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CONTENTS

CONTRIBUTION OF ORGANICALLY GROWN SPELT TO THE GRAIN QUALITY

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Abstract

Organic agriculture is capable to contribute to the production of healthier food. Organically produced cereals, such as spelt, represent the potential source of quality and healthy components for human food. Spelt wheat is one of the husked hexaploid wheat grown for centuries within a low-input technology. The nutritive value of spelt is high and it contains all the basic components which are necessary for human nutrition. Spelt is high in digestible proteins, vitamins, minerals and antioxidants. Differences may occur due to the growing place and season, cultivation, fertilizers etc. The content of nutritionally important minerals (Fe, Mg, Zn, Ca, Mn) and some antioxidants were analyzed in spelt wheat grown during four different seasons and in semiarid conditions. The spelt wheat was organically grown in the period 2012-2015, at Maize Research Institute Zemun Polje. The crop was sown on the area of 0.33 ha and after harvesting, grain yield was measured from all production area and calculated with 14% of moisture. Then, grains were milled and content of nutrients Mg, Fe, Mn, Ca and Zn, as well as inorganic phosphorus (Pi), phytic phosphorus (P_{phy}), phenolics, β-carotene and glutathione (GSH), were determined in grains. Obtained data were processed by analysis of variance (ANOVA) and differences with $p<0.05$ were considered as significant. Among growing seasons, the highest spelt yield was observed in 2012 (4200 kg ha^{-1}). The variations in content of minerals and antioxidants followed variations in sum of precipitation, as well as average air temperature. The content of Mg was significantly higher in 2015, while the Ca content was lower. Concentration of Zn and Mn continuously decreased from 2012 to 2015. The highest content of almost all investigated antioxidants was observed mostly in 2013. GSH and β-carotene content variations were also affected by the meteorological conditions of the growing season.

Keywords: *Triticum aestivum* subsp. *spelt*, organic, minerals, antioxidants.

Introduction

Organic agriculture as a component of sustainable systems is capable to contribute healthier food production in comparison to conventional and conservation agriculture (Azadi et al. 2011). This system is holistically defined by the American Public Health Association (APHA, 2007) as "one that provides healthy food to meet current food needs while maintaining healthy ecosystems that can also provide food for generations to come with minimal negative impact to the environment. A sustainable food system also encourages local production and distribution infrastructures and makes nutritious food available, accessible, and affordable to all". Organically produced cereals represent the potential source of quality and healthy components for human food (Dragičević et al. 2014), while conventionally produced row materials carry a continuous risk of unallowed content of pesticide residues or pathogens exudates. Spelt wheat (*Triticum aestivum* subsp. *spelta*) is one of the husked hexaploid wheats which possesses the same genomes as bread wheat (*Triticum aestivum* L.) (Yan et al. 2003). Spelt wheat is an old European crop, grown for centuries, and a low-input plant, suitable for growing without the use of pesticides. Even with low fertilizing, spelt wheat gives a good harvest and has a better mineral uptake in comparison with *Triticum aestivum* L. (Bojnanská and Francáková 2002). Due to the high consumption of wheat in a variety of food products all over the world, wheat is considered an important source of minerals. The nutritive value of spelt wheat is high and it contains all the basic components which are necessary for human beings (Bojnanská and Francáková 2002). Spelt is high in digestible proteins, vitamins and minerals. Differences may occur

due to the growing place and season, cultivation, fertilizers (Puumalainen et al. 2002) and higher levels of several minerals were observed in organically grown spelt in comparison to production carried out in conventional systems. This indicates that organic conditions with agroecologicaly adapted variety may enhance mineral concentration in spelt grain. Compared to wheat, spelt has higher concentration of proteins and vitamins, better content of amino acids, starch and sugar and more desirable fiber content (Kohajdova and Karovičova 2008). On average, spelt has 30-60% higher concentrations of Fe, Zn, Cu, Mg, and P, which is most pronounced in fine bran and coarse bran, where cereal minerals are naturally concentrated (Ruibal-Mendieta et al. 2005). Ranhorta et al. (1995) found that spelt grain was higher in P (by 19%), Fe (by 20%), K (by 7%) and Zn (by 91%) in comparison to the hard red winter wheat. In contrast to minerals, and especially P, the phytic acid content tends to be 40% lower in spelt than in wheat, as indicated by our data obtained in fine brans, where aleuronic cells, which naturally contain phytic acid (Lopez et al. 2002), are the most concentrated (Ruibal-Mendieta et al. 2005). The content of nutritionally important minerals (Fe, Mg, Zn, Ca, Mn) and some antioxidants were analyzed in spelt wheat grain grown during four different seasons and in semiarid conditions.

Material and methods

The spelt was organically grown in the period 2012-2015, at Zemun Polje (44°52'N 20°20'E), on a slightly calcareous chernozem, with 53.0 % sand, 30.0 % silt, 17.0 % clay, 3.3 % organic matter, 7.0 pH KCl and 7.17 pH H_2O. After conversion of soil, spelt wheat was rotated with maize and soybean. The crop was sown on October 25 2011, November 9 2012, November 14 2013, and again November 14 2014, on the area of 0.33 ha. After harvesting, grain yield was measured from all production area and calculated with 14% of moisture. Then, grains were milled and content of nutrients Mg, Fe, Mn, Ca and Zn, as well as inorganic phosphorus (Pi), phytate (Pphy) – as factor which affect availability of mineral nutrients, phenolics, β-carotene – as factor which promotes availability of mineral nutrients, and glutathione (GSH) were determined. Total glutathione (GSH) was determined by the method of Sari Gorla et al. (1993), water soluble phenolics were determined by the method of Simić et al. (2004) and expressed in µg of *3-hydroxy-4-methoxycinnamic acid* g^{-1} and yellow pigment (YP) was determined by the American Association of Cereal Chemists Method (AACC, 1995) and expressed in µg of β-carotene g^{-1}. Significant differences between means of treatments were determined by the Fisher's least significant difference (LSD) test at the 0.05 probability level, after the analysis of variance (ANOVA) which was conducted by using two-factorial RCB design. Differences with $p<0.05$ were considered as significant. Interdependence between the grain yield of spelt and examined antioxidants were processed by regression analysis. *Meteorological conditions:* The vegetative period for spelt production in the first year, 2011-2012, received the lowest amount of precipitation and had the lowest average temperature, Table 1.

Table 1. Meteorological conditions during period of investigation

Months	X	XI	XII	I	II	III	IV	V	VI	VII	Aver./Sum
Temperatures											
2011-2012	12.1	4.4	5.5	2.7	-2.5	10.1	14.4	17.9	24.6	27.1	11.6
2012-2013	15.4	11.1	2.0	3.3	4.6	6.6	14.9	19.7	21.9	23.8	12.3
2013-2014	15.3	10.1	3.2	5.3	7.8	10.8	13.7	17.4	21.1	23.2	12.8
2014-2015	14.1	9.7	3.8	3.3	4.2	8.1	12.9	19.1	22.1	26.4	12.4
Precipitation											
2011-2012	26.7	2.7	41.9	64.3	33.5	10.7	56.2	58.5	14.8	19.8	329.1
2012-2013	41.3	24.6	47.1	80.8	51.9	96.2	14.9	93.9	37.8	16.0	504.5
2013-2014	21.9	25.4	5.2	30.7	19.9	46.9	84.8	192.5	71.2	187.4	685.9
2014-2015	56.6	10.5	41.3	46.7	44.0	99.1	19.7	97.8	31.1	7.2	454.0

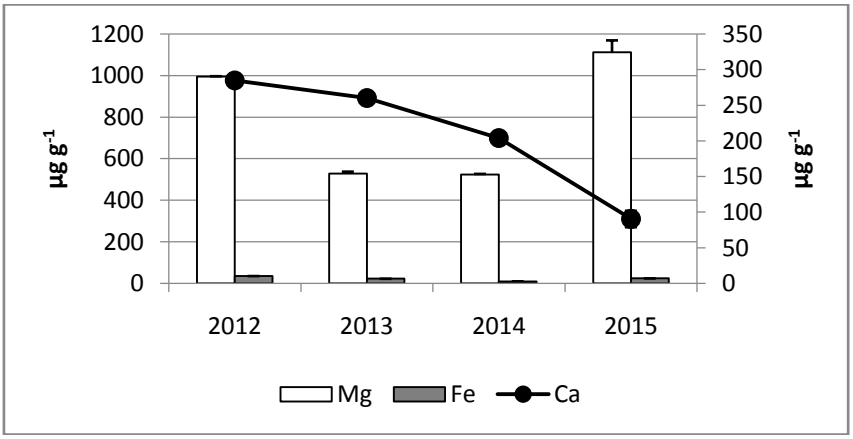
The 2011 year could be considered as a relatively moderate, with lower average temperatures and higher amounts of monthly precipitation than in 2012. The specific year, 2012, was unfavourable for

plant production generally and with unequal distribution of precipitation followed by the high average temperatures i.e. drought stress. Years 2013 and 2014 were pretty different and especially 2013-2014 period of spelt growth received a high amount of precipitation. Oppositely, 2015 could be considered as a relatively dry.

Results and discussion

The highest spelt yield was observed in 2012 (4200 kg per ha) while in three other years it was 3656.7 kg per ha in 2013, 2011.3 t per ha in 2014 and only 1800.0 kg per ha in 2015. The growing conditions of the cultivation year were segregating factor for grain yield and mineral content of elements (Mg, Fe, Ca, Mn and Zn) in spelt grain. This is also observed in previous studies (Wojtkowiak and Stepien 2015). Spelt wheat was observed to have significantly higher values for minerals and antioxidants in grains as a result of crop rotation and meteorological conditions, Figure 1 A and B.

A



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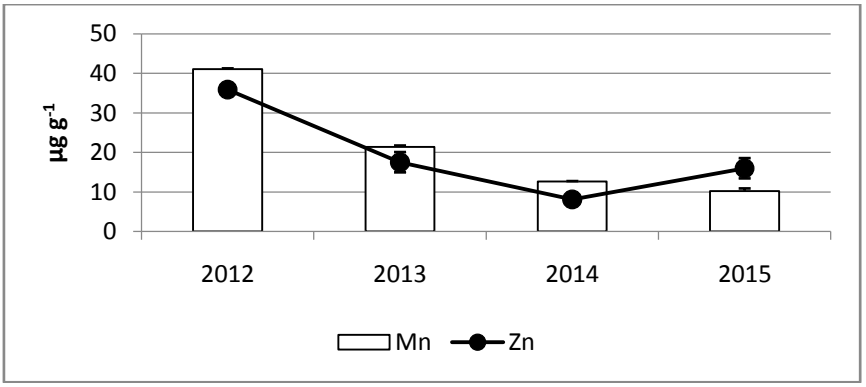
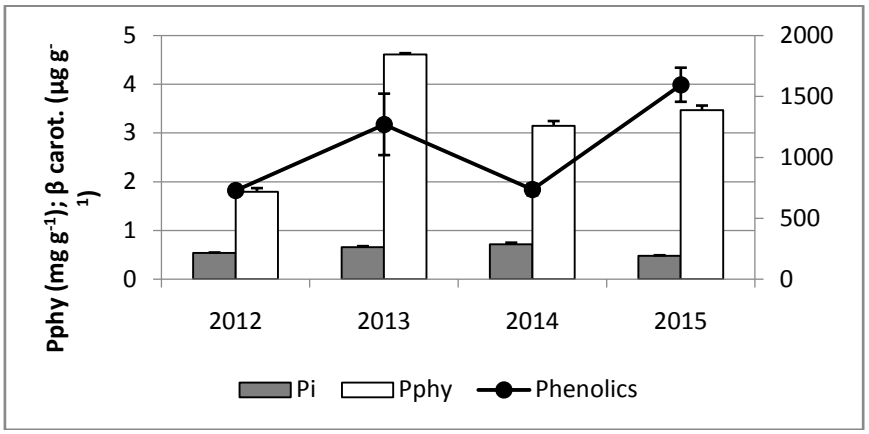


Figure 1 A and B. The content of analyzed minerals Fe, Mg, Zn, Ca and Mn in spelt grain in the period of 2012 to 2015

This study showed that a very high mineral concentration, close to daily requirements, can be produced by growing specific traditional wheat genotypes in an organic farming system. Thanks to spelt grain composition, the the main minerals are concentrated in fine bran and coares bran. Their content is 30-60% higher than in wheat (Riubal-Mendieta et al. 2005). The content of Mg was significantly increased in 2015 while the content of Ca was lowered. Fe was present in spelt grain at almost the same concentration in each year and had a lowest concentration together with Mg in 2014. The content of other two elements, Zn and Mn continuously decreased from 2012 to 2015 and Mn was the lowest in the last year. Lower Zn concentration in 2014 and 2015 was possible induced by better P absorption (Ryan et al., 2004), what is evidenced by the highest P_{phy} and Pi concentration in maize grain (Dragičević et al. 2014). Results also showed that even though phosphorus content

was higher phytic acid content showed the opposite trend and was 40% lower in spelt versus wheat fine bran, which may suggest that spelt has either a higher endogenous phytase activity or a lower phytic acid content than wheat (Riubal-Mendieta et al. 2005). Antioxidants level followed variations in sum of precipitation as well as average air temperature. The highest content of almost all investigated antioxidants was observed mostly in 2013. P_{phy} had the highest value in 2013 and phenolics in 2015, Figure 2 A. The concentration of phenolics and P_{phy} in spelt grain were increased in 2013 and 2014 compared to 2012 and 2015 which is probably connected with meteorological conditions and drought occurrence in 2012 and 2015. The concentration of inorganic phosphorus (Pi) in spelt grain was similar in all years but pretty lower than content of other two phytic components. GSH and β-carotene contents were also induced by the meteorological conditions of the year, Figure 2 B.

A



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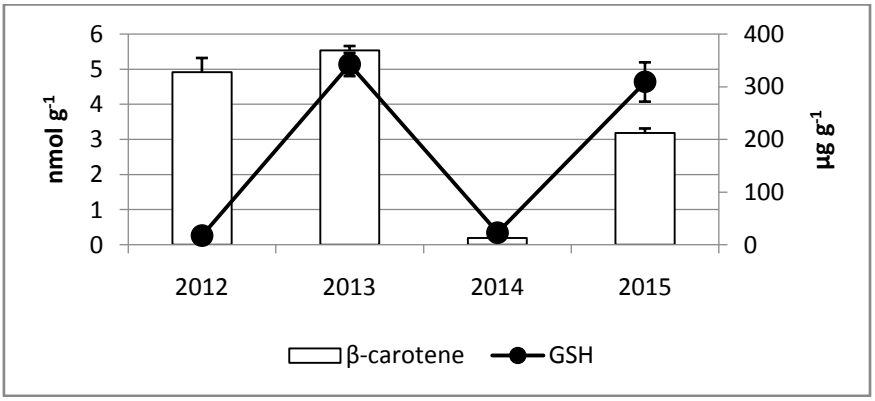

Figure 2 A and B. The content of analyzed minerals Fe, Mg, Zn, Ca and Mn in spelt grain in the period of 2012 to 2015

β-carotene which promotes bioavailability of mineral elements, was present in spelt grain in the higher content in 2012 and 2013, while its content significantly decreased in 2014 which was the season with the highest amount of precipitation – 685.9 mm. During next season, 2014-2015 content of β-carotene in spelt grain, have increased again. Plant products usually contain inhibitors, like phytate, polyphenolics, etc., which obstruct the absorption or utilization of mineral elements. According to results obtained from organic production of maize in the agroecological conditions of Zemun Polje, very important is to decrease content of inhibitors in foods and to increase content of promoters and the good way for that is balanced and adequate application of specific fertilizers (Dragičević et al. 2014).

