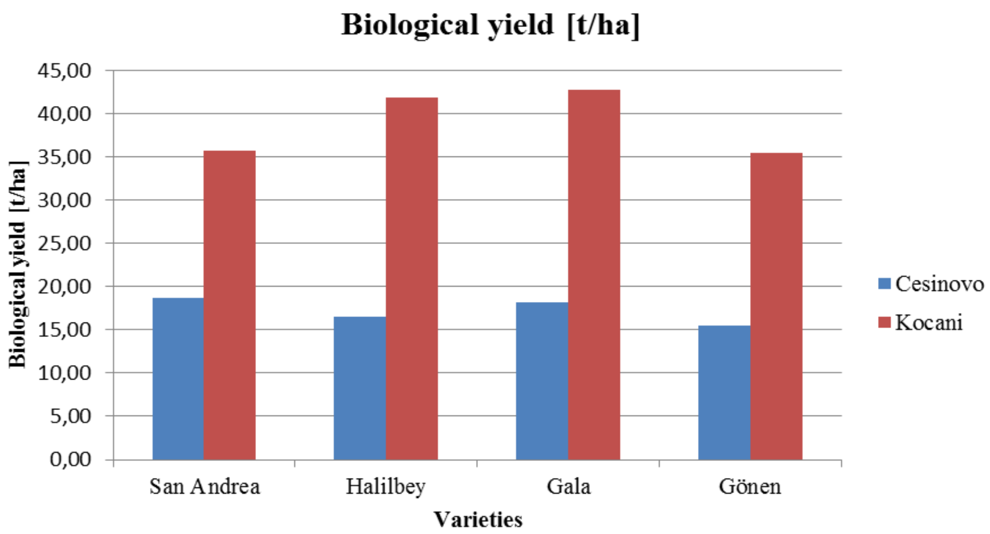

Figure 2. Biological yield of the investigated rice varieties

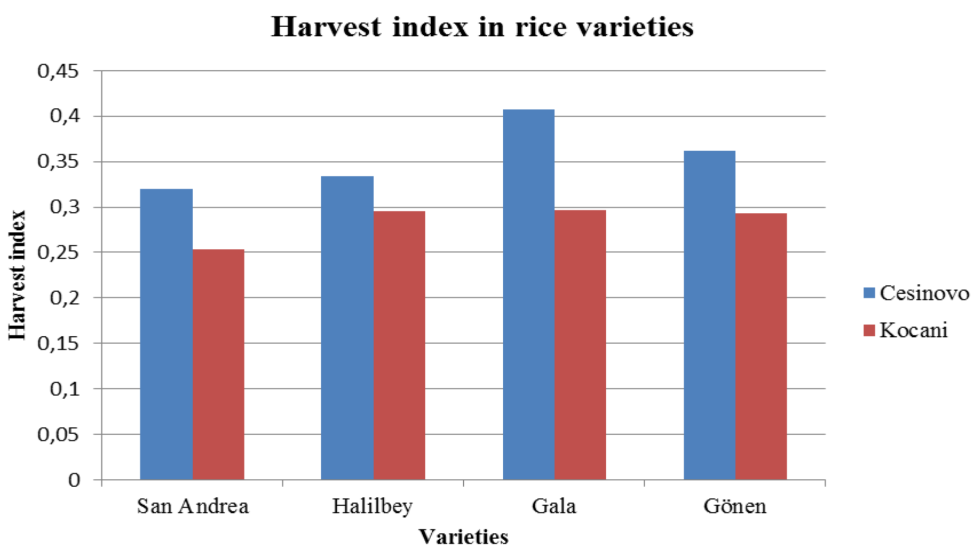

Figure 3. Harvest index in the rice varieties

The values of the harvest index varied between 0.25 (San Andrea, Kocani) and 0.41 (Gala, Cesinovo). The highest mean HI was observed in Gala (0.35) and the lowest (0.29) in San Andrea, but the differences among all cultivars were not significant. All the cultivars in the Cesinovo location gained better HI than in Kocani (Figure 3). In both locations, Gala achieved the highest and San Andrea the lowest harvest index. Harvest index (HI) was the only trait where the variation among cultivar was mainly influenced by the environment (location – highly significant at level $P \leq 0.01$), less than by genotype (significant at level $P \leq 0.05$) and no significantly influenced by interaction genotype × location (Table 3). These findings were in accordance of those of Turner et al. [23], related to the conditions of Australia, where the environment rather than genotype was seen as the major determinant of harvest index for most field crops for a given site × year × genotype. Li et al. [13] appointed that the crop harvest index is also highly influenced by environmental factors, such as soil condition and temperature. In their research on two locations, they obtained means of harvest index (expressed in %) of 30.44 % and 38.98 %. According to Jun [24], large variation was observed for harvest index in rice: about 0.25 among wild species, 0.30 among tall cultivars and more than 0.40 for semi-dwarf cultivars.

Plant height of the investigated varieties (Table 4) varied between 92.37 cm (Gala, Cesinovo) and 125.13 cm (San Andrea, Kocani). The tallest plants on average were observed in San Andrea (116.62 cm), while the shortest was in Gala (100.97 cm). Actually, two groups of cultivars differentiated regarding the plant height. San Andrea and Gönen were taller and did not differ significantly, Gala and Halilbey were shorter, also without significant differences between them. In the same time, among the means of each variety from a different group, significant differences were stated. Regarding different locations, both in Cesinovo and Kocani, the ranging for the plant height was the same: San Andrea > Gönen > Halilbey > Gala. The bigger values for plant height were observed in Kocani (Figure 4). The similar results were obtained in another study in the Kocani region during 2013 and 2014, conducted by Andov et al. [8] where the varieties Halilbey, Gala and Gönen were significantly shorter than San Andrea in both years of the research. As a conclusion, the registration of these three Turkish rice varieties (especially Halilbey and Gala), was a positive step from the aspect of including new rice genotypes with lower plant height in the Macedonian rice farming, suitable for intensive production systems.

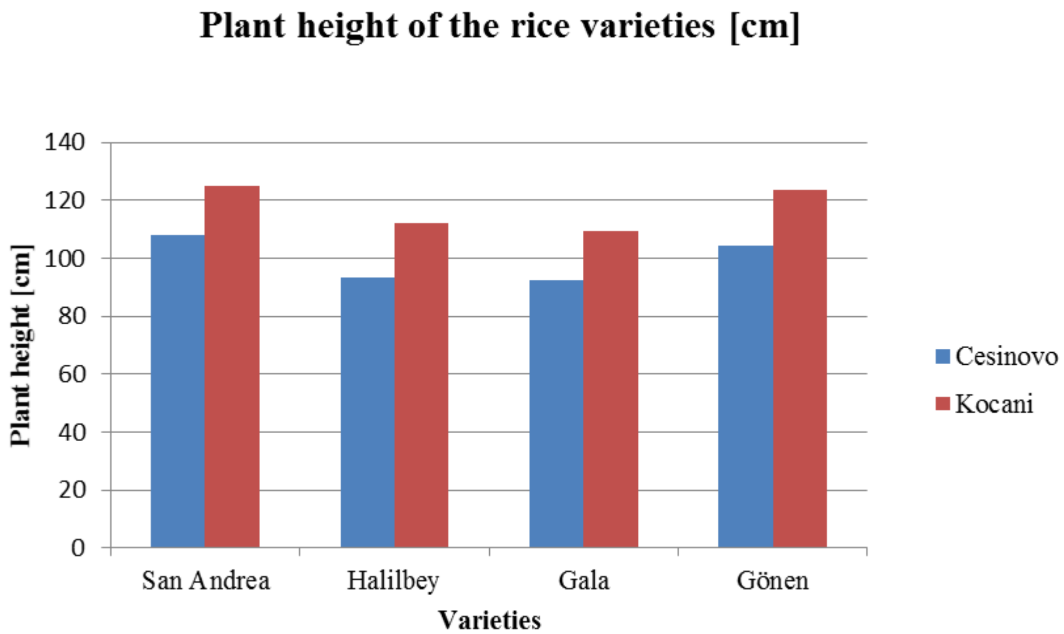

Figure 4. Plant height of the examined rice varieties

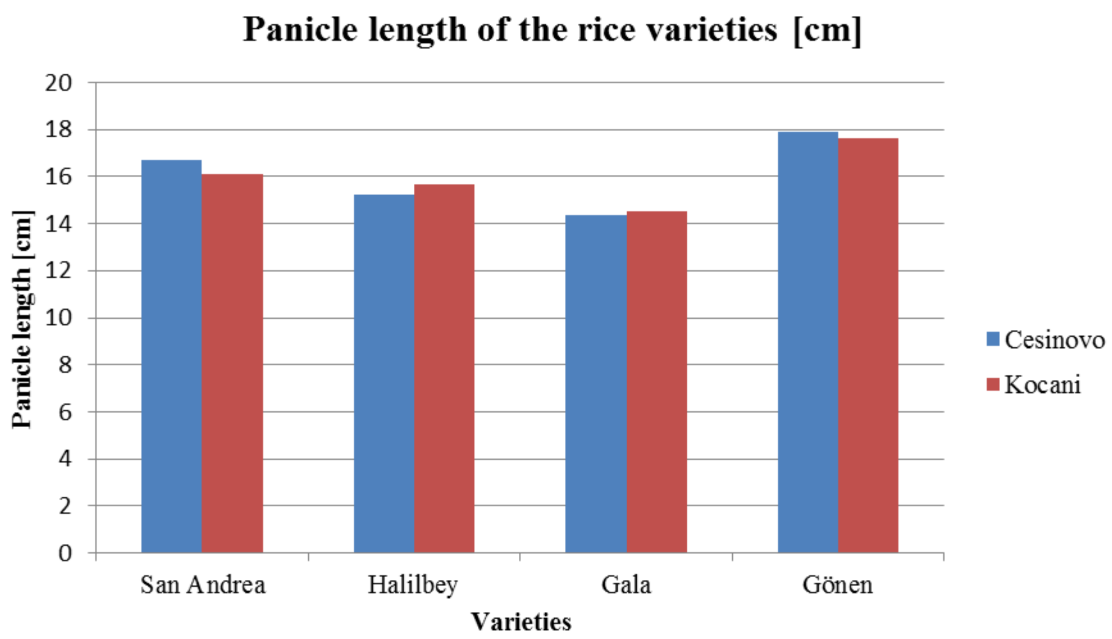

Figure 5. Panicle length of the investigated rice varieties

The number of panicles per m^2 in the investigated rice varieties (Table 4) varied from 232.67 (Halilbey, Cesinovo) to 516.33 (Gala, Kocani). Gala had the highest mean value (418.83) that was significantly higher than all the other varieties, while the significantly lowest was the mean number of panicles per m^2 in Halilbey (359.33). The comparison of the locations showed greater values in Kocani than in Cesinovo (Figure 6). Gala reached the highest values in both locations (321.33 in Cesinovo, 516.33 in Kocani). The lowest panicles number in Kocani (479.67) and Cesinovo (232.67), was observed for Halibey and Gönen, respectively. In this study, the analysis of variance for the number of panicles per m^2 showed significant genotype × location interaction, highly significant genotype and location variations (Table 3). In another two-years research conducted in the Kocani region, for San Andrea, it was observed a higher average number of panicles per m^2 (527.17) compared to Gala (492.00), Halilbey (489.17) and Gönen (448.50) [8].

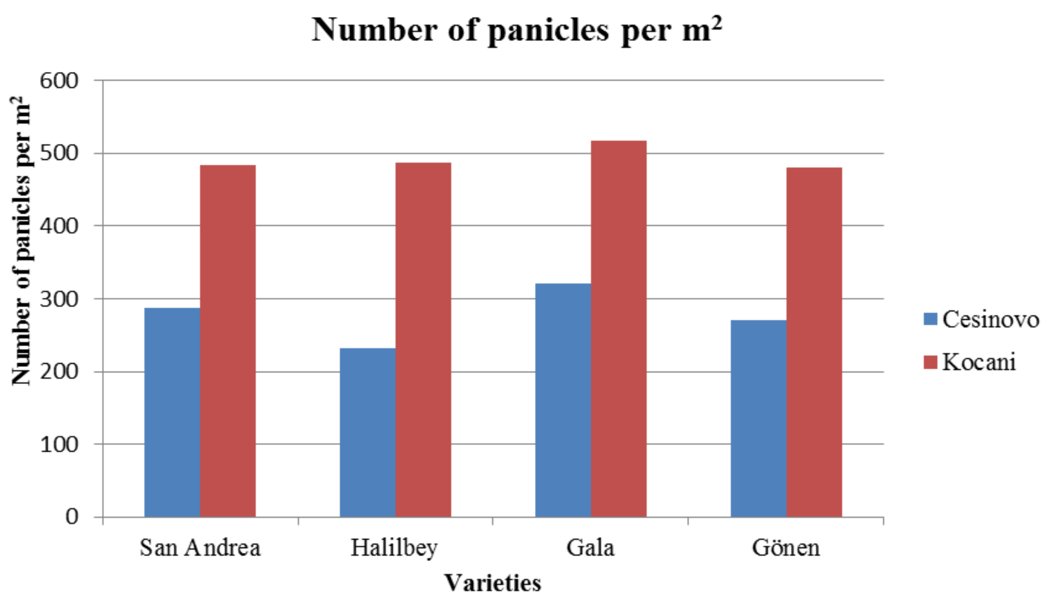

Figure 6. Number of panicles per m^2 in the examined rice varieties

Taking into account all the results obtained in this study, the general conclusion could be done in the sense that the genotype and location had a highly significant influence on paddy yield, biological yield, plant height and a number of panicles per m^2. Significant differences among genotypes by genotype × location interaction were found only for biological yield ($P \leq 0.01$) and a number of panicles per m^2 ($P \leq 0.05$).

The newly registered Turkish rice varieties Halilbey, Gala and Gönen, for most of the examined traits showed better results than the standard San Andrea. Especially for Gala, higher mean values for paddy rice yield, biological yield, harvest index and a number of panicles per m^2 were observed. Similar values were found also for Halilbey. These two varieties were significantly shorter – statured compared to San Andrea, which is a desirable characteristic. Gönen had the longest panicles among the varieties and also achieved some better results from San Andrea for other traits (even none significantly).

Regarding all the analyzed performances of Halilbey, Gala and Gönen in this and in some previous studies at the national level, their implementing in the entire rice production in the Republic of Macedonia can be recommended. Also, there is an interest for them to be included in some further investigations.

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ЕФЕКТИ НА ГЕНОТИПОТ И ЛОКАЦИЈАТА ВРЗ МОРФОЛОШКИТЕ СВОЈСТВА КАЈ СОРТИ ОРИЗ

Емилија Симеоновска*, Даница Андреевска, Добре Андов, Гордана Глаткова, Трајче Димитровски

[1]Земјоделски институт, Универзитет „Св. Кирил и Методиј“, Скопје, Република Македонија

Целта на испитувањето беше да се утврди влијанието на генотипот, локацијата и нивната интеракција на продуктивните и морфолошките својства на четири сорти ориз. Во 2014, два полски опита (рандомизирани блокови со три повторенија) беа поставени на две локации во кочанскиот регион со турските сорти Halilbey, Gala и Gönen и стандардот San Andrea. Податоците од својствата: принос на арпа, биолошки принос, жетвен индекс, висина на растенија, должина на метличка и број на метлички на m^2 беа обработени по методот комбинирана анализа на варијансата. Кај најголемиот број од својствата беа најдени високо сигнификантни разлики меѓу испитуваните генотипови и локации. Интеракцијата генотип × локација беше сигнификантна само за биолошкиот принос и бројот на метлички. Сортите Gala и Halilbey постигнаа највисоки просечни вредности за продуктивните својства и имаа пониско стебло, како погодни карактеристики. Заради покажаните позитивни особини на различни локалитети во ова испитување, сортите Halilbey, Gala и Gönen може успешно да бидат вклучени во македонското оризопроизводство.

Клучни зборови: ориз; генотип; интеракција генотип × локација; продуктивни својства; морфолошки својства

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Review

WEEDS AND WEED MANAGEMENT IN CONSERVATION AGRICULTURE (CA) INFLUENCED BY ITS THREE PILLARS AND HERBICIDES[#]

Zvonko Pacanoski

Faculty for Agricultural Sciences and Food, University Ss. Cyril and Methodius,
Skopje, Republic of Macedonia

e-mail: zvonkop@fznh.ukim.edu

CA is based on three large pillars: reducing or eliminating tilling, covering the soil, diversifying crop rotations. One of the most difficult management issues within this system are weeds and proper weed management. CA brings a shift in dynamics of the weed seed bank, weed populations, density and growth, requiring clear understanding and formulating a strategy for their management. Crop residues influence weed seed germination and seedling emergence by interfering with sunlight availability and suppression through physical and chemical allelopathic effects. Cover crops are fundamental and sustainable tools to manage weeds reducing their numbers physically and chemically. Diversification of the crop rotation help to disrupt the growing cycle of weeds, and prevent any weed species to dominate. Herbicides are an integral part of weed management in CA, as well. When herbicides are not used at appropriate rates or in rotation caused environmental pollution, weed shift, and resistance development in some weeds.

Key words: CA; weeds; crop residues; cover crops; crop rotation

REASONS FOR CA APPEARING AND ITS PILLARS

Conventional tillage (CT) has been an integral part of crop production since crops were first cultivated. Some historians have even evaluated the progress of agrarian societies by their developments in tillage. The premise for CT was to subdue or destroy native vegetation so desired plants might develop free from competition. From the onset of agriculture, CT was synonymous with seedbed preparation and weed control [1]. Apart this, CT is used for crop establishment in order to loosen and aerate the soil for planting, incorporate crop residues and nutrients, enhance the release of nutrients from the soil for crop growth, regulate the circulation of water and air within the soil [2, 3] and when carried out in autumn/winter expose the soil to frost in order to benefit soil structure [4].

Nevertheless CT practices often increase soil erosion rates leading to deteriorating soil physical, chemical and biological properties [5], reduced soil quality such as soil structure, with consequences for water infiltration [6], poor soil porosity, nutrient loss and low organic matter content [7], lead to increased greenhouse gas emissions [8] and create hard pans below the plough layer, as well [9]. Poor soil nutrient statuses in combination with poor weed management practices often contribute to decreased yields [10]. To alleviate this challenge, researchers have suggested a more sustainable method of farming, commonly referred to as Conservation Agriculture (CA).

CA (synonymous of zero tillage farming or no-tillage farming, ridge-till, mulch-till, and noninversion tillage) emerged historically as a response to soil erosion crises in the USA, Brazil, Argentina and Australia where currently, it spans over million hectares. The vulnerability of plough-based agriculture was exposed during the Dust Bowl era (1931-39); as the wind blew away the precious top soil from the drought-ravaged southern plains of the US, leaving behind the failed crops and farms. However, there was no answer then to solve the question of soil

[#]Dedicated to academician Gjorgji Filipovski on the occasion of his 100th birthday degradation. Then, what *Nature* magazine termed "an agricultural bombshell" was dropped by Edward Faulkner on July 5, 1943; with the first issue of his masterpiece book "*Plowman's Folly and A Second Look*" [11]. This book was a milestone in the history of agricultural practices—he questioned the wisdom of ploughing. Some of his statements are: "No one has ever advanced a scientific reason for plowing"; "There is simply no need for plowing in the first instance. And most of the operations that customarily follow the plowing are entirely unnecessary, if the land has not been plowed"; "There is nothing wrong with our soil, except our interference"; and, "It can be said with considerable truth that the use of the plow has actually destroyed the productiveness of our soils." The statements were questioned by both farmers and researchers because alternatives to ploughing at that time did not allow farmers to control weeds or plant into the residues [12]. The idea was widely embraced by farmers all across the world, particularly in the USA only after the Second World War, when the development of chemicals for agriculture allowed them to try it out. With the introduction of 2,4-D in the mid-1940s, producers were, for the first time, given an economical chemical alternative to tillage for preplant weed control. The introduction of numerous other herbicides in the succeeding decades allowed reduced and CA systems to become more feasible and popular [13]. CA systems are being advocated since the 1970s. However, the majority of CA expansion worldwide has occurred since the mid- to late-1990s [14]. This has been accelerated due to the development of efficient farm machinery and the availability of effective herbicides coupled with trained manpower, which have resulted in reduced production costs and higher profitability, besides several indirect benefits [15].

CA is a system designed to achieve agricultural sustainability by improving the biological functions of the agroecosystem with limited mechanical practices and judicious use of chemical inputs. According to FAO [16], CA is an approach to managing agro-ecosystems for improved and sustained productivity, increased profits and food security while preserving and enhancing the resource base and the environment. CA is characterized by three linked principles, namely:

1. Continuous no- or minimal mechanical soil disturbance (i.e., no-tillage and direct sowing or broadcasting of crop seeds, and direct placing of planting material in the soil; minimum soil disturbance from cultivation, harvest operation or farm traffic, in special cases limited strip tillage);

2. Permanent organic soil cover, especially by crop residues, crops and cover crops to protect and feed the soil, develop surface mulch; and

3. Diversification of crop species grown in sequence or associations through rotations or, in case of perennial crops, associations of plants, including a balanced mix of legume and non legume crops to help moderate possible weed, disease and pest problems, generate biomass, fix atmospheric nitrogen and serve as nutrient pumps [17–19].

These three principles converge towards one central and stated goal: to reduce soil degradation and improve soil fertility, by preserving its organic matter, flora and fauna [20].

CA, particularly no-till (NT) system as the core principle of CA, offers a way of optimizing productivity and ecosystem services, offering a wide range of economic, environmental and social benefits to the producer and to the society. At the same time, no-till farming is enabling agriculture to respond to some of the global challenges associated with climate change, land and environmental degradation, and increasing cost of food, energy and production inputs [21–23].

BENEFITS AND LIMITATIONS OF CA

CA technologies are essentially herbicide-driven, machine-driven and knowledge-driven, and therefore require vastly-improved expertise and resources for adoption in large areas. Like every technology, CA mainly is characterized as beneficiary oriented, but it is faced with many challenges and limitations, as well.

A host of benefits can be achieved through employing components of CA, including reduced soil erosion (96 % less erosion) [24] and water run-off (reduction of 70 % in the volume of run-off) [25, 26], increased productivity through improved soil quality, soil water holding capacity increased [27], water availability, water infiltration and water use efficiency [21], reduced sediment and fertilizer pollution in lakes and streams, increased biotic diversity, reduced labour demands [18, 28, 29] and improved quality of life. Advantages also include climate change mitigation through reduced emissions due to 60–70 % lower fuel use, 20–50 % lower fertilizer and pesticides use, 50 % reduction in machinery and labour requirement [30], C-sequestration 0.85 t ha^{-1} y^{-1} or more [31], and no CO_2 release as a result of no burning of residues. Overall, CA has a much lower carbon footprint than CT agriculture [32], and greenhouse gas (GHG) emissions of CO_2, CH_4 and NO_2 are all reduced with CA [33–35]. Based on experiences from North and South America, increased soil C sequestration is reported as the chief reason for improved soil productivity with time under CA compared to CT fields [36]. Improved soil moisture conservation [6], reduced erosion, and increased biological activity [37], are all associated with residue retention. Crop rotation increases crop diversity, reducing crop yield penalties associated with insect damage, diseases and weed infestations whilst improving nutrient cycling [38].

Despite both environmental and production advantages offered through CA, adoption rates have previously lagged in many countries due to several factors including: availability of required equipment, lack of information, producer mindsets, and, initially, greater weed control problems, herbicide dependency [38, 39] and increased risk of herbicide resistance [40]. Other disadvantages include reduced spring soil temperature [41], increased pest problems (incidence of rhizoctonia disease, for example), risk of increased N_2O emissions and increased dissolved reactive P leaching, reduced reliability of crop yields, risk of topsoil compaction [42], and reluctance of farmers to accept the new practice [43]. Several factors including biophysical, socio-economic and cultural limit the adoption of CA, particularly by resource-poor farmers. The current major barriers to the spread of CA systems are (i) competing use of crop residues in rainfed areas, (ii) weed management strategies, particularly for perennial species, (iii) localized insect and disease infestation, and (iv) likelihood of lower crop productivity if site-specific component technologies are not adopted [44, 45]

WEEDS IN CA SYSTEMS

Although benefits are context specific, CA has been identified as an effective tool for sustainably increasing yields in many parts of the world [21, 46], adopting CA will face several managerial changes, and weed control is perceived as one of the most challenging [5, 47–49].

CT has been a major agricultural weed control technique for several decades [50], so the development of CA systems that advocate NT or reduced tillage has significant implications for growers [51]. CT affects weeds by uprooting, dismembering, and burying them deep enough to prevent emergence, by changing the soil environment and so promoting or inhibiting the weeds' germination and establishment, and by moving their seeds both vertically and horizontally [52–54]. CT is also used to incorporate herbicides into the soil and to remove surface residues that might otherwise impede the herbicides' effectiveness. Reduction in tillage intensity and frequency, as practiced under CA, generally increases weed infestation and causes a variation in the dynamics of the weed populations [55].

Mishra and Singh [56] observed that over the course of time, an NT–NT sequence favoredrelatively higher weed growth over a CT–CT sequence in a rice–wheat system. While weed growth in the initial year was not higher under the NT–NT sequence, in the third year of experimentation total weed dry weight was significantly higher under the NT–NT than CT–CT tillage sequence. Total weed density was significantly lower (16.3 plants m^{-2}) under the CT than the other reduced tillage systems(36.7–39.2 plants m^{-2}). The main benefit of CT is a highly significant decline of perennial weeds. Only 2.6 perennial weed plants per quadrant in CT as compared to 7.5–9.0 in reduced tillage treatments were noted [57]. Tolimir *et al*. [58] also noted considerably lower weed infestation per square meter under CT (7 weeds) compared to reduced (39 weeds) and NT (46 weeds). Swanton et al. [59] found that tillage was an important factor affecting weed composition: *Chenopodium album* and *Amaranthus retroflexus* were associated with a CT system, whereas *Digitaria sanguinalis* was associated with NT.

Shifts in weed populations from annual large-seeded broadleaf to annuals grass and small-seeded broadleaf and perennials, as well have been observed in CA systems [60–63]. Perennial weeds thrive in reduced or NT systems [64, 65]. Most perennial weeds have the ability to reproduce from several structural organs other than seeds. Among them, perennial monocots are considered a greater threat than perennial dicots in the adoption of reduced tillage systems [11]. In practice, researchers have shown that small-seeded annual grass species and perennials become more difficult to manage as tillage is decreased, whereas large-seeded broadleaf weeds become easier to manage in production systems with less tillage [66]. For example, in ZT-DSR (zero-tillage- dry direct-seeding), weed flora often shifts towards more difficult to control and competitive grasses and sedges such as *Leptochloa chinensis* (L.) Nees, *Eragrostis* spp., *Echinochloa colona* (L.) Link., *Cyperus* spp., *Eclipta prostrata* (L.) L., *Ammannia* spp., *Sphenochloa zeylinica* Gaertn. [67]. Similar, in the Eastern Indo-Gangetic Plains, big and serious problems under NT are *Cyperus rotundus* L. and *Cynodon dactylon* (L.) Pers. [68]. According to Shaw *et al*. [1] winter annual and biennial weeds, and some brush species that were not problematic with CT can increase with NT, like *Cirsium arvense*, where NT leaves the roots undisturbed and *Cirsium arvense* populations can increase. Opposite, analysis of multiplied dominance ratio (MDR) of weeds in Japan showed that summer annual weeds, especially grass weeds, were much more abundant than perennial weeds in the NT fields [69].