Table 2. The significance of relations between investigated minerals and antioxidants in spelt grain (average 2012-2015)

Variables	Yield	Pi	Pphy	Phen.	GSH	β-carot.	Ca	Mg	Fe	Zn	Mn	Temp.
Pi	-0.008											
Pphy	-0.245	0.344										
Phen.	-0.377	-0.506*	0.620*									
GSH	-0.143	-0.253	0.816*	0.924*								
B - carot.	0.779*	-0.400	0.117	0.284	0.455							
Ca	0.880*	0.435	-0.220	-0.690*	-0.403	0.428						
Mg	-0.044	-0.975	-0.519*	0.346	0.048	0.245	-0.433					
Fe	0.711*	-0.708*	-0.455	0.055	0.034	0.808*	0.323	0.663*				
Zn	0.684*	-0.692*	-0.626*	-0.099	-0.159	0.683*	0.341	0.693*	0.979			
Mn	0.912*	-0.187	-0.620*	-0.539*	-0.439	0.601*	0.787*	0.214	0.790*	0.838*		
Temp.	-0.791*	0.578*	0.588*	0.177	0.178	-0.735*	-0.481	-0.570*	-0.973	-0.987	-0.904*	
Precip.	-0.645*	0.768*	0.472	-0.099	-0.045	-0.774*	-0.240	-0.728*	-0.996	-0.979	-0.746*	0.958

*- Values significant according to LSD-test

Statistical analysis showed positive and negative correlations between investigated components of spelt grain under the influence of meteorological parameters, Table 2. The content of almost all investigated antioxidant except β-carotene negatively correlated with grain yield of spelt while microelements content was positively connected with grain yield, except Mg concentration. Very interesting is that, in average for all years of investigation, content of Mg and inorganic phosphorous in spelt grain was negatively correlated (- 0.975) which suggested that if inorganic phosphorus is present in higher concentration, the content of Mg is lower. Concentration of Zn and Fe were correlated positively (0.979). And, concentrations of Zn and Fe were negatively correlated with average air temperature (-0.973 and -0.978) and sum of precipitation (-0.966 and -0.979) during period of investigation. It was already mentioned that GSH and phenolics play an important role in spelt nutritional quality (Dragičević et al. 2013). Phenolics and phytate participation in spelt grain were in significant, positive correlation (0.620*) while GSH content was significantly and highly connected to Pphy and phenolics (0.816* and 0.924*).

Conclusions

Organic conditions with agro-ecologically suitable variety may enhance mineral and antioxidants concentration in spelt wheat grain. The growing conditions of the cultivation year were segregating factor for grain yield, mineral content of elements (Mg, Fe, Ca, Mn and Zn) and content of antioxidants (Pi, Pphy, phenolics, β-carotene and GSH) in spelt grain. Statistical analysis showed positive and negative correlations between investigated components of spelt grain and under the influence of meteorological parameters such as Zn and Fe concentrations which were negatively correlated with average air temperature and sum of precipitation.

Acknowledgments

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EFFECTS OF pH ON HUE ANGLE AND VISIBLE ABSORPTION MAXIMA OF CYANIDIN

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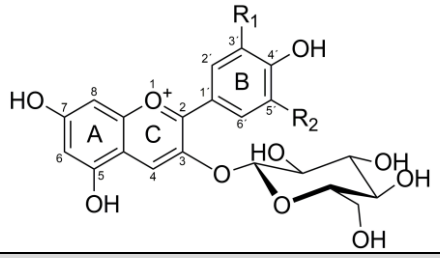
Abstract
As a major sub-group of flavonoids, anthocyanins are water soluble plant pigments responsible for the blue, purple and red color of many plant tissues. They occur primarily as glycosides of their respective aglycone anthocyanidin. Cyanidin is the most abundant anthocyanidin in fruits and vegetables (50%). In recent years, the interest in the properties and stability of anthocyanin extracts has increased. However, there remain little data in the literature relating to the properties and stability of pure anthocyanins, and especially of the anthocyanidins. The aim of the study was to compare under various pH conditions visible absorbance maxima ($\lambda_{max\text{-}vis}$) with the corresponding hue angle (h_{ab}) values of cyanidin as a color reference. Thus cyanidin aqueous solution was subjected to spectroscopic and colorimetric study to examine influence of pH value on h_{ab} and $\lambda_{max\text{-}vis}$. The cyanidin had reddish nuances at the lowest pH values. By stepwise pH increase the colour of cyanidin is gradually changed toward magenta and lilac tones, and then with further pH increase to more reddish nuances. In alkaline region cyanidin showed yellow green tones, which is gradually changed toward yellow nuances with further pH increases. For the $\lambda_{max\text{-}vis}$ the following tendency was observed: as pH increased, the $\lambda_{max\text{-}vis}$ values of cyanidin showed the bathochromic shift. The cyanidin solution exhibited variations of h_{ab}, although their $\lambda_{max\text{-}vis}$ remained stable. By contrast, solutions having shifted spectra share the same basic tonality. Measurement of the h_{ab} and the $\lambda_{max\text{-}vis}$ showed that these values were highly pH dependent. Thus we can conclude that the $\lambda_{max\text{-}vis}$ values of these cyanidin solutions at the various pHs correlate poorly with their corresponding h_{ab} and caution should be applied when using $\lambda_{max\text{-}vis}$ values for interpretation of colours.

Keywords: cyanidin, hue angle, visible absorbance maximum, spectrophotometry, colorimetry.

Introduction

Anthocyanins are the most important groupof pigments, after chlorophyll that is visible to the human eye. Chemically, anthocyanins (fromthe Greek *anthos*, a flower, and *kyanos*, dark blue) are flavonoids (flavan like). The anthocyanidins are the basic structures of the anthocyanins. The anthocyanidins (or aglycons) consist of an aromaticring [A] bonded to an heterocyclic ring [C] that contains oxygen, which is also bonded by a carbon-carbon bond to a third aromaticring [B]. When the anthocyanidins arefound in their glycoside form (bonded to a sugar moiety) they are known as anthocyanins(Figure 1) (Castañeda-Ovando et al. 2009; Delgado-Vargas et al., 2000). There is a huge variety of anthocyanins spread in nature. Up to now there are reports of more than 500 different anthocyanins and 23 anthocyanidins of which only six are the most common in vascular plants, pelargonidin, peonidin, cyanidin, malvidin, petunidin and delphinidin (Figure 1) (Castañeda-Ovando et al. 2009). The differences between individual anthocyanins are the number of hydroxyl groups in the molecule; the degree of methylation of these hydroxyl groups; the nature, number, and location of sugars attached to the molecule; and the number and the nature of aliphatic or aromatic acids attached to the sugars in the molecule (Galvano et al. 2004). The glycoside derivatives of the three non-methylated anthocyanidins (cyanidin, delphinidin and pelargonidin) are the most common in

nature, being found in 80% of pigmented leaves, 69% in fruits and 50% in flowers. The distribution of the six more common anthocyanidins in fruits and vegetables is: cyanidin 50%, delphinidin 12%, pelargonidin12%, peonidin 12%, petunidin 7%and malvidin 7% (Castañeda-Ovando et al. 2009). Cyanidins are considered the widest spread anthocyaninin the plant kingdom. They are largely distributed in the human diet through crops, beans, fruits, vegetables and red wines, suggesting that we daily ingest significant amounts of these compounds from plant-based diets (Galvano et al. 2004). Anthocyanins are of great nutritional interest because of the marked daily intake (180 to 215 mg/day in the United States), which is much higher than the intake (23 mg/day) estimated for other flavonoids, including quercetin, kaempferol, myricetin, apigenin, and luteolin. They have been reported to have positive effects in the treatment of various diseasesand are prescribed as medicines in many countries (Galvano et al. 2004). In plant tissues the anthocyanins produce blue, purple, red and intermediate hues, and appear "black" in some commodities. Their hue and structure are dependent on pH value and the presence of copigments. It has been recognised for many years that anthocyanins make a significant contribution to the colour, and hence acceptability, of many fruits, some vegetables and associated products, including beverages and preserves. Subsequently it was recognised that anthocyanin-rich extracts might have potential as food additives (Clifford, 2000). Anthocyanins have been reported to be strong antioxidants, inhibit the growth of cancerous cells, inhibit inflammation, be vasoprotectors, and have anti-obesity effects. Many of the health benefits associated with berry fruit may be due to the high concentrations of anthocyanins that they contain (McGhie and Walton 2007). The addition of natural anthocyanin extracts, to give colour toprocessed foodstuffs, could be regarded in this contextas maintaining current levels, if not actually redressing the balance, which may be desirable in view of their beneficial effects (Bridle and Timberlake 1997). Most food nowadays is processed in some way before reaching the consumer, and manufacturers have a need to replace colour lost during processing or to colour products which would otherwise be colourless and unappealing. With increasing public concern about the safety of synthetic colorants, natural pigment extracts are assuming greater prominence (Bridle and Timberlake 1997). Carotenoids and anthocyanins are amongst the most utilised vegetable colorants in the food industry. Anthocyanins are water-soluble and they are extracted from grapes, berries, red cabbage, apples, radishes, tulips, roses and orchids, amongst others (Castañeda-Ovando et al. 2009). Anthocyanins (E163) have been approved for use infoods based on very limited toxicological data (Clifford, 2000). The most common way to indicate anthocyanin colour is based on presentation of visible absorbance maxima ($\lambda_{max\text{-}vis}$) from visible absorption spectra (Fossen et al. 1998). In order to examine possibility of application spectrophotometric as well as colorimetric measurement in food industry and eventual difference between the obtained data, we compared the spectrophotometric and colorimetric parameters obtained under the same conditions.



Anthocyanin	R_1	R_2
Pelargonidin 3-glucopyranoside (Pg3Glc)	H	H
Cyanidin 3-glucopyranoside (Cy3Glc)	OH	H
Peonidin 3-glucopyranoside (Pn3Glc)	OCH_3	H
Delphinidin 3-glucopyranoside (Dp3Glc)	OH	OH
Petunidin 3-glucopyranoside (Pt3Glc)	OCH_3	OH
Malvidin 3-glucopyranoside (Mv3Glc)	OCH_3	OCH_3

Figure 1. Structure of the six most common anthocyanidin 3-monoglucosides (Cabrita et al. 2000)

Material and methods

Chemicals and reagents

The chloride salt of cyanidin (2-(3,4-dihydroxyphenyl)chromenylium-3,5,7-triol chloride, CAS Number: 528-58-5, $C_{15}H_{11}O_6Cl$, molecular weight 322.7 g/mol) was from Polyphenols Laboratories AS (Sandnes, Norway). Hydrochloric acid and sodium hydroxide were obtained from Merck (Darmstadt, Germany). Aqueous solutions were prepared from Milli-Q water (resistivity >18 MΩ cm) (Millipore, Bedford, MA, USA).

Spectrophotometric and colorimetric measurements

The chloride salt of cyanidin was dissolved in Milli-Q water to 2×10^{-4} mol dm^{-3}. This solution was equilibrated in the dark at 25 °C, following the procedure of Brouillard et al. (Brouillardet al. 1982; Brouillard et al. 1978). Successive pH jumps of around 0.5 pH units (from pH 0.5 to 13.1) were achieved by a modified procedure to that described previously (Brouillard et al. 1982; Brouillard et al. 1978; Heredia et al. 1998; Hurtado et al. 2009). Briefly, after each addition of an aliquot (a few µL) of HCl or NaOH, the solutions were equilibrated for 5 min on a magnetic stirrer, and the pH was measured using a Seven Easy pH meter (Mettler Toledo, Schwerzenbach, Switzerlend) equipped with an InLab micro electrode (Mettler Toledo, Schwerzenbach, Switzerlend). The visible absorption spectra (380-900 nm) of the cyaniding solution was recorded at each pH at 25.0 ± 0.1 °C, using a Cary 100 Bio UV-visible spectrophotometer (Varian, Mulgrave, Victoria, Australia) in a thermostated 10-mm-path-length quartz cell, with Milli-Q water as the reference. Each spectrum had the solvent spectrum subtracted and was multiplied by the dilution factor. The hue angle (h_{ab}) for 2×10^{-4} mol dm^{-3}cyanidin solution was determined at each pH using a Konica Minolta CR-400 Chroma meter (Sensing, Inc., Osaka, Japan).

Results and discussion

Color expression of anthocyanins is dependent on the pH value (Brouillard 1982; Fossen et al. 1998; Heredia et al. 1998; Hurtado et al. 2009). Anthocyanins in rather strong acid solutions occur only as flavylium forms (Brouillard et al. 1982; Brouillard 1982; Torskangerpoll and Andersen 2005). However, when pH increases, each anthocyanin occurs as a mixture of various equilibrium forms (Brouillard et al. 1982; Brouillard 1982).The hue angle (h_{ab}), is the qualitative attribute of colour (Hurtado et al. 2009). The hue angle and the visible absorbance maximum in the aqueous solutions of cyanidin (2×10^{-4} mol dm^{-3}) were studied across the whole pH range (from pH 0.5 to 13). The cyanidin showed a reddish hue (31.41°) at the lowest pH value (pH 0.5) (Figure 2). By stepwise pH increase until pH 7.5, the colour of cyanidin is gradually changed toward magenta and purple tones (until h_{ab}=359.34°), and then with further pH increase until pH 8.8 the colour of cyanidin is changed to more reddish nuances (until h_{ab}=16.92°). At pH 9.6cyanidin showed greenish tones (h_{ab}=152.55°), which is with further pH increases gradually changed toward yellow nuances (81.36° at pH 12.5). Figure 3 shows visible absorbance maxima ($\lambda_{max\text{-}vis}$) of 2×10^{-4} mol dm^{-3} aqueous solution of cyanidin at the various pH values. The following tendency was was observed: as pH increased, the $\lambda_{max\text{-}vis}$ values of cyanidin showed the bathochromic shift. The $\lambda_{max\text{-}vis}$ of cyanidinaqueous solution shifted from 517 nm (at pH 0.5) to 599 nm (at pH 12.0) (Figure 3). In pH region 3.5 to 4.6 these shift were dramatic (from 525 to 550nm). Further increase in pH resulted in very gradually increase of $\lambda_{max\text{-}vis}$, until pH 8.8-9.6, were another bathochromic shift is observed (from 571 to 594nm). It has been postulated that as long as the $\lambda_{max\text{-}vis}$ is not shifting, the hue values is not changing. Our colorimetric analysis of cyanidin solutions (Figure 2) in comparison to the $\lambda_{max\text{-}vis}$ (Figure 3) resulted in different conclusions, the cyanidin solutions displaying huge variations of chromatic tonalities, although their spectral $\lambda_{max\text{-}vis}$ remained stable.

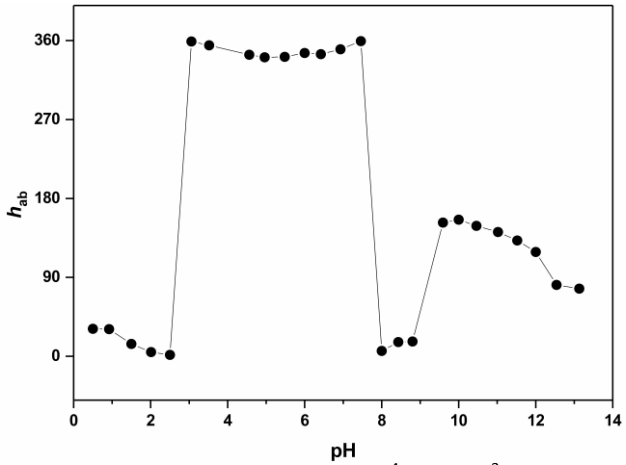


Figure 2. The hue angle (h_{ab},°) as a function of pH for the 2×10^{-4} mol dm^{-3}cyanidin aqueous solution.

For example, perceptually, the most remarkable variation for cyanidin solutions was the extent of the hue gamut covered by the cyanidin solution at pH 0.50-1.50 (sharing the same visible $\lambda_{max\text{-}vis}$, 517 nm): from orange-red hue (h_{ab}=31.41°) to a red basic hue (h_{ab}=14.05°) (Table 1). Solutions in the pH range 2.0-3.1 sharing the same $\lambda_{max\text{-}vis}$, (522-523 nm) while hues changes from red hue (h_{ab}=4.76°) to a magenta hue (h_{ab}=358.99°). With further pH increase at pH 7.5-8.0 solution again sharing the same visible $\lambda_{max\text{-}vis}$, (565 nm) while his hues ranged from magenta (h_{ab}=359.34°) to a red hue (h_{ab}=6.20°). In pH range 10.0-12.0 solutions sharing the same visible $\lambda_{max\text{-}vis}$, (599 nm) and his hues vary from green hue (h_{ab}=155.88°) to a yellow green hue (h_{ab}=118.89°). By contrast, solutions having shifted spectra share the same basic tonality. The cyanidin solutions with $\lambda_{max\text{-}vis}$ at 523 nm and 565 nm (pH 3.1 and 7.5 respectively) have the same basic tonality (h_{ab} 358.99-359.34°), and with $\lambda_{max\text{-}vis}$ at 550 nm, 559 nm and 562 nm (pH 4.6; 6.0 and 6.4 respectively) have the similar hues (h_{ab} 343.89-345.89°).

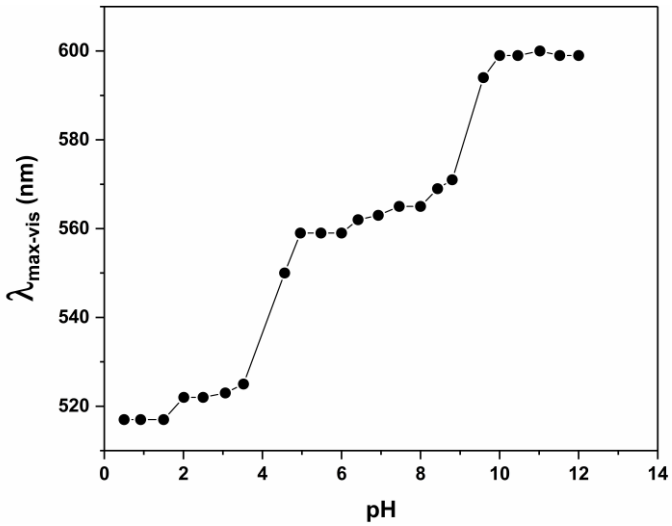


Figure 2. The visible absorbance maximum ($\lambda_{max\text{-}vis}$) values as a function of pH for the 2×10^{-4} mol dm^{-3}cyanidin aqueous solution.

Table 1. The influence of different pH values on visible absorbance maximum ($\lambda_{max\text{-}vis}$) and hue angle (h_{ab}) of $2 \cdot 10^{-4}$ mol·dm^{-3}cyanidin aqueous solution.

pH	$\lambda_{max\text{-}vis}$(nm)	h_{ab} (°)
cyanidin		
0.50	517	31.41
0.92	517	30.92
1.50	517	14.05
2.01	522	4.76
2.50	522	1.65
3.06	523	358.99
3.52	525	354.62
4.56	550	343.89
4.96	559	341.01
5.48	559	341.51
6.00	559	345.89
6.42	562	344.59
6.93	563	350.05
7.46	565	359.34
8.00	565	6.20
8.43	569	16.22
8.80	571	16.92
9.59	594	152.55
10.00	599	155.88
10.46	599	148.73
11.02	600	141.73
11.52	599	132.08
12.00	599	118.89
12.54		81.36
13.13		77.19

Conclusions

Measurement of the hue angle and the visible absorbance maximum showed that their values were highly dependent on the pH. The hue angle measurements of cyanidin aqueous solution showed that some measurable spectral variations did not correspond to a colour variation perceptible by the human visual system. Regarding the attribute of hue, the previous works unanimously considered a unique reference for anthocyanin solutions at different pH, their visible $\lambda_{max\text{-}vis}$. Consequently, when the $\lambda_{max\text{-}vis}$ remains stable, the "reference hue" was probably considered so. The colorimetric analysis of hue angle cyaniding aqueous solutions resulted in different conclusions. The solutions show huge variations in their chromatic tonalities, although their spectral $\lambda_{max\text{-}vis}$ effectively remained stable; then, *vice versa*, these solutions with shifted spectra share the same basic tonality, so $\lambda_{max\text{-}vis}$ of the cyanidin solutions at the various pHs correlated poorly with the corresponding h_{ab} and caution should be applied when using $\lambda_{max\text{-}vis}$ values for interpretation of colors.

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THE FREQUENCY OF MEAT CONSUMPTION AND BONE MINERAL DENSITY IN FEMALE POPULATION

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Abstract
The objective was to investigate the impact of the frequency of meat consumption on bone mineral density (BMD) in females. BMD was measured in 210 females by DEXA denzitometar. For the manner of nutrition a Questionnaire was used. The females were divided into 4 age groups, and 4 subgroups: those consuming meat on daily bases; 3-5 times/week; 1-2 times/week; and no consumers. Data analysis was performed by statistical program Statistica 7.1 for Windows and SPSS Statistics 17.0. The significance was determined by $p < 0.05$. 40-49 years old females, did not have significant differences in BMD no matter of the meat consumption frequency. 50-59 years old females who consumed meat 3-5 times weekly had significantly lower BMD compared to those with 1-2 weekly meat consumption ($p<0.001$) and to no consumers ($p<0.01$), while BMD was not significantly different between 1-2 weekly consumers and no consumers ($p>0.05$). 60-69 years old females on 3-5 times consumption had significantly lower BMD compared to no consumers ($p<0.001$) and 0.33 g/cm2 lower BMD compared to no consumers, which was significant difference ($p<0,001$). However, 1-2 weekly consumers had significantly (0.18 g/cm2) lower BMD compared to no consumers ($p<0.01$). Every day meat consumers > 69 had significantly lower BMD compared to 1-2 weekly consumers ($p<0,001$). Despite this, there was no significant difference in BMD between 1-2 and 3-5 weekly consumers and no consumers ($p>0.05$).The results suggest that frequency of consumption in age of 40-49, does not have negative impact on the BMD of females. In age of 50-59 and 60-69 frequent meat consumption significantly correlated with lower BMD. However, in age over 69, despite the findings that BMD of females on 1-2 weekly meat consumption was significantly higher than of those on everyday consumption, the correlation between BMD and meat consumption frequency was insignificant.

Keywords: Densitometry, questionnaire, age groups, meat consumption.

Introduction

Meat and its numerous species and products are important part of human diet and culture worldwide as a source of lipids, proteins with high biological capacity, trace elements and vitamins (Zang et al. 2010,Wyness et al. 2013). High levels of SFA were linked with cardiovascular diseases in the past, but resent data of Siri-Tarino et al. (2010), found no significant evidence for SFA and cardiovascular diseases association. Losses in high quality protein, especially in older adults, cause sarcopenia and sarcopenic obesity by replacing lost skeletal muscle into fat (Paddon-Jones and Leidy 2014). It has been demonstrated that collagen has a positive influence on the delivery and bioactivity of bone morphogenic protein-2 and ectopic bone formation, enhancing bone healing (Bhakta et al. 2013). Other beneficial effects on health by meat peptides include antihypertensive, antioxidant, antithrombotic, immunomodulatory, anticancer, and antimicrobial activities (Di Bernardini et al. 2011). However, what is the potential role of proteins, amino acids and peptides from meat and other type of food in bone health and bone mineral density (BMD) maintenance

through aging; whether recommended protein intakes should be increased in order to prevent or reduce osteoporosis and sarcopenia is the issue for scientists, clinitians, nutritionists and public health professionals. Bone health is multifactorial issue, it depends on genetics, BMD peak, sun exposure, lifestyle and exercise (Kanis, 2013). Amount and type of protein influences bone health. Protein has been identified as being both detrimental and beneficial to bone health, depending on a variety of factors, including the level of protein in the diet, the protein source, calcium intake, weight loss, and the acid/base balance of the diet. Loss of bone mass (osteopenia) or its heavier form (osteoporosis) and loss of muscle mass (sarcopenia) that occur with age are closely related and track together over the life span. Calcium and protein intake interact constructively to affect bone health. Intakes of both calcium and protein must be adequate to fully realize the benefit of each nutrient on bone. Concerns about dietary protein increasing urinary calcium appear to be offset by increases in absorption. Likewise, concerns about the impact of protein on acid production appear to be minor compared with the alkalinizing effects of fruits and vegetables. Perhaps more concern should be focused on increasing fruit and vegetable intake rather than reducing protein sources (Heaney and Layman 2008). Clinical studies do not support the idea that animal protein has a detrimental effect on bone health or that vegetable-based proteins are better for bone health (Kerstetter et al. 2003). There is increasing evidence that a higher protein intake may have beneficial effects on BMD (Heaney, 2001, Rapuri, 2003), and may reduce bone loss in patients with recent hip fracture (Schurch et al. 1998), particularly in the elderly (Hannan, 2000), and in those consuming adequate calcium levels. The researchers identified a positive association between dietary protein intake and change in bone mass density in those with the highest intake of protein who were supplemented with calcium and vitamin D. There was no benefit from supplementation among those with lower intakes of protein. (Dawson-Huges and Harris 2002, Whiting et al. 2002, Dawson-Hughes, 2003). The higher protein content in the calcium-rich DASH diet and sodium reduction improve the markers of bone turnover and calcium metabolism in adults (Lin et al. 2003). While protein seems to have a direct anabolic effect on bone, the relation between protein intake and bone is further complicated by the potential negative effect of overall dietary acid-base balance. A Western-type diet has been reported to be associated with osteoporosis and urinary calcium loss (Mauer et al. 2003). Urinary calcium has been found to be increased with acid-forming foods, such as meat, fish, eggs, and cereal, and negatively associated with plant foods and is likely determined by the acid-base status of the total diet. Bone loss may be attributable, in part, to the mobilization of skeletal salts to balance the endogenous acid generated from acid-forming foods (Spence and Weaver 2003). Moreover, it has been suggested that animal protein–based diets might have a greater negative effect on skeletal health than do vegetable-based diets (Sellimeyer et al. 2001) because dietary animal protein induces a greater increase in urinary calcium excretion than vegetable protein. In a large group of middle-aged and elderly women in China, urinary excretion of calcium was correlated positively with intake of animal protein (Zhao et al. 1993). Several studies examining the effect of meat have found no effect on either bone mineral density (BMD) or BMD markers. A 16-wk randomized crossover study of healthy postmenopausal women found that consuming a high-meat diet (297 g/d of meat), providing 117 g of protein, did not adversely affect urinary calcium excretion, calcium retention, or clinical indicators of bone formation and resorption compared with a low-meat diet (45 g/d of meat and 68 g of protein) (Roughead et al. 2003). Considering the contradictory findings in literature about the effect of animal proteins on BMD of female and male population; no data about the impact of nutrition on BMD; nor specific clinical trials conducted about the association of meat consumption and BMD of Macedonian population, the focus of this study was to investigate the link between the frequency of meat consumption and BMD in female population of Republic of Macedonia, and possibly answer to the question whether frequent meat consumption, might be recommended or denied for prevention of osteoporosis in females or in some of the female age subgroups.

Material and methods

BMD (in g/cm^2 and t-score) at lumbar spine was measured by Dual Energy X-ray Absorptiometry (DEXA) in 210 females. For the manner of nutrition a Questionnaire was used. The females were divided into 4 age groups (40-49; 50-59; 60-69; and > 69 years old), and 4 subgroups according to meat intake: those consuming meat on daily bases; 3-5times/week (t/w); 1-2 t/w; and no consumers (used as reference data). Data analysis for the whole female group no matter of age, and for every age subgroup separately was performed by statistical program Statistic 7.1 for Windows and SPSS Statistics 17.0. Numerical data (age), were analyzed by descriptive statistics (Mean; Std. Deviation; ±95. 00% CI; Minimum; Maximum). Data distribution was tested with Kolmogorov-Smirnov test; Lilliefors test; and Shapiro-Wilks test (p); BMD as dependent phenomenon; and age and frequency of meat consumption as independent phenomena ratio was tested by Multiple Regression analysis (R). The significance was determined by $p < 0.05$.

Results and discussion

Females categorized in 4 subgroups according to the meat consumption frequency (MCF) were analyzed in context of number/percentage (n/%) of females within a frequency subgroup who had normal level (NL) of BMD according to t-score values which are compatible with their age (t-score of 1 to -1); osteopenia (t-score of -1 to -2.5); osteoporosis (t-score bellow -2.5 to -3.5): and a heavy form (HF) of osteoporosis prone to spontaneous fractures (lower than -3.5 to -5) -Table 1 below.

Table 1. BMD level related to meat consumption frequency

MCF	NL		Osteopenia		Osteopor.		HF		Total	
	n	%	n	%	n	%	n	%		
Everyday	3	25	3	25	3	25	3	25	12	100
3-5 t/ w	14	16.9	25	30.1	28	33.7	16	19.3	83	100
1-2 t/w	22	22	47	47	12	12	19	19	100	100
No consum	6	40	3	20	6	40		/	15	100

Results showed that no meat consumers had the highest percentage of normal BMD level compared to females on everyday consumption, 3-5 t/w and 1-2 t/w consumption (40 % v. 25% v. 16.9% v. 22%); as well as they had the lowest percentage of osteopenia compared to other consumers subgroups (20% v. 25% v. 30.1% v. 47%). However, the percentage of osteoporosis occurrence was highest in no consumers subgroup but there was no evidence of heavy form of osteoporosis. The highest percentage of heavy form of osteoporosis was found in everyday meat consumes (25%). When we summarized the percentage of osteoporosis occurrence and its heavy form, the highest percentage was found in 3-5 t/w consumers. Therefore, these results suggest that frequent meat consumption on everyday basis and 3-5t/w does not prevent occurrence of osteoporosis and its heavy forms, thus the fracture risk. However, it should be noted that the level of calcium intake was not investigated in those females. Feskanich et al (1996) reported that the risk of forearm fractures with a high intake of protein (> 90 g/d) is exacerbated by a low calcium intake (< 541 mg/d). Similar observations were made by Meyer et al (1997) who reported an elevated risk of fracture in elderly men and women with a high intake of protein from non dairy sources and calcium intakes < 400 mg/d. However, both authors did not find a positive effect of high calcium and high protein intakes on fracture risk. The results (Table 2) are related to investigated ratio between BMD in g/cm^2 as dependent phenomenon; and meat consumption frequency (every day, 3-5 t/w, 1-2 t/w and no consumers) like independent phenomena.

Table 2. BMD & Meat Consumption Frequency

MCF	Beta	Std.Err. of Beta	B	Std.Err. of B	t(206)	p-level
Intercept			1.14	0.03	32.87	0.000
Everyday	-0.43	0.07	-0.38	0.06	-6.17	0.000
3-5 t/w	-0.82	0.11	-0.29	0.04	-7.55	0.000
1-2 t/w	-0.49	0.11	-0.17	0.04	-4.45	0.000

Moderately strong correlation was determined for the ratio investigated for R=0.53 and p<0.001(p=0.000). No consumer female subgroup was used as referent category. 3-5 t/w meat consumption had the highest influence on BMD (Beta=-0.82), weaker in 1-2 t/w consumer subgroup (Beta=-0.49), while the weakest influence had meat intake on everyday basis (Beta=-0.43). Females on everyday meat consumption had significantly lower BMD for p<0,001(p=0,000) i.e. 0.38 g/cm^2 (B=-0.38) lower BMD compared to no consumers as well as all other MCF subgroups showed significantly lower BMD compared to no consumers. These results suggest that neither frequent nor rear consumption of meat was beneficial for BMD. Moreover, those females had significantly lower BMD compared to no consumer. Therefore, protein intake of those females might be insufficient from other food protein source than meat, which could be more beneficial for BMD, or they had lower calcium intake and higher calcium loss through urinary excretion. These results go in favor of the finding of Mauer et al (2003) who determined that Western-type diet (high in animal protein and fat) was associated with osteoporosis and urinary calcium loss. Spencer and Weaver (2003) found that increased urinary calcium was linked to acid-forming foods, such as meat, fish, eggs, and cereal, and negatively associated with plant foods and is likely determined by the acid-base status of the total diet. Moreover, it has been suggested that animal protein–based diets might have a greater negative effect on skeletal health than do vegetable-based diets (Sellimeyer et al. 2001) because dietary animal protein induces a greater increase in urinary calcium excretion than vegetable protein. Results of BMD and its correlation with MCF for each age group separately (40-49, 50-59, 60-69 and above 69) are presented in further tables bellow and they slightly differ from previously mentioned age no stratified results.