Different tillage systems disturb the vertical distribution of weed seeds in the soil, in different ways. Studies have found that moldboard plowing buries most weed seeds in the tillage layer, whereas chisel plowing leaves most of the weed seeds closer to the soil surface [70]. In reduced or NT systems, depending on the soil type, 60–90 % of weed seeds are located in the top 5 cm of the soil [52, 54, 71]. Similar, the highest number of weed seed species was found in the treatments with reduced and NT treatments in a soil layer of 0–5 cm. In deeper soil layers (5–10, 10–20 cm), no differences in weed seed species number were found [72]. Chauhan *et al.* [73] reported that a low-soil-disturbance single-disc system retained more than 75 % of the weed seeds in the top 1-cm soil layer, whereas the high-soil-disturbance seeding system buried more than 75 % of the seeds to a depth more than 5 cm. Further, the accumulation of the weed seeds at the soil surface increases their chance to germinate in one season with suitable moisture and temperature, and they are exposed to insect predation, (vertebrates and invertebrates) [74] fungal and bacterial attack [75], rodents and birds consume [76] and decay thus depletion of the weed seed bank is high [77] as well as weed density [78]. Sagar and Mortimer [79] found that weed seed survival across time is lowest when seeds remain on the soil surface because of exposure to environmental extremes and predation. Similar, Usman *et al*. [80] concluded that there is a rapid loss of viability of weed seeds in addition to predation on the soil surface in NT compared to CT where seeds are buried in the soil and prevented from environmental hazards. Even in the absence of seed predators, weed seeds from species as diverse as *Panicum miliaceum* L., *Amaranthus retroflexus* L., and *Solanum sarrachoides* Sendtner have been shown to lose viability at a greater rate when positioned near the soil surface than when buried below the emergence zone [81, 82].

Although some studies found that weed seed bank, weed density and weed infestation are smaller in NT compared with CT, other studies claimed the opposite [83]. According to Singh *et al*. [11] the presence of weed seeds on the upper soil surface, due to no tillage operation, leads to higher weed infestation in the NT system. Cardina *et al*. [63] studied the weed seed bank size and composition after 35 years of continuous crop rotation and tillage system and concluded that weed seed density was highest in NT and generally declined as tillage intensity increased. The soil weed seed bank was 1.5 and 2.2 times greater in the shallow ploughing and shallow ploughless tillage treatments, compared with the CT treatment (deep ploughing). In the shallow ploughing and shallow ploughless tillage treatments, there were found 25.5 % and 41.5 % more weed seed species in the soil, compared with the CT treatment [84]. According to Menalled *et al*. [85], above ground weed biomass, species density, and diversity were lowest in the CT system, intermediate in the NT system, and highest in the low-input and organic systems. Higher weed seed densities in NT systems may be the result of reduced herbicides availability because of adsorption to near surface organic matter [86].

COVER CROPS AND THEIR RESIDUES

The use of cover crops in CA offers many advantages, one of which is weed suppression through physical as well as chemical allelopathic effects [87, 88], while actively growing or after termination [89]. Prior to termination, cover crops can compete with weed species for necessary resources such as light, water, and nutrients; cover crops can also release allelochemicals into the soil which may be detrimental to nearby competing weed species, particularly for small-seeded weeds [90]. Cereal rye (*Secale cereale* L.) and soft red winter wheat (*Triticum aestivum* L.) used as cover crops also contain allelopathic compounds that inhibit weed growth [91]. Yenish *et al*. [92] reported increased short-term weed control using a rye cover crop in NT corn (*Zea mays* L.) but not season-long control. In southern Brazil, black oat (*Avena strigosa* Schreb.) is the predominant cover crop on millions of hectares of NT soybean [*Glycine max* (L.) Merr.] because, in part, of its weed-suppressive capabilities [93]. In Japan, a possible goal of a cover crop system in NT soybean is to eliminate or greatly reduce the use of herbicides in association with the implementation of reduced tillage and the appropriate use of cover crops [69]. Two annual medic species [burr medic (M. *polymorpha* cv. Santiago) and barrel medic (M. *truncatula* Gaertn. cv. Mogul)], berseem clover (*Trifolium alexandrinum* L. cv. Bigbee), and medium red clover (*Trifolium pratense* L.) were NT seeded as cover crops into winter wheat (*Triticum aestivum* L.) stubble in a winter wheat/corn (*Zea mays* L.) rotation system. The density of winter annual weeds was between 41 and 78 % lower following most cover crops when compared with no cover control in 2 out of 4 site years, while dry weight was between 26 and 80 % lower in all 4 trial site years [94].

In CA systems, the presence of crop residue acting as mulches on the soil surface, influence soil temperature and moisture regimes that affect weed seed germination and emergence patterns over the growing season. Crop residues can influence weed seed germination and seedling emergence [95, 96]. Several mechanisms may contribute to reduced weed emergence and growth where surface cover crop residues are present, including reduction in light penetration to the soil [97], physical obstruction resulting in seed-reserve depletion before emergence [98], increased seed predation or decay [74, 99], decreased daily soil temperature fluctuations [101], or the production of allelopathic compounds [97].

Weed emergence generally declines with increasing residue amounts. However, the emergence of certain weed species is also favored by some crop residue at low amounts. For example, germination and growth of *Avena fatua* L. and *Avena sterilis* L. may get stimulated with low levels of wheat residue [11]. Further, late emerging weed plants produce fewer seeds than the early emerging ones [77]. For example, the residue of *Vicia villosa* Roth and *Secale cereal* L. reduced total weed density by more than 75 % compared with the treatments with no residue [100]. The presence of rye mulch in corn significantly reduced the emergence of *Chenopodium album* L., *Digitaria sanguinalis* (L.) Scap., and *Portulaca oleracea* L. and total weed biomass [101]. Tuesca *et al.* [102] attributed the lower densities of *Chenopodium album* L. under NT systems to the inhibitory effect of crop residues on light interception. Saini [103] found that rye residue provided 81–91% control of *Diodia virginiana* L. and *Jacquemontia tamnifolia* [L.] Griseb., *Digitaria sanguinalis* [L.] Scop. control was only 11 % in cotton and peanut.

For significant suppressive effects of mulch on the emergence and growth of *Echinochloa crus-galli* (L.) P. Beauv. and *Eclipta prostrata* (L.) L., 6 t ha^{-1} rice residue as mulch was needed, whereas, the emergence of *Echinochloa colona* (L.) Link and *Dactyloctenium aegyptium* Willd. was reduced with as little as 1 to 2 t ha^{-1} [68]. Chhokar *et al.* [104] observed that 2.5 t ha^{-1} rice residue mulch was not effective in suppressing weeds, but 5.0 and 7.5 t ha^{-1} residue mulch reduced weed biomass by 26 to 46 %, 17 to 55 %, 22 to 43 %, and 26 to 40 % of *Phalaris minor* Retz., *Oxalis corniculata*. L., *Medicago polymorpha* L. and *Setaria viridis* (L.) P.Beauv., respectively, compared with ZT without residue. Similar, Chauhan and Abugho [96] reported that 6 t ha^{-1} crop residues reduced the emergence of *Echinochloa colona* (L.) Link, *Dactyloctenium aegyptium* Willd. and *Cyperus iria* L. by 80–95 %, but only reduce the emergence of *Echinochloa crus-galli* (L.) P. Beauv. by up to 35 %. However, crop residues alone may not be able to fully control weeds, e.g. *Vicia villosa* residues suppressed weeds early in the growing season but herbicide was needed to achieve season-long weed control [11].

Unlike in the CT system, crop residues present at the time of herbicide application in CA systems may decrease the herbicide's effectiveness as the residues intercept the herbicide and reduce the amount of herbicide that can reach the soil surface and kill germinating seeds [51]. According to Chauhan *et al.* [105], crop residues can intercept 15–80 % of the applied herbicides and this may result in reduced efficacy of herbicides in CA systems.

DIVERSIFIED CROP ROTATION

Crop rotation increases crop diversity, reducing crop yield penalties associated with insect damage, diseases and weed infestations whilst improving nutrient cycling [38]. Crop rotations are arguably the most effective way to control weeds. It limits the build-up of weed populations and prevents weed shifts as the weed species tend to thrive in a crop with similar growth requirements. Different crops require different cultural practices, which help to disrupt the growing cycle of weeds and prevent any weed species to dominate [11]. In this way, any given crop can be thought of a filter, only allowing certain weeds to pass through its management regime [106]. Rotating crops will rotate selection pressures, preventing one weed from being repeatedly successful, and thus preventing its establishment [107]. Weed diversity has been shown to increase under crop rotation compared to monoculture [108, 109]. Greater diversity prevents the domination of a few problem weeds. Murphy *et al.* [110] observed the highest weed species diversity in NT fields with a three-crop rotation of corn–soybean–winter wheat. Weed species composition would be affected by rotation design, and weed population dynamics are very dependent on the crops included in the rotation [111]. The diversification of the system even for a short period and intensification by including summer legumes/green manuring decreased the weed menace [112]. The integration of red clover in the sweet corn–pea–wheat rotation led to a 96% reduction in the seed bank density of winter annuals [113]. Further, including perennial forages, such as alfalfa in rotation, has been shown to contribute weed control for up to three years, and can be particularly effective in NT systems [114, 115]. In NT systems of the Northern Great Plains of the United States (US) and Canada, stacked rotation designs offer superior weed control compared to yearly rotations [116]. Anderson and Beck [117] found that warm-season weeds were more prevalent in rotations with two warm-season crops in 3 years, whereas these species were rare in rotations that included 2-year intervals of cool-season crops or fallow. Similar, weed community density declined across time with NT when rotations consisted of two cool-season crops followed by two warm-season crops; in contrast, weed community density was 13-fold greater with a two-crop rotation and NT [118]. In that context, in the semiarid Great Plains, producers who rotated cool-season and warm-season crops reduced weed community density and could grow some crops without needing herbicides to achieve optimum yields. With these diverse rotations, producers are using 50% less herbicide to manage weeds compared with that in less-diverse rotations [118].

HERBICIDES IN CA

Herbicides have an important role in weed control under CA systems [55]. Restricting tillage reduces weed control options and increases reliance on herbicides in such production systems, particularly the recent development of post-emergence broadspectrum herbicides provides an opportunity to control weeds in CA [119]. Therefore, CA is presently a common farming system in many countries, principally because many types of herbicides are available [120]. The presence of weed seeds on the upper soil surface, due to no-tillage operation, leads to higher weed infestation in CA, and so far herbicides are the only answer to deal with this problem [11]. They play an important role, particularly in controlling weeds during the first years after the adoption of conservation agriculture [121]. Herbicides are effective weed control measures and offer diverse benefits, such as saving labor and fuel cost, requiring less human efforts, reducing soil erosion, saving energy, increasing crop production, reducing the cost of farming, allowing flexibility in weed management, and tackling difficult-to-control weeds [122, 123]. The use of herbicides to facilitate weed control and soil cover management is an option to reduce production costs and to avoid the aforementioned negative effects associated with soil tillage, including the stimulation of further weed emergence and spread. In Canada adoption of NT has not increased herbicide use significantly [124], and in the US Great Plains, NT wheat systems have controlled weeds using cultural tactics and reduced herbicide usage by 50% compared to CT [118]. Similar, in some West European countries [125] and some areas in Australia [126], which have agroecological conditions similar to Europe, herbicide use per tonne of output is lower in CA systems with integrated weed management than in CT farming [127]. Published research on NT corn and soybean showed that preemergence herbicide use for summer annual weed control could be reduced 50 % by banding herbicides over crop rows and substituting between row mowing for herbicides [128–130]. This equaled a 50 % reduction in preemergence herbicide use and a 25 % reduction in total herbicide use in no-till. In corn and soybean, crop yields were statistically indistinguishable among weed-free checks, broadcast preemergence herbicide treatments, and some treatments using banded preemergence herbicide followed by between-row mowing. Similarly, NT with effective herbicide weed control was more remunerative in the soybean–wheat system [131]. Further, the application of a burndown herbicide such as paraquat or glyphosate at planting followed by a herbicide such as Harmony Extra (Thifensulfuron-methyl+ Tribenuron-methyl) in the spring excellent solve the problems with broadleaved weeds no-till wheat [132]. Herbicide treatments such as glufosinate, mesotrione, and dicamba + diflufenzopyr are effective in suppressing *Taraxacum officinale* Web. competition in no-tillage corn [133].

However, to sustain CA systems, herbicide rotation and/or integration of weed management practices is preferred as continuous use of a single herbicide over a long period of time may result in the development of resistant biotypes, shifts in weed flora and negative effects on the succeeding crop and environment [11, 134]. Selection pressure imparted by herbicide tactics can result in weed shifts attributable to the natural resistance of a particular species to the herbicide or the evolution of herbicide resistance within the weed population [55]. Reddy [135] found that continuous bromoxynil-resistant cotton production resulted in weed species shift toward *Portulaca oleracea* L., *Senna obtusifolia* [L.] Irwin and Barneby, and (*Cyperus esculentus* L.).

A major criticism of CA is its enhanced reliance on herbicides as compared to tilled systems. In particular, glyphosate may be heavily used, especially to control perennial weeds [136]. When herbicides are not used at appropriate rates or in rotation [40], it can lead to the development of herbicide resistance among major weed species [137,138]. Herbicide resistance and weed control problems are clearly the main reasons given by adopters for past or intended reductions in NT use [139]. Herbicide resistance and weed control issues are the major reason why some NT adopters are reducing their use of NT as the core principle of CA.

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ВЛИЈАНИЕ НА ТРИТЕ СТОЛБА НА КОНЗЕРВАЦИСКОТО ЗЕМЈОДЕЛСТВО И ХЕРБИЦИДИТЕ ВРЗ ПЛЕВЕЛИТЕ

Звонко Пацаноски

Факултет за земјоделски науки и храна, Универзитет „Св. Кирил и Методиј“, Скопје, Република Македонија

Конзервациското земјоделство е засновано на три големи столба: редуцирана обработка или целосно нејзино елиминирање, покриеност на почвата и разновиден плодоред. Еден од најголемите проблеми со кои се соочува овој систем се плевелите и правилната борба против нив. Конзервацискиот систем предизвикува промена во динамиката на плевелните семиња во почвата, плевелната популација, заплевеленоста и растот на плевелите, со што се наметнува потребата од усогласување и изработување стратегија за борба против нив. Растителните остатоци влијаат врз 'ртењето на плевелите и нивните поници преку попречување на достапноста на светлината, физичкото задушување и хемиското (алелопатско) дејствување. Покривните култури се основен и одржлив начин за физичко и хемиско (алелопатско) намалување на бројноста на плевелите. Разновидноста на културите во плодоредот помага во прекинување на циклусот на пораст на плевелите и ја спречува појавата на доминација на некои плевелни видови. Хербицидите, исто така, претставуваат интегрален дел од борбата против плевелите во конзервациското земјоделско производство. Но, кога не се употребуваат во препорачаните дози или во хербициден плодоред, можат да предизвикаат загадување на животната средина, промени во плевелната популација и појава на резистентност кај некои плевелни видови.

Клучни зборови: конзервациско земјоделско производство; плевели; растителни остатоци; покривни култури; плодоред

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Original scientific paper

THE INFLUENCE OF TREE ROW STRIP MULCHING IN PEAR ORCHARD ON SOIL TEMPERATURE AND MOISTURE, GROWTH AND YIELD OF THE TREES[#]

Marjan Kiprijanovski[1*], Vjekoslav Tanaskovic[1], Sonja Bojkovska[2]

[1]Faculty of Agricultural Sciences and Food, Ss. Cyril and Methodius University,
Skopje, Republic of Macedonia
[2]Agency for Financial Support of the Agriculture and the Rural Development,
Skopje, Republic of Macedonia

[*]e-mail**:** kiprijanovski.marjan@yahoo.com

The paper presents results of the investigations of the influence of various mulching and covering materials in orchard tree-row on soil characteristics (temperature and moisture) and tree behavior (growth, yield and fruit quality) of Bartlet pear grafted on a quince rootstock. The experimental orchard was established with planting distance 3.2 m × 1.3 m and irrigated with drip irrigation system. The soil surface between the rows was maintained by grassing. The follow mulching materials were used as treatments: straw, conifer sawdust, peat, black plastic foil, cloth of polyester fabric (geotextile). Clean cultivation was used as a control treatment. The obtained results indicated that mulching material has effect on reduction of the soil temperature during summer as well as improvement of soil water conservation. Mulching of the soil improved vegetative growth of the trees, yield, and fruit quality of Bartlet pear.

Key words: pear; mulching materials; water conservation; growth; yield

INTRODUCTION

The maintenance of the soil in orchards under canopy is a big problem because the approach of the machinery to the trunk is obstructed by branches. On the other hand, it is known that most of the roots are located in the vicinity of the trees. The fruit tree absorbs water and nutrients mostly from that area and deterioration of soil quality or weed competition cannot be allowed there. The maintenance of the soil in the orchards should be practicable, without weeds competition, with good structure and reduced erosion potential, the soil should not be a suitable habitat for insects and other pests etc. A way of soil maintaining which meets all these requirements does not exist, but a certain balance of these factors should be made [1]. Usage of synthetic herbicides for reduction of weeds is the most commonly used method for soil maintenance in the rows. The negative side of this method is that the soil structure worsens, the hummus reduces, pH decreases, the root grows in the surface layers where it absorbs the applied soil herbicides which transfer to the fruits [2]. Maintenance of the soil under canopy by tillage is a commonly used method. In such a way, weeds are controlled, the water infiltration in the soil is improved, the soil is aerated and the moisture conservation is increased. However, after several years of usage, the content of hummus is reduced and the soil requires additional organic manure. In the orchards, this strip can be cultivated with side cultivators [3].

Weed management in orchards should favor the safety of the environment, including the quality of soil, and should take into account the effectiveness, costs, and influence on yielding of the crops. Synthetic herbicides are the most effective way of controlling weeds within orchards. The small number of registered herbicides for use in the orchards is an issue in practice [4]. The excessive use of glyphosate based herbicides leads to toxicity, presence of residues in the environment, plants and fruits [5].

[#]Dedicated to academician Gjorgji Filipovski on the occasion of his 100th birthday

The usage of postemergence herbicides facilitated the growth and the yield of plum trees and may be considered a convenient method for floor management under trees in conventional orchards [6].

The increased pressure to reduce pesticide use, fruit growers are forced to require alternatives for control of weeds in orchards. One option is the use of mulches, which may have advantages in improving soil physical properties and tree growth [7].

Mulching the soil in the row with various organic matters has next advantages: prevents erosion, increases water conservation, prevents weed occurrence, increases the activity of the soil microorganisms and earthworms, and increases the content of hummus and nutrients in the soil. Different mulch material in pear orchard have positive effects on soil water conservation, evapotranspiration and water physical characteristics of the soil [8]. Organic mulches are of benefit to soils because they increase their productivity and organic matter content and protect them from excessive solar radiation and moisture loss [9]. The weeds in orchards are successfully controlled with synthetic mulches such as polyethylene plastic, woven polypropylene fabric and nonwoven polyacrylic fabric [10]. Straw, sawdust, compost and peat moss [11], waste and textiles (linen, jute, wool) [12], are used as natural mulches in practice.

Covering the soil with black foil keeps the soil moisture, but there is not possibility of aeration and the temperature of the soil during the summer is very high [13]. Under plastic mulch, soil properties like soil temperature, moisture content, bulk density, aggregate stability and nutrient availability improved. Plant growth and yield are also positively influenced by the plastic mulch due to the modification of soil microclimate. Even though it has many advantages, high initial cost, removal and disposal of plastic materials are some of the limitations experienced by the farmers. To overcome these limitations photo and biodegradable plastic mulches can be used for sustaining the productivity as well as controlling environmental pollution due to the use of plastics [14].

The protection of the environment, should be priorities when the weeds are treated. Also the efficiency, the price and the impact on the yield should be taken into consideration. The integral weed control should be based on rational use of herbicides and alternative methods [4]. Given the limited number of herbicides available for organic production, orchard floor management takes on a more critical role for organic fruit growers [15]. In the organic orchards, the so-called Swiss sandwich system is often used, which covers different ways of maintaining the surface in the form of strips [16].

The problems that occur from the excessive use of agrochemicals in the fruit production make the use of integrated and organic production necessary. The soil maintenance is also part of the program of these concepts of fruit production. The aim of the research was to determine the effect of mulching the soil under canopy in pear orchards on growth, yield and fruit quality, as well as examination of the influence of different mulch materials on the temperature of the soil and water conservation as an alternative way of production adaptation to the climate changes and the lack of water for irrigation.

MATERIAL AND METHODS

The study was conducted in pear orchard located in Kumanovo region, north-eastern part of R. Macedonia. The soil type of the experimental field was Fluvisol. Bartlet pear orchard was established in 2010, with distance of 3.2 × 1.3 m (2400 trees/ha). The trees were grafted on quince rootstock MA and Beurre Hardy was used as an interstock. The tree crowns were trained as a slender spindle. White anti-hail net with shading of 15 % was installed in the orchard. The irrigation was scheduled according to long-term average daily evapotranspiration of pear orchards for Kumanovo region (Table 1). The long-term average (LTA) crop evapotranspiration was calculated by FAO software CROPWAT using crop coefficient (K_c) and stage length adjusted for the local conditions. Drip irrigation system was installed in the pear orchard and daily evapotranspiration was decreased by 30-35% (coefficient of the coverage-application of the water only on part of the total surface).

Table 1. Long-term average daily and monthly evapotranspiration for pear orchard in Kumanovo region calculated by FAO software CROPWAT

Months	May	June	July	August
Evapotranspiration, mm/day	2.12	3.94	5.82	5.0
Evapotranspiration, mm/month	65.81	118.21	180.64	150.15

The soil between rows was maintained by grassing and multiple mulching. Manually cultivation at depth of 10 cm had been used for maintaining tree row in orchard (a strip with a width of 0.9 m) till we have established the field trial. Investigation of influence of various soil mulching materials on temperature and water conservation in the soil as well as growth, yield and fruit quality of pear trees was conducted in the two consecutive years (6^{th} and 7^{th} leaf of the trees) at the cultivar Bartlet.

Mulching of the soil in row under the trees, in a 0.9 cm wide strip was made at the beginning of first year of the experimentation. The following treatments were included in the experimental field:

1. Mulching with wheat straw with layer of 15 cm (Straw),
2. Mulching with conifers sawdust with layer of 10 cm (Sawdust),
3. Mulching with peat with layer of 10 cm (Peat),
4. Covering with black polyethylene foil with thickness 0.07 mm (Foil),
5. Covering with gray colored polyester fabric (Geotextile) with thickness 3 mm (Geotextile),
6. Clean cultivation on 10–12 cm depth, used as control (Control).

The treatments were applied in completely randomized blocks with 3 replications and 8 trees on the plots (24 trees per treatment). Mineral fertilization and plant protection were carried out according to current recommendations for commercial pear orchards.

Potential evapotranspiration in the treatments (ETP) was determined by the soil water balance method using direct measurements of soil moisture in the soil layer 0–100 cm [17, 18]. Monitoring of soil water income during the growing period and the active soil moisture at the end of vegetation period was used in this estimation. The soil water income was determined by estimation of the initial or active soil moisture at the beginning of vegetation (Wi), the irrigation water requirements (I) and the effective precipitation during the vegetation period (P). The incomes of water from precipitation (P) was taken from the Hydrometeorological Service of the Republic of Macedonia. The effective rainfall was calculated on the basis of total incomes (reduction from 30 to 50 %, depending on the period of vegetation) [17]. Irrigation water requirements (I) for all treatments were calculated according to results in table 1. All treatments have received same quantity of irrigation water during the growing period. Also, as a result of controlled irrigation practice, surface runoff (RO) and deep percolation (DP) were excluded from this estimation. The subsurface water and water transported upward by capillary rise (CR) didn't have influence on water income in the root zone, and they were ignored. The difference between the water content relevant to MSM (momentary soil moisture) and PWP (permanent wilting point) at the end of vegetation is the active soil moisture at the end of the vegetation period (We). The potential evapotranspiration (ETP) was determined by the equation: $ETP = (P + I + Wi) - We$. Conservated water in the soil is calculated on the basis of the difference between evapotranspiration in the control threatment and evapotranspiration in examined threatments.

The soil temperature was measured in the period May-September by digital thermometer, once a month in the afternoon, in three places by treatment on three depths: at the soil surface, at a depth of 3 cm and 10 cm. Only average values are presented here.

The growth of the trees was estimated through measurement of the trunk diameter at a height of 30 cm above the soil surface by caliper at the beginning and the end of the experimental years. The trunk cross-sectional area (TCSA) was calculated from those measurements. The bearing of the trees was computed at the harvest data through the number of the fruits, yield per tree and estimated yield per unit area (ha). The yield efficiency was calculated as kg/cm^2 TCSA. The fruit quality was determined based on average fruit weight and classifying as the extra class of fruit (diameter > 60 mm) and lower classes (diameter < 60 mm).

The statistical analysis of the results was conducted by analysis of variance (ANOVA) and significance of differences between means of treatments was calculated by LSD test. Results were expressed at the $P < 0.05$ level of significance. Statistical program SPSS version 11.0 was used.

RESULTS AND DISCUSION

Ecological conditions

Soil is the physical medium in which the trees are anchorage, which makes it an important factor for their existence, growth, productivity and quality of the fruits. To achieve optimal yield, the pear trees needs sufficient amounts of nutrients and water throughout the vegetation. For pear orchards, the most suitable are deep, structural, drained, carbonless, light soils, with a humus content of at least 2 % and pH 5.5–7 [19].

The soil type of the experimental field was Fluvisol with average field capacity at 60 cm depth of 17.3 %, permanent wilting point of 8.3 %, and soil bulk density of 1.52 g/cm^3.

Table 2. Chemical and water - physical properties of the soil (depth of 0–60 cm)

Parameter	Value
Reaction (pH in water)	6.7
$CaCO_3$, (%)	0.0
Organic matter (%)	2.4
Total nitrogen (%)	0.14
Available phosphorus (mg/100 g soil)	61.7
Available potassium (mg/100 g soil)	59.4
Wilting point (soil moisture retention at 15 bars), volume %	8.6
Field capacity (soil moisture retention at 0,33 bars), volume %	17.3
Bulk density, g/cm^3	1.53

Water- physical properties of the soil such as field capacity (soil moisture retention at 0.33 bars), wilting point (soil moisture retention at 15 bars) and bulk density have a crucial role in determining the irrigation regime of the fruit plantations, i.e. for determining the time and the irrigation application rate [20]. The average soil pH at 0 to 60 cm depth was 6.70. The contents of easily accessible P and K are 61.7 mg/100g and 59.4 mg/100 g, respectively (Table 2). This data shows that the chemical properties of the soil can provide favorable conditions for the pear cultivation.

Successful cultivation of pear orchards is closely related to the climate conditions in the region. Pear is a fruit species with specific needs for several climatic elements, especially for the temperature regime, the schedule of precipitation and the relative air humidity. Based on these parameters, the cultivar and cultivation technology are usually determined, with particular reference to the irrigation. From the data in Table 3 it can be concluded that the climate is semi-arid, suitable for pear growing. A strong change in temperature conditions can be noticed. In terms of precipitation, there is a slight increase in quantities, but their intensity and patterns is unfavorable for fruit production. The increase in temperature and the unfavorable precipitation patterns indicate an increased need for irrigation during vegetation and search for opportunities for water conservation in the soil.

Table 3. Meteorological condition in Kumanovo region

Period	Temperature, °C			Rainfalls, mm			Annually air
	Annually	IV – X	VI – VIII	Annually	IV – X	VI – VIII	humidity, %
2015	13.4	19.5	23.3	629	389	153	73
2006/15	13.1	19.2	23.8	577	477	189	74
1960/90	11.8	17.5	21.6	542	331	132	74

Water balance and evapotranspiration in experimental orchard

The water balance represents all changes in the water content in a certain volume of soil [18]. In practice, the water balance is used to determine the crop water requirements and the evapotranspiration of agricultural crops during vegetation [17, 21]. The results of the water balance are presented in the table 4. Based on them, the evapotranspiration (ETP) in different ways of maintaining the soil in the row was obtained. From the results obtained in this research, it can be concluded that the active moisture at the end of vegetation in the treatment with black foil is the highest, 1199 m^3/ha, followed by the threatments where the surface is mulched with peat, sawdust, geotextile and straw. The lowest amount of active moisture at the end of the vegetation was found in the control variant, 424 m^3/ha. The highest evapotranspiration during vegetation was noted in the control variant. When we present the ETP data in comparative values, then it can be seen that mulching with black foil shows 18.9 % less evapotranspiration compared to the control treatment, followed by other treatments. These data simultaneously present the water conservation capacity depending on the material used to cover the surface in the row. The treatment with black foil had the highest amount of conserved water, 775 m^3/ha more than the control treatment, followed by peat, sawdust, geotextile and straw, respectively. According that we can concluded that covering the surface in the row can reduce the irrigation for four application rates in the treatment with black foil, two application rates in the treatment with a peat and one in the other treatments, without any consequences at the trees. In other field trial with application of organic mulch materials in pear orchard in Kumanovo region, is noted 12 % higher ETP in control treatment without mulch material in comparison with sawdust mulch treatment and 2 % higher ETP in comparison with grass scraping mulch material [8].

Table 4. Water balance and ETP in pear orchard in m^3/ha

Treatment	Initial active moisture (Wi)	Income from precipitation (P)	Income from irrigation (I)	Total incomes	Active moisture to end of vegetation (We)	ETP (P+I+Wi)-We	Conservation of soil water, m^3/ha
Straw	550	1380	2600	4530	593	3937	169
Sawdust	550	1380	2600	4530	651	3879	227
Peat	550	1380	2600	4530	848	3682	424
Foil	550	1380	2600	4530	1199	3331	775
Geotextile	550	1380	2600	4530	632	3898	208
Control	550	1380	2600	4530	424	4106	0.0

Soil temperature in experimental orchard

The growth of the roots and their absorption power is directly dependent on the soil temperature. Very low and very high temperatures in the soil negatively affect the development of the overall processes in the plant. Evaporation is directly dependent on the soil temperature. At higher temperatures the soil loses moisture faster, which requires more frequent irrigation. On the surface part of the soil in the peat treatment temperature was lower 7.6 °C compared to the control treatment (Figure 1). At a depth of 3 cm, a temperature of 30.8 °C was measured in control treatment, and in peat 25.0 °C, or a difference of 5.8 °C. Black foil and geotextile had the smallest deviations in the measurements compared to the control or the approximate temperature with small differences, which leads to the conclusion that these two variants almost completely transmit heat. In the straw and sawdust treatments there was a decrease in the soil temperature at all measurements at different depths. On average, the mulch and cover material reduces the soil temperature, and on the surface of the soil by 4.88 °C, on the soil layer of 3 cm by 3.68 °C, while the soil layer of 10 cm has a lower temperature by 3.46 °C. The soil temperature depends largely on the way the soil is maintained. In the warm summer months, soil mulched with black foil had the highest temperature, and soils mulched with straw and sawdust had much lower temperatures [13]. The organic mulches kept the soil temperature cooler compared with bare soil, while soil under black plastic had a higher mean temperature than bare soil throughout the season [22]. In spring and sumer months the soil temperatures with straw mulch were lower than under cultivation and geotextile treatments [6].

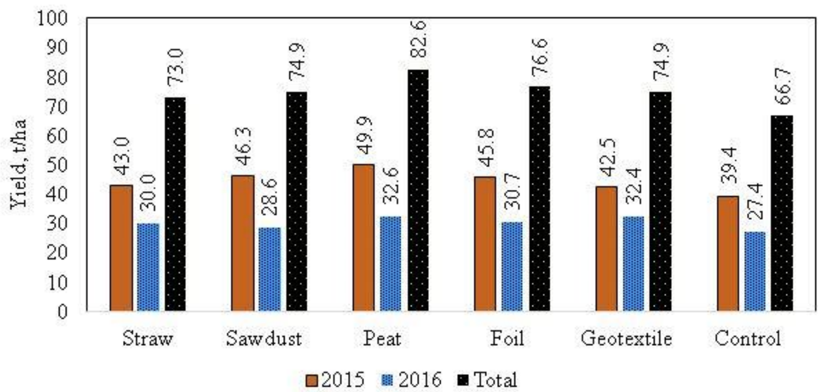

Figure 1. The effect of mulching of the soil temperature

The growth of the tree

The trunk is the most important and integral indicator of the overall activities of the tees [2]. Its growth in a certain period is a significant indicator of the optimal agrotechnical measures applied in the orchards. The activity of the root system and the productivity of the assimilation apparatus is most precisely registered through the dimensions of the trunk. With the increased absorption of water and nutrients from the soil, the productivity of the asymilates in the crown of the trees increases. All these products contribute to the formation of elements of the xylem and the phloem in the trunk, which eventually affects trunk diameter increase. From the data presented in Table 5, it can be concluded that there is a low growth of the trunk in all treatments in the two experimental years. The weak growth of the trunk is primarily due to the dwarfing rootstock on which the trees are grafted, but also on the fairly high yield of the trees. However, the greatest increment of the trunk for the entire test period was determined in trees where the soil was mulched with a sawdust (4.61 cm^2), and the lowest increment was measured in the trees of the control (3.49 cm^2). The increment of the trunk diameter of the trees of the other treatments is in the range between the growth of the fruits of extreme treatments. There were statistically significant differences among the growth of TCSA in treatment mulched by sawdust and in straw treatments, treatment covered with geotextile and control treatment.

The usage of geotextile or straw mulches over the ground cover had significant advantages in terms of increased soil water and tree growth in apple orchard [7]. The growth trunk diameter at apple trees was different between the applied mulches, however, no positive effects was observed compared to the control. No significant differences were observed in TCSA between differ mulches [11]. The application of straw mulch in apple orchard and had higher efficiency toward the elimination of weeds, the improving growth parameters as well as the yield and the quality of the fruit compared with the clean cultivation [23]. The data given in our study are not fully consistent with previous findings concerning the effects of differ mulches on overall growth parameters. That is because the tree growth depends on many factors among with tree bearing in the propriate growing season.

Table 5. Trunk cross section area (TCSA)

Treatment	TCSA, cm^2			Growth, cm^2		
	2015 Beginning	2015 End	End of 2016	2015	2016	Total
Straw	22.80	25.39	26.47	2.55ab	1.09b	3.64b
Sawdust	25.22	28.24	29.84	3.01a	1.60a	4.61a
Peat	21.10	23.61	25.19	2.42ab	1.57a	4.00ab
Foil	22.49	25.24	26.75	2.64ab	1.45ab	4.09ab
Geotextile	22.37	24.90	26.17	2.43ab	1.21ab	3.64b
Control	22.17	24.65	25.81	2.38b	1.11b	3.49b

Values followed by the same letter in a column were not statistically different according to LSD test ($P < 0.05$).

Yield per tree and unit area

The income, the profitability and the economic justification for pear cultivation depends on the yield of the trees. The yield can be shown through several parameters such as: the number of fruit per tree, the yield per tree and unit area, and through relative indicators such as yield per stem size. The yield differs between experimental years (Table 6 and Figure 2), due to damage on the trees caused by late spring frosts in 2016.

The highest total yield for the two experimental years was obtained in the treatment where the soil was mulched with peat (35.5 kg/tree or 82.6 t/ha). The lowest yield per tree was obtained in the control treatment (28.6 kg/tree or 66.7 t/ha). A statistically significant difference compared to the control treatment were determined in the treatments of peat mulching and foil covering. In the period of full bearing of the pear tree, in the Skopje region, determined an average annual yield of 14.3 kg/fruit or 23.7 t/ha [24].

Table 6. The yield per tree

Treatment	Yield per tree, kg		
	2015	2016	Total
Straw	17.9ab	12.5ab	30.4b
Sawdust	19.3ab	11.9b	31.2ab
Peat	20.8a	13.6a	34.4a
Foil	19.1ab	12.8ab	31.9a
Geotextile	17.7ab	13.5a	31.2ab
Control	16.5b	11.4b	27.9b

Values followed by the same letter in a column were not statistically different according to LSD test ($P < 0.05$)

The yield per tree does not differ greatly compared to current results, but there considerably larger difference in the yield per unit area. This is primarily due to the difference in the number of trees per unit area (2400 versus 1666). At the cultivar Bartlet, on generative rootstock obtained average yield of 31.6 t/ha [25]. Compared to our results, the yields was lower, which is primarily due to the large difference in the intensity of the experimental plantations. The mulch treatments had not significant differences in TCSA at the trees. Similarly, the overall yield, total number of fruits, fruit firmness and total soluble solids content were not significantly affected by the use of different mulches. No statistically significant differences were observed between sizes among the fruit collected from trees grown under various mulches, but sawdust contributed to the significant increase of the fruit diameter [11]. The various way of floor management (clean cultivation, herbicides, straw mulching, mowing) under canopy, in plum orchard, had no statistically significant differences on the tree growth, but the cumulative yield for six year had been the greatest at the spraying with herbicides treatment, and the yield had been the lowest at the mulching treatment [6].

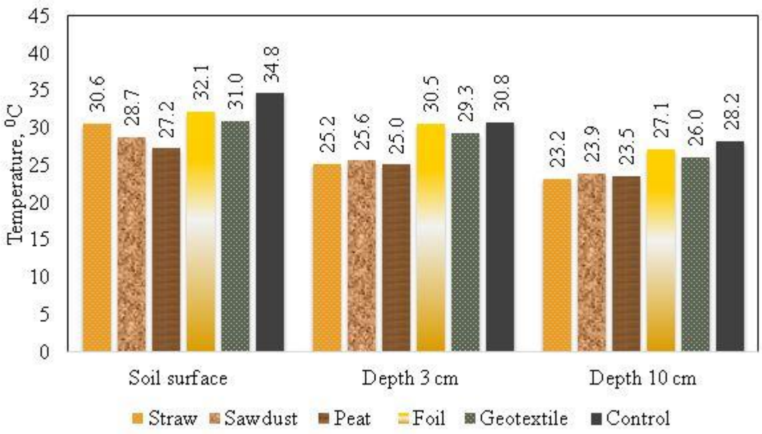

Figure 2. Yield per unit area

Yield efficiency is an important indicator of trees bearing. From the data given in table 7, it can be concluded that the average yield according to this parameter is greatest in trees where the soil is mulched with peat (0.73 kg/cm^2), while the lowest yields was obtained in sawdust and control treatments, 0.56 and 0.57 kg/cm^2 , respectively. According to literature data, dwarfing trees give more fruits when this parameter is taken into consideration. In our case, the trees of the variants with sawdust are most developed, but also with the smallest yield compared to other variants.

Table 7. Yield efficiency, kg/ cm^2 TCSA

Treatment	Yield, kg/ cm^2 TCSA		
	2015	2016	Average
Straw	0.75	0.47	0.61
Sawdust	0.72	0.40	0.56
Peat	0.93	0.54	0.73
Foil	0.78	0.48	0.63
Geotextile	0.75	0.52	0.63
Control	0.70	0.44	0.57

The quality of the fruits

The results for the average fruit weight, number and weight of fruits by classes, as well as their percentage participation in certain classes are presented in table 8. It can be concluded that the average weight of fruits in all treatments was within the characteristic of Bartlet cultivar. The average weight of the fruits in treatments did not differ greatly, but still the fruits of the treatment where the soil was mulched with straw reach the highest values (235.0 g), and the fruits of the control treatment had the lowest value (215.0 g). There was no significant difference between the control variant and variant straw in terms of the number of fruits per tree, and the difference in the weight of the fruits can be explained with the fact that by mulching the water content of the soil improves and thus provides better conditions for the development of the trees in the course of vegetation. It was found that there was no statistically significant difference between the control and the other treatments. At the Bartlet cultivar multiyear average weight of the fruits of 241.2 g is determined [26], which is very close to the results of our trials. The mulcing in the tree rows had significant effects on the trees growth and the yield compared to tillage, but there was no effect on fruit quality parameters in pears [15].

In all treatmens there was a very high percentage of extra class fruits. On average, the largest mass of fruits of extra class was in treatment mulching with peat (15.7 kg/ tree). In control treatment the smallest percentage of extra-class fruits and fruits weight was noticed. This is primarily due to less favorable conditions for the development of trees because of poor water availability in the zone of the root system. In other treatments the index of water conservation increases, the conditions for development of the root system were improved, through which higher yield and improved quality characteristics of the fruits were provided.

Table 8. The weight of the fruits and the mass and % of fruits per classes (average 2015–2016)

Treatment	Fruit weight, g	The mass of fruits per tree, per classes, kg		Number of fruits per classes, %	
		Extra class	Lower classes	Extra class	Lower classes
Straw	225.0a	14.1	1.1	85.4	14.6
Sawdust	222.0a	14.3	1.3	83.9	16.1
Peat	221.6a	15.7	1.5	83.5	16.5
Foil	217.5a	14.7	1.2	83.6	16.4
Geotextile	220.6a	14.3	1.3	84.9	15.1
Control	210.0a	12.5	1.4	81.1	18.9

CONCLUSIONS

Mechanical cultivation of the soil in the tree row minimizes weed competition during growing period of the trees. However, it carries several disadvantages for soil structure, low water conservation and high soil temperature, resulting in weak tree growth and lower yield. Mulching with organic matter or soil coverage with synthetic materials improves conservation of moisture and successfully controls the growth of weeds. With the soil conditions improvement activity of the root system is ensured, resulting in better growth and fruiting of the trees. The application of this measure can mitigate the consequences of insufficient rainfall during vegetative period of the trees.The simplest and cheapest way of soil mulching is by straw. Over time the straw rot and should be replaced every year. In this way the organic matter of the soil constantly increases. Lack of straw as mulching material is that it is easy and can be spread by wind, soil remains bare and it occurs weeds. The best mulch material is sawdust from coniferous plants. Its disadvantage is lack of sufficient quantities for mass application. Peat is quite effective as mulch material, but it is very expensive. Geotextile is very effective for covering of the soil, it takes a long time, has a good water permeability of precipitation, do not allow growth of weeds, has good moisture conservation. The only disadvantage is its high cost. The black foil as a covering material has many shortcomings because of can not be recommended for practical usage in the orchards.

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ВЛИЈАНИЕ НА МУЛЧИРАЊЕ НА ПОВРШИНАТА ВО РЕДОТ ВО НАСАД ОД КРУША НА ТЕМПЕРАТУРА И ВЛАЖНОСТ НА ПОЧВАТА, ПОРАСТ И ПРИНОС НА ОВОШКИТЕ

Марјан Кипријановски[1], Вјекослав Танасковиќ[1], Соња Бојковска[2]
Факултет за земјоделски науки и храна, Универзитет „Св. Кирил и Методиј“,
Скопје, Република Македонија
[2]Агенцијата за финансиска поддршка во земјоделството и руралниот развој,
Скопје, Република Македонија

Во трудот се презентирани резултатите од испитувањата за влијанието на разни видови мулч и покривен материјал на површината во редот во насад од круша врз температурата и влажноста на почвата, порастот, приносот и квалитетот на плодовите кај сортата *виљамовка* на подлога дуња МА. Експерименталниот насад е посаден на растојание на садење 3.2 × 1.3 m и наводнуван со систем капка по капка. Површината на почвата помеѓу редовите е одржувана со затревување. Во испитувањето беа опфатени следниве видови мулч материјал:

слама, пилевина од четинари, тресет, црна фолија, ткаенина од полиестерски влакна и окопување на почвата како контролна варијанта. Резултатите од испитувањето покажуваат дека материјалот за мулчирање има ефект врз редукција на температурата на почвата во текот на летните месеци, како и врз подобрување на конзервација на влагата во почвата. Со мулчирањето на почвата во редот се подобруваат вегетативниот пораст, приносот и квалитетот на плодовите кај сортата *виљамовка.*

Клучни зборови: круша; мулч материјали; конзервирање на вода; пораст; принос

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Original scientific paper

ASSESSING THE INFLUENCE OF SOIL PROPERTIES ON OPTIMAL PRODUCTION STRUCTURE AT VEGETABLE FARMS[#]

Ivana Janeska Stamenkovska[1*], Aleksandra Martinovska Stojcheska[1], Mile Markoski[1], Jaka Zgajnar[2]

[1]Faculty of Agricultural Sciences and Food, University Ss. Cyril and Methodius, Skopje, Republic of Macedonia
[2]Biotechnical Faculty, University in Ljubljana, Domzale, Slovenia

e-mail: ijaneska@fznh.ukim.edu.mk

The variability in soil properties influences the farm management decisions in reference to the fertilizers' optimization strategy and crop productivity. The aim of this research is to assess the influence of chemical soil properties on optimal production plan at vegetable farms in R. Macedonia, utilizing optimization potential of mathematical programming techniques. The study simulates the economic performance of a typical vegetable farm in four scenarios based on different soil contents of key nutrients; hence four fertiliser management strategies are defined. Main results point to the optimal scenario, where the solution provides highest gross margin, lowest number of enterprises, highest labour engagement, but relatively modest working capital. Vegetable crops included in the optimal production plan have more need for potassium, nevertheless the nitrogen and phosphorus are also important. The offered options for basic fertilization and additional nutrition can be considered as reasonable and realistic solution that can be applied in practice.

Key words: soil properties; optimal production structure; multi-criteria decision making; vegetable production

INTRODUCTION

Variation of soil chemical and physical properties influences both nutrition and crop management efficiencies. The variability in soil properties can cause uneven crop growth and decrease the effectiveness of the uniformly applied fertilizers on the field [1]. It can also influence the farm management decisions in reference to the fertilizers' optimization strategy and crop productivity. On the other side, the intensive agricultural practices with an excessive use of fertilizers have negative impact on the environment and due to unused nutrients, it also has negative influence on economic efficiency since they present additional unnecessary costs. Together with the sediment run-off, manure and production chemicals, soil nutrients are considered as one of the most common environmental pollutants from agriculture [2]. Consequently, these negative farm externalities are reflected on farmers' profit. As profit-maximizers on one site and risk averse behavior on the other, farmers aim to achieve stable income, through economically efficient production practices. Also, high fertilizers' prices urge to their rational application and not excessive use.

Operations research proved to be an adequate approach in assisting farmers in production planning. Such an application concerns the decision whether to use certain chemical fertilizers or to introduce alternative practices leading to more sustainable agriculture. Further, we find examples where mathematical programming techniques could be applied in preparing fertilization plan as an important task in the context of crop production [3]. Besides, such analysis based on optimization paradigm can also help policy makers to evaluate the appropriateness of agro-environmental policies [4] and is therefore common approach in estimating models for policy impact assessment.

[#]Dedicated to academician Gjorgji Filipovski on the occasion of his 100th birthday

The core issue in nutrition management is at what time, in which form, at what amount and what combination of manure and fertilizers to apply, in order to meet the estimated nutrition requirements considering both soil fertility and minimizing the negative consequences on the environment [3]. From methodical viewpoint, it is a common allocation problem that could be supported with mathematical programming based on constrained optimization. In that context, the linear programming (LP) approach is most commonly applied in different studies optimizing total production while minimizing the environmental impact [5].

However, classical LP technique based on single criterion optimisation (most often maximization of profit or minimizing nutrition costs) has been criticized mostly due to the emphasized rigidity of the constraints [6]. In case of developing a crop production fertilization plan, rigid constraints are impossible especially if we consider robustness of nutrition requirements estimation and what in relative terms means exceeding such a plan for one unit. On the other side farmers could be strongly motivated towards the intrinsic satisfactions of their work, rather than simply towards economic goals.

Although [7] argue that the LP optimization simplifies the agricultural production planning in practice; however it does not consider farmer's preferences in relation to efficient use of resources, minimizing the environment pollution, and stable income. In addition, a number of studies analyse the linkages between economic and environmental aspects, where beside the classical objective of profit maximisation, the most recurrent criteria are minimization of agri-chemical inputs, minimization of nutrients resulting from chemical or manure fertilizers, etc. [2].

Considering the development of agricultural technology and the diversification of farming, farmers' decisions have also increased in complexity. As a result, multi-criteria decision making (MCDM) has become an important approach in planning the agricultural production. Goal Programming (GP) is one of the most used MCDM techniques that overcome some of the listed LP drawbacks [8–10]. In the literature, we can find different examples utilising the GP paradigm in fertilisation planning and nutrition management. Such examples could be [11] who have applied GP approach supported with penalty functions (PFs). [12] applied similar approach based on Euclidean distance for the problem of sugarcane fertilizer mix. [13] have applied priority goal programming in nutrition management for rice production. For the same type of production, [14] have applied fuzzy goal programming (FGP) and genetic algorithm (GA) based on fuzzy GP approach. [15] have extended common LP with binary variables into mixed integer program (MIP) and in such a manner enables considering additional technical constraints. [3] have utilised weighted goal programming (WGP) supported with penalty functions (PF) to improve LP solution and to find compromise solution of optimal fertilisation plan.

Within the crop subsector in Macedonia, vegetable production takes place on around 60 thousand hectares (or around 10% of arable land) and is the most important in terms of contribution to the gross value added (around one-third). Characteristically in the country, the production structure includes wide range of different vegetable farm enterprises and different production technologies (open field and plastic tunnels being typical for the dominant small-scale farmers).

Taking into account the above considerations, the aim of this research is to assess the influence of chemical soil properties on the optimal production plan at vegetable farms, utilising the weighted goal programing technique. This study simulates the economic performance of vegetable farms in four scenarios based on different soil contents of the key nutritive elements; hence four different fertiliser management strategies are defined. Beside the potential applicability of the MCDM innovative tool, a significant contribution of this paper is that the results confirm the theoretical and empirical benefits of the multidisciplinary approach.

MATERIAL AND METHOD

Weighted goal programming

Instead of using the traditional linear programing approach with single criterion optimization, the multi-criteria decision making (MCDM) is better suited to situations with more than one objective (or goals), such as the typical situation in which the farmer acts as a manager facing different production, technological and economic decisions.

WGP is a MCDM technique that has become a widely used approach in management science [12] and is also often applied in nutrition management which is also the case of this study. It enables analysis of decision making considering several contradictive objectives at once and searches for the best compromise solution. Therefore, the crucial objectives that are in contradiction are converted into goals, while others are usually considered as constraints in the optimization model [16]. In its mathematical formulation (1), WGP minimizes the sum of weighted undesired deviations (*min a*) from aspired values of set goals, and does not minimize or maximize the goals themselves [17]. Contrary to the classical LP model, WGP enables determination of positive and negative deviation variables, defined for each goal. Negative deviation variables (n_q) refer to underachievement, while positive deviation variables (p_q) for overachievement of the goal value. The deviations within the WGP model are calculated as a ratio, thus any marginal change of the goal is of equal importance regardless how distant it is from the aspired value [18]. The authors also argue that in order to keep the deviations in controlled margins, the WGP model should be upgraded with the system of penalty functions (PF), which will be considered in further research.

Considering that the goals are measured in different units of measurement, the selection of preferential weights determining the relative importance of each goal is crucial. The weight itself contributes to normalization of different scales the goals are expressed in (k_q), but also to ranking the decision-makers' preferences (u_q and v_q) [9, 19, 20]; In other words, this specification allows for modelling which goal should be satisfied first (prioritised) or in larger scope.

$$\min a = \sum_{q=1}^{Q}\left(\frac{u_q n_q}{k_q} + \frac{v_q p_q}{k_q}\right)$$

$$s.t.$$

$$f_q(x) + n_q - p_q = b_q \qquad \text{for } q = 1 \text{ to } Q$$

$$x \in F$$

$$n_q \geq 0, p_q \geq 0$$

Since preferential weights are of crucial importance in applied approach, it is very important how they are defined. In the literature, different methods are applied for calculating the weights (u_q and v_q) [10, 21–23]. In this study, we will apply Analytical Hierarchy Process (AHP) to calculate consistent weights for defined goals [24].

Model

This work attempts to assess the influence of different levels of soil properties on the optimal production plan at the farm level. In order to determine the optimal production structure by satisfying the preferences of the vegetable producers in Macedonia, a two stage modular model is constructed. It is a general production model based on mathematical programming techniques. The model is developed as a spread-sheet in MS Excel, enabling integration and complementarity of its modules, and can be easily adapted on different situations at the vegetable farm. In fact, the current model specification is built upon a previous base version of such model [25], specifically adapted and for the purpose of this study. The first module is supported by normative linear programming (LP) approach and it is used for calculating the aspiration values of the specific goals. These are needed for the second module where they enter as goal values. In this context, four different objective functions are calculated, (i) maximisation of the farm gross margin, (ii) minimisation of the farm working capital, (iii) minimisation of farm labour needs and (iv) minimisation of water requirements for crop production. The aim of the second module is to determine the optimal vegetable production plan at the farm level, utilising the WGP technique, considering the above mentioned four conflicting goals (i) to (iv).

The constructed model includes 214 decision variables, divided in four aggregated group of activities: (1) crop activities including 15 vegetable crops, whereas each production activity is supported by detailed enterprise budgets; this group of activities also covers the production technology and crop rotation; (2) input related activities capturing land, labour and fertilizers; (3) infrastructure activities referring to investments in plastic tunnels or irrigation systems and (4) balanced activities ensuring integrity of the solutions. Farmers are expected to make decisions under a number of constraints. The constraints in the model are determined with the typical vegetable farm characteristics. In this context, the first group comprises the endogenous constraints dealing with the production factors scarcity, including land, labour and working capital, while another group refers to the agro-technical constraints assuring that mineral nutrient requirements are met. Nitrogen, phosphorus and potassium needs per crop are included in the model to register the nutrients flow. The irrigation systems applied per crop are also subject of the agro-technical constraints. Farm decision making is influenced by external factors affecting the production structure, as market or policy constraints. A set of balance constraints is incorporated into the model through the maximum available land per crop and minimum number of crop enterprises.

Input data

Different sources of data support the optimization model. Primary data for calculating the enterprise budgets are obtained from direct interviews with vegetable producers in 2013, using a structured questionnaire. Considering the type of production and the geographical region, 60 farms from the South-East region of R. Macedonia were included in the survey.

This region is characterized with altitude of 50 to 500 m. In this first sub-Mediterranean region is the region of Gevgelija where most of the surveyed farms are located. Based on the pedological map sized 1:50.000 [26–28], different soil types and complexes are determined in this area. Alluvial and colluvial soils are of particular importance taking into consideration the biological and production-technological specifics of most vegetable crops.

The data are supplemented with Farm Monitoring System (FMS) data for the period 2005–2011, whereas FMS as an annual survey carried out by the National Extension Agency collects production, income and costs related data per farm enterprise from 600 farms in the country. In order to obtain enterprise budgets representing the current average farming behaviour, and not that of the best and most progressive farmers, a panel of relevant experts was consulted for assessing the average current farming approach [29].

The model takes into consideration a constructed case study based on a typical vegetable farm from the South-East region of R. Macedonia [25]. The typical vegetable farm was constructed upon FMS data using the cluster analysis as a multivariate statistical technique. Four factors were used to derive the clusters (farm size in hectares, gross margin, total number of crop farm enterprises and total number of vegetable activities), utilising a hierarchical procedure and Ward's minimum variance algorithm. The similarity among the objects is measured with squared Euclidean distance as a distance measure [30]. This method optimises the minimum variance within the clusters thus creating groups of relative equal sizes and shapes [31]. The cluster analysis resulted in three clusters (very small farms, small farms and medium farms). This study's typical case farm is based on the "very small farms" cluster, which represents around 70 % of the total sample size (the constrains are set at maximum 2 hectare of utilised area under open field production plus 0.5 hectares under plastic tunnels, 2200 labour hours and 1496 euros farm working capital).