Females aged 40-49. No significant difference in average value of BMD (g/cm^2), (H=2.26) and p>0.05(p=0.52), related to MCF, was found. There was no significant difference (p>0.05) in the results of multiple comparison of p values related to BMD of females and MCF. The results presented in Table 3. bellow are related to the ratio between BMD in g/cm^2 like dependent phenomenon, and MCF on every day basis, 3-5 t/w and 1-2 t/w consumption; and the age of females like independent phenomena. Strong insignificant correlation for R=0.60 and p>0.05(p=0.48) was found. No consumers were used as referent category. The highest influence to this ratio had 3-5 t/w consumption (Beta=-0.51), than everyday consumption (Beta=-0.41), age had even weaker influence (Beta=-0.29), while the weakest influence had 1-2 t/w meat consumption (Beta=-0.23). Under condition of unchanged other parameters, females on 3-5 t/w meat consumption had averagely 0.21g/cm^2 (B=-0.21) insignificantly lower BMD than no consumers p> 0. 05 (p= 0. 42).The results between the other MCF and no consumers were similar.

Table 3. BMD/ Age & Meat Consumption Frequency

	Beta	Std.Err.of Beta	B	Std.Err.of B	t(206)	p-level
Intercept			2.29	1.27	1.80	0.11
Age	-0.29	0.36	-0.02	0.03	-0.81	0.44
Everyday	-0.41	0.44	-0.30	0.32	-0.93	0.38
3-5 t/w	-0.51	0.60	-0.21	0.24	-0.85	0.42
1-2 t/w	-0.23	0.55	-0.10	0.24	-0.41	0.70

All results suggest that MCF insignificantly correlated with BMD of females aged 40-49. Frequent meat consumption was associated with insignificantly lower BMD compared to 1-2 t/w meat

consumption or to no consumers BMD. Data about the link between MCF and BMD, and the range of protein intakes for optimizing bone health among premenopausal women is unclear. Kerstetter et al (2005) studied protein-induced effects on net bone balance in young women and showed increased gastrointestinal calcium absorption as well as insignificant trend toward decreased bone resorption with a high-protein diet. Data from one large population-based cohort study performed by Beasley et al (2010) provided evidence that protein intake in the upper range of typical consumption in the United States did not affect bone mass of premenopausal women negatively. **Females aged 50-59.** There was significant difference for H=31.72 and p < 0. 00 1(p=0.000) of females BMD (g/cm^2) at age of 50-59 on 3-5 t/w consumption and those on 1-2 t/w consumption and no consumers (0.84 v. 1.01 v. 1.117). For p<0.001(p=0.000) females on 3-5 t/w meat consumption had significantly lower BMD compared to those on 1-2 t/w consumption and to no consumers for p<0.01(p=0.003). However, there was no significant BMD difference for p>0.05 (p=0.081) between no consumers and 1-2 t/w consumers.

The results presented in Table 4. bellow are related to the ratio between BMD in g/cm^2, and MCF (3-5 t/w and 1-2 t/w consumption and no consumption); and the age of females. Moderately strong significant correlation for R=0.67 and p< 0.001 (p=0.000) was found for this ratio. No consumer subgroup was a referent category. The highest influence to BMD values had 3-5 t/w consumption (Beta=-1.09), than 1-2 t/w consumption (Beta=-0.51), and age the weakest one (Beta=-0.11). Under unchanged other parameters, females on 3-5 t/w meat consumption had significantly lower BMD за p<0.001(p=0.000), compared to no consumers for 0.33 g/cm^2 (B=-0.33); as well as females on 1-2 t/w meat consumption for p<0.05 (p=0.03) i.e. 0.15 g/cm^2 (B=-0.15) lower BMD compared to no consumers, under unchanged other parameters; For each year increasing in age BMD was insignificantly lower for averagely 0,006 g/cm^2 (B=-0.006).

Table 4. BMD/ Age and Meat consumption frequency

	Beta	Std.Err. of Beta	B	Std.Err. of B	t(206)	p-level
Intercept			1.48	0.29	5.06	0.000
Age	-0.11	0.10	-0.006	0.01	-1.10	0.27
3-5 t/w	-1.09	0.23	-0.33	0.07	-4.74	0.000
1-2 t/w	-0.51	0.23	-0.15	0.07	-2.20	0.03

The results of postmenopausal women at 50-59 years age strongly suggest that frequent meat consumption correlated with lower BMD. Frequent consumption of meat (3-5 t/w) was followed with higher percentage of osteoporosis of the spine detected by densitometry compared to those on 1-2 t/w consumption (76.47% v. 26.92%). The calcium intake was not measured in this study, but these results are consistent with the finding of Fescanhich et al (1996) who suggested that, as a result of increased urinary calcium excretion with high protein intake, there was an increased risk of fractures or osteoporosis. May be females in our study did not consume enough vegetables and fruits in parallel with high meat consumption. Barzel et Massey (1988) suggest that excess in dietary protein can adversely affect the bone because a diet, which is high in protein and low in fruits and vegetables, generates a large amount of acid, mainly as sulfates and phosphates. The kidneys respond to this dietary acid challenge with net acid excretion. Concurrently, the skeleton supplies buffer by active resorption of bone. Calciuria is directly related to net acid excretion. On contrary, Heaney and Layman (2008) suggested that higher protein diets were actually associated with greater bone mass and fewer fractures when calcium intake was adequate.

Females aged 60-69. For H=13.67 and p<0.01(p=0.001), significant BMD (g/cm^2) difference in females aged 60-69 on 3-5 t/w meat consumption compared to those on 1-2 t/w consumption and no consumers was found (0.83 v. 0.97 v.1.15). Females no consumers, had significantly higher BMD for p<0,001(p=0,000) compared to BMD of 3-5 t/w consumers, while the difference of BMD between no consumers and 1-2 t/w meat consumers was insignificant for p>0.05(p=0.14). The results shown on Table 5. bellow are related to BMD; and MCF (3-5 t/w, 1-2 t/w & no consumers) & age of the females. Moderately strong significant correlation was found for this ratio for R=0.56 and

p<0.001(p=0.0006). No consumers subgroup was used as referent data. The highest influence to this ratio had 3-5 t/w meat consumption (Beta=-0.70), than 1-2 t/w consumption, and the weakest one had the females age (Beta=0.14). Under unchanged other parameters, females on 3-5 times/week meat consumption had 0.33 g/cm^2 (B=-0.33) lower BMD compared to no consumers, which was significant difference for p<0.001(p=0.000); Females on 1-2 t/w meat consumption had approximately 0.18 g/cm^2 (B=-0.18) lower BMD compared to no consumers, which was significant difference as well for p<0.01(p=0.005); and with each increasing of the age for one year, BMD was insignificantly higher for p>0.05(p=0.26).

Table 5. BMD / Age & Meat consumption frequency

	Beta	Std.Err.of Beta	B	Std.Err.of B	t(206)	p-level
Intercept			0.50	0.57	0.88	0.38
Age	0.14	0.12	0.01	0.01	1.14	0.26
3-5 t/w	-0.70	0.15	-0.33	0.07	-4.56	0.000
1-2 t/w	-0.44	0.15	-0.18	0.06	-2.96	0.005

Females at age of 60-69 on 3-5 t/w meat consumption had higher percentage of osteoporosis detected compared to those females on 1-2 t/w consumption (51% v. 42.85%). These results together with the previously mentioned results suggest that frequent meat consumption was not beneficial for BMD of older women above 60. It has to be noted that the quantity of meat intake could not be precisely estimated due to subjectivity of the participants, thus it was not known whether these females had abundant protein intake, accompanied with low vegetable intake. The calcium intake was not considered as well. It is interesting that these results are more consistent with older research findings. Sellmeyer et al (2001) and Dawson-Huges et al (1990) concluded that elderly women who had relatively high dietary animal protein intakes and limited vegetable protein intakes had more rapid bone loss at the femoral neck and a greater risk of hip fracture than did those with lower dietary animal protein intakes and higher vegetable protein intakes, thus suggesting that abundant dietary protein may also be harmful in older persons. Barzel and Massey (1998) noted that a high intake of dietary protein may adversely affect bone through effects on calcium excretion and acid-base metabolism.

Females aged over 69. For H=15.99 and p<0.01(p=0.001) a significant difference of BMD (g/cm^2) in females aged above 69 related to MCF on everyday basis compared to 3-5 t/w, 1-2 t/w and no consumers ((M=0.73 v. 0.84 v. 0.92 v. 0.95). In this age subgroup only the females above 69 years age who were on 1-2 t/w meat consumption had significantly higher BMD for p<0.01(p=0.002) compared to those who were on every day consumption. Therefore these results suggest that 1-2 t/w meat consumption is not harmful for BMD of females above 69 years old. Results presented on Table 6. are related to the ratio between BMD (g/cm^2) like dependent phenomenon and meat consumption frequency; and females age as independent phenomena. Moderately insignificant correlation for this ratio was found for R=0.44 and p<0.01(p=0.002). No consumer subgroup was used as referent data. The highest influence to this ratio had everyday meat consumption (Beta=-0.42), than 3-5 t/w consumption (Beta=-0.37), the age (Beta=0.08), while 1-2 t/w consumption had the weakest influence (Beta=-0.07). Under condition of unchanged other parameters, females on everyday meat consumption had significantly lower BMD for p<0.05 (p=0.04), i.e. 0,21 g/cm^2 (B=-0.21) lower BMD as compared to no consumers; 3-5 times/week meat consumers had averagely 0.10 g/cm^2 (B=-0.10) lower BMD compared to no consumers, which was insignificant difference for p>0.05(p=0.27; With each increasing in age for one year BMD was increased for averagely 0.003 g/cm^2 (B=0.003), insignificantly for p>0.05 (p=0.43); and 1-2 times/week meat consumers had averagely 0.02 g/cm^2 (B=-0.02) lower BMD compared to no consumers, insignificantly for p>0.05(p=0.84).

Table 6. BMD / Age & Meat Consumption Frequency

	Beta	Std.Err.of Beta	B	Std.Err.of B	t(206)	p-level
Intercept			0.75	0.27	2.76	0.007
Age	0.08	0.10	0.003	0.003	0.79	0.43
Everyday	-0.42	0.21	-0.21	0.10	-2.05	0.04
3-5 t/w	-0.37	0.33	-0.10	0.09	-1.11	0.27
1-2 t/w	-0.07	0.34	-0.02	0.09	-0.20	0.84

Females at age over 69 on 3-5 t/w meat consumption had higher percentage of osteoporosis detected compared to those females on 1-2 t/w consumption (58.33% v. 37.04%). The results of the females aged over 69 strongly suggest that frequent meat consumption on every day basis or 3-5 t/w is not beneficial for their BMD which is compatible with the findings of 60-69 years old subgroup analyzed previously and with the findings of Sellmeyer et al (2001), Dawson-Hughes et al (1990), and Barzel and Massey (1998).

Conclusions

Based upon the results of this study, frequent meat consumption on everyday basis and 3-5 t/w consumption was followed with higher incidence of osteoporosis, and significantly lower BMD compared to 1-2 t/w consumers and no consumers, thus it is not recommendable for prevention of osteoporosis, when females were not categorized in age subgroups. The results were slightly different when females were divided in subgroups according to their age: 40-49; 50-59; 60-69: and >69 years old. MCF was insignificantly correlated to BMD of females aged 40-49 suggesting that other factors like estrogens levels, physical activity, and lifestyle might played more important role rather than meat consumption. However, in age of 50-59 and 60-69 there was a moderately strong significant correlation between BMD and MCF, and higher incidence of osteoporosis, suggesting that frequent meat consumption had negative impact on BMD of these females. In the age over 69, despite the findings in this study of significantly higher BMD in 1-2 t/w meat consumption compared to BMD of females on everyday consumption, the influence of MCF on BMD of females over 69 age was insignificant, suggesting that other factors might have higher positive or negative impact on their BMD. Therefore we recommend avoidance of meat consumption or its decrease to 1-2 t/w combined with more fruit and vegetables with adequate food derived calcium intake in elderly population.

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THE EFFECT OF TAPIOCA-STARCH EDIBLE COATING ON QUALITY OF FRESH-CUT CAULIFLOWER DURING STORAGE

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Abstract

The purpose of this study is to determine preventing browning of cut surface of fresh-cut cauliflower using edible coating. Three different contrentration of tapioca-starch solution (5, 10 and 20 g/L) was used, and gelatine was added at the stable concentration (2.5 g/L). The fresh-cut cauliflower stalk was dipped these solutions for 5 minutes, then dried, packaged and stored at 4°C and 85-90% RH for 28 days. Poliphenol oxydase (PPO) activity, total soluble solids (TSS), color *L*a*b*,* and h° values, weight loss, and browning rate were determined seven days intervals during storage. According to the results; PPO activity of edible coated samples were found to be higher than control. Also, hue angle values of coated samples were lower than control group. However, weight losses and browning rate of samples treated with 2,0% of tapioca-starch coating were the lowest compared to the other treatments. Also, TSS of fresh-cut cauliflower stalks coated with tapioca-starch at all doses were determined higher than control group. In conclusion, the edible coating with tapioca-starch was not effect to prevent browning. But this coating increased TSS of samples and decreased weight loss.

Keywords: *Brassica oleraceae* L. var. botrytis, starch-based coating, browning, minimally processed.

Introduction

The consumption of fresh-cut vegetables has been increasing in recent years due to their health benefits. Fresh-cut fruits and vegetables represent a rapidly growing segment of the produce industry as more consumers demand fresh, convenient, and nutritious foods. This is due to the lifestyles of modern consumers that prefer a fresh product that is easier and faster to eat, and desire natural products that can promote health benefits. Quality of fresh-cut fruit products determines their value to consumers and is a combination of attributes, properties, or characteristics including appearance, texture, flavor, and nutritional value. A major challenge faced by the produce industry is to manipulate the quality of fresh-cut produce that the shelf-life is long enough to ensure efficient marketing. Fresh-cut produce deteriorates faster than intact produce because of internal and external browning of the cut surfaces (Gonzalez-Aguilar et al. 2005). One of the latest alternatives to reduce the deleterious effect brought by minimal processing is the application of edible coatings. Acting as a barrier to gases, they are expected to generate a sort of modified atmosphere in each coated fruit piece, and along with relative humidity and optimum refrigeration temperature, they contribute to achieve a reasonable shelf-life in fresh-cut products (Rojas-Grau et al. 2008) The semipermeable barrier provided by edible coatings is aimed to extend the shelf-life by reducing the transfer of moisture, aroma and flavor compounds, gas exchange, respiration and oxidative reaction rates, as well as suppress physiological disorders on fresh-cut fruits (Baldwin et al. 1996, Park, 1999, Wong et al. 1994). In addition, edible coatings can be used as carriers of active compounds, such as antimicrobial agents, which can be used to decrease the population of spoilage and pathogenic microorganisms (Glass and Johnson 2004). One major advantage of using edible coatings is that several active ingredients can be incorporated into the polymer matrix and consumed with the food. Traditionally, edible coatings have been used in the fresh-cut industry as a strategy to reduce the undesired effects that minimal processing produces on intact fruit tissues (Giacalone et al. 2010). Polysaccharides generally present a good barrier to oxygen at low relative humidity (RH) due to their

tightly packed structure and low solubility. Polysaccharide-based coatings have been used to extend the shelf-life of fresh-cut fruits and vegetables by reducing respiration rate and gas exchange due to selective permeabilities to O_2 and CO_2 (Rojas-Grau et al. 2009). One shortcoming of polysaccharides is they provide poor moisture barrier due to their hydrophilic character. Chitosan, starch, cellulose, alginate, carrageenan, gelatin, zein, gluten, whey, carnauba, beeswax and fatty acids are the most commonly used compounds to form edible coatings (Baldwin et al. 2011, de Aquino et al. 2015, Shit and Shah 2014). Polysaccharide-based edible coatings may include cellulose derivatives; starch and its derivatives, alginate, pectin, and gellan gum (Olivas and Barbosa-Canovas 2005, Valencia-Chamorrosa et al. 2011). Starch based films have been particularly considered for the reason that they exhibit physical characteristics similar to synthetic polymers: transparent, odorless, tasteless, semi-permeable to CO_2 and resistant to O_2 passage (Nisperos-Carried, 1994). Tapioca starch, naturally or modified, is increasing its utility in food industry because it has some inherent properties that are demanded. The purpose of this study is to determine the effect of tapioca starch-based edible coating on some biochemical characteristics of fresh-cut cauliflower stalks.