Analyzed scenarios

In order to assess the influence of different levels of soil properties over the optimal production structure and farm profitability, the model assumes four different scenarios. Each scenario represents the level of average soil contents of nitrogen, phosphorus and potassium determined as kg/ha at soil depth of 20 cm [32]. In addition to the baseline scenario, three different scenarios are included for analysing the effect of the soil enrichment with soil nutrients on the optimal production structure at vegetable farms (Table 1).

For instance, the soil is optimally secured with macro nutrients in quantities of 161 kg nitrogen, 400 kg phosphorus and 400 kg of potassium.

Table 1. Level of soil nutrients in four different scenarios

Scenarios	**Level of soil enrichments with soil nutrients**	**P_2O_5 (kg/ha)**	**K_2O (kg/ha)**	**N (kg/ha)**
Baseline scenario	Medium level	250	250	91
Scenario M_Low	Low level	150	150	50
Scenario M_Optimal	Optimal level	400	400	161
Scenario M_High	High level	650	650	310

RESULTS AND DISCUSSION

Weighted goal programming as a method based on MCDM paradigm enables obtaining more positive results, closer to the practice at farm level. The obtained results confirm that the level of soil chemical properties have strong influence on the farm optimal production plan and its economic performances.

In this section, we begin with reporting the optimized production structure under the four different scenarios, followed by nutrients and fertilizer recommendations. In the end, we present the economic effect of each scenario, with discussion.

The production structure obtained with all scenarios corresponds to the most often cultivated vegetable crops in Macedonia, thus confirming that the Macedonian farmers avoid monoculture and produce different vegetable crops in order to distribute the market risk and to use the labour efficiently. The production plan determined with the baseline scenario (Table 2) includes diversified structure of eleven vegetable crops, cultivated on total area of 0.38 ha. Around 58 % of land is used for open field production, while the remaining are crops produced under plastic tunnels. The production of watermelon dominates the production structure with 28 % of total land and highest gross margin per crop (279.2 EUR), followed by lettuce (15 %) and cabbage (13 %).

Table 2. Optimal production structure determined with the baseline scenario

Crops	Land per crop (ha)	Share of land (%)	Yield (kg)	GM per crop (€)
Carrot 1-1	0.02	5 %	750.0	90.5
Bean 1-1	0.02	5 %	28.1	36.6
Potatoes 1-1	0.01	3 %	349.4	26.5
Watermelon 2-1	0.10	28 %	4950.0	279.2
Onion 1-2	0.00	0 %	7.9	0.9
Beetroot 1-1	0.04	10 %	937.5	16.1
Lettuce 2-2	0.06	15 %	2812.5	204.9
Melon 1-1	0.02	5 %	581.3	351.4
Cabbage 1-1D	0.05	13 %	1874.8	79.4
Spinach 1-2D	0.04	10 %	225.0	83.8
Eggplant 1-2D	0.02	6 %	1240.7	63.3
Total	0.38	100 %	–	1232.6

Note: In the respective vegetable enterprises, the first part of the code "n-nD" refers to open field production (1) or plastic tunnel production (2); the second part refers to first crop in annual rotation (1) or second crop on same land (2); and the D refers to drip irrigation (if no D in the end, it means using standard furrow irrigation)

In the case of farms cultivating crops on soil with low level of nutrients, the optimal production plan also includes eleven crops. However, the structure itself differs from the baseline scenario. Total cultivated area under this scenario captures 0.50 ha, out of which 0.29 ha are open field production and the remaining of 0.21 ha refer to the plastic tunnel production. The production of watermelon again dominates the production plan with 27 %. In this respect, it is important to mention that the model offers a possibility for stricter market constraints; for instance, if there is a projected limitation of the possible market absorption of certain crop, than a market constraint can be activated in the model. For the moment, such constraint is set for cabbage, as in many years there are large market surpluses, so the farmer cannot rely on producing that crop only. Further in the production structure, follows onion with 19 %, lettuce (15 %) and garlic (13 %). Highest gross margin in this scenario (S_Low) is evidenced for garlic production (612.7 EUR), followed by production of melon with gross margin of 464.9 EUR.

Table 3. Optimal production structure determined with the low nutrition content scenario

Crops	Land per crop (ha)	Share of land (%)	Yield (kg)	GM per crop (€)
Pepper 1-1	0.00	0 %	0.0	0.0
Carrot 1-1	0.02	5 %	992.2	118.3
Bean 1-1	0.02	5 %	37.2	48.4
Potatoes 1-1	0.00	1 %	148.8	11.2
Watermelon 2-1	0.14	27 %	6548.8	359.0
Onion 1-2	0.09	19 %	3582.0	418.3
Garlic 1-2	0.06	13 %	620.2	612.7
Lettuce 2-1	0.07	15 %	3720.9	264.5
Melon 1-1	0.02	5 %	769.0	464.9
Cabbage 1-1D	0.00	0 %	0.0	0.0
Spinach 1-2D	0.05	10 %	297.67	110.9
Total	0.50	100 %	–	2408.2

Note: In the respective vegetable enterprises, the first part of the code "n-nD" refers to open field production (1) or plastic tunnel production (2); the second part refers to first crop in annual rotation (1) or second crop on same land (2); and the D refers to drip irrigation (if no D in the end, it means using standard furrow irrigation)

The optimal production plan determined with optimal scenario (S_Optimal) (Table 4) shows that different content of nitrogen, phosphorus and potassium result in different production structure compared to the other scenarios. This is the solution with least diversified production structure, since the optimal level of soil chemical attributes leads to production of nine vegetable crops. Although with lower number of crop enterprises, the area under these crops is higher compared to the baseline scenario. The vegetable production within this scenario is organized on in total 0.49 ha, with 57 % as open field production and the remaining of 43 % are vegetable crops under plastic tunnels. As in the other cases, four major crops in this production plan are watermelon (28 %), onion (19 %), lettuce (15 %) and garlic (13 %). The production of garlic is also the most profitable crop with a gross margin determined at 605 EUR.

Table 4. Optimal production structure determined with the optimal nutrition content scenario

Crops	Land per crop (ha)	Share of land (%)	Yield (kg)	GM per crop (€)
Carrot 1-1	0.02	5 %	978.0	118.7
Bean 1-1	0.02	5 %	36.7	47.7
Potatoes 1-1	0.00	1 %	146.7	11.3
Watermelon 2-1	0.13	28 %	6455.1	365.7
Onion 1-2	0.09	19 %	3530.7	422.7
Garlic 1-2	0.06	13 %	611.3	605.0
Lettuce 2-1	0.07	15 %	3667.7	276.5
Melon 1-1	0.02	5 %	758.0	458.2
Spinach 1-2D	0.05	10 %	293.4	109.3
Total	0.49	100 %	-	2415.2

Note: In the respective vegetable enterprises, the first part of the code "n-nD" refers to open field production (1) or plastic tunnel production (2); the second part refers to first crop in annual rotation (1) or second crop on same land (2); and the D refers to drip irrigation (if no D in the end, it means using standard furrow irrigation)

Similar production structure is determined with the fourth scenario (S_High) where a situation with richer soil properties content is assumed. Table 5 present the optimal production plan including ten crops produced on total land of 0.44 ha. Considering the given constraint related to the maximum share of land each crop can have, again most represented is the production of watermelon with 28 %, followed by onion with 19 %, lettuce (15 %) and garlic (13 %). The production of garlic is similarly as in the low and optimal scenarios, single most profitable activity, with a gross margin of 540.6 EUR.

Table 5. Optimal production structure determined with the high nutrition content scenario

Crops	Land per crop (ha)	Share of land (%)	Yield (kg)	GM per crop (€)
Tomatoes 1-1	0.00	0 %	0.0	0.0
Carrot 1-1	0.02	5 %	873.8	106.0
Bean 1-1	0.02	5 %	32.8	42.6
Potatoes 1-1	0.00	1 %	131.1	10.1
Watermelon 2-1	0.12	28 %	5767.2	326.7
Onion 1-2	0.08	19 %	3154.5	377.7
Garlic 1-2	0.05	13 %	546.1	540.6
Lettuce 2-1	0.07	15 %	3276.8	247.2
Melon 1-1	0.02	5 %	677.2	409.4
Spinach 1-2D	0.04	10 %	262.1	97.7
Total	0.44	100 %	-	2158.0

Note: In the respective vegetable enterprises, the first part of the code "n-nD" refers to open field production (1) or plastic tunnel production (2); the second part refers to first crop in annual rotation (1) or second crop on same land (2); and the D refers to drip irrigation (if no D in the end, it means using standard furrow irrigation)

In the model, we determine for each scenario and for each crop production activities the required need for additional nutrients that cannot be supplied from the soil contents [32].

In Table 6, based on the model results, different fertilization strategies are suggested for each scenario. In general, soils that have poorer content of nutrients require higher fertilization. Actually, in the case of the optimal and rich soil scenario, there is no projection for application of the basic NPK fertilizer. On the other side, the low nutrient content soils and the baseline medium content soil require significant application of NPK, as the model chose the combination 8:26:26 (1162.9 kg for total land of 0.50 ha in the low content scenario and 402.1 kg for total land of 0.38 ha in the baseline scenario). Urea is also included in the fertilization program for these two soil scenarios. In the case of optimal soil nutrient availability, there is no need for basic fertilization with NPK fertilizers, except for Urea (46 %). Additionally, potassium sulfate (K_2SO_4) is foreseen for the low and baseline scenarios, while the only type of fertilizer, given the richness of soil with the required nutrients, which is proposed for the high soil properties level is superphosphate (26 %), to compensate for the lacking of this element in the soil.

Table 6. Cost and quantities of different fertilization strategies

Scenario	**S_Baseline**		**S_Low**		**S_Optimal**		**S_High**	
Fertilizers	**Total Cost (€)**	**Q (kg/land)**	**Total Cost (€)**	**Q (kg/land)**	**Total Cost (€)**	**Q (kg/land)**	**Total Cost (€)**	**Q (kg/land)**
NPK 8:26:26	196.2	402.1	567.3	1162.9				
K_2SO_4	12.3	34.5	61.0	170.5				
UREA N 46	3.6	10.9	1.7	5.0	0.43	1.3		
Super phosphate 26 %							0.0	0.0

The economic impact of different levels of soil attributes presented in four different scenarios is reported in Table 7. Small-scale farms cultivating vegetable crops on soil with medium nitrogen, phosphorus and potassium content, as determined with the baseline scenario, reveal lowest profitability, expressed as total farm gross margin, due to the optimized crop production structure and the associated higher costs for fertilizers necessary to satisfy the selected crop nutrients needs. The economic performance improves within the other three scenarios. Farms cultivating their crops on land with low level of soil properties reveal higher gross margin for about 40% compared with the baseline, due to the larger amount of land included in the solution, as well as the derived production structure. Although the working capital requirements within this scenario is also higher, the total land under vegetable crops increases from 0.38 ha in the baseline to 0.50 hectares in S_Low scenario, leading to higher gross margin at farm level.

Highest farm gross margin is evidenced for farms with optimal level of soil properties (4929 EUR/ha) and not within the S_High scenario (4904.5 EUR/ha), which is even though more economically efficient in terms of EUR per hectare utilised than S_Low scenario (3050 EUR/ha). In that case, all production factors on the case farm are optimised to achieve the highest gross margin, which also enables highest level of labour productivity, when converted to hour of engaged workforce (4 EUR/h). The relatively low difference in the farm gross margin per hectare in the optimal and high level scenarios is due to the isolated effect of the soil nutrients requirements and the similar need for fertilizers for the obtained optimal production structure.

With regard to the physical resources, the results confirm that vegetable production is labour-intensive, whereas in the S_Low scenario there is an additional need for renting seasonal labour. Highest labour requirements are noted in the optimal scenario, which interestingly is the scenario yielding highest farm return, since more labour intensive and profitable crops are included in the solution.

The water requirements for irrigating the vegetable crops are determined for both furrow and drip irrigation systems. In all four scenarios the water requirements for drip irrigation are higher confirming that this irrigation strategy is most efficient for vegetable production and should be spread more not only in plastic tunnels, but also on the open field production.

Table 7. Multi-criteria decision making results in baseline and different soil attributes impact scenarios

Scenario	S_Baseline	S_Low	S_Optimal	S_High
Economic indicators				
Farm GM (€)	667.0	1525.0	2415.0	2158.0
Farm GM/h (€)	2.3	3.1	4.0	4.0
Farm WC (€)	1584.5	2490.4	1543.0	1378.2
Land (ha)				
Total land (ha)	0.38	0.50	0.49	0.44
Open field (ha)	0.22	0.29	0.28	0.25
Plastic Tunnel (ha)	0.16	0.21	0.21	0.19
Labour (h)				
Own labour (h)	291.0	259.0	601.3	537.2
Rented labour (h)	0.0	234.1	0.0	0.0
Water (m^3)				
Furrow irrigation (m3)	326.6	309.1	304.7	272.2
Drip irrigation(m3)	712.4	800.7	789.2	705.1
Goals achieved values (€)				
Max farm GM (€)	667.0	1525.0	2415.0	2158.0
Min farm WC (€)	1584.5	2490.4	1543.0	1378.2
Min farm LAB cost (€)	291.0	259.0	601.3	537.2
Min farm WAT cost (€)	1039.0	1109.7	1093.9	977.3
Total deviations (%)	8 %	160 %	280 %	200 %
Goals deviations (%)				
Max farm GM (%)	0 %	0 %	0 %	0 %
Min farm WC (%)	8 %	103 %	74 %	41 %
Min farm LAB cost (%)	0 %	0 %	132 %	107 %
Min farm WAT cost (%)	0 %	56 %	73 %	52 %

The second stage goal programing reported in the end of Table 7 stems from the original LP solutions. We can see that in the baseline scenario, with lowest farm gross margin, there is no or very little deviation from the set goals, whereas in the highest economic impact scenario, the goals are significantly exceeded. In this farm case exercise, stretching the goals actually leads to higher profit for the farmers – with highest deviation of the labour factor and relatively low working capital involved, this scenario yields good compensation between the set goals and hence gives the highest performance.

CONCLUSIONS

Having good basis and analysis of the soil-climatic conditions in certain areas, coupled with the knowledge of the crop biology and production technology specifics can improve the development of vegetable production strategies in Macedonia in terms of value added, quantities, assortment, technology (open field production, tunnel or glasshouses) as well as the intensity of land use. In addition to the standard economic parameters in determining and optimizing the production plan at farm level, in this study we introduce deeper assessment on some soil properties (total nitrogen, phosphorus and potassium) and in that respect nutrition balance at farm level.

The main results point to the optimal scenario as the one with most efficiently allocated and utilized production resources. In this scenario, the solution provides highest gross margin, lowest number of enterprises (9 as compared to 10 or 11 in the other scenarios), highest labour engagement (double than the baseline scenario), but relatively modest working capital. It is interesting to note that the set goals in the optimal scenario are most intensively stretched, but this compromise solution nevertheless produced the best economic output.

The content of soil with macro and micro nutrients is one of the main factors for obtaining high yields, but also product quality. Vegetable crops have more need for potassium (those that were in the solution, but also in general), but nevertheless the nitrogen and phosphorus are also important. The offered options for basic fertilization and additional nutrition can be considered as reasonable and realistic solution that can be applied in practice. This is providing evidence that the theoretical approach applied in the construction of the model, when containing up-to-date technological and economic data, gives a good platform for evaluating different management practices as a very valuable farm management tool, but can also be used on macro level for sector decisions or policy related impact assessment analysis. In this regard, having a multidisciplinary approach is very useful, so the results can be as close (positive) to the reality as possible.

The model can be further improved by enhancing the plant nutrition related aspects, such as the application time of certain fertilizers (as basic or supplementary fertilization), the potential use of manure (also seen as a circular economy practice), deeper information and selection of organic fertilizers, aspects of prices and different procurement sources of fertilizers etc… These kinds of additions would add to the sophistication of the model and contribute to make the fertilization plan more realistic; from technical point of view this would mean adding additional inequality constraints [3]. Last but not least, in order to increase the applicability of the model and its positive character and to minimize the normative assumptions, an introduction of WGP with penalty function in future efforts would add to the reflected reality of the model results.

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ОЦЕНУВАЊЕ НА ВЛИЈАНИЕТО НА ПОЧВЕНИТЕ СВОЈСТВА ВРЗ ОПТИМАЛНАТА ПРОИЗВОДНА СТРУКТУРА КАЈ ГРАДИНАРСКИ ФАРМИ

Ивана Јанеска Стаменковска[1], Александра Мартиновска Стојческа[1], Миле Маркоски[1], Jaka Zgajnar[2]

[1]Факултет за земјоделски науки и храна, Универзитет „Св. Кирил и Методиј“, Скопје, Република Македонија
[2]Biotechnical Faculty, University in Ljubljana, Domzale, Slovenia

Променливоста на почвените својства влијае на одлуките на земјоделските производители во врска со утврдување на стратегија за оптимизирање на ѓубривата, како и продуктивноста на растителното производство. Целта на ова истражување е да се оцени влијанието на хемиските почвени својства врз оптималниот план за производство кај градинарски фарми во Р. Македонија, со употреба на техники на математичко програмирање. Студијата ги симулира економските перформанси на типично земјоделско стопанство со градинарско производство во четири сценарија засновани на различна содржина на клучните нутритивни елементи во почвата; според тоа, дефинирани се четири различни стратегии за примена на ѓубрива. Главните резултати се поврзуваат со сценариото со оптимална обезбеденост на хемиски својства, каде што се добива највисока бруто маржа, најмал број на линии на производство, најголема ангажираност на работна сила, но при релативно скромен работен капитал. Градинарските култури вклучени во оптималната производна структура имаат поголема потреба од калиум, но сепак значајна е улогата и на азотот и фосфорот во почвата. Понудените опции за основно ѓубрење и дополнителна прихрана претставуваат реално решение кое може да се применува во практиката.

Клучни зборови: почвени својства; оптимана производна структура; повеќекритериумско донесување на одлуки; градинарско производство

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Original scientific paper

CHECKLIST OF LARGER ASCOMYCETES IN THE REPUBLIC OF MACEDONIA[#]

Mitko Karadelev[1*], Katerina Rusevska[1], Iskra Kajevska[2], Danijela Mitic Kopanja[1]

[1]Institute of Biology, Faculty of Natural Science and Mathematics, Ss Cyril and Methodius University, Skopje, Republic of Macedonia
[2]Macedonian Mycological Society, Skopje, Republic of Macedonia

[*]e-mail: mitkok@pmf.ukim.mk

A comprehensive checklist of all up to date registered ascomycetes from Macedonia was established for the first time. The aim of this study was to summarize all data on ascomycetes and contribute to the knowledge of the presence of these fungi in the country. Information for this checklist are based on data from field mycological research trips, species documented from existing literature, and examined exsiccatae from the Macedonian Collection of fungi (MCF) accompanied with data noted in the MACFUNGI database of fungi. A total number of 255 taxa belonging to larger (macroscopic) ascomycetes were recorded among which 107 species, three varieties and three forms were new records for Macedonia. Among listed taxa18 are already red-listed in addition to approximately fifty species which are estimated to be rare in the country.

Key words: fungi check list; Ascomycota; taxonomy; nomenclature; Macedonia

INTRODUCTION

The territory of the Republic of Macedonia is defined with high ecosystems and species diversity due to its specific geographic position combined with diverse relief structure, complex geology, substantial water resources and modified sub-Mediterranean, continental and mountainous climate. Combination of these parameters contributes to a considerably rich mycodiversity in the country. Past studies of Macedonian mycobiota in last 20 years were mainly focused on basidiomycetes (Karadelev & Rusevska [1]), while ascomycetes as phylogenetically and phenotipically the most diverse and the largest taxonomic group of fungi remained understudied. The first contribution to Macedonian ascomycetes diversity was published in 1988 by Tortić [2]. Some valuable data of Ascomycota from Macedonia with special emphasis on phytopathogenic species has been documented in the following papers: Bryner *et al.* [3]; Papazova-Anakieva *et al.* [4–6] and Sotirovski *et al.* [7–11]. The country's first checklist of ascomycetes was compiled in 1998 by Karadelev & Nastov [12] listing a total of 52 species. Individual or non-targeted data for ascomycetes presence was also found in Grujoska [13], Grujoska & Papazov [14], Irbe *et al.* [15], Karadelev [16], Karadelev [17], Karadelev *et al.* [18–25], Karadelev & Murati [26], Karadelev & Spasikova [27–29], Karadelev & Rusevska [30, 31], Rusevska & Karadelev [32], Tortić & Cekova [33] and Pilat & Lindtner [48]. A comprechensive listing had targeted only few taxonomic groups, such as Helvellaceae, Morchellaceae, Pyronemataceae and hypogeous ascomycetes by Karadelev *et al.* [34], Chavdarova *et al.* [35] and Kajevska *et al.* [36]. Hitherto, a total of 18 ascomycetes have been taken into consideration for conservation actions and proposed in the supplemented edition of the National red list of fungi in Karadelev & Rusevska [1].

The aim of this study is to summarize an up to date checklist of all registered and unpublished ascomycetes from Macedonia which is expected to significantly enrich the knowledge of the presence of these fungi in the country and contribute to determining conservation status of listed taxa.

[#]Dedicated to academician Gjorgji Filipovski on the occasion of his 100th birthday

EXPERIMENTAL SECTION

Species records, locations, and their ecology were recorded from available literature, cited along each record in the list. Data was also extracted from exiccata notes and records in Macedonian Collection of fungi (MCF) accompanied with data noted in the MACFUNGI database.

Key microscopic features for ascomycetes (dimensions, shape and ornamentation of spores, asci, paraphyses and other elements) of majority of available specimens have been preferentially examined in their fresh state in a tap water (Baral [37]). Herbarium exiccata had been firstly rehydrated in tap water prior to miscroscopic examination. Observation of the material has been conducted using light microscope (LW Scientific, Inc., Lawrenceville, Georgia, USA). Standard chemical reagents have been used: Lugol's solution, Melzer's reagent, Cotton Blue and 5% KOH have been used mostly for the representatives of the class Pezizomycetes while Congo red had been primarelly used for the representatives of Leotiomycetes. For identification at morphological species level we have used available identification keys in Hawker [38], Dennis [39, 40], Moser [41], Breitenbach & Kränzlin [42], Hansen & Knudsen (eds.) [43], Montecchi & Sarasini [44] and Medardi [45]. Listed species names follow the Index Fungorum [46] and the MycoBank [47] databases and are listed in the alphabetical order of a valid current name. Most common synonims are also given, where available. References are given for published species while we supplemented unpublished collections with a locality, a forest type, a confirmed host species (if known) and with a date of collecting.

CHECKLIST OF LARGER ASCOMYCETES IN THE REPUBLIC OF MACEDONIA

1. ***Aleuria aurantia*** (Pers.) Fuckel
References: Tortić [2], Karadelev & Nastov [12], Tortić & Cekova [33], Kajevska *et al.* [36].

2. ***Anthracobia macrocystis*** (Cooke) Boud.
References: Karadelev & Murati [26], Kajevska *et al.* [36].

3. ***Anthracobia subatra*** (Rehm) M.M. Moser
Syn. *Lachnea subatra* Rehm
References: Kajevska *et al.* [36].

4. ***Apiognomonia veneta*** (Sacc. & Speg.) Höhn.
Syn. *Diaporthe veneta* Sacc. & Speg.
References: Papazova-Anakieva *et al.* [5, 6].

5. ***Arachnopeziza aurata*** Fuckel
Karadzhica Mt.: deciduous forest, *Populus tremula* (rotten wood), 14.07.2011; Pelister NP: oak forest, *Quercus* sp. (rotten wood), 19.04.2002.

6. ***Arachnopeziza aurelia*** (Pers.) Fuckel
Syn. *Peziza aurelia*Pers.
Galichica NP: oak forest, *Quercus* sp. (rotten wood), 22.04.2002; Skopska Crna Gora Mt.: oak forest, *Quercus* sp. (acorn and rotten wood), 07.05.2018.

7. ***Ascocoryne cylichnium*** (Tul.) Korf
Syn. *Peziza cylichnium* Tul.
References: Tortić [2], Karadelev & Nastov [12].

8. ***Ascocoryne sarcoides*** (Jacq.) J.W. Groves & D. E. Wilson
Syn. *Helvella sarcoides* (Jacq.) Dicks.
Anamorph syn. *Coryne dubia* (Pers.) Gray
References: Karadelev & Nastov [12], Karadelev *et al.* [18], Karadelev & Murati [26].

9. ***Ascotremella faginea*** (Peck) Seaver
Kozhuf Mt.: beech and fir forest, *Fagus sylvatica* (rotten wood), 13.07.2004; mixed beech and pine forest *Fagus sylvatica* (rotten wood), 07.05.2005; Osogovski Planini Mt.: beech forest, *Fagus sylvatica* (rotten wood), 09.06.2007.

10. ***Balsamia vulgaris*** Vittad.
References: Chavdarova *et al.* [35].

11. ***Barlaea constellatio*** (Berk. & Broome) Sacc.
Syn. *Pulvinula convexella* (P. Karst.) Pfister
Mavrovo NP: mixed forest, 25.10.2016; Plachkovica Mt.: riparian vegetation, soil, 06.10.2014.

12. ***Belonidium mollissimum*** (Lasch) Raitv.
Syn.: *Trichopeziza mollissima* (Lasch) Fuckel
Shar Planina Mt.: beech forest, *Petasites albus* (dry stem), 24.04.2018.

13. ***Bertia moriformis*** (Tode) De Not.
Syn. *Psilosphaeria moriformis* (Tode) Stev.
References: Karadelev *et al.* [18], Karadelev & Murati [26].

14. ***Biscogniauxia nummularia*** (Bull.) Kuntze
Karadzhica Mt.: beech forest, *Fagus sylvatica* (fallen branch), 29.07.1997, 11.10.2011; Shar Planina Mt.: beech forest, *Fagus sylvatica* (bark, rotten wood), 16.07.1997; Jakupica Mt.: beech forest, *Fagus sylvatica* (fallen branch), 08.07.1999; Maleshevski Planini Mt.: beech and pine forest, *Fagus sylvatica* (fallen branch), 18.09.2001; Kozhuf Mt.: beech forest, *Fagus sylvatica* (bark), 15.07.2014; Pelister NP: beech

forest, *Fagus sylvatica* (fallen branch), 20.10.2005; Kitka Mt.: beech forest, *Fagus sylvatica* (bark), 03.05.2007; Lisec Mt.: beech forest, *Fagus sylvatica* (rotten wood), 16.05.2007; Osogovski Planini Mt.: beech forest, *Fagus sylvatica* (bark, fallen branch), 20.10.2007; 31.03.2018, 29.10.2014, 22.06.2018; Galichica NP: beech forest, *Fagus sylvatica* (fallen branch), 14.07.2010; Jasen Reserve: beech and pine forest, *Fagus sylvatica* (bark), 05.10.2010.

15. ***Biscogniauxia repanda*** (Fr.) Kuntze
Syn. *Sphaeria repanda* Fr.
Galichica NP: deciduous forest, *Sorbus aucuparia* (fallen branch), 27.07.1988.

16. ***Bisporella citrina*** (Batsch) Korf & S.E. Carp.
Syn. *Calycina citrina* (Hedw.) Gray
References: Karadelev & Nastov [12], Karadelev [17], Karadelev *et al.* [19, 23], Karadelev & Murati [26].

17. ***Bisporella pallescens*** (Pers.) S. E. Carp. & Korf
Korab Mt.: beech and fir forest, *Fagus sylvatica* (rotten wood), 15.07.2013.

18. ***Brunnipila clandestina*** (Bull.) Baral
Syn. *Lachnum clandestinum* (Bull.) P. Karst.
Skopska Crna Gora Mt.: oak and hornbeam forest, deciduous tree (fallen twig), 10.05.2018.

19. ***Bulgaria inquinans*** (Pers.) Fr.
Syn. *Bulgaria polymorpha* Oeder ex Wettst.
References: Tortić [2], Karadelev & Nastov [12], Grujoska [13], Rusevska & Karadelev [32], Tortić & Cekova [33].

20. ***Caloscypha fulgens*** (Pers.) Boud.
Syn. *Pseudoplectania fulgens*(Pers.) Fuckel
References: Karadelev *et al.* [22].

21. ***Camarosporidiella laburni*** (Pers.) Wanas.
Syn. *Cucurbitaria laburni* (Heyder ex Pers.) Ces. & De Not.
Syn. *Gibberidea laburni* (Pers.) Kuntze
Vodno Mt.: mixed deciduous forest, *Laburnum anagyroides* (branches), 10.11.2007.

22. ***Capitotricha bicolor*** (Bull.) Baral
Syn. *Dasyscyphus bicolor* (Bull.) Fuckel
Syn. *Lachnum bicolor* (Bull.) P. Karst.
References: Karadelev & Nastov [12], Karadelev *et al.* [18].

23. ***Catinella olivacea*** (Batsch) Boud.
Syn. *Patellaria olivacea* (Batsch) W. Phillips
Mavrovo NP: beech forest, soil, 19.09.2010.

24. ***Cistella acuum*** (Alb. & Schwein.) Svrcek
Syn. *Dasyscyphus acuum* (Alb. & Schwein.) Sacc.
Shar Planina Mt.: spruce forest, *Picea abies* (cones and rotten wood), 01.07.2010.

25. ***Chaetosphaerella phaeostroma*** (Durieu & Mont.) E. Müll. & C. Booth
Syn. *Byssosphaeria phaeostroma* (Durieu & Mont.) Stev.
Kozhuf Mt.: beech forest, *Fagus sylvatica* (bark), 04.08.1984.

26. ***Cheilymenia stercorea*** (Pers.) Boud.
Syn. *Peziza stercorea*Pers.
References: Kajevska *et al.* [36].

27. ***Cheilymenia vitellina*** (Pers.) Dennis
References: Karadelev *et al.* [24], Kajevska *et al.* [36].

28. ***Chlorencoelia versiformis*** (Pers.) J.R. Dixon
Syn. *Chlorosplenium versiforme* (Pers.) P. Karst.
Galichica NP: beech and fir forest, *Fagus sylvatica* (rotten wood), 15.09.2006; Prespa Lake area: pine plantation, *Pinus nigra* (rotten wood), 09.07.2006.

29. ***Chlorociboria aeruginascens*** (Nyl.) Kanouse
Syn. *Chlorosplenium aeruginascens* (Nyl.) P. Karst.
References: Tortić [2], Karadelev & Nastov [12].

30. ***Chlorociboria aeruginosa*** (Oeder) Seaver ex C. S. Ramamurthi, Korf & L. R. Batra
Syn. *Chlorosplenium aeruginosum* (Oeder) De Not.
References: Karadelev & Nastov [12], Karadelev [17].

31. ***Chloroscypha alutipes*** (W. Phillips) Dennis
Skopje: tree nursery, *Cupressus* (dry branches), 23.05.2018.

32. ***Chlorosplenium sericeum*** (Alb. & Schwein.)
Galichica NP: oak forest, deciduous tree (rotten wood), 30.07.2007

33. ***Choiromyces meandriformis*** Vittad.
Mavrovo NP: beech forest, soil, 10.09.2008.

34. ***Ciboria rufofusca*** (O. Weberb.) Sacc.
References: Karadelev *et al.* [20].

35. ***Ciboria viridifusca*** (Fuckel) Höhn.
Belchisko Blato: alder forest, *Alnus glutinosa* (conelike flowers), 19.10.2014.

36. ***Claviceps purpurea*** (Fr.) Tul.
References: Karadelev & Spasikova [27–29].

37. ***Colpoma quercinum*** (Pers.) Wallr.
Syn. *Colpoma nigrum* Tode) Höhn.
Galichica NP: oak forest, *Quercus frainetto* (branch), 21.07.1988.

38. ***Coryne atrovirens*** (Pers.) Sacc.
Syn. *Leotia atrovirens* Pers.
References: Karadelev *et al.* [24].

39. ***Crocicreas dolosellum*** (P. Karst.) S. E. Carp.
Syn. *Cyathicula dolosella* (P. Karst.) Dennis
Shar Planina Mt.: beech forest, ?*Petasites albus* (dry stem), 24.04.2018.

40. ***Cryphonectria parasitica*** (Murrill) M. E. Barr
Syn. *Endothia parasitica* (Murrill) P. J. Anderson & H. W. Anderson
References: Bryner *et al.* [3], Papazova-Anakieva *et al.* [4, 6], Sotirovski *et al.* [7–11].

41. ***Cudonia circinans*** (Pers.) Fr.
References: Karadelev & Rusevska [1], Karadelev *et al.* [20].

42. ***Cudoniella clavus*** (Alb. & Schwein.) Dennis
Syn. *Helotium clavus* (Alb. & Schwein.) Gillet
References: Karadelev *et al.* [24].

43. ***Cudoniella tenuispora*** (Cooke & Massee) Dennis
Karadzhica Mt: beech and fir forest, herbaceous stem, 25.04.2018.

44. ***Cyathicula cyathoidea*** (Bull.) Thüm.
Syn. *Peziza calyculus* Sowerby
Syn. *Hymenoscyphus calyculus* (Sowerby) W. Phillips
Bistra Mt.: beech forest, *Fagus sylvatica* (rotten wood), 14.10.2006; mixed beech and oak forest, deciduous tree (fallen branch), 11.10.2008; Galichica NP: beech forest, *Fagus sylvatica* (fallen branch), 02.11.2008; Kozhuf Mt.: beech forest, *Fagus sylvatica* (rotten branches), 10.10.1983; Osogovski Planini Mt.: beech forest, *Fagus sylvatica* (fallen twigs), 29.10.2014; Plachkovica Mt.: beech forest, *Fagus sylvatica* (fallen twig), 21.10.2014.

45. ***Cyclaneusma niveum*** (Pers.) DiCosmo, Peredo & Minter
Syn. *Propolis nivea* (Pers.) Fr.
Osogovski Planini Mt.: pine plantation, *Pinus nigra* (fallen needles), 21.10.2007.

46. ***Daldinia concentrica*** (Bolton) Ces. & De Not.
Syn. *Hemisphaeria concentrica* (Bolton) Klotzsch
References: Karadelev & Nastov [12].

47. ***Dasyscyphella nivea*** (R. Hedw.) Raitv.
Syn. *Dasyscyphus niveus* (R. Hedw.) Sacc.
Syn. *Peziza nivea* (R. Hedw.) Fr.
Galichica NP: oak-hornbeam forest, *Quercus* sp. (rotten wood), 22.04.2002.

48. ***Diatrype bullata*** (Hoffm.) Fr.
Plachkovica Mt.: riparian vegetation, *Salix* sp. (branch), 03.09.2014.

49. ***Diatrype disciformis*** (Hoffm.) Fr.
References: Karadelev & Nastov [12], Karadelev [17], Karadelev *et al.* [18, 23], Karadelev & Murati [26].

50. ***Diatrype stigma*** (Hoffm.) Fr.
References: Karadelev & Nastov [12], Karadelev [17], Karadelev *et al.* [18, 19, 25], Karadelev & Murati [26].

51. ***Diatrypella favacea*** (Fr.) Ces. & De Not.
References: Karadelev *et al.* [18].

52. ***Diatrypella verruciformis*** (Ehrh.) Nitschke
Syn. *Diatrypella verrucaeformis* (Ehrh.) Nitschke
References: Karadelev & Nastov [12].

53. ***Disciotis venosa*** (Pers.) Boud.
Syn. *Peziza venosa* Pers.
References: Karadelev & Nastov [12], Karadelev & Rusevska [1], Karadelev *et al.* [34].

54. ***Dothistroma septosporum*** (Dorog.) M. Morelet
Syn. *Mycosphaerella pini* Rostr.
References: Papazova-Anakieva *et al.* [5].

55. ***Dumontinia tuberosa*** (Bull.) L.M. Kohn
Shar Planina Mt.: meadow, *Anemone nemorosa* (rhizomes), 07.05.2016; Pelister NP: meadow, wet place with *Anemone nemorosa* (rhizomes), 13.04.2018.

56. ***Elaphomyces granulatus*** Fr.
Syn. *Hypogaeum cervinum*(L.) Pers.
References: Karadelev *et al.* [25], Karadelev & Spasikova [27–29], Chavdarova *et al.* [35].

57. ***Elaphomyces reticulatus*** Vittad.
Syn. *Lycoperdastrum reticulatum* (Vittad.) O. Kuntze
References: Karadelev & Spasikova [27–29], Chavdarova *et al.* [35].

58. ***Encoelia fascicularis*** (Alb. & Schwein.) P. Karst.
Smrdesh: riparian vegetation, *Salix* sp. (bark of a living branch), 03.09.2014.

59. ***Eutypella scoparia*** (Schwein.) Ellis & Everh.
Skopje (vicinity): deciduous forest, *Robinia pseudoacacia* (fallen branch), 02.01.2016, deciduous tree (fallen branch), 20.02.2018; Vod-

no Mt.: deciduous forest, deciduous tree (fallen branch), 21.03.2018.

60. ***Genea fragrans*** (Wallr.) Paol.
References: Chavdarova *et al.* [35], Kajevska *et al.* [36].

61. ***Genea hispidula*** Berk. ex Tul.
References: Chavdarova *et al.* [35], Kajevska *et al.* [36].

62. ***Genea verrucosa*** Klotzsch
References: Chavdarova *et al.* [35], Kajevska *et al.* [36].

63. ***Geoglossum nigritum*** Cooke
References: Karadelev & Nastov [12], Rusevska & Karadelev [32].

64. ***Geopora arenicola*** (Lév.) Kers
Syn. *Lachnea arenicola* (Lév.) Gillet
References: Chavdarova *et al.* [35], Kajevska *et al.* [36].

65. ***Geopora arenosa*** (Fuckel) S. Ahmad
Vodno: deciduous forest (soil), 04.05.2016

66. ***Geopora sumneriana*** (Cooke) M. Torre
Syn. *Lachnea sumneriana* (Cooke) W. Phillips
Syn. *Sepultaria sumneriana* (Cooke) Massee
References: Karadelev & Nastov [12], Chavdarova *et al.* [35], Kajevska *et al.* [36].

67. ***Glyphium elatum*** (Grev.) H. Zogg
Slan Dol: mixed deciduous forest (aspen, silver poplar and black locust), deciduous tree (bark of fallen branches), 23.12.2014.

68. ***Graddonia coracina*** (Bres.) Dennis
Shar Planina Mt.: beech forest, deciduous tree (fallen branch), 24.04.2018.

69. ***Guignardia aesculi*** (Peck) V.B. Stewart
References: Papazova-Anakieva *et al.* [5].

70. ***Gyromitra esculenta*** (Pers.) Fr.
Syn. *Helvella esculenta* Pers.
References: Karadelev [16], Karadelev *et al.* [34].

71. ***Gyromitra infula*** (Schaeff.) Quél.
Syn. *Helvella infula* Schaeff.
References: Tortić [2], Karadelev & Nastov [12], Karadelev *et al.* [20, 21, 34].

72. ***Gyromitra gigas*** (Krombh.) Cooke
Pelister Mt.: molika pine forest, soil, 16.04.2018; Karadzica Mt.: mugo pine forest, soil, 21.04.2018.

73. ***Gyromitra parma*** (J. Breitenb. & Maas Geest.) Kotl. & Pouzar
Syn. *Discinaparma*J. Breitenb. & Maas Geest.
References: Karadelev & Rusevska [1, 31], Karadelev & Murati [26].

74. ***Gyromitra perlata*** (Fr.) Harmaja
Syn. *Discina perlata* (Fr.) Fr.
Skopje: pine plantation, *Pinus sylvestris* (rotten wood), 07.03.2018; Vodno Mt.: pine plantation, *Pinus sylvestris* (rotten wood), 03.04.2018;

75. ***Helvella acetabulum*** (L.) Quél.
Syn. *Paxina acetabulum* (L.) Kuntze
Syn. *Macroscyphus acetabuliforme* Gray
References: Karadelev & Murati [26], Karadelev *et al.* [34].

76. ***Helvella atra*** Oeder
Syn. *Leptopodia atra* (Oeder) Boud.
References: Karadelev & Rusevska [1], Karadelev & Nastov [12], Karadelev *et al.* [34].

77. ***Helvella crispa*** Bull.
Syn. *Craterella crispa* (Bull.) Pers.
References: Tortić [2], Karadelev & Nastov [12], Rusevska & Karadelev [32], Karadelev *et al.* [34].

78. ***Helvella costifera*** Nannf.
Skopje (vicinity): hornbeam forest, soil, 26.05.2016.

79. ***Helvella elastica*** Bull.
Syn. *Leptopodia elastica* (Bull.) Boud.
References: Karadelev *et al.* [20, 34], Rusevska & Karadelev [32].

80. ***Helvella sublicia*** Holmsk.
Syn. *Helvella ephippium* Lév.
References: Karadelev & Rusevska [1], Tortić [2], Karadelev & Nastov [12], Karadelev *et al.* [34].

81. ***Helvella fibrosa*** (Wallr.) Korf,
Syn. *Cyathipodia villosa* (Hedw.) Boud
Skopska Crna Gora Mt.: oak forest, soil, 18.06.2011.

82. ***Helvella lacunosa*** Fr.
References: Tortić [2], Karadelev & Nastov [12], Karadelev [16], Karadelev *et al.* [19, 34], Karadelev & Murati [26], Rusevska & Karadelev [32].

83. ***Helvella leucomelaena*** (Pers.) Nannf.
Syn. *Acetabula leucomelaena* (Pers.) Sacc.
References: Rusevska & Karadelev [32], Karadelev *et al.* [34].

84. ***Helvella monachella***(Scop.) Fr.
Syn. *Helvella leucopus* Pers.

Kumanovo (vicinity), along river Pchinja: riparian vegetation (willow and poplar), soil, 10.04.2007; Gradishtanska Planina: poplar forest, soil, 12.04.2018.

85. ***Helvella macropus*** (Pers.) P. Karst.
Syn. *Peziza macropus* Pers.
Bistra Mt.: beech forest, *Fagus sylvatica* (rotten wood), 14.10.2006.

86. ***Helvella solitaria*** P. Karst.
Syn. *Helvella queletii* Bres.
References: Karadelev *et al.* [34].

87. ***Herpotrichia juniperi*** (Duby) Petr.
References: Papazova-Anakieva *et al.* [5].

88. ***Heyderia abietis*** (Fr.) Weinm.
Syn. *Gymnomitrula abietis*(Fr.) S. Imai
References: Karadelev & Rusevska [1], Tortić [2], Karadelev & Nastov [12].

89. ***Humaria hemisphaerica*** (Hoffm.) Fuckel
Syn. *Lachnea hemisphaerica* (F. H. Wigg.) Gillet
References: Tortić [2], Karadelev & Nastov [12], Irbe *et al.* [15], Karadelev *et al.* [20, 23, 24], Karadelev & Murati [26], Kajevska *et al.* [36].

90. ***Hyaloscypha hyalina*** (Pers.) Boud.
Syn. *Octospora hyalina* (Pers.) Gray
Jasen Reserve: pine and oak forest, *Quercus* sp. (rotten wood), 05.10.2010; Shar Planina Mt.: spruce forest, *Cornus mas* (rotten wood), 01.07.2010.

91. ***Hymenoscyphus fructigenus*** (Bull.) Gray
Syn. *Phialea fructigena*(Bull.) Gillet
Skopska Crna Gora Mt.: oak forest, *Quercus* sp. (acorn), 10.09.2005, 02.10.2005; Bistra Mt.: oak forest, *Quercus* sp. (acorn), 01.06.2006, 10.07.2011; Pelister NP: beech forest, *Fagus sylvatica* (beechnuts), 18.09.2006; Osogovski Planini Mt.: beech forest, *Fagus sylvatica* (beechnuts), 16.10.2007, *Corylus avellana* (nut), 05.07.2018; Galichica NP: deciduous forest, *Corylus avellana* (nut), 18.10.2008; Mavrovo NP: beech forest, *Fagus sylvatica* (beechnuts), 30.06.2010; Korab Mt.: beech forest, *Fagus sylvatica* (beechnuts), 15.07.2013; Maleshevski Planini Mt.: oak forest, *Quercus* sp. (acorn), 19.09.2014; Plachkovica Mt.: beech forest, *Fagus sylvatica* (beechnuts), 21.10.2014; Karadzhica Mt.: beech and fir forest, *Fagus sylvatica* (beechnuts), 25.04.2018.

92. ***Hymenoscyphus scutula*** (Pers.) W. Phillips
Shar Planina Mt.: deciduous forest, deciduous tree (fallen twig), 27.10.2017.

93. ***Hymenoscyphus serotinus*** (Pers.) W. Phillips
Syn. *Lanzia serotina* (Pers.) Korf & W. Y. Zhuang
Bistra Mt.: beech forest, *Fagus sylvatica* (rotten wood), 14.10.2006; Jasen Reserve: mixed forest (beech, pine, maple, ash and oak), *Fagus sylvatica* (fallen branches), 15.10.2010; Osogovski Planini Mt.: beech forest, *Fagus sylvatica* (fallen twigs), 29.10.2014.

94. ***Hymenoscyphus syringicolor*** Svrček
Shar Planina Mt.: beech and oak forest, wet place, 24.04.2018, 30.04.2018.

95. ***Hypomyces aurantius*** (Pers.) Tul.
Pelister NP: pine planting, *Pinus sylvestris* (log), 17.07.1989; Belasica Mt.: basidiocarp of *Xerocomus subtomentosus*, 30.09.2010; Shar Planina Mt.: spruce forest, mushroom fruitbody, 01.07.2010.

96. ***Hypomyces chrysospermus*** Tul. & C. Tul.
Osogovski Planini Mt.: beech forest, bolets (fruitbody), 20.06.2018; Karadzhica Mt.: mixed forest, bolets (fruitbody), 08.09.2018.

97. ***Hypomyces lateritius*** (Fr.) Tul. & C. Tul.
Syn. *Peckiella lateritia* (Fr.) Maire
Kozhuf Mt.: beech forest, basidiocarp of *Lactarius deliciosus*, 23.10.2005; Pelister NP: molika pine forest with *Abies borisii-regis*, basidiocarp of *Lactarius salmonicolor*, 07.10.2001.

98. ***Hypomyces rosellus*** (Alb. & Schwein.) Tul. & C. Tul.
Vodno Mt.: pine plantation, *Pinus* sp. (rotten wood), 20.05.2018.

99. ***Hypomyces spadiceus*** Fr. ex Cooke
Syn. *Hypolyssus spadiceus*(Fr.) Kuntze
Pelister NP: beech and fir forest, basidiocarp of *Lactarius torminosus*, 19.09.2006.

100. ***Hypocrea citrina*** (Pers.) Fr.
Jakupica Mt.: oak and hornbeam forest, plant remnants, 12.07.1999; Nidze Mt.: pine forest, plant remnants, 17.07.2002;

101. ***Hypocrea gelatinosa*** (Tode) Fr.
Syn. *Creopus gelatinosus* (Tode) Link
Demir Hisar: oak forest, deciduous tree (rotten wood), 15.10.2011; Jakupica Mt.: beech forest, *Fagus sylvatica* (stump), 11.07.1999; Karadzhica Mt.: beech forest, *Fagus sylvatica* (rotten wood), 11.10.2001.

102. ***Hypocrea pulvinata*** Fuckel
References: Karadelev & Rusevska [30].

103. ***Hypocrea rufa*** (Pers.) Fr.
Dobra Voda Mt.: oak forest, deciduous tree (fallen branch), 10.03.2008; Mariovo: Macedonian oak forest, basidiocarp of *Xerocomus* sp., 11.10.2005; Nidze Mt.: mixed forest (pine, beech and fir), basidiocarp of *Fomitopsis pinicola*, 17.07.2002; Plachkovica Mt.: beech forest, *Fagus sylvatica* (rotten wood), 06.10.2014; Skopje: pine planting, *Suillus* sp. (basidiocarp), 10.10.2005; Pelister NP: beech forest, *Fagus sylvatica* (fallen branch), 18.09.2006.

104. ***Hypoxylon fragiforme*** (Pers.) J. Kickx f.
References: Tortić [2], Karadelev & Nastov [12], Karadelev *et al.* [18], Karadelev & Murati [26].

105. ***Hypoxylon fuscum*** (Pers.) Fr.
References: Karadelev & Nastov [12], Karadelev *et al.* [18], Karadelev & Murati [26], Karadelev & Rusevska [30].

106. ***Hypoxylon howeanum*** Peck
Karadzhica Mt.: beech forest, *Fagus sylvatica* (bark), 29.07.1997; Shar Planina Mt.: beech forest, *Fagus sylvatica* (bark), 11.07.1998.

107. ***Hypoxylon rubiginosum*** (Pers.) Fr.
Syn. *Hypoxylon rubiginosum* var. *rubiginosum*(Pers.) Fr.
References: Karadelev & Nastov [12], Karadelev *et al.* [18].

108. ***Hypoxylon serpens*** (Pers.) J. Kickx f.
Syn. *Nemania serpens* var. *serpens*
References: Karadelev & Nastov [12], Karadelev *et al.* [18].

109. ***Hysterographium fraxini*** (Pers.) De Not.
Syn. *Hypoderma fraxini* (Pers.) DC.
References: Rusevska & Karadelev [32].

110. ***Jackrogersella multiformis*** (Fr.) L. Wendt
Syn. *Hypoxylon multiforme* (Fr.) Fr.
Syn. *Hypoxylon multiforme* var. *multiforme*(Fr.) Fr.
Syn. *Annulohypoxylon multiforme*(Fr.) Y.M. Ju, J.D. Rogers & H.M. Hsieh
References: Karadelev & Nastov [12], Karadelev & Rusevska [30].

111. ***Kabatina thujae*** R. Schneid.
References: Papazova-Anakieva *et al.* [5].

112. ***Kretzschmaria deusta*** (Hoffm.) P.M.D. Martin
Syn. *Sphaeria deusta*Hoffm.
Syn. *Ustulina deusta* (Hoffm.) Maire
References: Tortić [2], Karadelev & Nastov [12], Karadelev *et al.* [18].

113. ***Lachnellula suecica*** (de Bary ex Fuckel) Nannf.
References: Karadelev & Nastov [12], Karadelev & Rusevska [31].

114. ***Lachnum tenuissimum*** (Quél.) Korf & W.Y. Zhuang
Syn. *Dasyscyphus tenuissimus* (Quél.) Dennis
References: Karadelev & Nastov [12].

115. ***Lachnum virgineum*** (Batsch) P. Karst.
Syn. *Dasyscyphus virgineus*(Batsch) Gray
References: Karadelev & Nastov [12], Karadelev *et al.* [18].

116. ***Lasiosphaeria ovina*** (Pers.) Ces. & De Not.
References: Karadelev & Nastov [12], Karadelev *et al.* [18].

117. ***Lasiosphaeria spermoides*** (Hoffm.) Ces. & De Not.
Karadzhica Mt.: beech forest, *Fagus sylvatica* (rotten wood), 11.10.2001; Mavrovo NP: beech forest, *Fagus sylvatica* (fallen branch), 19.06.2010.

118. ***Leotia lubrica*** (Scop.) Pers.
References: Tortić [2], Karadelev & Nastov [12], Karadelev *et al.* [23, 25], Tortić & Cekova [33].

119. ***Leptosphaeria acuta*** (Moug. & Nestl.) P. Karst
Karadzhica Mt.: beech and fir forest, herbaceous stem, 25.04.2018.

120. ***Leptosphaeria doliolum*** (Pers.) Ces. & De Not.
References: Karadelev & Nastov [12], Karadelev *et al.* [18].

121. ***Lirula nervisequia*** (DC.) Darker
Syn. *Hypoderma nervisequum* DC.
References: Papazova-Anakieva *et al.* [5].

122. ***Lophodermium pinastri*** (Schrad.) Chevall.
References: Papazova-Anakieva *et al.* [5].

123. ***Lophodermium seditiosum*** Minter
References: Papazova-Anakieva *et al.* [5].

124. ***Melanomma pulvis-pyrius*** (Pers.) Fuckel
Mavrovo NP: beech-fir forest, *Abies borisii-regis* (bark), 19.06.2010; Shar Planina Mt.: beech forest, *Fagus sylvatica* (rotten wood), 11.07.1997.

125. ***Melastiza chateri*** (W.G. Sm.) Boud.
References: Kajevska *et al.* [36].

126. ***Melastiza flavorubens*** (Rehm) Pfister & Korf
References: Kajevska *et al.* [36].

127. ***Microglossum viride*** (Pers.) Gillet
References: Karadelev & Rusevska [1], Tortić [2], Karadelev & Nastov [12], Tortić & Cekova [33].

128. ***Microglossum griseoviride*** V.Kučera
Bistra Mt.: beech forest (soil), 25.09.2017; Pelister NP: beech and fir forest (soil), 18.09.2017.

129. ***Microsphaera alphitoides*** Griffon & Maubl.
References: Papazova-Anakieva *et al.* [5, 6], Karadelev & Murati [26], Karadelev & Rusevska [30].

130. ***Microsphaera juglandis*** Golovin
Kozhuf Mt.: roadsides, *Juglans regia* (leaves), 15.07.2005; Prespa Lake area: *J. regia* (leaves), 08.07.2004.

131. ***Microstoma protracta*** (Fr.) Kanouse
References: Karadelev & Rusevska [1].

132. ***Mitrophora semilibera*** (DC.) Lév.
References: Karadelev & Murati [26], Karadelev *et al.* [34].

133. ***Mitrula paludosa*** Fr.
References: Karadelev & Rusevska [1], Karadelev *et al.* [34].

134. ***Mollisia cinerea*** (Batsch) P. Karst.
References: Karadelev & Nastov [12], Karadelev *et al.* [18, 23].

135. ***Mollisia discolor*** (Mont. & Fr.) W. Phillips
Galichica NP: oak forest, deciduous tree (rotten wood), 30.07.2007.

136. ***Mollisia ligni*** (Desm.) P. Karst.
Syn. *Pyrenopeziza ligni* (Desm.) Sacc.
Deshat Mt.: beech forest, *Fagus sylvatica* (rotten wood), 12.05.2007; Jakupica Mt.: beech forest, *Fagus sylvatica* (fallen branch), 12.07.1999.

137. ***Mollisia rivularis*** (Svrček) L.G. Krieglst.
Karadzhica Mt.: beech and fir forest, herbaceous stem, 25.04.2018.

138. ***Morchella conica*** Pers.
References: Karadelev *et al.* [25, 34], Karadelev & Murati [26].

139. ***Morchella deliciosa*** Fr.
References: Karadelev *et al.* [25, 34].

140. ***Morchella elata*** Fr.
References: Karadelev & Rusevska [1], Karadelev *et al.* [34].

141. ***Morchella esculenta*** (L.) Pers.
References: Karadelev & Rusevska [1], Karadelev [16], Karadelev & Murati [26].

142. ***Morchella esculenta*** var. ***umbrina*** (Boud.) S. Imai
Syn. *Morchella umbrina* Boud.
Vodno Mt.: deciduous forest, soil, 03.03.2018.

143. ***Morchella steppicola*** Zerova
References: Karadelev *et al.* [34].

144. ***Mycosphaerella pyri*** (Auersw.) Boerema
Unknown locality and date, *Pyrus amygdaliformis* (leaves).

145. ***Nectria cinnabarina*** (Tode) Fr.
References: Papazova-Anakieva *et al.* [5], Karadelev & Nastov [12], Karadelev *et al.* [18].