Material and methods

Plant Material

Cauliflower (*Brassica oleraceae* L. var. botrytis) were obtained from Kocaeli Wholesale Distribution Center and immediately brought to the laboratory. The cauliflower were screened for uniformity such as being free from any mechanical damages and diseases, and also for similar stage of maturity. The cauliflower separated into stalks. This stalks was used to investigate coating.

Preparation of tapioca starch-based edible coating

For this purpose tapioca starch which is extracted from cassava root (*Manihot esculenta*) was used as polysaccharide material. The tapioca starch-based (TS)-solution at the doses of 5 g/L, 10 g/L and 20 g/L was prepared. Since TS did not form gel at the low temperature, solution was heated until 65^{o}C temperature. For providing flexibility of coating, gelatin was added into all starch-solutions at the 2.5 g/L doses. After solution prepared, the cauliflower stalks dipped into solution at the 40^{o} temperature for 3 min. Then all samples were dried at room temperature for fifteen minutes.

Packaging and Storage Conditions

200 g of fresh-cut cauliflower stalks of each replicate was placed in a plastic box (polyethylene terephthalate (PET)) with cover and 110x110x50 mm in size. All treated samples were stored in a cold room at 5 ±1°C and a relative humidity of 85-90% for 28 days.

Color measurements

Color measurements (L*, a*, and b* values) were performed using a chromometer CR-400 (Konica Minolta, Inc. Osaka, Japan) equipped with illuminant D65 and 8 mm aperture of the instrument for illumination and measurement. The instrument was calibrated with a white reference tile (L* = 97.52, a* = −5.06, b* = 3.57) prior to measurements. The L* (0 = black, 100 = white), a* (+ red, − green), and b* (+ yellow, − blue) color coordinates were determined according to the CIELab coordinate color space system (Radzeviciu et al. 2014). Hue angle (ho = tan-1 (b*/a*) when a* > 0 and b* > 0 or ho=180o+ tan-1 (b*/a*) when a*< 0 and b > 0) was calculated from the a* and b* values (Lancester et al. 1997). Color measurement was done three different point of each stalks of each replicate.

Polyphenol oxidase activity (PPO)

To measure polyphenol oxidase activity, 5 g of homogenized fresh-cut cauliflower stalk was extracted with 0.1 M phosphate buffer, pH 7 containing 5 g of polyvinylpyrrolidone using magnetic stirrer for 15 min. The homogenate was filtered through Whatman No. 1 filter paper, and the filtrate collected as an enzyme extract. PPO activity was determined by a spectrophotometric method based

on an initial rate of increase in absorbance at 410 nm (Soliva et al. 2000). Phosphate buffer pH 7 (0.1 M, 1.95 mL), 1 mL of 0.1 M catechol (substrate) and 50 µL of the enzyme extract were pipetted into a test tube and mixed thoroughly. The mixture was rapidly transferred to a cuvete of path lenght 1-cm. The absorbance at 410 nm was recorded continuously at 25°C for 5 min using ultraviolet-visible (UV-VIS) spectrophotometer (UV Mini 1240, UV-VIS Spectrophotometer, Shimadzu, Japan) (Arnnok et al. 2010)

Browning Rate (%)
In each analysis period, the number of the browning samples is to rate to the total number of the samples in the box, and calculated as a percentage (%).

Total soluble solids (TSS)
TSS were determined for each sample fruit in three replications using an Atago DR-A1digital refractometer (Atago Co. Ld., Japan) at 20 °C and expressed as percent value (%) (Kasım and Kasım 2015)

Weight losses
Weight of each sample with three replication of each treatment group was recorded on the day of harvest and on the sampling dates. Cumulative weight losses were expressed as percentage loss of original weight.

Statistical analysis
Experiments were conducted in acompletely randomized design with a minimum of-three replications per storage treatments per sampling date. Data were analyzed by ANOVA and differences among means were determined by the Duncan's multiple range test with significance level at $p<0.05$.

Results and discussion
Total Soluble Solids (TSS)

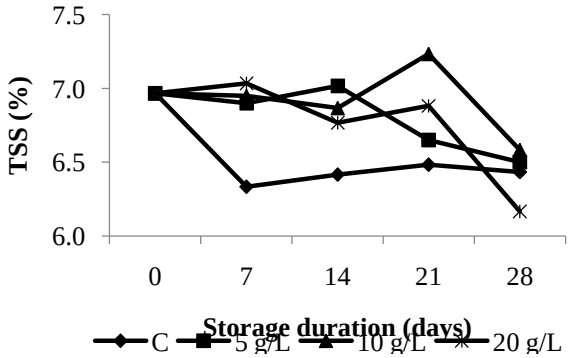

Figure 1. The TSS (%) values of cauliflower stalks that fresh-cut and coated with tapioca starch at different doses. C: control

TSS values of samples in all treatment groups were shown in Fig. 1. As shown Fig. 1, while TSS of control group decreased, tapioca starch-based coating significantly ($p<0.05$) increased TSS of samples in all doses. But, among the treatments there was no seen evident changes. In previously study, harton plantain (*Musa paradisiaca*) was coated with cassava starch-based coating material, but the authors did not found significant difference with a confidence level of 95% in the concentration of total soluble solids (TSS) (Cardozo et al. 2015). The result of the present study is inappropriate with this result, but we used the fresh-cut samples, however, the authors used intact banana fruits. So, the cauliflower stalks could be metabolized the coating material, therefore it can

be said that the TSS of the samples found to be high. When banana is considered, since the fruit bark peeled so the coating material did not effect on the TSS value of banana.

Polyphenol oxydase (PPO) Enzyme Activity

Browning due to oxidation of phenols, which is often catalyzed by the polyphenol oxidase enzyme to form colored melanins, decreases the nutrient content in fruits (Vamos-Vigyazo, 1981). The PPO enzyme activity of fresh-cut and coated with TS, was higher than control group, in all doses of coatings (Fig. 2).

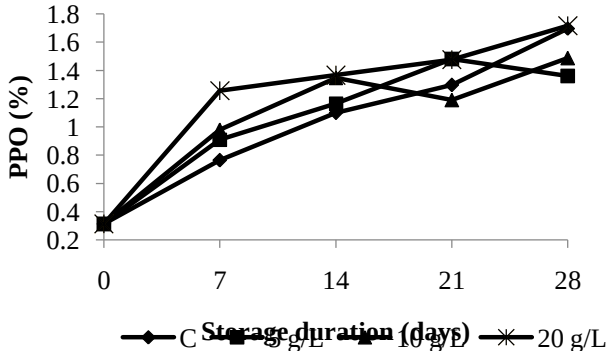

Figure 2. The PPO (%) values of cauliflower stalks that fresh-cut and coated with tapioca starch at different doses. C: control

The highest PPO values were obtained by the coating treatment 20 g/L, and also the differences between this treatment and the other coatings and control group were significant, statistically ($p<0.05$). The PPO enzyme activity of cauliflower stalks fresh-cut and coated with TS, was higher than control group, in all doses of coatings. Therefore, it can be said that the starch-based coating material caused the increase of PPO. This reason is due to the starch metabolize by the samples, and cutting of the samples caused increase the respiration rate so that the other secondary metabolizm rate is increase. Combination of citric acid dipping (5 g/L) and cassava starch coating (10 g/L), with and without glycerol (10 g/L), delayed carotenoid formation and browning reactions of fresh-cut mango during storage was found by Chiumarelli et al. 2010. Similarly, de Moura et al. (2016) concluded that, the lowest PPO activity of mangaba fruit (*Hancornia speciosa*) was found in the pulp of fruit coated with the highest starch concentration tested (4%). But in the present study is not agree with this findings.

Browning Rate

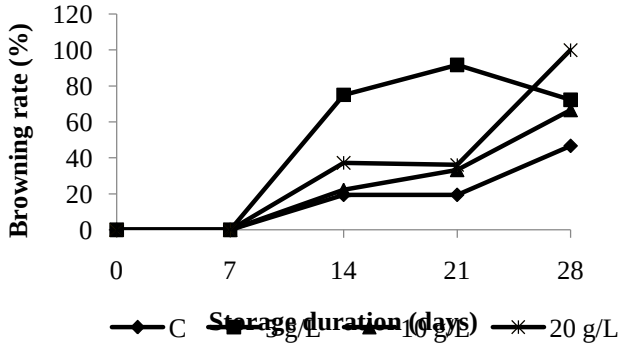

Figure 3. The browning rate (%) values of cauliflower stalks that fresh-cut and coated with tapioca starch at different doses. C: control

Fruits are living tissues that undergo enzymatic browning, texture decay, microbial contamination and undesirable volatile production, highly reducing their shelf-life, if they are in any way wounded

(Tapia et al. 2008). Although, the PPO activity of samples coated with 20 g/L TS was high, the browning rate of cauliflower coated with the 5 g/L TS was found higher than the other coating treatments and control (Fig. 3). However, the differences between these two treatment was not significant at the level of $p<0.05$. But the differences between 5 g/L TS treatment and 10 g/L TS and control is significant. Therefore, it could be concluded that the coating treatments were not effect on browning rate of samples.

L values*

*L** values of samples showed a decreasing trend for the first 7 days of storage, but after that time it started to increase, and they showed sharp increase especially the samples treated with 10 g/L and 20 g/L TS at the day of 21. Also differences among the storage time was significant statistically ($p<0.05$). Furthermore, the differences between samples treated wit 5 g/L TS and the other treatment groups were found to be significant.

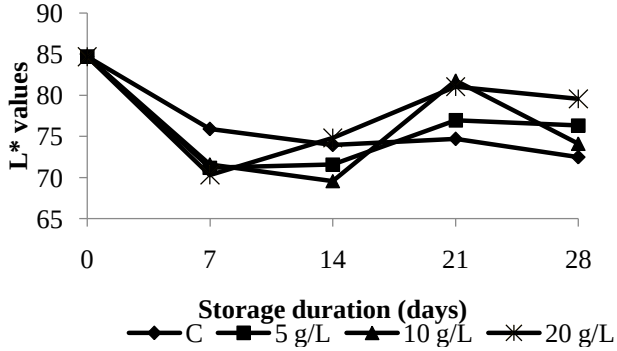

Figure 4. The *L** values of cauliflower stalks that fresh-cut and coated with tapioca starch at different doses. C: control

In a study, it was found that the alginate coatings of fresh cut nectarine were effective on delaying the evolution of the parameters related to postharvest ripening, such as color (Hue, L*) and loss of acidity (Chiabrando and Giacalone 2013). In the present study, coatings of fresh-cut cauliflower stalks with 20 g/L TS was effect on luminosity of samples, both 21th day and at the end of storage periods. The samples into this treatment were stayed brighter than the other treatments.

h^o values

Color, flavor, texture, and nutritive value are generally recognized as the four quality factors of fruits and vegetables. The natural pigments, chlorophylls, carotenoids, and anthocyanins, form the chemical basis of color. Enzymatic and non-enzymatic browning contributes to coloring of certain processed fruits and vegetables (Jen, 1989). The h^o values of fresh-cut cauliflower stalks treated with tapioca starch with different doses were given in Fig. 5. According to the Fig. 5, the h^o values of samples in all treatment groups decreased during the storage. But this decrease was found the lowest in control group, and followed bay 10 g/L TS, 20 g/L TS and 5 g/L TS treatments.

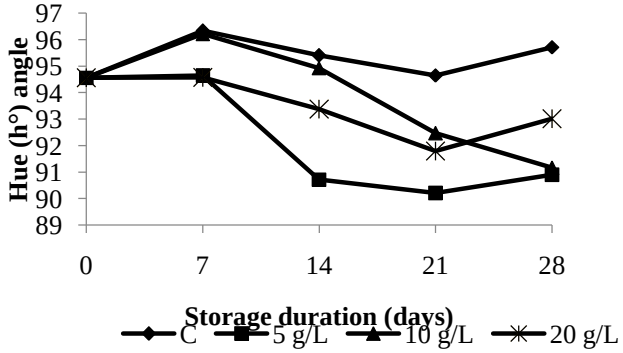

Figure 5. The hue (h^o) angle values of cauliflower stalks that fresh-cut and coated with tapioca starch at different doses. C: control

Also differences among the treatments were found to be significant statistically at the level of $p<0.05$. The h^o values of samples treated with 5 g/L TS, was lower than those of the other treatment during storage. H^o values is shown true color of samples. Therefore it can be said that, the color of samples treated with tapioca starch was darkened compared to control. Also, browning rate and PPO activity of coated samples is higher than conrol group. Furthermore, it was found in a study that the hue angle of surface color of minimally processed pummelo coated with the two starches did not differ from the control (Kerdchoechuen et al. 2011). So, this result supported to our findings, too.

Weight Losses

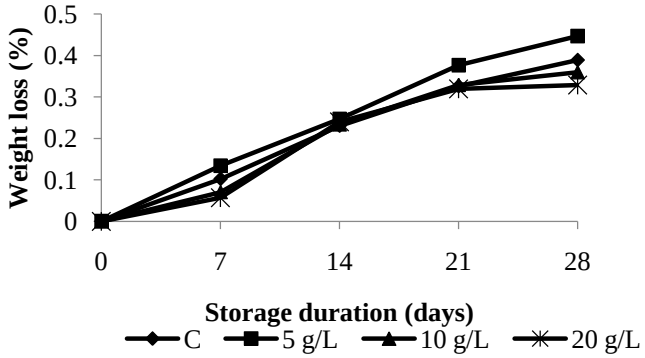

Figure 6. The weight loss (%) values of cauliflower stalks that fresh-cut and coated with tapioca starch at different doses. C: control

Weight loss values of samples in all treatment gruops increased during storage, but tapioca starch based coating reduce weight losses especiall high dose (20 g/L) compared to other treatments, and also differences between this treatment and the other treatments were significant statistically ($p<0.05$). The weight loss during the storage period, implying quality loss, and, consequently, consumer rejection. According to Han and Gennadios, 2005, the edible coatings can protect fruits and vegetables from dehydration due to their moisture barrier property, resulting in an extension of the shelf life of minimally processed products. But, Placido et al., (2015) concluded that, in pequi fruit, the vitamin C, titratable acidity, soluble solids contents and weight loss showed that starch-based coatings did not achieve satisfactory results. Despite these results, it was found in the present study that the tapioca-starch based coatings reduced weight loss in the case of fresh-cut cauliflower.