146. ***Neobulgaria pura*** (Pers.) Petr.
References: Tortić [2], Karadelev & Nastov [12].

147. ***Neobulgaria pura*** var. ***pura*** (Pers.) Petr.
Syn. *Coryne bresadolae* Rehm
References: Karadelev [17].

148. ***Neodasyscypha cerina*** (Pers.) Spooner
Vodno Mt.: deciduous forest, deciduous tree (fallen branch), 18.03.2018; Karadzhica Mt.: beech and fir forest, *Fagus sylvatica* (rotten wood), 25.04.2018.

149. ***Neonectria ditissima*** (Tul. & C. Tul.) Samuels & Rossman
Syn. *Necria galligena* Bres.
References: Papazova-Anakieva *et al.* [5].

150. ***Neophloeospora maculans*** (Berenger) Videira & Crous
Syn. *Mycosphaerella mori* (Fuckel) F.A. Wolf
References: Papazova-Anakieva *et al.* [6].

151. ***Ophiognomonia leptostyla*** (Fr.) Sogonov
Syn. *Gnomonia leptostyla* (Fr.) Ces. & De Not.
References: Papazova-Anakieva *et al.* [6].

152. ***Ophiostoma ulmi*** (Buisman) Melin & Nannf.
References: Papazova-Anakieva *et al.* [6].

153. ***Orbilia xanthostigma*** (Fr.) Fr.
Syn. *Calloria xanthostigma*(Fr.) W. Phillips
Mavrovo NP: mixed deciduous forest (alder, hazel and sycamore), deciduous tree (rotten wood), 18.09.2010; Osogovski Planini Mt.: beech forest, *Fagus sylvatica* (rotten wood), 29.10.2014; Shar Planina Mt.: beech forest, *Fagus sylvatica* (rotten wood), 24.04.2018.

154. ***Otidea abietina*** (Pers.) Fuckel
Syn. *Aleuria abietina*(Pers.) Gillet
References: Karadelev *et al.* [20, 22], Kajevska *et al.* [36].

155. ***Otidea alutacea*** (Pers.) Masse
References: Kajevska *et al.* [36].

156. ***Otidea auricula*** (Schaeff.) Sacc.
References: Kajevska *et al.* [36].

157. ***Otidea bufonia*** (Pers.) Boud.
References: Kajevska *et al.* [36].

158. ***Otidea cantharella*** (Fr.) Quél.
Syn. *Flavoscypha cantharella* (Fr.) Harmaja
References: Karadelev *et al.* [23], Kajevska *et al.* [36].

159. ***Otidea cochleata*** (L.) Fuckel
References: Kajevska *et al.* [36].

160. ***Otidea concinna*** (Pers.) Sacc.
References: Karadelev *et al.* [22], Karadelev & Murati [26], Kajevska *et al.* [36].

161. ***Otidea leporina*** (Batsch) Fuckel
Kichevo: pine plantation, soil, 01.09.2006; Mavrovo NP: mixed forest, 13.10.2009; Shar Planina Mt.: spruce forest, soil, 11.07.2013.

162. ***Otidea onotica*** (Pers.) Fuckel
Syn. *Peziza onotica* Pers.
References: Tortić [2], Karadelev & Nastov [12], Kajevska *et al.* [36].

163. ***Pachyella violaceonigra*** (Rehm) Pfister
References: Karadelev & Rusevska [1].

164. ***Perrotia flammea*** (Alb. & Schwein.) Boud.
Syn.*Lachnella flammea* (Alb. & Schwein.) Fr.
Galichica NP: oak forest, deciduous tree (rotten wood), 30.07.2007; Jasen Reserve: mixed pine and oak forest, deciduous tree (fallen branch), 15.10.2010.

165. ***Pestalotiopsis funereoides*** Steyaert
Syn. *Pestalotia funerea* Desm.
References: Papazova-Anakieva *et al.* [5].

166. ***Peziza arvernensis*** Boud.
Belasica Mt.: beech forest, soil, 01.05.2002; Dobra Voda Mt.: oak forest, soil, 30.05.2012; Kitka Mt.: beech forest, soil, 11.05.2002; Kozhuf Mt.: beech forest, soil, 03.08.1984; Kozjak Mt.: mixed beech and pine forest, soil, 16.05.2004; Osogovski Planini Mt.: beech forest, soil, 10.06.2007, 22.06.2018; Mavrovo NP: mixed forest, soil, 03.06.2018, Maleshevski Planini Mt.: beech and fir forest, *Fagus sylvatica* (rotten wood), 21.06.2018.

167. ***Peziza badia*** Pers.
References: Karadelev & Nastov [12].

168. ***Peziza buxea*** Quél.
Syn. *Humaria buxea* (Quél.) Sacc.
Bistra Mt.: beech and fir forest, *Fagus sylvatica* (decaying wood), 05.06.2009.

169. ***Peziza celtica*** (Boud.) M.M. Moser
Syn. *Pachyella celtica* (Boud.) Häffner
References: Karadelev *et al.* [23], Karadelev & Murati [26].

170. ***Peziza cerea*** Sowerby
Syn. *Pustularia cerea* (Sowerby) Rehm
References: Tortić [2], Grujoska & Papazov [14].

171. ***Peziza depressa*** Pers.
Bistra Mt.: beech forest, soil, 09.09.2017.

172. ***Peziza domiciliana*** Cooke
Syn. *Aleuria domiciliana* (Cooke) McLennan & Halsey
References: Karadelev & Murati [26].

173. ***Peziza howsei*** Roze & Boud.
Syn. *Galactinia howsei*(Roze & Boud.) Boud.
Bistra Mt.: oak forest with hazel and maple, soil, 10.05.2007.

174. ***Peziza indiscreta*** W. Phillips & Plowr.
Vodno Mt.: mixed forest, soil, 11.05.2016.

175. ***Peziza praetervisa*** Bres.
Bogdanci: oak forest, *Quercus frainetto* (stump), 03.09.1982; Pchinja river: riparian vegetation, soil, 29.04.2014.

176. ***Peziza repanda*** Pers.
References: Karadelev *et al.* [23].

177. ***Peziza saniosa*** Schrad. ex J.F. Gmel.
References: Karadelev & Nastov [12].

178. ***Peziza sicula*** Inzenga
Slan Dol: fallow land, soil, 17.03.2018.

179. ***Peziza succosa*** Berk.
Syn. *Otidea succosa* (Berk.) Thüm.
Syn. *Aleuria succos*a (Berk.) Gill.
Shar Planina Mt.: spruce forest, soil, 29.08.2018.

180. ***Peziza varia*** (Hedw.) Alb. & Schwein.
References: Karadelev [17], Karadelev & Rusevska [30].

181. ***Peziza vesiculosa*** Bull.
Syn. *Helvella vesiculosa* (Bull.) Bolton
References: Karadelev & Murati [26].

182. ***Pezizella alniella*** (Nyl.) Dennis
References: Karadelev *et al.* [22].

183. ***Phyllactinia corylea*** (Pers.) P. Karst.
Syn. *Phyllactinia guttata* (Wallr.) Lév.

Kozhuf Mt.: beech forest, *Fagus sylvatica* (leaves), 24.10.2005.

184. ***Phialina separabilis*** (P. Karst.) Huhtinen & Scheuer
Syn. *Hymenoscyphus separabilis* (P. Karst.) Dennis
References: Karadelev & Murati [26].

185. ***Pithya cupressina*** (Batsch) Fuckel
References: Karadelev & Rusevska [1].

186. ***Pithya vulgaris*** Fuckel
References: Karadelev *et al.* [20].

187. ***Plectania melastoma*** (Sowerby) Fuckel
Vodno Mt.: deciduous forest, deciduous tree (branch), 05.05.2017.

188. ***Polydesmia pruinosa*** (Berk. & Broome) Boud.
Galichica Mt.: oak forest, Sordariomycetes (ascocarp), 10.10.2000.

189. ***Poronia punctata*** (L.) Fr.
References: Karadelev & Rusevska [1], Karadelev *et al.* [22], Karadelev & Rusevska [31].

190. ***Propolis farinosa*** (Pers.) Fr.
Syn. *Propolis versicolor* (Fr.) W. Phillips
References: Karadelev & Nastov [12], Irbe *et al.* [15], Karadelev *et al.* [18, 23].

191. ***Pseudoplectania nigrella*** (Pers.) Fuckel
Jablanica Mt.: oak forest with planted pines, soil, 11.03.2017; Pchinja river: riparian vegetation, soil, 29.04.2014.

192. ***Ptychoverpa bohemica*** (Krombh.) Boud.
Syn. *Verpa bohemica* (Krombh.) J. Schröt.
References: Karadelev & Murati [26], Rusevska & Karadelev [32], Karadelev *et al.* [34].

193. ***Rhabdocline pseudotsugae*** Syd.
References: Papazova-Anakieva *et al.* [5].

194. ***Rhytisma acerinum*** (Pers.) Fr.
References: Karadelev & Nastov [12], Karadelev *et al.* [18, 23], Karadelev & Murati [26].

195. ***Rhytisma salicinum*** (Pers.) Fr.
Shar Planina Mt.: beech forest, *Salix caprea* (leaves), 08.07.1998.

196. ***Rosellinia callosa*** G. Winter
Skopje: tree nursery, deciduous tree (rotten wood), 09.03.2018.

197. ***Rutstroemia bolaris*** (Batsch) Rehm
References: Karadelev *et al.* [19].

198. ***Rutstroemia bulgarioides*** (Rabenh.) P. Karst.
References: Karadelev & Rusevska [1], Karadelev *et al.* [22].

199. ***Rutstroemia conformata*** (P. Karst.) Nannf.
Syn. *Ciboria conformata* (P. Karst.) Svrcek
Skopje: deciduous forest, *Populus* sp. (fallen leaves), 03.05.2018

200. ***Rutstroemia echinophila*** (Bull.) Höhn.
Demir Hisar: oak forest with chestnut plantation, *Castanea sativa* (fruits), 15.10.2011; Shar Planina Mt.: chestnut forest, *Castanea sativa* (fallen fruits), 17.10.2009; Skopska Crna Gora Mt.: oak forest with chestnut plantation, *Castanea sativa* (fallen fruits), 15.10.2006.

201. ***Rutstroemia firma*** (Pers.) P. Karst.
Mavrovo NP: oak forest, *Quercus cerris* (fallen branch), 10.10.2010.

202. ***Rutstroemia petiolorum*** (Roberge ex Desm.) W. L. White
Mavrovo NP: oak forest, woody debris, 02.10.2010; Skopje: deciduous forest, *Populus* sp. (fallen leaves and stems), 27.04.2018.

203. ***Sarcoscypha austriaca*** (Beck ex Sacc.) Boud.
Deshat Mt.: beech forest, *Fagus sylvatica* (rotten wood), 12.05.2007; Matka: deciduous forest, *Quercus* sp. (fallen branch), 1.04.2000; Vodno Mt.: deciduous forest, deciduous tree (fallen branch), 15.03.2018.

204. ***Sarcoscypha coccinea*** (Scop.) Lambotte
Syn. *Peziza aurantiaca* Bull
References: Tortić [2], Karadelev & Nastov [12], Karadelev & Murati [26], Rusevska & Karadelev [32].

205. ***Sarcosphaera coronaria*** (Jacq.) J. Schröt.
Syn. *Sarcosphaera crassa* (Santi) Pouzar
References: Karadelev & Rusevska [1], Karadelev *et al.* [19], Rusevska & Karadelev [32].

206. ***Sawadaea bicornis*** (Wallr.) Miyabe
References: Karadelev *et al.* [23].

207. ***Schmitzomia radiata*** (L.) W. Phillips
Syn. *Stictis radiata* (L.) Pers.
Skopje: tree nursery, deciduous tree (fallen branch), 08.02.2018.

208. ***Sclerotinia pseudotuberosa*** (Rehm) Rehm
Syn.*Ciboria batschiana* (Zopf) N.F. Buchw.
Syn. *Ciboria pseudotuberosa*Rehm
Dobra Voda Mt.: oak forest, *Quercus* sp. (fallen twigs), 23.10.2007; Mavrovo NP: oak forest, rotten wood, 10.10.2010; Skopje (vicinity): oak forest, *Quercus* sp. (fallen acorns),

24.10.2007; Suva Gora Mt.: oak forest, *Quercus* sp. (fallen acorns), 12.10.2011.

209. ***Sclerotinia sclerotiorum*** (Lib.) de Bary
References: Karadelev *et al.* [24].

210. ***Scutellinia cejpii*** (Velen.) Svrček
References: Kajevska *et al.* [36].

211. ***Scutellinia kerguelensis*** (Berk.) Kuntze
Syn. *Lachnea kerguelensis* (Berk.) Sacc.
References: Kajevska *et al.* [36].

212. ***Scutellinia crinita*** (Bull.) Lambotte
Skopje: deciduous forest, deciduous tree (rotten wood), 26.04.2018; Skopska Crna Gora Mt.: oak and hornbeam forest, deciduous tree (rotten wood), 05.05.2018, Kitka Mt.: oak and hornbeam forest, deciduous tree (rotten wood), 13.05.2018; Goleshnica Mt.: riparian vegetation, deciduous tree (fallen branch), 16.05.2018.

213. ***Scutellinia scutellata*** (L.) Lambotte
References: Karadelev & Nastov [12], Karadelev *et al.* [19, 23], Kajevska *et al.* [36].

214. ***Scutellinia umbrorum*** (Fr.) Lambotte
Syn. *Peziza umbrorum* Fr.
References: Kajevska *et al.* [36].

215. ***Seiridium cardinale*** (W. W. Wagener) B. Sutton & I.A.S. Gibson
Syn. *Coryneum cardinale* W. W. Wagener
References: Papazova-Anakieva *et al.* [5, 6].

216. ***Sowerbyella radiculata*** (Sowerby) Nannf.
Skopje: *Cupressus* plantation (soil, among fallen leaves), 19.02.2018.

217. ***Spathularia flavida*** Pers.
References: Karadelev & Rusevska [1], Karadelev *et al.* [20].

218. ***Tapesia fusca*** (Pers.) Fuckel
Syn. *Mollisia fusca* (Pers.) P. Karst
References: Karadelev & Nastov [12].

219. ***Taphrina deformans*** (Berk.) Tul.
Dojran Lake area: kermes oak forest, *Prunus webbi* (leaves), 06.05.2013; Mariovo: orchard, *Prunus persica* (leaves), 15.05. 2005 and 12.06.2005; Kitka Mt.: orchard, *Prunus persica* (leaves), 03.05.2007; Kumanovo: garden in the monastery, *Prunus persica* (leaves), 02.06.2005; Star Dojran: city park, *Prunus persica* (leaves); 25.04.2004.

220. ***Taphrina pruni*** Tul.
Karadzhica Mt.: beech forest (fruits of *Prunus domestica*), 29.05.2011; Shar Planina Mt.: deciduous forest, *Prunus* sp. (fruits), 25.05.2009.

221. ***Tarzetta catinus*** (Holmsk.) Korf & J. K. Rogers
References: References: [12], [36].

222. ***Tarzetta cupularis*** (L.) Svrcek
References: Kajevska *et al.* [36].

223. ***Tarzetta gaillardiana*** (Boud.) Korf & J. K. Rogers
Shar Planina Mt.: beech forest, *Petasites albus* (dry stem), 24.04.2018.

224. ***Terfezia terfezioides*** (Mattir.) Trappe
Taorska Klisura and Badar: oak forest (hypogeous), 10.12.2015.

225. ***Tolypocladium ophioglossoides*** (Ehrh. ex J. F. Gmel.) Quandt
Syn. *Cordyceps ophioglossoides* (Ehrh.) Link
Syn. *Torrubia ophioglossoides* (Ehrh.) Tul. & C. Tul.
Kozhuf Mt.: beech forest, *Elaphomyces* (ascocarps), 24.10.2005.

226. ***Trichoglossum hirsutum*** (Pers.) Boud.
References: Karadelev & Rusevska [1], Karadelev *et al.* [24].

227. ***Trichophaea hemisphaerioides*** (Mouton) Graddon
Syn. *Humaria hemisphaerioides* (Mouton) Eckblad
References: Kajevska *et al.* [36].

228. ***Trichophaeopsis bicuspis*** (Boud.) Korf & Erb
References: Kajevska *et al.* [36].

229. ***Tuber aestivum*** (Wulfen) Spreng.
References: Karadelev *et al.* [22].

230. ***Tuber aestivum*** var. ***uncinatum*** (Chatin) I. R. Hall
Syn. *Tuber uncinatum* Chatin
Mavrovo: beech forest (hypogeous), 07.11.2017

231. ***Tuber belonei*** Quél.
Syn. *Tuber bellonae* Quél.
Shar Planina Mt.: beech forest (hypogeous), 10.02.2017

232. ***Tuber borchii*** Vittad.
Taorska Klisura and Badar: oak forest (hypogeous), 10.01.2015; Mavrovo NP: beech forest (hypogeous), 07.11.2017.

233. ***Tuber brumale*** Vittad.
Karadzhica Mt.: oak forest (hypogeous), 10.01.2014.

234. ***Tuber brumale*** var. ***moschatum*** (Bull.) I. R. Hall
Syn. *Tuber moschatum* Bull.
Kitka Mt.: oak forest (hypogeous), 20.11.2016.

235. ***Tuber dryophilum*** *Tul. & C. Tul.*
Mavrovo NP: beech and fir forest; 11.01.2014.

236. ***Tuber excavatum*** Vittad.
Karadzhica Mt.: oak forest (hypogeous), 10.01.2014; Kitka Mt.: oak forest (hypogeous), 09.11.2014; Mavrovo NP: beech forest (hypogeous), 07.11.2017.

237. ***Tuber foetidum*** Vittad.
Gradishtanska Planina Mt.: oak and hornbeam forest (hypogeous), 01.03.2016.

238. ***Tuber fulgens*** Quél.
Mavrovo NP: beech forest (hypogeous), 07.11.2017.

239. ***Tuber macrosporum*** Vittad.
Vardar valley, deciduous forest (hypogeous), 20.12.2016.

240. ***Tuber magnatum*** Pico
Vardar valley, deciduous forest (hypogeous), 28.11.2016.

241. ***Tuber melanosporum*** Vittad. – planted
Taorska Klisura and Badar: planted *Corylus avelana* (hypogeous), 28.01.2015.

242. ***Tuber mesentericum*** Vittad.
Mavrovo NP: beech and fir forest (hypogeous), 11.01.2014.

243. ***Tuber puberulum*** Berk. & Broome
Kozhuf Mt.: beech forest (hypogeous), 13.01.2014; Skopje (vicinity): oak forest (hypogeous), 25.12.2014.

244. ***Tuber rufum*** f. ***ferrugineum*** (Vittad.) Montecchi & Lazzari
Syn. *Tuber ferrugineum*Vittad.
Kitka Mt.: oak forest (hypogeous), 09.11.2014.

245. ***Tuber rufum*** f. ***lucidum*** (H. Bonnet) Montecchi & Lazzari
Kitka Mt.: oak forest (hypogeous), 09.11.2014.

246. ***Tuber rufum*** f. ***nitidum*** (Vittad.) Montecchi & Lazzari
Syn. *Tuber nitidum* Vittad.
Valandovo: kermes oak forest (hypogeous), 10.12.2008; Galichica NP: oak forest (hypogeous), 03.06.2010.

247. ***Urnula craterium*** (Schwein.) Fr.
References: Karadelev & Rusevska [1].

248. ***Valsa sordida*** Nitschke
References: Papazova-Anakieva *et al.* [5].

249. ***Venturia inaequalis*** (Cooke) G. Winter
Kichevo: roadside, *Malus* sp. (leaves of a living tree), 11.07.2010; Pelister NP: beech forerst, *Prunus cerasifera* (fruit), 20.10.2005.

250. ***Verpa conica*** (O.F. Müll.) Sw.
Syn. *Verpa digitaliformis* Pers.
References: Karadelev & Rusevska [1], Karadelev *et al.* [34].

251. ***Xylaria carpophila*** (Pers.) Fr.
Karadzhica Mt.: beech and fir forest, *Fagus sylvatica* (fallen beechnuts), 29.05.2011; Mavrovo NP: beech forest, *Fagus sylvatica* (fallen beechnuts), 30.06.2010, 30.04.2018.

252. ***Xylaria filiformis*** (Alb. & Schwein.) Fr.
References: Karadelev *et al.* [22].

253. ***Xylaria hypoxylon*** (L.) Grev.
References: Tortić [2], Karadelev & Nastov [12], Karadelev [17], Karadelev *et al.* [18, 23], Karadelev & Murati [26], Tortić & Cekova [33].

254. ***Xylaria longipes*** Nitschke
Mavrovo NP: beech forest, *Fagus sylvatica* (rotten wood), 30.06.2010; Plachkovica Mt.: beech forest, *Fagus sylvatica* (rotten wood), 10.07.2014; Slan Dol: riparian vegetation, deciduous tree (rotten wood), 23.12.2014; Mavrovo NP.: beech forest, *Fagus sylvatica* (rotten wood), 04.04.2018.

255. ***Xylaria polymorpha*** (Pers.) Grev.
References: Tortić [2], Karadelev & Nastov [12], Grujoska [13], Karadelev [17], Karadelev *et al.* [18], Pilat & Lindtner [48].

RESULTS AND DISCUSSION

Presented species checklist establishes the current knowledge of the diversity of so far registered ascomycetes taxa in the Republic of Macedonia. The phylum Ascomycota is the most diverse and the largest taxonomic category in *Fungi* with approximately 64000 known taxa (Kirk *et al.* [49]). Unfortunately, compared to Basidiomycota it is the least explored fungal group in Macedonia with total number of 255 registered taxa estimated up to date. A majority of the listed ascomycetes belong to the class Pezizomycetes (106), followed by the Leotiomycetes (70) and Sordariomycetes (47), while the Dothideomycetes, Eurotiomycetes, Geoglossomycetes, Orbiliomycetes, Lecanoromycetes and Taphrinomycetes were each represented by little or few species only. Families with highest species diversity are Pyronemataceae with total number of 35 taxa, followed by 20 Helotiaceae species including one variety and 18 Pezizaceae species. The most abundant genera are: *Peziza* with 16 registered species, *Helvella* represented by 12 species and *Tuber* represented by 18 taxa (14 species) including two varieties and three forms. Among the taxa listed, 107 species, three varieties and three forms are reported for the first time for the country. Regarding their trophic affiliation, most newly recorded ascomycetes have been registered as humicolous, lignicolous and plant debris saprobes (124), mycorrhizal taxa are represented with 86 species and there are 45 fungal and plant parasites. To date, the following 18 ascomycetes are categorized in the Macedonian Red List of Fungi (MRLF): *Cudonia circinans, Discina parma, Disciotis venosa, Helvella atra, H. ephippium, Heyderia abietis, Microglossum viride, Microstoma protractum, Mitrula paludosa, Morchella elata, Pithya cupressina, Poronia punctata, Rutstroemia bulgaroides, Sarcosphaera crassa, Spathularia flavida, Trichoglossum hirsutum, Urnula craterium* and *Verpa conica* (Karadelev & Rusevska [1]). The majority data for the ascomycetes in Macedonia are from the most visited localities, such as: Shar Planina Mt., Pelister NP, Karadzhica Mt., Mavrovo NP and the wider area of Skopje. Current biogeographical data and many records of apparently rare and noteworthy species indicate that the diversity of ascomycetes in Macedonia is still incompletely known and underlines the need for a research in a greater depth.

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ЛИСТА НА АСКОМИЦЕТИ ВО РЕПУБЛИКА МАКЕДОНИЈА

Митко Караделев[1*], Катерина Русевска[1], Искра Кајевска[2], Данијела Митиќ-Копања[1]

[1]Институт за биологија, Природно-математички факултет,
Универзитет „Св. Кирил и Методиј",Скопје, Република Македонија
[2]Македонско миколошко друштво, Скопје, Република Македонија

Во оваа студија за првпат е претставен детален список на сите познати и досега регистрирани видови асцомицети од Македонија. Целта на овој труд е да се сумираат сите податоци за видови од типот Ascomycota (торбести габи), со што ќе се придонесе кон познавањето на оваа голема група габи во Македонија. Информациите за овој список се добиени врз основа на податоци собрани од досегашните миколошки истражувачки терени, податоци на видови документирани во постоечката научна литература, податоци од ревидирано испитување на суви примероци од Македонската колекција на габи (Macedonian Collection of Fungi, MCF) како и податоци од базата MACFUNGI. Од досегашните истражувања е забележано присутво на вкупно 255 таксони од кои 107 вида, три варијетета и три форми претставуваат нови наоди за земјата, но во споредба со претставниците на Basidiomycota, овие габи се многу послабо истражувани кај нас. Во Црвената листа на габи на Македонија се вклучени 18 вида аскомицети. Според податоците презентирани во овој труд, педесетина видови се ретки и можат да послужат за дополнување на Црвената листа на габи на Македонија.

Клучни зборови: листа на габи; Ascomycota; таксономија; номенклатура; Македонија

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Original scientific paper

CHARACTERIZATION OF A LOCUS *LECH13* IN DIFFERENT TOMATO VARIETIES USING FRAGMENT ANALYSES

Elizabeta Miskoska – Milevska*, Zoran T. Popovski, Tome Nestorovski

Faculty of Agricultural Sciences and Food,
University Ss. Cyril and Methodius, Skopje, Republic of Macedonia

*e-mail: miskoska@yahoo.com

The microsatellite markers are routinely used to investigate the genetic structure of natural populations. The microsatellite polymorphisms are important for estimation diversity among varieties and for evaluation of the efficiency of microsatellite for establishing varieties relationships. The locus *LECH13* was tested in six tomato varieties in order to evaluate its usefulness in the genetic differentiation among six morphologically different tomato varieties. The fragment analyses were performed using the *Applied Biosystems* DNA analyzer. The number of detected alleles for the microsatellites locus *LECH13* was four in researched tomato varieties (134-136-138-146 bp). The allele138 bp was noticed only in *Lycopersicon esculentum* subsp. *spontaneum*var. *racemigerum*. The average PIC value for the locus *LECH13* was 0.3677 and it belongs to the group of modest informative markers. The present study showed that the locus *LECH13* could be used in the genetic differentiation of tomato varieties, but in combination with other polymorphic microsatellite loci.

Key words: DNA microsatellites, fragment analyses, locus *LECH13*, tomato, varieties

INTRODUCTION

The simple sequence repeats (SSRs) or DNA microsatellite markers are important molecular tools for the phylogenic estimations and determination of the genetic distance among different systematic categories. They are short tandem repeats (2-10 bp), middle repetitive, tandemly arranged, hypervariable DNA sequences distributed in the plant, animal and human genomes. According to Zane *et al*. [1] microsatellites are present in both coding and noncoding regions and are usually characterized by a high degree of length polymorphisms. The informativeness of microsatellites as genetic markers has already been shown with great success in several plant species [2]. According to He *et al.* [3], the allelic variation may be correlated with the number of repeats within a particular microsatellite locus. In other words, the repeat length may correlate with the polymorphism information content (PIC). The usually high variability of microsatellites might lead to inconsistencies due to the high chance of independently arising, equally sized alleles (homoplasies) [2]. Such microsatellites may generate polymorphisms useful for the analysis of genetic diversity and relationships within the genus *Lycopersicon*. When choosing new microsatellite loci for identification purposes or for studies on genetic variation, both the level of polymorphism and the scorability of the banding patterns are important [4]. Molecular marker must be very informative, especially in a crop like *Lycopersicon esculentum*, where genetic diversity seems very limited [5]. In our previous research precise dendrogram was created based on the data genetic distance among investigated tomato [6]. In this study, only locus LECH13 was in the focus.

The aim of the present study was to survey the applicability of the locus *LECH13* in genetic differentiation among six morphologically different tomato varieties of *Lycopersicon esculentum* Mill.

EXPERIMENTAL SECTION

Plant material

Six tomato varieties of *Lycopersicon esculentum* Mill. (var. *grandifolium* from subsp. *cultum*; var. *cerasiforme* – red and yellow, var. *pruniforme* and var. *pyriforme* from subsp. *subspontaneum*; and var. *racemigerum* from subsp. *spontaneum*) were involved in this research. There are many classifications of the genus *Lycopersicon*, but in this research was used classification by Brezhnev [7]. A comparison between the used nomenclature and nomenclature of Peralta *et al*. [8] is presented in Table 1.

Table 1. Comparison between different tomato nomenclatures

Tomato names (Peralta *et al.*)	Lycopersicon equiualent
Solanum habrochaites S. Knapp and D.M Spooner	Lycopersicon hirsutum Dunal
Solanum peruvianum L.	part of Lycopersicon peruvianum (L.) Miller
Solanum arcanum Peralta	part of Lycopersicon peruvianum (L.) Miller

The plant material was obtained from the GeneBank of the Agricultural Institute in Skopje

DNA isolation and PCR conditions

Fresh leaves were collected from ten individual plants per each variety. DNA was isolated using Promega's Wizard ® Genomic DNA purification kit. Also, DNA was extracted from pooled seeds (received from the fruits of 10 individual plants) of each variety using modified CTAB method [9–11]. The quality of the isolated DNA was examined by running on 0.8 % agarose gel. The optimization of the PCR conditions for amplification of the locus *LECH13* was carried out using appropriate primers (Operon, Huntsville, AL). Some general data for the locus *LECH13* and appropriate primer are given in Table 2. The PCR products were visualized by running on 2 % agarose gel, stained with ethidium bromide and photographed under UV light by using a G-Box system (Sygene).

Table 2. General data for microsatellite locus *LECH13* and primers used in this study

Locus	Repeat motif	Primer sequences (5'-3')
LECH13	$(TA)_{6-1}(GA)_4$	F: M13-taa caa tca aaa gaa ctt cgc R:atc ccc tta ttg att aca tcc

F - Forward primer (5'-3')
R - Reverse primer (5'-3')
M13 tail: 5'-cac gac gtt gta aaa cga c-3'

The DNA isolation and optimization of the PCR conditions were done in the Laboratory for biochemistry and molecular biology within the Department of Biochemistry and Genetic Engineering at the Faculty of Agricultural Sciences and Food – Skopje [12].

Data analyses

The fragment analyses were realized using the *Applied Biosystems* DNA analyzer (*ABI 3130*) and *GeneMapper®Software program* (v. 3.2). The data were analyzed using the specific program *Power Marker Software* (v. 3.25).

RESULTS AND DISCUSSION

The microsatellites are specific for each individual genome or species. They were used to evaluate genetic diversity and relationships within the genus *Lycopersicon*. The locus *LECH13* was used in many research, but in different tomato cultivars and accessions [2–4, 12, 14]. The main objective of this work was to examine the potential of the locus *LECH13* in genetic differentiation among six morphologically different tomato varieties of *Lycopersicon esculentum* Mill., received from Gene Bank of the Agricultural Institute in Skopje.

The analyzed microsatellite primer set gave good amplification across the six tomato varieties and was used for the fragment analysis. The results from fragment analysis were shown as electropherograms of homozygous (Figure 1 a) and heterozygous samples (Figure 1 b and c).

A. very important part of the fragment analyses is an interpretation of the obtained electropherograms. Namely, the additional problem of the fragment analyses can be a determination of the peak (or peaks). This step is important for the relevant conclusion regarding the homozygous or heterozygous profile of the samples. In that sense, it is necessary to be careful in electropherograms' analyzing because further statistical analyses are based on these results.

It is important to select the major allelic peaks and to ignore the stutter peaks. The stutter peaks are small peaks that appear before, rarely after major allelic peaks and are side effects during the amplification of the microsatellite loci. They could be recognized according to their sizes and locations. It is recommended to take in consideration peaks higher than 100 RFU (relative fluorescence units) and lower than 2000 RFU. The peaks lower of 100 RFU must be interpreted very carefully.

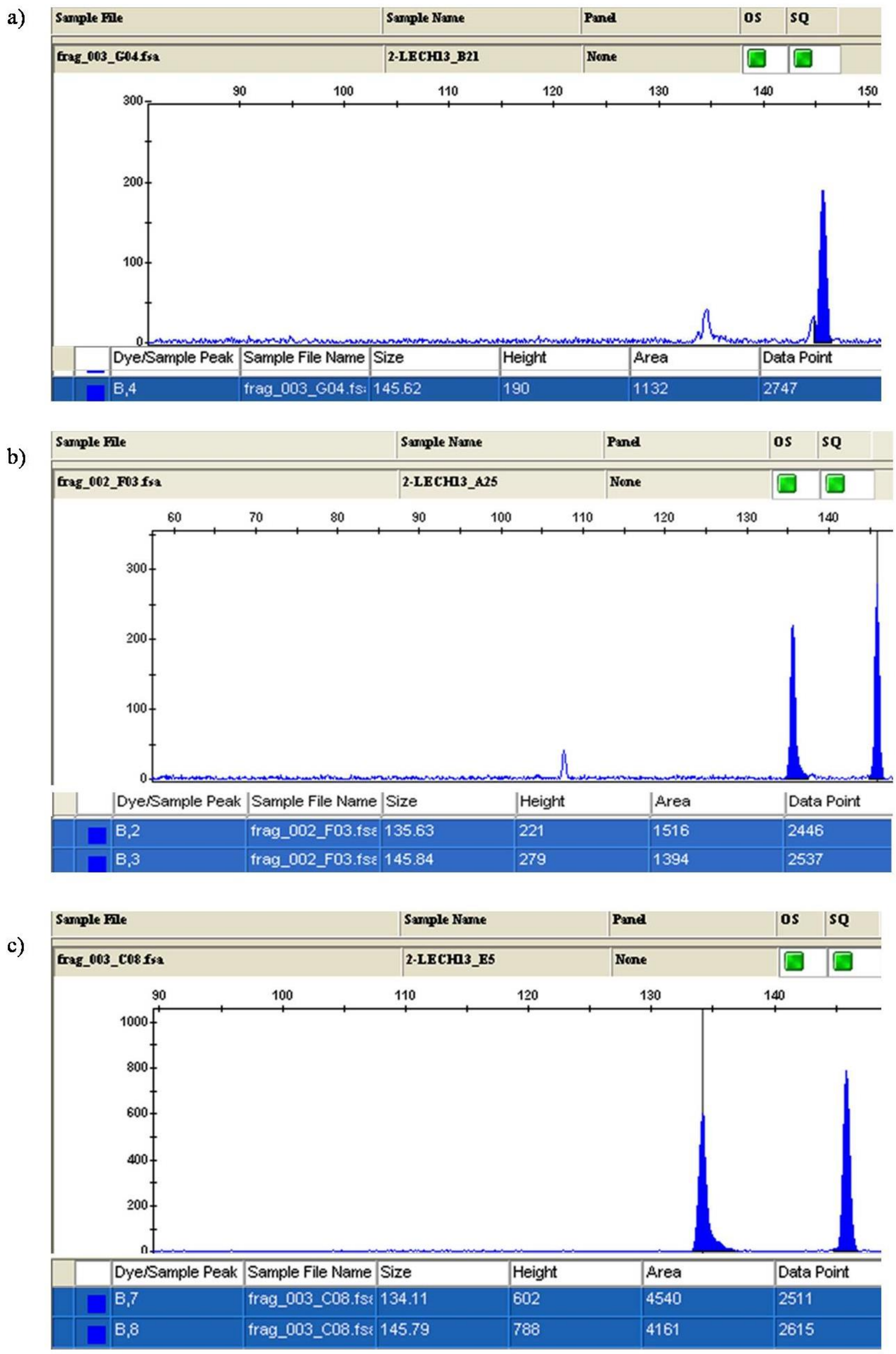

Figure 1. Electropherograms of locus *LECH13*: a) homozygous; b) and c) heterozygous

The fragment analyses of the locus *LECH13* (Figure 1) showed 4 allelic variants (134-136-138-146 bp). The allelic variants and their frequencies are presented in Figure 2. One of these alleles (138 bp) was specific for *Lycopersicon esculentum* subsp. *spontaneum* var. *racemigerum*.

For the same locus Smulders *et al.* [4] noticed 2 different alleles in researched tomatoes (124-128 bp), while Bredemeijer *et al.* [12] found only one allele (126 bp). Alvarez *et al.* [2] detected 5 different alleles among the investigated tomatoes (124-126-128-130-132 bp), and only one of them was a specific allele. According to He *et al.* [3], only one allele was noticed on this microsatellite locus. Garcia-Martinez *et al.* [14] found two alleles (124-128 bp) on the same locus.

It can be concluded that data, related to allele number and size, obtained in this research were different from previous results published by Smulders *et al.* [4], Bredmeijer *et al.* [13], Alvarez *et al.* [2], He *et al.* [3], Garcia-Martinez *et al.* [14]. One of the reasons for this could be the different plant material used in each research. For instance, Bredmeijer *et al.* [13] and He *et al.* [3] researched only cultivated tomato accessions, whereas in this study, tomato varieties that belong to subsp. *cultum*, subsp. *subspontaneum* and subsp. *spontaneum* were included.

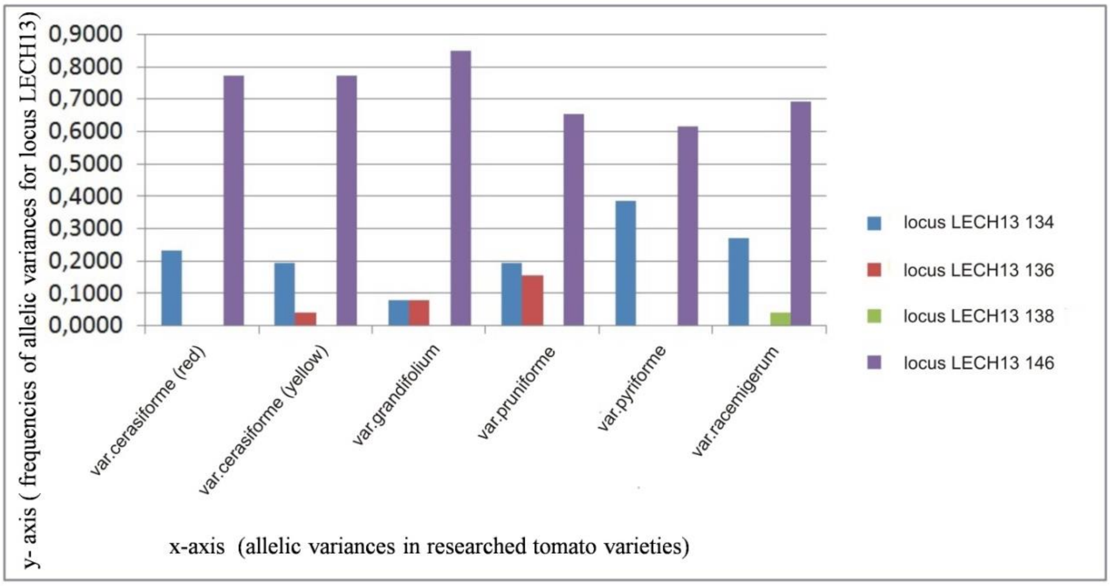

Figure 2. Allelic variances and their frequencies for locus *LECH13*

The difference in allele size could be related to the methodological approach. Namely, different DNA analysers, as well and different work conditions on the same DNA analyser (for ex. different capillary length, different type of polymer) could be the reason for receiving such differences in allele size. This means that doing analyses on the same DNA analyser and in the same working conditions (for ex. same capillary length, same type of polymer) is the best approach.

From the data presented in Figure 2, it can be concluded that the allelic variants in size of 134 and 146 bp appeared on the locus *LECH13* among all researched varieties, while allele of 136 bp is present only in *Lycopersicon esculentum* subsp. *subspontaneum* var. *cerasiforme* (yellow), *Lycopersicon esculentum* subsp. *cultum* var. *grandifolium* and *Lycopersicon esculentum* subsp. *subspontaneum* var. *pruniforme*. The allele (138 bp) was noticed only in DNA isolated from the seed of *Lycopersicon esculentum* subsp. *spontaneum* var. *racemigerum*. This allele was not noticed in the fragment analyses of DNA received from leaves of *Lycopersicon esculentum* subsp. *spontaneum* var. *racemigerum*, neither in the fragment analyses of DNA received from seed, respectively from leaves of the other varieties. This conclusion is probably due to the fact that in the fragment analyses of DNA from leaves of *Lycopersicon esculentum* subsp. *spontaneum* var. *racemigerum*, were not included plants that contain this allele, while the seed material was mixed.

Based on the obtained data, it could be concluded that the individual approach, using DNA isolation from individual plants, is better for fragment analyses. If we decide to use DNA isolated from pooled seeds, probably we will have to include a much bigger number of samples.

In the researched varieties, the highest allele frequency was found for the allelic variant of 146 bp, and its values were: (0.7692) for *Lycopersicon esculentum* subsp. *subspontaneum* var. *cerasiforme* (red) and *Lycopersicon esculentum* subsp. *subspontaneum* var. *cerasiforme* (yellow), (0.8462) for *Lycopersicon esculentum* subsp. *cultum* var. *grandifolium*, (0.6538) for *Lycopersicon esculentum* subsp. *subspontaneum* var. *pruniforme*, (0.6154) for *Lycopersicon esculentum* subsp. *subspontaneum* var. *pyriforme* and (0.6923) for *Lycopersicon esculentum* subsp. *spontaneum* var. *racemigerum*.

For the locus LECH13, average observed heterozygosity (Ho = 0.5513) was higher than average expected heterozygosity (He = 0.4229), meaning the increased level of heterogeneity in the researched tomato varieties. Also, the observed heterozygosity was higher than the expected heterozygosity and it indicates a high level of allogamy.

The informativeness of polymorphic DNA markers could be quantitatively measured by a statistic called the polymorphism information content or PIC. In the researched tomato varieties, the average PIC value for the locus *LECH13* was 0.3677. According to the classification of Botstein *et al.* [15], the locus *LECH13* showed modest informativeness for all researched varieties.

The genetic differentiation test in the researched tomato varieties showed minor differentiation for the locus *LECH13* (0.0256). On the other hand, in the estimated tomato subspecies, this locus showed modest differentiation (0.0896) [16].

The present data show that this microsatellite locus gave amplification and polymorphism across six tomato varieties. However, data from a number of microsatellite loci will have to be combined to provide a unique DNA profile for individual varieties. Therefore, a combination of the locus *LECH13* with other more polymorphic microsatellite loci will be necessary to allow distinguishing tomato varieties.

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КАРАКТЕРИЗАЦИЈА НА *LECH13*-ЛОКУСОТ ВО РАЗЛИЧНИ ВАРИЕТЕТИ ДОМАТИ СО УПОТРЕБА НА ФРАГМЕНТ-АНАЛИЗИ

Елизабета Мискоска-Милевска, Зоран Т. Поповски, Томе Несторовски

Факултет за земјоделски науки и храна,
Универзитет „Св. Кирил и Методиј“, Скопје, Република Македонија

Микросателитските маркери се користат рутински за испитување на генетската структура на природната популација. Микросателитските полиморфизми се важни за процена на разновидноста меѓу вариететите и за евалуација на ефикасноста на микросателитите во утврдување врски меѓу вариететите. *LECH13*-локусот беше тестиран кај шест вариетети на домати со цел да се оцени неговата корисност во генетската диференцијација меѓу шесте морфолошки различни вариетети на домати. Фрагмент-анализите беа изведени со ДНК-анализатор на *Applied Biosystems*. Во испитаните сорти домати, на микросателитскиот локус *LECH13* беа забележани четири алелни варијанти (134-136-138-146 bp). Алелот (138 bp) беше забележан само кај *Lycopersicon esculentum* subsp. *spontaneum* var. *racemigerum*. Просечната вредност на PIC за *LECH13*-локусот беше 0.3677 и тој припаѓа на групата умерено информативни маркери. Ова истражување покажа дека *LECH13*-локусот може да се користи во генетска диференцијација на вариетети на домати, но во комбинација со други полиморфни микросателитски локуси.

Клучни зборови: ДНК-микросателити; фрагмент-анализи; *LECH13*-локус; домат; вариетети

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Original scientific paper

DENDROFLORA OF THE GALIČICA MOUNTAIN RANGE AND ISLAND OF GOLEM GRAD IN THE REPUBLIC OF MACEDONIA

Jane Acevski[1], Dejan Mandžukovski[2]

[1]Faculty of Forestry, Ss. Cyril and Methodius University, Skopje, Republic of Macedonia
[2]Public enterprise Makedonski šumi, Skopje, Republic of Macedonia

[*]e-mail: jane.acevski@live.com

This article presents data regarding the tree species and their forms of habitus, areal types and ecological valence on Galičica Mountain and the island of Golem Grad which are part of National Park Galičica. Our research was conducted during the period from 1995 to 2018, during which we registered much higher diversity and richness in dendroflora compared to other mountains on the Balkan Peninsula. According to this research, autochthonous dendroflora of Galičica and the island of Golem Grad consist of 180 taxa which is 56 % of the entire dendroflora of the Republic of Macedonia.

Kew words: dendroflora; form of habitus; areal types; ecological valency; Galičica Mountain; island of Golem Grad

INTRODUCTION

The mountain Galičica is situated between two natural lakes, Ohrid and Prespa, in the Republic of Macedonia and covers an area of 39.250 ha. The island of Golem Grad is situated on the southwestern part of Prespa lake and it is the only natural island in the Republic of Macedonia. Both Galičica Mountain and the island Golem Grad are constituents of the National Park Galičica (NPG). It is characterized with significant floristic richness, [1–16] due to many factors: geographical position, geology, petrography, soil composition, orography, climate, hydrography, florogenesis and antropo-zoogenic influence [17–25]. This mountain range, although relatively smaller compared to others in the south of the country, is considered to be among the most diverse and rich in dendroflora in the Balkan Peninsula [1, 8, 9, 15–17].

The specificity of the dendroflora is manifested not only by the presence of a large number of plant species, but also by the fact that many of them are endemic, relict, rare plants and some species reach the borders of their natural ranges within NPG.

Figure 1. Investigated area on Galičica Mt.

Most relict species are relics from the Tertiary, which are scattered mainly in the deep valleys and the slopes of Galičica. Beside endemic and relict species, Galičica and the island of Golem Grad are characterized by rare plants which are found only on other one or two mountains within the Republic of Macedonia [1, 26–28].

The great and specific floristic richness of the mountain range Galičica and the island of Golem Grad attracted the attention of many botanists, phytogeographers and florists, from the 19th century to the present [1, 3–16].

In order to protect and preserve the extraordinary natural wealth of this mountain range, in October 1958, with the decision of the Assembly of the Republic of Macedonia, part of this massif (22.700 ha) was declared as National Park, including the island of Golem Grad in Prespa lake.

The study of dendroflora as part of the rich flora of this mountain, from a scientific point of view, is a special interest, of course, due to the large number of woody species, represented by lower taxonomic categories which were not the subject of these studies, but the position to taxa of the species.

Figure 2. The island of Golem Grad in Prespa lake

Besides determining of the total number of woody species, their inventory, it is also of interest were to be solved the systematic affiliation and bio-ecological characteristics of all kinds.

With the study of the dendroflora of this massif and the island, a clear presentation of the horizontal and vertical distribution of the individual woody species, and some other ecological circumstainces are obtained.

The obtained results and recognition for the dendroflora of the mountain massif of Galičica, besides the fact that have special importance for the science and forestry practice, will also find wider application in the ecology, protection of biodiversity, raising the education of the civil society for the beautiful and rare natural heritage, as well as for the bigger valorization of those heritage within the National Park Galičica.

METHODS

We used standard methodology for conducting floristical research. Collected plant material was deposed in the herbarium of the Faculty of Forestry in Skopje -Herba SF.

The specimens were determined using reference literature: The Flora of the Republic of Macedonia (Micevski, 1995–2010), Flora Europaea (Tutin et al. eds., 1964–1980), Prodromus florae peninsulae Balcanicae (Hayek, 1927–1933), Mountain flora of Greece (Strid & Tan, eds., 1987, 1991), Flora Helenica (Strid & Tan, eds., 1997, 2002). The nomenclature of the taxa is presented according to Euro+Med PlantBase [29] and for some taxa The Plant List [30]. Areal types of tree species were determined according to Gajić [31] and Pignatti [32].

RESULTS AND DISCUSSION

According to our research conducted from 1995 to 2018, autochthonous dendroflora of Galičica and the island of Golem Grad consist of 180 taxa (species and subspecies), thus representing 58% of the entire dendroflora of the Republic of Macedonia [13]. These 180 taxa are within 86 genera, 44 families and 3 subfamilies, 27 orders, 4 subclasses, 2 classes and 1 division (Table 4).

By form of habitus (Table 1), of the total dendroflora of 180 taxa, 76 (42.2 %) belong to the category of trees, 62 taxa (34.5 %) are shrubs, 25 taxa (14 %) are half shrub, 14 species (7.7 %) are lianas and 3 species (1.6 %) are semiparasites-epiphytes.

Table 1. Participation of tree species according to the form of habitus

Form accord. habitus	tree			shrub			halfshrub	lianas	epiphyte	Total
	high tree (HT)	medium tree (MT)	low tree (LT)	high (HSh)	medium (MSh)	low (LSh)	Halfshrub (HSh)	Lianas (l)	Epiphyte (E)	
N	13	39	24	16	21	25	25	14	3	180
%	7.2	21.7	13.3	8.9	11.7	13.9	14	7.7	1.6	100
Total		76			62		25	14	3	180
%		42.2			34.5		14	7.7	1.6	100

The autochthonous tree species of Galičica Mountain belong to 9 bigger floristic elements, and as the most numerous is Euro-Asian with 58 species (32.2 %), then the Boreal with 30 species or (16.6 %) and the Euri Mediterranean with 25 species (6.6 %). The share of the Balkan and sub-Balkan endemic floristic element with 23 species (12.8 %) gives special significance to this mountain and sets it in Europe as one of the few with such numerous endemic species in the composition of dendroflora.

Less numerous are the Steno Mediterranean floristic element with 15 species (8.3 %), the Mediterranean-mountainous with 12 species (6.6 %), The floristic element with wider distribution is represented with 11 species (6.1 %), and the Atlantic floristic element with only 1 representative i.e. 0.56 %.

Table 2. Percentage in participation of tree species according to the floristic elements

floristic element	Balkan and subBalk.	Steno Mediterannean	Euri Mediterannean	Mediterannean Mountain	Euro Asian	Boreal	Orophyl-South Europe	Atlantic	Floristic element with wider distribution	Total
N	23	15	25	12	58	30	5	1	11	180
%	12.78	8.33	13.89	6.67	32.22	16.67	2.78	0.56	6.11	100.00

According to the ecological valence of Galičica, the most frequent are the thermophytic taxa with 36 % or 64 species, then thermomesophytic with 18.8 % or 33 taxa, with 17 %, or 30 species are the thermoxerophytic, mesophilic with 10.8 % or 19 species and least represented are xerophyte species with 4.5 % or 8 species which is shown in Table 3. This relationship is the result of the ecological conditions, primarily the basic substrate and the influence of the submediterranean climate, and the small percentage of the xerophyte species is due to higher relative air humidity, which is conditioned by the immediate proximity of the lakes.

Table 3. Participation of tree species according to the ecological valence

ecological valency	T	K	M	TK	TM	TH	Total
N	65	9	20	30	33	23	180
%	36.1	5	11.1	16.7	18.3	12.8	100

On this mountain and island of Golem Grad too, there is a large number of rare tree types i.e. they can be seen only on Galičica or maybe on other 1 to 2 mountains but except on those, they cannot be found anywhere else in Macedonia: *Prunus prostrata, Genista radiata, Pinus heldreichii, Euphorbia veneta, Euphorbia glabriflora, Celtis planchoniana, Acatholimon ulicinum, Cotoneaster parnasicus, Staphylea pinnata* and *Aesculus hippocastanum;*

The largest number of autochthonous tree species are assumed to be directly descended from the Tertiary. At present, the following species are considered as remnants of the old Tertiary dendroflora on Galalichica: *Berberis croatica*, *Pinus peuce*, *Aesculus hippocastanum* and *Staphyllea pinnata.*

For a certain number of woody species, the mountain massif of Galičica represents their land range. Such woody species whose near-southernmost boundary spreads on this mountain are: *Juniperus sabina, Staphylea pinnata* and *Pinus peuce*, while the species that reach their northernmost border here are: *Acantholimon ulicinum, Euphorbia characias subsp. characias* and *Prunus prostrata*. Woody species with easternmost boundary are: *Prunus coccomilia, Genista radiata* and *Laburnum alpinum*, and with the westernmost boundary: *Celtis planchoniana* (island of Golem Grad).

On Galičica Mountain, there are sites which are characterized with great diversity and large floristic wealth in relatively small areas. As such we would mention the following: "Zli Dol", with 65 and "St. Zaum" (Osoj) with 56 woody species. The majority of these woody species are Balkan endemics, Tertiary relics or belong to the group of rare woody plants.