Conclusions

In the present study it was studied the effect of coating with tapioca starch (TS)-based coating film on some quality characteristic of fresh-cut cauliflower during storage. As findings of research, coatings with TS decreased weight losses especially high dose, and the TSS of fresh-cut caulifower in all coating doses was higher than control. While the coating treatment was not effect darkening of the samples, brightness of samples increased by coatings.

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THE EDIBLE COATING TREATMENTS ON COLOR QUALITY FRESH-CUT LEEK DURING COLD STORAGE

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Abstract

This study was carried out to determine the effect of plant-based edible coating on color changes of fresh-cut leek during storage. For this aim 0.5, 1 and 2% solutions of tapioca-starch were prepared and 0,25% gelatine was added to each dose. The fresh-cut leek samples were dipped these solutions for 5 minutes, then dried, packaged and stored at 4 C and %85-90 RH for 28 days. The color values (*L*a*b** and h^o), discoloration rate (%), poliphenol oxydase (PPO) activity, total soluble solids (TSS) and weight losses of samples were determined in seven days intervals during storage. The results of the research shoved that, L values of coated samples were higher than that of the control group while the h^o values of control group were higher. PPO activity of samples increased in all treatment groups, but did not show evident differences among the treatments. The TSS of samples treated with 0,5% tapioca-starch edible coating were the lowes, whereas it remained the same for the other doses of edible coatings ofthe control group. The weight losses of the samples treated with 1% were the lowest among the treatment groups. Discoloration rate of samples treated with 2% edible coating, however, were found to the lowest. Therefore it could be said that, the coating with tapioca-starch of fresh-cut leek was found to be effective in preventing discoloration especially at the higher dose (2%).

Keywords: *Allium porrum*, browning, postharvest , starch-based coating, minimally processed.

Introduction

Edible coatings may be defined as a thin layer of material that covers the surface of the food and can be eaten as part of the whole product. The composition of edible coatings must therefore conform to the regulations that apply to the food product concerned (Guilbert et al. 1995). Polysaccharides are the most widely used components found in edible coatings for fruits (Kester and Fennema 1986; Krochta, 1997), as they are present in most commercially available formulations. Polysaccharides show effective gas barrier properties although they are highly hydrophilic and show high water vapor permeability in comparison with commercial plastic films. The main polysaccharides that can be included in edible coating formulations are starch and starch derivates, cellulose derivates, alginate, carrageenan, chitosan, pectin, and several gums (Vargas et al. 2008). Starch is the natural polysaccharide most commonly used in the formulation of edible coatings because it is inexpensive, abundant, biodegradable, and easy to use. Native granular starch is converted into a thermoplastic material by conventional methods in the presence of plasticizers, such as water and glycerol (Thire et al. 2003). Coatings made from starch become brittle in dry atmospheres and lose strength and barrier properties in high humidity (Peterson and Stading 2005). The addition of plasticizers overcomes their flexibility and extensibility (Mali et al. 2002). Leek (*Allium porrum* L.) is a popular vegetable due to its nutritional values. The white sheaths of leek contain on average 83-90% water, 1.5-2.0% protein, 0.3% lipids, 5.0-14.2% carbohydrates, and 1.8% fiber. Leeks also contain vitamin A, vitamin C, carotenoids, flavonoids; and the major flavor compounds of leek are nonprotein sulfur-containing amino acids (Nunes, 2009). Minimal processing of leek stalks includes root trimming, removal of outer damaged or decayed leaves and trimming to a desired length. Postharvest quality deterioration of minimally processed leeks includes inner leaf growth and discoloration of the cut surface, as well as fresh weight loss. Such deterioration is significantly reduced by storage at 0°C

temperature (Tsouvaltzis et al. 2008). On the other hand, storage at 0 C is practically inapplicable, since in commercial practice minimally processed produce is most commonly prepared, shipped, and stored at 5–10°C (Watada et al. 1996). The major cause of quality loss of minimally processed leeks is inner leaf growth and to a lesser extent dehydration and discoloration at the higher storage temperature. Therefore, the prevention of these changes of fresh-cut leek is important, and edible coating of fresh-cut fruit and vegetables delays such quality losses. Thus, the aim of this study is to determine the effect of starch-based edible coating on color quality of fresh-cut leeks.

Material and methods

Plant Material

Leeks (*Allium porrum* L. cv. İnegöl) were obtained from Kocaeli Wholesale Distribution Center and immediately brought to the laboratory. The leeks were screened for uniformity such as being free from any mechanical damages and diseases, and being at a similar stage of maturity. The 3-4 cm diameter leeks were used forthis study. The outer, yellowed leaves of leeks were hulled, and the compressed roots were cut. Then, the yellow leaves of leeks were cut with a sharp knife at the length of 10 cm, and washed with tap water.

Preparation of starch-based edible coating

For purposes of this research, tapioca starch which is extracted from cassava root (*Manihot esculenta*) was used as polysaccharide material. The tapioca starch-solution at the doses of 0.5% (5 g/L) , 1% (10 g/L) and 2% (20 g/L) were prepared. Since starch did not form gel at the low temperature, solution was heated until 65^{o}C temperature. For providing flexibility of coating, gelatin was added into all starch-solutions at the 2.5 g/L (0.25%) doses. After the solutions were prepared, the leek samples were dipped into the solutions at the 40^{o} temperature for 3 min. Then all samples were dried at room temperature for fifteen minutes.

Packaging and Storage Conditions

200 g of fresh-cut leek of each replicate was placed in a plastic box (polyethylene terephthalate (PET)) with cover and 110x110x50 mm in size. All treated samples were stored in a cold room at 5 ±1°C and a relative humidity of 85-90% for 28 days.

Color measurements

Color measurements (L*, a*, and b* values) were performed using a chromometer CR-400 (Konica Minolta, Inc. Osaka, Japan) equipped with illuminant D65 and 8 mm aperture of the instrument for illumination and measurement. The instrument was calibrated with a white reference tile (L* = 97.52, a* = −5.06, b* = 3.57) prior to measurements. The L* (0 = black, 100 = white), a* (+ red, − green), and b* (+ yellow, − blue) color coordinates were determined according to the CIELab coordinate color space system (Radzevičius et al. 2014). Hue angle (ho = tan-1 (b*/a*) when a* > 0 and b* > 0 or ho=180o+ tan-1 (b*/a*) when a*< 0 and b > 0) was calculated from the a* and b* values (Lancester et al. 1997)

Polyphenol oxidase activity (PPO)

To measure polyphenol oxidase activity, 5 g of homogenized fresh-cut leek was extracted with 0.1 M phosphate buffer, pH 7 containing 5 g of polyvinylpyrrolidone using magnetic stirrer for 15 min. The homogenate was filtered through Whatman No. 1 filter paper, and the filtrate collected as an enzyme extract. PPO activity was determined by a spectrophotometric method based on an initial rate of increase in absorbance at 410 nm (Soliva et al. 2000). Phosphate buffer pH 7 (0.1 M, 1.95 mL), 1 mL of 0.1 M catechol (substrate) and 50 µL of the enzyme extract were pipetted into a test tube and mixed thoroughly. The mixture was rapidly transferred to a cuvete of path lenght 1-cm. The absorbance at 410 nm was recorded continuously at 25°C for 5 min using ultraviolet-visible (UV-

VIS) spectrophotometer (UV Mini 1240, UV-VIS Spectrophotometer, Shimadzu, Japan) (Arnnok et al. 2010)

Discoloration Rate (%)
In each analysis period, the number of color-changing samples was calculated as a ratio to the total number of samples and expressed as (%).

Total soluble solids (TSS)
TSS were determined for each sample fruit in three replications using an Atago DR-A1digital refractometer (Atago Co. Ld., Japan) at 20 °C and expressed as percentage value (%) (Kasım and Kasım 2015)

Weight losses
The weight of each sample with three replications of each treatment group was recorded on the day of the harvest and on the sampling dates. Cumulative weight losses were expressed as percentage loss of original weight.

Statistical analysis
Experiments were conducted in an aentirely randomized design with a minimum of-three replications per storage treatments per sampling date. Data wasanalyzed by ANOVA and differences among means were determined by the Duncan's multiple range test with significance level at $p<0.05$.

Results and discussion
L values

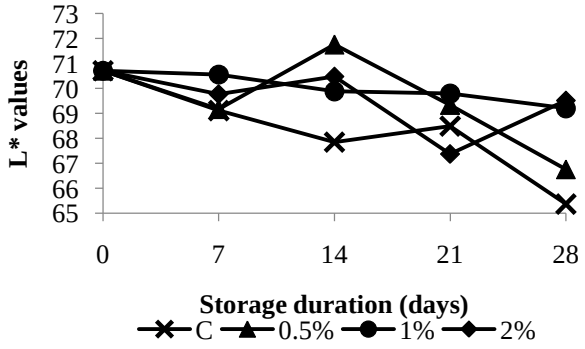

Figure 1. *L** values of fresh-cut and coated leeks.

L values of fresh-cut leeks in all samples decreased during the storage. But this decrease was minimum in the leek samples coated with 1% tapioca-starch (TS), and the differences among the treatments were found to be significant statistically at the level of $p<0.05$. Furthermore, no evident changes were obtained up to 14. days of stage, but after that time L values of samples in all treatments decreased. Therefore, it can be said that coating fresh-cut leek with 1% TS was effective to retain white color and also to bring brightness to the samples. But neither lower (0.5%) nor higher (2%) doses of coating were effective on white color of samples. Also, with extending storage duration, the coating material lost its protective effect, and the L color of samples decreased. In green vegetables, the senescence process usually leads to a yellow coloration of the tissues, normally considered the major consequence of chlorophyll degradation (Toivonen and Brummell, 2008). Also, in some minimally processed green vegetables, the synthesis of pheophytin, an olive-colored pigment, appears when the chlorophyll loses its bond with the magnesium atom and substitutes it with a hydrogen atom. The maintenance of a low temperature and a high relative

humidity, combined with atmospheres lowered in O2 and moderately rich in CO2, are shown to be the main advisable techniques to delay this disorder (Artes et al. 2007). So, in the present study, the tapioca starch-based edible coating materials is providing high relative humidity on the surrounding ofthe product, but also barrier to gas penetration. Therefore the L values of fresh-cut leeks with coated edible films is found to be higher than that of the control group.

Color a, b* and hue (h^o) angle*

The a* values of fresh cut and coated with TS leeks, shown at Fig. 2. According to the Fig. 2, a* values of samples is -12.5 in all samples at the beginning of the storage, but this values decreased during storage, and it varied between -6.5 and -7.8 at the end of the storage. However, differences among the coating treatments did not find significant statistically ($p<0.05$).

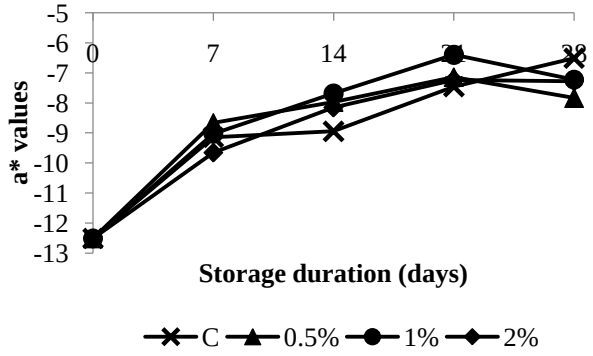

Figure 2. *a** values of fresh-cut and coated leeks.

b* values of coated fresh-cut leek pieces was high at initial measurement (41.6, Fig.3), while after that time these decreased until the end of the storage, like a* values. But, the differences between the samples coated with 2% and the other treatments were significant at the level of $p<0.05$. According to this result, it was suggested that 2% TS coating maintained the color of samples compared to that of the other treatments. Whereas, when the hue angle values are examined, it was seen that the hue angles values of samples in control were higher than the other treatments (Fig.4). The differences among the treatments, however, were not significant, statistically ($p<0.05$). Similar results were obtained by (Kerdchoechuen et al. 2011), the authors found that the hue angle of surface color of minimally processed pummelo coated with the two starches did not differ from the control. Also, Riberio et al. (2007) studied that the ability of polysaccharide-based (starch, carrageenan and chitosan) coatings to extend the shelf-life of strawberry fruit (*Fragaria ananassa*) and found that the edible coating did not cause significant colour differences.

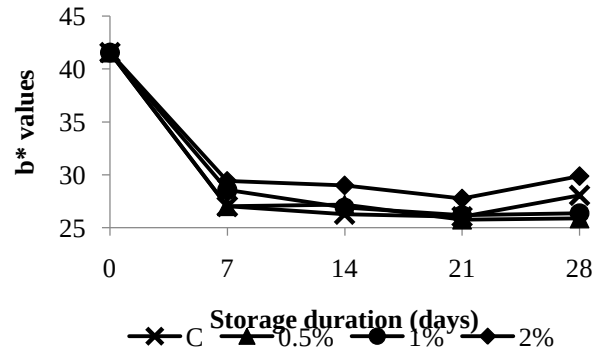

Figure 3. *b** values of fresh-cut and coated leeks.

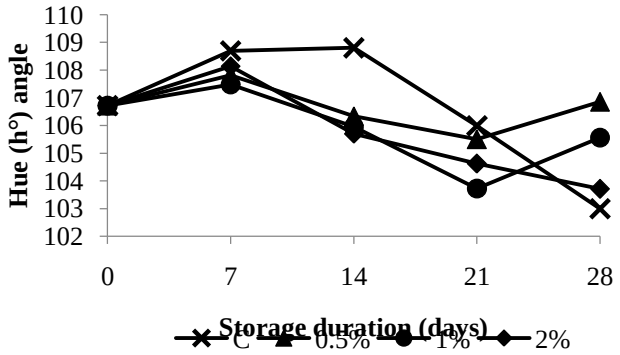

Figure 4. Hue (h^o) angle values of fresh-cut and coated leeks.

Polyphenol Oxidase (PPO) Activity

The PPO activity of samples in all treatments groups increased throughout the storage (Fig. 5). The first 7 days of storage, the PPO activity of control and 0.5% TS was lower compared to the other treatments, but after that time it was increased in all treatments. However, no significant differences among the treatments were determined ($p<0.05$). The PPO activity of treatments was 0.83%, 0.88%, 0.95% and 0.93% for C, 0.5% TS, 1% TS and 2% TS, respectively, at the end of the storage duration. Polyphenol oxidase is an enzyme that naturally found in many fruit cells and responsible for enzymatic browning on the wounded tissues (Alandes et al. 2009). During peeling and cutting process, an undesirable brown color is produced as a result of enzymatic browning reaction due to intermixing of polyphenol oxidase with phenolics compounds. In the present study starch-based edible coating was not effective on decreasing PPO activity. However, there was no darkening observed on the cut surface of the fresh-cut leeks, but the discoloration of cut surface only occured depending on dehydration.

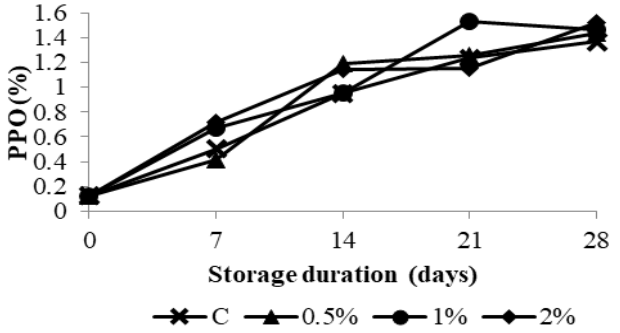

Figure 5. The PPO activity of fresh-cut and coated leeks.

Discoloration Rate (%)

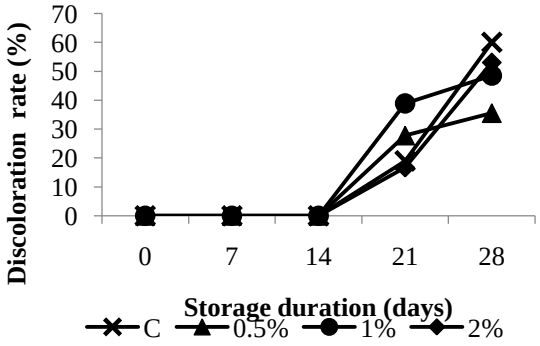

Figure 6. Discoloration rate of fresh-cut and coated leeks.

Discoloration rate (DR) of samples that obtained by visual scoring increased the last two weeks of storage (Fig 6). That is, for the first two week no external discoloration was observed in the samples. The DR of samples in control and 2% TS was found lower than the other treatments at the day of 21, whereas, after that time it increased in all treatment groups. But, the differences among the treatments were not significant statistically ($p<0.05$). Edible films are commonly used to protect perishable food products from deterioration by slowing dehydration, providing a selective barrier to moisture, oxygen and carbon dioxide, improving textural quality, reducing microbial growth (Fan et al., 2009). In the present study, with the usage of apioca starch-based edible coating, the discoloration of the leek was retarded for two weeks of storage. But after that time this preservative effect of edible coating was not enough the prevent color changes.

Total Soluble Solids (TSS, %)

The TSS (%) values of samples measured as 7.0% at the initial stage of storage (Fig. 7), however, they decreased in all treatment groups during the storage. Meanwhile, the most decreasing was found in 0.5% TS treatment for the first two weeks of storage. But no significant differences were found among the treatments ($p<0.05$). Therefore it can be concluded that, the coating treatments did not effect the TSS content of samples.

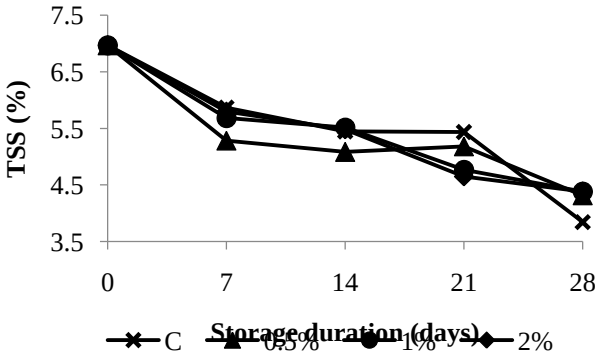

Figure 7. TSS values of fresh-cut and coated leeks.

Weight Loss

Weight losses of samples in all treatment groups increased during storage as seen in Fig 8. Weight loss of fresh cut leek decreased by 1% TS significantly ($p<0.05$), compared to control and 2% TS, throughout storage. But, the weight losses of samples varied between 0.64%-0.77%, and did not exceed 1%. So, the fresh-cut leek samples did not lose their weight too much according to initial weight, although, the TS treatment at the 1% dose was found effective to maintain the weight of leeks, more than the other treatments. It was previously found that, the starch-based edible coating of minimally processed pummelo had a lower weight loss of 4.8–7.7% compared to the control (Kerdchoechuen et al. 2011). The results of present study is compatible with this finding.

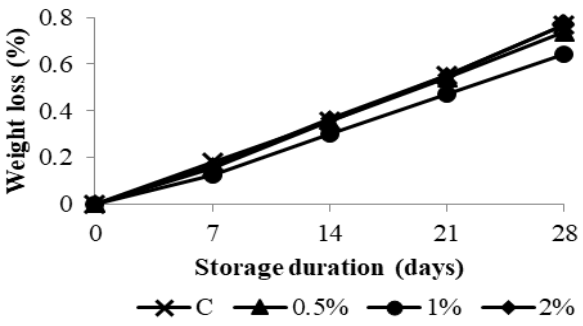

Figure 8. Weight loss of fresh-cut and coated leeks.

Conclusions

The present study aimed to, evaluate the effect of the tapioca starch-based edible coating on the color and the other biochemical characteristics of fresh-cut leek pieces. For this purpore,s three different starch-based edible coating were used, and the color L* a* b* and hue angle values, PPO activitity, discoloration rate, TSS and weight losses of samples were calculated in weekly intervals during storage. According to the results, the tapioca starch-based edible coatings has no evident effect on the color values; however the brightness of samples as measured by L values, was found to be higher in all coating treatments. Similarly, using edible coating did not decrease PPO activity. Despite that, discoloration rate of samples in 2% the TS was lower than the other treatments. TSS values of samples in all coating treatments was not show significant changes depends on edible coating treatment, so it could be concluded that the coating treatment did not effect on TSS values. But, the 1% tapioca starch-based edible coating decreased the loss of weight. In conclusion, it could be said that the 1% and 2% doses of edible coating is found to be succesful in the preservation of color quality of fresh cut leek pieces.

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THE FUNGAL DISEASES IN KIWIFRUIT STORAGE, AND NON-CHEMICAL METHODS USING TO PREVENT THESE DISEASES

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Abstract

The kiwifruit is originated Southeast Asia whose production and consumption is increased each day. The production quantity has reached 3.447.605 tonnes around the world. It is also important to protect the quality of kiwifruit which has a high vitamin C, in the postharvest period. One of the most important problems of kiwifruit is infections originated by fungus during storage. The most common fungal disease in kiwifruit storage is *Botrytis cinerea*. The first symptom is seen after a few week storage. Because the pathogen can develope in the cold storage conditions, it causes decay and serious economical damage. Although the chemical methods is used to prevent this disease, with the increasing consumer awareness and due to caused the risk both environmental and human health, the tendency towards non-chemical methods of combat has been increased. The aim of this study is to compile the studies on non-chemical struggle methods used in *Botrytis cinerea* struggle. It was found that the most common non-chemical treatments are curing, heat applications, ultraviolet light applications, gamma radiation applications, ozone application and essential oil applications. Because these physical methods are not risk to the human health, it will guide for future research.

Keywords: Kiwifruit, storage, *Botrytis cinerea.*

Introductıon

Kiwi was not a well-known fruit until the end of the 20th century. But nowadays, it is a specie that is recognized all over the world and its production and consumption increases every year. The origin is Southeast Asia. The kiwi is a plant belonging to the genus *Actinidia* in *Actinidiaceae* family. The genus of *Actinidia* contains more than 50 species, all of which are of Asian origin (Koday 2000). Some of these are grown as ornamental plants in various parts of the world. In this genus, only five species can be eaten as fruits. These species are *Actinidia deliciosa*, *A. chinensis*, together with the species that fruits are small and their shells are hairless such as *A. arguta*, *A. colomikta* or *A. eriantha*. The cultivated species is *A. deliciosa* (Koday, 2000). Species of *A.deliciosa* and *A.chinensis* have economic importance. The Hayward variety occupies almost 95% of commercially cultivated areas. Over the world, the fruit species that have increased the most in the last 100 years in terms of production area and consumption are the kiwifruit. The Hayward variety with green fruit is the most preferred variety by producers and consumers for its delicious taste, its high nutritional value and yield, and its long shelf life. For this reason, a large part of the world's kiwi production is composed by the Hayward variety (Yılmaz, 2016). In 2014, China is in the 1st place in world kiwi production. Turkey is in the 8th place in world production with 31,795 tons (Table 1).

Damages in kiwi storage

a) Fungal Diseases

1.Botrytis cinerea; It has a very broad host directory. The pathogen acts as saprophyte in aged and injured plant tissues. *Botrytis cinerea* can cause serious losses in more than 200 plant species worldwide. The disease occurs during kiwi storage and it causes infections between 0-35˚C temperatures. The diseased fruit flesh is wet and glassy, from the outside the affected area seems that is darker than the firm part of the fruit. The first sign of the infection appears a few weeks after

the fruit is placed in a cold storage. Pathogen causes serious economic damage in the last stages of production, but it also developes during storage and lead to decay.

Table 1: The kiwi production in the world (FAO 2014)

	Countries	Production(ton)		Countries	Production (ton)
1	China	1.840.000	13	Korea	9.158
2	Italy	506.958	14	Australia	4.239
3	New Zealand	410.746	15	Israel	3.500
4	Chili	266.017	16	Switzerland	501
5	Greece	171.510	17	Montenegro	500
6	France	620.00	18	Kirghizistan	400
7	Iran	43.165	19	Slovenia	260
8	Turkey	31.795	20	Bulgaria	208
9	Japan	31.600	21	Cyprus	100
10	USA	25.855	22	Tunusia	32
11	Spain	20.881	23	Kanada	30
12	Portugal	18.150			
	Total	3.447.605			

2. Alternaria Rot; Alternaria alternata is a fungal pathogen which occurs mainly in ripe fruits as saprophytes. The pathogen causes decay by entering fruits by wounds on the fruit after harvest.

3. *Penicillium spp.*; *Penicillium* species (especially *Penicillium expansum*) are common in apples and pears, but also in kiwifruits. It is a fungal pathogen that causes the blue mold disease. This disease occurs in apple, pear and kiwi after harvest and is also called soft or wet decay.

4. *Phoma destructiva*; The pathogen can affect all parts of the plant above the ground. The pathogen forms stains on the leaves ranging in color from dark brown to gray. As these stains develop, they are seen as rings. The pathogen is primarily effective in old leaves whereas all leaves are susceptible to disease. Leaves can fall when the disease is severe. Stains on the leaf are similar to early blight, but there are numerous picnidiums in Phoma lesions. Dark brown lesions in the form of intervening rings form on the stems and both green and mature fruit may be infected. Disease symptoms often occur as small submerged lesions on the sepal end of the fruit, and then a large number of picnidium occurs in the middle of them.

b) Mechanical Disorders

1. Vibration damage necrosis; During the transportation of the product, when the inner flesh is exposed to fibrillation and the fruit flesh is watered and softened.

2. Freeze damage; This status is occured when the temperature of the warehouse is below the freezing point. Cells break apart and become jelly-like.

3. Water Loss; It is occures due to the excessive decrease of relative humidity of storage rooms. On the surface of the fruit, firstly shrinkage shown and is followed by desiccation.

4. Ammonia damage; Discoloration caused by ammonia leaks on the surface of the fruit, it can be seen in cold room cooled by ammonia.

5.Granulation; It is also called sac drying and crystallization. The granulation occurs due to the fruit juice sac in the stem part of fruit appears to be enlarged and hardened and discolored.

The methods of kiwi storage

1. Ventilated Cold Storages (Traditional, Simple, Ordinary); The cooling air of the night is taken in by convection or ventilation and used for cooling the product.

2. Conventional cooled storages In cooling the storage room, the change of situation of the refrigerant is utilized. This provides the desired degree of cooling.

3. Controlled Atmposphere (CA) Storages; Storage in the CA prevents the binding of auto-catalytic ethylene which is required for maturation to the receptors, thereby prolonging the shelf life of the

product and ensuring that the fruit does not lose quality after storage. Storage in CA Kiwi is carried out in commercially developed countries. In the CA storage, with the increase of CO_2 and the reduction of O_2 is slow down the operation of the enzymes, the ethylene synthesis and respiration rate is decreased and therefore storage life and fruit quality of kiwifruit are increased (Öz and Eriş 2009). Hayward kiwi variety was stored in controlled (CA) and normal atmospheric (NA) storage for 180 days at 0±0.5°C temperature and 90-95% relative humidity. As a result of the study, kiwi was found to be successfully stored for 6 months in storage at CA in combination of 5:5 and 5:2 (%CO_2:%O_2) when compared to NA warehouse (Eris et al. 1996). 1-methylcyclopropene (1-MCP) was applied to Hayward kiwifruit fruit and stored in controlled atmosphere (KA) and normal (NA)+ethylene controlled (EC) conditions. As a result of the study, especially CA (2% O_2, 5% CO_2) + EC application had positive effects on the preservation and shelf life of 'Hayward' kiwi variety fruits. 1-MCP treatment caused deterioration of taste and foreign smell formation. The 'Hayward' kiwifruit fruits were stored successfully at 0°C and 95% relative humidity at CA (2% O_2; 5% CO_2) and NA + ethylene controlled conditions for 6 months without loss of quality (Yıldırım, 2010).

4. Modified Atmosphere Packaging; Modified Atmosphere Packing (MAP) is the process whereby products are packaged with a passive or active gas composition in a package that limits carbon dioxide, oxygen and water vapor permeability. MAP application in kiwis; not only reduces the rate of fruit softening but also prolongs the storage period because of the increase of CO_2 and the decrease of O_2 in the environment. In one study, the Hayward kiwi variety was packaged in three different packages: consumer packaging, classical packaging, modified packaging, and maintained at 0°C temperature and 90-95% relative humidity conditions. According to the result of this research, the modified packaging reduced the weight loss during storage. In classical packaged fruit, weight loss has increased. The level of total soluble solids (TSS) increased while the shell thickness of the fruit, the fruit stiffness, the vitamin C content and the titratable (TE) acidity level decreased during the cold storage in general. It was found that the effect on the skin thickness of packaging types is negligible. Fungal rotting factors were increased in the modified packaged fruit, but decreased in the consumer packaged fruit. Color lightening was occured in fruit flesh and skin during the storage. Flavor characteristics of the fruit decreased during storage. As a result, it was determined that Hayward kiwi fruit has been successfully preserved for 6 months with the modified packaging and 5 months with the consumer and classic packaging (Namdar, 2005). The effects of different post harvest practices and different packaging types on kiwi storage time and fruit quality were investigated. In this study, 3-dose 1-MCP such as 312.5 ppb, 625 ppb and 1250 ppb were applied to some of the kiwifruits. Some of them are packed with LDPE and PVC packaging material. The kiwi were then stored for 2, 4 and 6 months in cold air storage at 1°C and 95% relative humidity. The results obtained from the research showed that the applications of PVC and LDPE were successful in terms of the criteria examined. Among the 1-MCP applications, the application of 1250 ppb 1-MCP showed more positive results than the other doses. This application was followed by application of 625 ppb 1-MCP and 312.5 ppb insufficient in terms of preservation of the desired fruit quality (Duman, 2011).

Prevention methods of diseases

Heat Treatments

It is required that before all harvested crops are stored, the disease agents must be free from bugs and dirt and dust. The sensitivity of the harvested product to postharvest diseases and the physiological changes that cause the pathogens to develop in the fruit are increased during long-

term storage. This shortens the life of the stored product. Nowadays, disinfecting methods are preferred so as not to leave residue on the products. Temperature treatments are also the most preferred method among them economically (Fallik, 2004). These applications kill pathogens causing surface degradation, while preserving fruit quality during long-term storage and marketing. Three different methods are used in temperature applications. These are hot water applications, hot steam applications and hot air applications. In these three applications, the first objective is to reduce the microorganism load, while at the same time stimulating the skin structure and increasing the resistance.

Curing-hot air applications

The curing application is done just before the storage of many vegetables and fruits just after the harvest. This practice prevents the germination of spores on the products. Products are kept in relatively high temperature conditions for a while. Thus, the fruit and vegetables are stored in a way that gives them resistance. In a study conducted, the effects of temperature and humidity on curing *Botrytis cinerea* infection levels were investigated. After harvesting, each fruit was inoculated with 17 ml of Tween 20 containing 25 000 spores in the drop. The best curing effect was obtained at 10°C; The disease intensity was highest at 0°C, whereas the curing effect was reduced at temperatures above 10°C (Bautista-Banos et al. 1997). In another study, flower bud decay in kiwi fruit inoculated with *Botrytis cinerea* showed a decrease with increase in ambient temperature holding time between harvest and packaging / classification. The holding period at 0°C prior to packing / classification was effective in reducing decay. When the effect of the holding time was examined, the degree of decay was reduced by 20% with 7 days of waiting at 0°C, while not decreasing at 4°C for 4 days. In non-inoculated kiwi fruit subjected to the same treatments, *Botrytis* decay intensity was similarly reduced. The crack rate in packed and chilled fruits immediately after harvest was higher than in other applications. This is due to the pre-cooling and the interaction between the polyethylene coated plates.(Pennycook and Manning, 1992).