Figure 3. *Juniperus foetidissima* (left –bark on a trunk; right - habitus)

Figure 4. *Acantholimon ulicinum* (left) *Euphorbia glabriflora* (right)

Figure 5. *Aesculus hippocastanum* (left) *Cotoneaster parnassicus* (right)

Figure 6. *Prunus prostrata*

Figure 7. *Euphorbia characias* L. subsp. *characias* (left) *Celtis planchoniana* (right)

Table 4. Overview of the autochthonous dendroflora of Galičica Mt and island Golem Grad

Nº	taxa	Synonym	Common name Macedonian
1	2	3	4
1	*Ephedra foeminea* Forssk.	*Ephedra campylopoda* C. A. Mey.	кревка коситерница
2	*Ephedra major* Host		kоситерница
3	*Taxus baccata* L.		tиса
4	*Abies borisii-regis* Mattf.	*Abies alba* var. *acutifolia* Turrill	ела
5	*Pinus peuce* Griseb.		молика, елов бор
6	*Pinus nigra* J. F. Arnold		црн бор
7	*Pinus heldreichii* H. Christ	*Pinus leucodermis* Antoine	муника
8	*Juniperus communis* L.		модра смрека
8	*Juniperus communis* subsp. *nana* Syme		планинска смрека
9	*Juniperus oxycedrus* L.		црвена смрека
10	*Juniperus sabina* L.		смрделика
11	*Juniperus excelsa* M. Bieb.		дива фоја
12	*Juniperus foetidissima* Willd.		питома фоја
13	*Clematis vitalba* L.		повит
14	*Clematis flammula* L.		скребут
15	*Clematis viticella* L.		халожина
16	*Berberis croatica* Horvat		/
17	*Berberis vulgaris* L.		жолтика
18	*Iberis sempervirens* L.		/
19	*Platanus orientalis* L.		чинар
20	*Ulmus minor* Mill.		полски брест
21	*Ulmus glabra* Huds.	*Ulmus glabra* Huds.	планински брест
22	*Ulmus laevis* Pall.	*Ulmus effusa* Willd.	вез
23	*Celtis australis* L.		копривка
24	*Celtis planchoniana* K. I. Chr.	*Celtis glabrata* Planch.	/
25	*Morus alba* L.		бела црница
26	*Morus nigra* L.		црна црница
27	*Ficus carica* L.		смоква
28	*Fagus sylvatica* L.		бука
29	*Castanea sativa* Mill.		костен
30	*Quercus trojana* Webb	*Quercus macedonica* A. DC.	македонски даб
31	*Quercus cerris* L.		цер
32	*Quercus frainetto* Ten.	*Quercus farnetto* Ten.	плоскач
33	*Quercus petraea* (Matt.) Liebl.		горун
34	*Quercus pubescens* Willd.		благун
35	*Quercus pubescens* Willd. subsp. *pubescens*	*Quercus virgiliana* (Ten.) Ten.	/
36	*Quercus robur* subsp. *pedunculiflora* (K. Koch) Menitsky	*Quercus pedunculiflora* K. Koch	стежер
37	*Betula pendula* Roth		бреза
38	*Alnus glutinosa* (L.) Gaertn.		евла
39	*Corylus avellana* L.		леска
40	*Corylus colurna* L.		мечја леска

Table 4 (continuation)

41	*Carpinus betulus* L.		воден габер
42	*Carpinus orientalis* Mill.		бел габер
43	*Ostrya carpinifolia* Scop.		црн габер
44	*Juglans regia* L.		орев
45	*Acantholimon ulicinum* (Schult.) Boiss.	*Acantholimon androsaceum* (Jaub. & Spach) Boiss.	/
46	*Fumana ericoides* (Cav.) Gand.		сунчец
47	*Helianthemum canum* (L.) Baumg.		/
48	*Helianthemum nummularium* subsp. *grandiflorum* (Scop.) Schinz & Thell.	*Helianthemum grandiflorum* (Scop.) DC.	сунчаница
49	*Salix alba* L.		бела врба
50	*Salix × fragilis* L.		кршлива врба
51	*Salix triandra* L.		прашлика
52	*Salix pentandra* L.		/
53	*Salix eleagnos* Scop.		црна врба
54	*Salix amplexicaulis Bory*		ракита
55	*Salix caprea* L.		ива
56	*Salix cinerea* L.		лагушка
57	*Populus tremula* L.		Јасика
58	*Populus alba* L.		бела топола
59	*Populus nigra* L.		црна топола
60	*Vaccinium myrtillus* L.		боровинка
61	*Tilia cordata* Mill.	*Tilia parvifolia* Hoffm.	липа
62	*Tilia grandifolia* Hoffm.	*Tilia platyphyllos* Scop.	големолисна липа
63	*Tilia tomentosa* Moench	*Tilia argentea* DC.	сребренолисна липа
64	*Buxus sempervirens* L.		шимшир
65	*Euphorbia glabriflora* Vis.		млечка
66	*Euphorbia characias* L. subsp. *characias*	*Euphorbia veneta* Willd.	вулфанова млечка
67	*Daphne mezereum* L.		див јоргован
68	*Daphne laureola* L.		лисец
69	*Daphne oleoides* Schreb.		бријачица
70	*Rubus idaeus* L.		малина
71	*Rubus sanctus* Schreb.	*Rubus sanguineus* Friv.	капина
72	*Rubus ulmifolius* Schott		капина
73	*Rubus canescens* DC.	*Rubus tomentosus*	капина
74	*Rubus hirtus* aggr		капина
75	*Rubus wahlbergii* Arrh.		капина
76	*Rubus caesius* L.		капина
77	*Rosa canina* L.		шипка
78	*Rosa arvensis* Huds.		шипка
79	*Rosa gallica* L.		шипка
80	*Rosa tomentosa* Sm.		шипка
81	*Rosa pendulina* L.		шипка
82	*Rosa spinosissima* L.		шипка
83	*Prunus dulcis* (Mill.) D. A. Webb	*Amygdalus communis* L.	бадем
84	*Prunus webbii* (Spach) Vierh.	*Amygdalus webbii* Spach	див бадем
85	*Prunus mahaleb* L.	*Cerasus mahaleb* (L.) Mill.	горупла

Table 4 (continuation)

86	*Prunus avium* (L.) L.	*Cerasus avium* (L.) Moench	цреша
87	*Prunus spinosa* L.		трнинка
88	*Prunus cerasifera* Ehrh.		џанка
89	*Prunus cocomilia* Ten.		дива слива
90	*Prunus prostrata* Labill.		/
91	*Crataegus orientalis* M. Bieb.		бел глог
92	*Crataegus heldreichii* Boiss.		глог
93	*Crataegus sericea* Dzekov		глог
94	*Crataegus monogyna* Jacq.		глог
95	*Cotoneaster integerrimus* Medik.		мушмулица
96	*Cotoneaster tomentosus* (Aiton) Lindl.		мушмулица
97	*Cotoneaster parnassicus* Boiss. & Heldr.	*Cotoneaster mariana* And. A and Andonovski V.	мушмулица
98	*Amelanchier ovalis* Medik.		рушвица
99	*Sorbus domestica* L.		скоруша
100	*Sorbus aucuparia* L.		јаребика
101	*Sorbus torminalis* (L.) Crantz		брекиња
102	*Sorbus aria* (L.) Crantz		мукиња
103	*Sorbus umbellata* (Desf.) R. M. Fritsch		/
104	*Sorbus graeca* (Spach) Schauer		/
105	*Malus pumila* Mill.		киселачка
106	*Malus florentina* (Zuccagni) C. K. Schneid.		дива јаболка
107	*Pyrus communis* subsp. *pyraster* (L.) Ehrh.	*Pyrus pyraster* (L.) Burgsd.	дива круша
108	*Pyrus spinosa* Forssk.	*Pyrus amygdaliformis* Vill.	горница
109	*Ribes multiflorum* Roem. & Schult.		рибизла
110	*Ribes alpinum* L.		планинска рибизла
111	*Ribes petraeum* Wulfen		рибизла
112	*Genista radiata* (L.) Scop.		омелика
113	*Genista sericea* Wulfen		/
114	*Genista carinalis* Griseb.		/
115	*Genista januensis* Viv.		/
116	*Genista subcapitata* Panc.		/
117	*Genista sagittalis* L.		/
118	*Lembotropis nigricans* (L.) Griseb.	*Cytisus nigricans* L.	/
119	*Cytisus hirsutus* L.		заечка
120	*Cytisus procumbens* (Willd.) Spreng.		/
121	*Chamaecytisus pseudojankae* Pifko et Barina		/
122	*Chamaecytisus absinthioides* (Janka) Kuzmanov.		/
123	*Laburnum alpinum* (Mill.) Bercht. & J. Presl		доброцвет
124	*Hippocrepis emerus* subsp. *emeroides* (Boiss. & Spruner) Lassen	*Coronilla emerus* L.	заечка
125	*Colutea arborescens* L.		плускавец
126	*Ononis spinosa* L.		грмотрн
127	*Dorycnium herbaceum* Villar		/
128	*Astragalus angustifolius* Lam.		/
129	*Pistacia terebinthus* L.		смрделика
130	*Cotinus coggygria* Scop.		руј

Table 4 (continuation)

131	*Rhus coriaria* L.		гроздест руј
132	*Staphylea pinnata* L.		клокоч
133	*Acer tataricum* L.		жестил
134	*Acer pseudoplatanus* L.		горски јавор
135	*Acer heldreichii* Orph.		планински јавор
136	*Acer obtusatum* Willd.		глувач
137	*Acer hyrcanum* subsp. *intermedium* (Pančić) Bornm.	*Acer intermedium* Pančić	/
138	*Acer platanoides* L.		млеч
139	*Acer monspessulanum* L.		маклен
140	*Acer campestre* L.		клен
141	*Aesculus hippocastanum* L.		див костен
142	*Cornus mas* L.		дрен
143	*Cornus sanguinea* L.	*Thelycrania sanguinea* (L.) Fourr.	песји дрен
144	*Hedera helix* L.		бршлен
145	*Euonymus europaeus* L.		курика
146	*Euonymus latifolius* (L.) Mill.		широколисна курика
147	*Euonymus verrucosus* Scop.		брадавичеста курика
148	*Rhamnus cathartica* L.		кркавина
149	*Rhamnus rhodopea* Velen.		кршика
150	*Rhamnus alpina* subsp. *fallax* (Boiss.) Maire & Petitm	*Rhamnus fallax* Boiss.	љигавина
151	*Rhamnus saxatilis* Jacq.		кркавиња, камењарка
152	*Frangula rupestris* Schur	*Rhamnus rupestris* Scop.	кршика
153	*Frangula alnus* Mill.		трушлика
154	*Paliurus spina-christi* Mill.		чалија
155	*Vitis vinifera* L.	*Vitis sylvestris* C. C. Gmel.	дива лоза
156	*Viscum album* subsp. *austriacum* (Wiesb.) Vollm.	*Viscum laxum* Boiss. & Reut.	имела
157	*Loranthus europaeus* Jacq.		имела
158	*Arceuthobium oxycedri* (DC.) M. Bieb.		имела
159	*Comandra umbellata* subsp. *elegans* (Spreng.) Piehl	*Comandra elegans* (Spreng.) Rchb.	/
160	*Elaeagnus angustifolia* L.		дафина
161	*Fraxinus ornus* L.		црн јасен
162	*Fraxinus excelsior* L.		бел јасен
163	*Fraxinus angustifolia* Vahl.		полски јасен
164	*Phillyrea latifolia* L.	*Phillyrea media* L.	грипа
165	*Ligustrum vulgare* L.		калина
166	*Jasminum fruticans* L.		сурџел
167	*Vinca major* L.		/
168	*Satureja montana* L.		чубрика
169	*Viburnum lantana* L.		црна удика
170	*Viburnum opulus* L.		црвена удика
171	*Lonicera formanekiana* Halácsy		/
172	*Lonicera caprifolium* L.		анамски раце
173	*Lonicera etrusca* Santi		заплетина
174	*Sambucus nigra* L.		бозел

Table 4 (continuation)

175	*Solanum dulcamara* L.		пасквица
176	*Humulus lupulus* L.		хмељ
177	*Artemisia alba* Turra	*Artemisia lobelii* All.	пелин
178	*Asparagus acutifolius* L.		спаражина
179	*Ruscus aculeatus* L.		кострика
180	*Hyssopus officinalis* subsp. *aristatus* (Godr.) Nyman		/

CONCLUSIONS

The occurrence of a large number of woody species on Galičica Mountain and the island of Golem Grad is due to many factors: geographical position, the north-south stretching direction, influence of the Mediterranean climate coming through the valley of the river Crn Drim, demographic, antropozoogenic, cultural, historical and other factors.

Stemming from data of numerous previous works, as well as from the results obtained in this paper, some ideas for future tasks of dendrofloristics research related to this mountain massif have come up:

– Because of the large number of woody species and in some cases their morphological variability, there are many uncertainties left regarding the taxonomy, especially of lower taxa, such as subspecies, varieties and forms; further research is needed to generate relevant data in order to resolve these issues;

– in certain taxa such as *Sorbus* sp, *Rosa* sp, *Crataegus* sp, *Rubus* sp etc. the systematics to the level of species within each genus is very complex, due to the large variability of morphology and other characteristics of the species. But with new technologies and scientific knowledge, such as DNA analysis, in the future, it should be easier and safer to determine the nature of the variability, and consequently the systematic affiliation of these taxa.

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ДЕНДРОФЛОРАТА НА ПЛАНИНАТА ГАЛИЧИЦА И ОСТРОВОТ ГОЛЕМ ГРАД ВО РЕПУБЛИКА МАКЕДОНИЈА

Јане Ацевски[1], Дејан Манцуковски[2]

[1]Шумарски факултет, Универзитет „Св. Кирил и Методиј“, Скопје, Република Македонија
[2] Јавно претпријатие „Македонски шуми“, Скопје, Република Македонија

Во овој труд се презентирани податоци за застапеноста на дрвните видови по форма на хабитус, флорен елемент и еколошка валенца присутни на планината Галичица и островот Голем Град кои се составен дел од Националниот Парк Галичица. Податоците презентирани во трудот се темелат на истражувањата преземени во период од 1995 до 2018 година. Според овие истражувања автохтоната дендрофлора на планината Галичица и Островот Голем Град се состои од 180 видови, што претставува 56 % од вкупната дендрофлора на Република Македонија.

Клучни зборови: дендрофлора; форма на хабитус; флорен елемент; еколошка валенца; Галичица; остров Голем Град

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Original scientific paper

ANISANTHA DIANDRA (ROTH) TUTIN AND *OCHLOPOA INFIRMA* (KUNTH) H. SCHOLZ - NEW SPECIES OF POACEAE FAMILY IN REPUBLIC OF MACEDONIA

Mitko Kostadinovski[1], Renata Ćušterevska[1], Vlado Matevski[1,2]

[1]Institute of Biology, Faculty of Natural Sciences and Mathematics, Sts. Cyril and Methodius University, Skopje, Republic of Macedonia
[2]Macedonian Academy of Sciences and Arts, Skopje, Republic of Macedonia

e-mail: makostadinovski@gmail.com

The horological data for two new plant species of the flora of the Republic of Macedonia are presented - *Anisantha diandra* (Roth) Tutin and *Ochlopoa infirma* (Kunth) H. Scholz (Poaceae). The species *Anisantha diandra* is registered in several localities in the Republic of Macedonia (Skopje, Ohrid, Tetovo), while the species *Ochlopoa infirma* was found only in the vicinity of Bogdanci. A short description is given of the differential morphological characteristics of both species, as well as the habitats to which they are registered. Both species can be considered as native autochthonous for the flora of the Republic of Macedonia.

Key words: *Anisantha diandra*, *Ochlopoa infirma*, Poaceae, Flora, Distribution, Republic of Macedonia

INTRODUCTION

The Poaceae family is not yet processed in the edition "The Flora of the Republic of Macedonia". It is one of the unresearched groups of vascular plants on the territory of the Republic of Macedonia. Besides the comprehensive contributions to the genera *Poa* and *Festuca* [1–5], in the last 30 years, certain data on the distribution of several rare taxa from this family are cited in the literature [6–9].

In the paper, new horological data of the species *Anisantha diandra* (Roth) Tutin and *Ochlopoa infirma* (Kunth) H. Scholz for the flora of the Republic of Macedonia are presented. Both genera have complicated nomenclatural history [10–13] and cover a relatively small number of annual plants, mainly prevalent in the wider area of the Mediterranean.

EXPERIMENTAL SECTION

Herbarium specimens of the species *Anisantha diandra* and *Ochlopoa infirma* from the Macedonian National Herbarium at the Faculty of Natural Sciences and Mathematics in Skopje (MKNH) were used, which provide basic geographical and environmental data about the localities and habitats where they were collected.

When determining the herbarium material, appropriate literature was consulted (Prodromus Florae peninsulae Balcanicae, III [14]; Flora Europaea, V [15, 16], and other regional floras). The taxonomy and nomenclature is consistent with Euro+Med Plant base [17, 18]).

Besides that, an attempt has been made to determine the level of nativeness of each species according to the CABI site - Invasive Species Compendium [19], as well as their status of endangerment in Europe, according to IUCN [20].

RESULTS AND DISCUSSION

Anisantha diandra(Roth) Tutin

[Syn.: *Bromus diandrus* Roth; *Bromus gussonei* Parl.]

Mk Skopje: Settlement Aerodrom, Reonski Centar, ruderal habitat; 41°58'40.71"N, 21°28'37.54"E, 23.05.2018 (leg. et det. M. Kostadinovski) (MKNH);

Skopje: Settlement Aerodrom, Reonski Centar, in front of the market "Kam". 41°58'47.82"N; 21°28'28.87"E 235 m. 4.06.2010 (leg. et det. M. Kostadinovski) (MKNH); Ohrid: Ljubanište, ruderal habitat next to the camp, 40°55'24.85"N; 20°45'52.06"E, 25.06.2008 (leg. V. Matevski & M. Kostadinovski) (MKNH); Tetovo: village Sarakino, the right bank of the river Vardar. 12.09.2010; 41°58'45.48"N; 21° 2'54.35"E 4016 m (leg. et det. M. Kostadinovski) (MKNH).

The genus *Anisantha* is widely distributed in Europe, which includes annual herbaceous plants. Key differential morphological characteristics in which the genus *Anisantha* is differentiated from the genus *Bromus* is the appearance of the spikelet (which are wider towards the top than in the middle), as well as the length of the awn (which is longer than the length of the karyopsis). The genus *Anisantha* in Europe is represented by 10 species, from which the species *A. sterilis* and *A. tectorum* have been known in Macedonia so far [15, 17].

The species *Anisantha diandra* spreads to the Mediterranean and Southwestern Europe (Baleares, Corsica, Crete, France, Greece, Spain, Italy, the former Yugoslavia), Portugal, Sardinia, Sicily, Tunisia [*Az Be Br Rs (K)] [15, 17]. This species is also cited as a native for North Caucasus, the north of the European part of the Russian Federation and Ukraine [17]. In some countries, such as England, Germany, Ireland, Belgium and Luxembourg and others, the species is introduced, and in other countries it may also have invasive status (Syria, Jordan, Australia, Mexico and others) [19].

So far there is no assessment of the status on the IUCN Red List for Europe [20].

Anisantha diandra is very similar to the widespread species *A. sterilis*, which often comes to the same habitat. This similarity may be the reason that so far the species *A. diandra* has been unnoticed. *A. diandra* from *A. sterilis* separates with the larger dimensions of the elements of the spikelet and the flower, especially with larger glume, lemma and lemma's awn. Quite clear differences are also found in the characteristics of sheaths indumentum and the leaves, as well as the stem directly below the inflorescence.

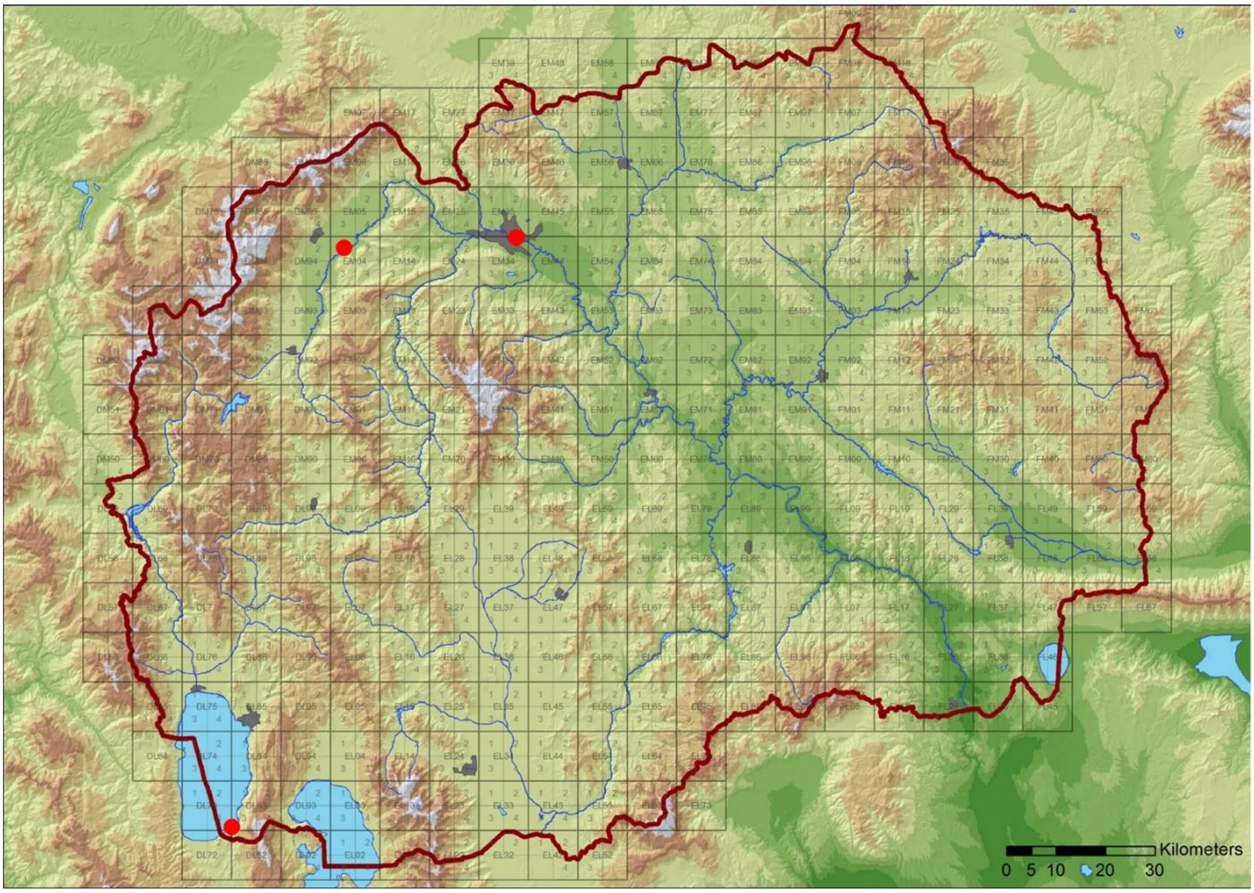

Figure 1. *Anisantha diandra* – Map of distribution in the Republic of Macedonia

Herbarium specimens of *A. diandra* were collected in the city of Skopje (Settlement Aerodrom, the district of Reonski Centar), in the vicinity of the village of Sarakino (Tetovo) and along the coast of Ohrid Lake near the village of Ljubaniste (Ohrid) (Figure 1). It grows in ruderal habitats, often near to the water bodies. In pioneering ruderal communities in which it occurs in Skopje, dominant species are *Cynodon dactylon*, *Chenopodium album*, *Anisantha sterilis*, *A. tectorum*, *Diplotaxis muralis* and others. For now, it is not possible to determine with certainty the degree of nativity, although its range in Europe ([15], [17]), and the environmental requirements, suggest that the species is considered as native in the flora in Macedonia.

***Ochlopoa infirma* (Kunth) H. Scholz**

[Syn. *Poa infirma* Kunth in Humb.; *P. annua* subsp. *exilis* (Tommasini) Murb.)].

Mk Bogdanci: near to the damp Paljurci (13.04.2001; 12.04.2003 leg. V. Matevski); Bogdanci: next to the river Gabroška Reka in the zone with *Quercus coccifera*; (41°13'11.16"N; 22°35'56.44"E; 117 m, 12.03.2011, leg. M. Kostadinovski).

The genus *Ochlopoa* in Europe is represented by eight species of annual plants [16], [17]. Interestingly, in Euro+Med Plant Base [17], not a single species of this genus is mentioned for Macedonia, although the species *O. annua* is very often plant in various grassland phytocenoses in our country ([21], [22], [23], [24]).

The species *Ochlopoa infirma* spreads mainly in the Mediterranean region of Europe, Africa and Asia (including the Black Sea), Portugal and the United Kingdom. With the status of introduced species is in Austria [19].

The species is still not endangered according to IUCN Red List in Europe.

In the Republic of Macedonia, the species has been registered on several occasions in the vicinity of Bogdanci. It develops on trampled habitats and other open habitats in the pseudomaquis belt, which are strongly with Mediterranean influence. From the nearby *O. annua* it differs with the spikelets with rather distant florets, and with the smaller anthers [16].

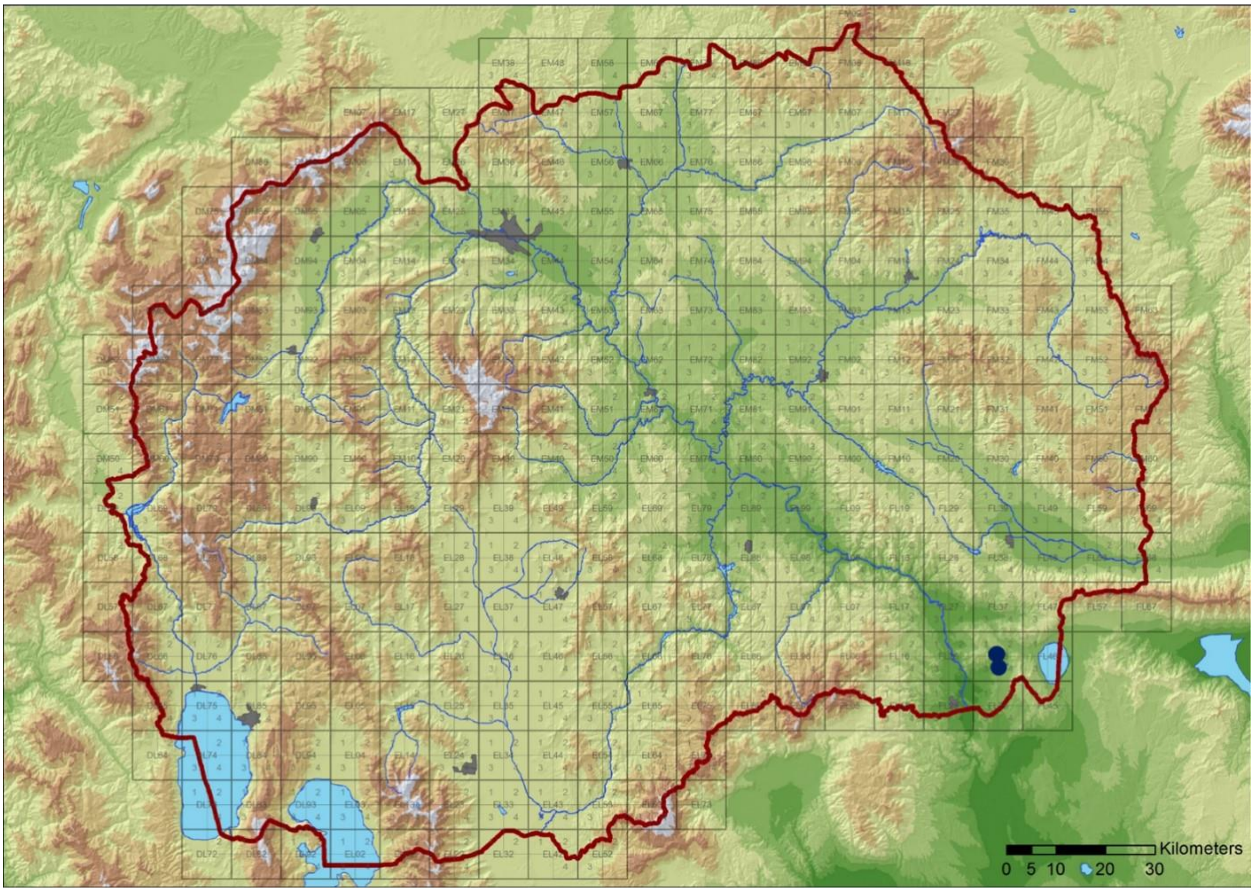

Figure 2. *Ochlopoa infirma* – Map of distribution in the Republic of Macedonia

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ANISANTHA DIANDRA (ROTH) TUTIN И *OCHLOPOA INFIRMA* (KUNTH) H. SCHOLZ - НОВИ ВИДОВИ ОД ФАМИЛИЈАТА POACEAE ВО ФЛОРАТА НА РЕПУБЛИКА МАКЕДОНИЈА

Митко Костадиновски[1], Рената Ќуштереска[1], Владо Матевски[1,2]

[1]Институт за биологија, Природно-математички факултет, Универзитет „Св. Кирил и Методиј“, Скопје, Република Македонија

[2]Македонска академија на науките и уметностите, 1000 Скопје, Република Македонија

За прв пат за флората на Република Македонија се наведуваат податоци за хорологијата на видовите *Anisantha diandra* (Roth) Tutin и *Ochlopoa infirma* (Kunth) H. Scholz (Poaceae). Видот *Anisantha diandra* е регистриран на неколку локалитети во Република Македонија (Скопје, Охрид, Тетово), додека видот *Ochlopoa infirma* е најден само во околината на Богданци. Даден е краток опис на диференцијалните морфолошки карактеристики на двата вида, како и на живеалиштата (хабитатите) на кои се регистрирани. И двата вида можат да се сметаат за автохтони за флората на Република Македонија.

Клучни зборови: *Anisantha diandra*; *Ochlopoa infirma*; Poaceae, флора; распространување; Република Македонија