Hot water and steam applications

Hot water application is usually used to control fungal disease and it is necessary to dry the product after application. Hot steam applications are mostly used for insect control whereas hot air applications are applied against both fungal agents and insects. The last two applications can be carried out in different forms, hot air is stationary or circulated, and humidity control can also be performed during application (Bal, 2009). When the effect of hot water treatments on decaying of Galia melon in long term storage is investigated, it is stated that decay caused by *Alternaria alternata* and *Fusarium* spp. is less in hot water applied fruits compared to fruits that untreated or dipped to water. Gray moldy inoculated strawberries were stored in water at temperatures of 40º, 42º, 44º, 46º, 48º and 50ºC for 15 minutes after inoculation. This application delayed the development of *Botrytis cinerea* in strawberry fruit. Waiting in water at temperatures of 44º and 46ºC is the best result in terms of preservation of fruit flesh stiffness and internal quality. At this temperature range, there was no negative effect on the external color and taste. The kiwi were kept in the water at 46, 48 and 50˚C temperature for 4, 8 and 15 minutes and the effectivity against Botrytis cinerea of this application was examined. After these applications, the kiwi were stored at 0°C for 13 weeks. According to the results of the study, 15 min at 46˚C and 8 min at 48˚C were found to be more effective in *Botrytis cinerea* control than other applications. These applications did not have a positive effect on the flesh severity and the application of 15 minutes at 48˚C caused damage to the kiwifruits (Cheah et al. 1992). In another study, the Kiwi inoculated with *Botrytis cinerea* was dipped in hot water (45, 50 and 55°C) for 2, 4 and 8 minutes after 3 weeks. All fruits were stored at 0.5˚C and 85-90% relative humidity for 18 weeks. Hot water treatments increased weight loss and weight loss was two fold higher than control samples in 55^{o}C hot water samples. In contrast, the infection rate was significantly inhibited at 6 and 12 weeks of storage in hot-water treated kiwi. In addition, hot water applications slow down the softening of the fruit. At the end of storage,

however, there were no significant differences between applications and control. Hot water applications did not have any positive or negative effects in terms of other quality parameters (Moghadam and Ebadi 2012). The hot water immersion treatment at 50°C and 1.5°C for 1.5 minutes after storage to kiwifruit was being positively effect on preservation of fruit quality after prolongation of shelf life and storage (8 days at 20°C temperature and> 90% relative humidity). With the hot water immersion treatment for 1.5 minutes at 55°C temperature, it is provided that preservation from *Botrytis cinerea* of Hayward kiwi fruits, and quality was maintained during marketing. Therefore, this application suggests that it provides an effective, non-chemical method for protecting the kivin from infection after harvest (Koukounaras et al. 2008).

Gamma Rays and UV-C Applications

Fruits and vegetables are deteriorated due to physical, chemical and biological factors. Several methods of conservation have been developed to reduce this deterioration effect. One of these preservation methods is to store fruits or vegetables by irradiating them. The radioactive materials emit alpha, beta, gamma, X-rays into the environment during the continuous disintegration of their atoms. These rays cause electric charged ions to form in the material they hit. These rays are called ionizing rays. Ionized beam has more energy than non-ionized visible light, television and radio waves and microwave (Acar, 1999). UV irradiation has advantages such as no residue, no legal limitations, and no need for a large safety area to be used, compared to other methods. It is necessary to act in accordance with some rules in food irradiation applications. Firstly, the irradiation method is not applied to the degraded products, but it does not provide an alternative to the Good Manufacturing Practices (Atasever and Atasever 2007). Ultraviolet irradiation is performed using UV-C lamps with the most lethal effect on microorganisms and with a wavelength of 254 nm. At the same time, this wavelength creates a slight stress response, increasing the post-harvesting resistance of the product (Kasım and Kasım 2007). It is stated that UV-C heat has two positive effects on the control of the storage decay of fruits and vegetables. These;

1. The deadly effect of pathogen damage to DNA structure,
2. It encourages the accumulation of antimicrobial compounds that will provide resistance to pathogens in fruit shells. (Bal, 2009).

The labels of irradiated foods must contain the symbol known as radura.

Figure 1: Symbol indicating that the food is irradiated

In a study of the effect of gamma irradiation on kiwi quality has shown that the practitioner can inactivate three pathogens of *Botrytis cinerea*, *Diaporthe actinidiae* and *Botryosphaeria dothidea*. Irradiation application reduced the L* and b* values while increasing the a* values of kiwifruit during storage. In contrast, the irradiated kiwi were softer than the unirradiated kiwi. Irradiation application reduces the amount of total soluble solids during storage while the kiwifruit has no effect on the organic matter content. The amount of vitamins C of irradiated kiwifruit was found to be lower than that of non-irradiated control fruits. The antioxidant activity of kiwi irradiated with 2 and 3 kGy radiation was lower than in control fruits and in treated with 1 kGy radiation. In addition, 3 kGy gamma radiation application also increased sensory quality. In general, gamma irradiation positively affects sensory and hygienic quality by controlling the microbiological population, while cause adverse effects on the vitamin C content, antioxidant activity and structural properties of kiwi (Yook, 2009). UV-C (between 0.01 and 1.50 J/cm^2) and temperature applications (temperatures between 35

and 48°C for 3, 5, 10, 15 and 20 minutes) against *Botrytis cinerea* and *Monilinia fructicola* fungi that causing postharvest decay on kiwifruit were investigated. *Botrytis cinerea* spores were inactivated by UV-C application at 45˚C for 15 minutes (1.00 J/cm^2) and *M. fructigena* spores at 45˚C for 3 minutes (0.50 J/cm^2) (Marquenie et al. 2002).

4. Ozone Application: The shelf life of products that are subjected to ozone treatment and stored become long. Because ozone has antimicrobial effect. Ozone treatment slows the softening of many fruit tissues and reduces weight loss. It has been reported that high doses of ozone adversely affect the sensory properties of foods such as color and flavor (Tan et al. 2005) The effect of ozone application varies according to the product variety. The effect of ozone gas on blossom end rot of Kiwifruit that caused by *Botrytis cinerea* (*Actinidia deliciosa*, cv Hayward) investigated. Artificially inoculated kiwis were kept at 0°C and 95% relative humidity in conventional cold storage for 4 months. The ethylene in the medium was removed by catalytic oxidation and the ozone gas was applied to the cold storage chamber by the continuous flow method (0.3 µL L-1). Ozone treatment reduced the severity of the disease by 56% and the development of the disease on the infected fruit was stable. Pathogen spores did not occur in the presence of ozone while the infected fruits formed sclerotia. In a study that to determine the cause of ozone suppressive effect, kiwi fruits were exposed to ozone (0.3 µL L-1) before and after inoculation on a traditional cold storage for 0, 2, 8, 24, 72 and 144 hours, and the effectivity of ozone treatment on severity of diseases has been monitored. As the duration of exposure to inoculation increased for the fruits exposed to ozone before inoculation, the disease was markedly suppressed and applications after inoculation remained ineffective. Pre-inoculation ozone application is highly recommended because it increases the resistance of kiwifruit to pathogen and prevents the formation of the disease. Measures of antioxidant and antioxidant activity in fruits that were exposed to ozone for a certain period showed a strong negative correlation between disease intensity or severity and phenol content (Minas et al. 2010).

5. Essential Oil Applications: Essential oils; which are obtained by different ways from plants, are terpene-like oil-like natural substances which can be transported by burning intense smelling, water vapor. Essential oils obtained from some medicinal aromatic plants have antioxidant and antimicrobial effects. The most common and reliable methods used in food preservation include heat treatment, freezing, drying, irradiation. In cases where these methods can not be applied or are inadequate, the addition of antimicrobial agent to food is a problem. Antimicrobial agents are used to destroy microorganisms that are undesirable in food but for any reason, and prevent them from multiplying (Cerit, 2008). Extracts from 345 plants and 49 essential oils were evaluated for antifungal activity against *Botrytis cinerea*. At the end of this study, lemongrass (*Cymbopogon martini*), thyme (*Thymus zygis*), cinnamon (*Cinnamomum zeylanicum*) and carnation buds (*Eugenia caryophyllata*) from the 49 essential oils showed the highest antifungal activity against *Botrytis cinerea* (Wilson et al. 1997). The effects of moth, thyme, mother-of-pearl, marjoram, lavender, rosemary, sage and ylang essential oils on *Botrytis cinerea* were investigated. As a result, it was determined that essential oils such as mint, thyme, sedum, marjoram applied at relatively low concentrations (85-300 µg/mL) prevent *Botrytis cinerea* development. In contrast, lavender, rosemary, sage, and yarn essential oils have been found to have less inhibitory effect (Daferera et al. 2002). The antifungal effect of essential oils of *Carnation carnium* (*Carum carvi*) and anise (*Pimpinella anisum*) on kiwifruit was investigated. For this purpose *Botrytis cinerea* spores, which are propagated and pre-disinfected in tissue culture, are inoculated with kiwi fruit. Four different doses of essential oils (200, 400, 600 and 800 µL/L) were then applied. As a result it was determined that blackcurrant caraway and aniseed essential oils increased kiwi shelf life and completely inhibited gray mold formation when compared to the control group. It has been determined that the antifungal effect increases as the dose treated increases (Fatemi et al. 2013).

Conclusıons

In many fresh fruits and vegetables, chemical substances are used against fungal pathogens. These substances are known to affect human health negatively. In this study, it has been shown that non-chemical physical and natural substances can be used as antifungal agents.

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DETERMINATION OF VITAMINS AS ADDITIVES FOR FORTIFICATION OF REFRESHING SOFT DRINKS

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Abstract
Soft drinks are sweetened, based on water, usually contain a certain amount of fruit juice, fruit pulp or other natural ingredients and they have a balanced acidity. Their nutritional and energy value is derived mainly from the content of sugars, but besides them there are also mineral elements, vitamins, enzymes and amino acids in minor amounts. Soft drinks are classified as fruit juices and refreshing beverages. Refreshing beverages can be clear or with pulp. The main ingredient is water, then sugar or artificial sweetener, fruit juice or fruit base or plant based extracts or based on cereals, with addition of carbon dioxide and allowed additives in the prescribed amounts. In recent times a trend is to fortify the refreshing beverages with certain vitamins or minerals. Ascorbic acid is commonly added in food as an antioxidant and stabilizer. The vitamins from B group are water soluble and played a significant role in human metabolism. In addition, it is important to consider the amount of the microelements that are necessary to meet our daily needs as nutrients. The research was made on refreshing beverages fortified with vitamins. The analysis was made on 20 different beverages in 2014, 2015 and 2016. The quantitative determination of the water soluble and fat-soluble vitamins in the refreshing beverages was performed by using by HPLC-DAD method. The vitamin C was determined by iodometric method. In the examined samples were usually present the following water soluble vitamins: B_1 (from 0.18 to 0.3 mg/100ml), B_3 (from 2.40 to 3.20 mg/100ml), B_5 (from 0.8 to 1.04 mg/100ml), B_6 (from 0.20 to 0,37 mg/100ml) and the vitamin C (from 9.06 to 16.41 mg/100ml), and more rarely were present vitamin B_2 (from 0.22 to 0.28 mg/100ml) and the liposoluble vitamin E (from 0.7 to 1.33 mg/100ml).

Keywords: refreshing beverages, microelements, HPLC method.

Introduction

Consumer demand is becoming more fastidious when choosing a beverage, of which they expect besides good sensory properties, to also have health benefits (http://hexagonnutrition.com/published-articles/03.06.2017). In order to meet the consumer's needs and to give contribution in improving their health, it is necessary to use all the advantages of the technology and science in order to produce "novel" food that will be in function for human health. This type of food may contain different macronutrients and micronutrients, which can reduce the risk of certain diseases (Mandic, 2007). According to the Rulebook on requirements regarding the quality of soft drinks (Official Gazette of the R. of Macedonia No. 15/2013) "Soft drinks" are products obtained by a special technological procedure from drinking water, natural mineral water or natural spring water in which can be added: aromas, sugars, sweeteners, starch hydrolysates, fruit juice, concentrated fruit juice, fruit syrup, plant extracts, fruit extracts, tea

extracts, cereals and other products, with or without the addition of carbon dioxide, mineral salts or vitamins. Soft drinks are available everywhere in the world, almost in the same forms, bottles, cans, paper laminated packaging, cups and many other forms of packaging (Ashurst, 2005). The properties of soft drinks are mainly related to the properties of the constituent components. Nutrition and energy value originates mainly from the sugar content. The biological significance is determined based on the content of minerals, vitamins, enzymes and amino acids. The presence of these ingredients is quite variable and primarily is conditioned by the composition of the basic and auxiliary raw materials, as well as from the very way of production. Vitamin C is the most frequently added vitamin and it must be labeled in the product declaration (Каракашова, 2008). The vitamins have a different chemical nature, they are essential for the human organism and need to be ingested through the diet because they cannot be synthesized in the body. The vitamins are active even in minimal quantities, usually they are present in different amounts in food products, and each of them is absolutely necessary for proper growth and evolution of the organism and maintenance of good health. The vitamins, according to solubility are divided into: water-soluble vitamins (hydro soluble) and fat-soluble vitamins (liposoluble) (Ѓорѓев и сор., 2008). Fat-soluble vitamins (vitamin A, D, E and K) are absorbed into the fats from food and stored in various tissues. In contrast, vitamins that are soluble in water (vitamins from group B and vitamin C), are not stored in the body in significant amounts (Katalinić, 2007). There are various reasons and goals for fortification of the soft drinks. By fortification of soft drinks there is the possibility of simultaneously overcoming a deficiency of more than one micronutrient. A wide variety of beverages are suitable for fortification, including fruit juices, fruit nectars, vegetable juices, fortified water, carbonated drinks, energy drinks and sports drinks. The quantity of the nutrients to be added to the fortified food corresponds to an amount generally recognised as both safe and effective by the Food and Drug Administration or the Recommended Daily Allowances-RDA. Soft drinks are usually fortified by more vitamins in order to gain greater health impact. On a weight basis, as a percentage of the total dry beverage formulation, these additional nutrients can make up from about 0 to 20 %, in terms of total dry matters. The choice of nutrients used to fortify beverages is based on a number of factors such as the chemical form of the micronutrient in terms of its bioavailability, the effects on the organoleptic characteristics of the particular beverage and cost (http://hexagonnutrition.com/published-articles/ 3.6.2017). Soft beverages can be preserved mainly in two ways: by heating and by adding chemical preservatives, as additives. The primary processing ie. the production of semi-products (juices, concentrates, puree, etc.), is performed usually separately or in another part of the factory, and most often from another producer. The technological procedure depends on the final product that we want to produce and is mainly performed in two phases. The first phase takes place in the department for syrup making, in tanks, with stirrers. Sugar syrup is prepared as a concentrated (cca 60%) sugar solution and contains 30 to 50 % final volume of water. It is prepared by cold or hot procedure or with a temperature higher than 60 ºC (by air removing and inactivating the eventual enzymes, getting more stable, better quality syrups). The syrup is pasteurized at 80 ºC, for 2 minutes, in a plate pasteurizer and it must be filtered. The fruit components are added to the sugar syrup and then follows the addition of regulators of acidity, colors, aroma, preservatives, vitamins and other ingredients. Mixing sugar syrup with other ingredients can be carried out in tanks with stirrers. The components that are previously dissolved separately are added and mixed in the tanks (Levaj, 2013). The content of the additives should not exceed the allowable quantity prescribed in the Rulebook on additives that can be used for food production (Official Gazette of the R. of Macedonia No. 31/2012, Amendments No. 114/2013). The fortification of the beverages with vitamins is planned in such a way so that the nutritive ingredients for fortification result in a desired quantity, solubility, bioavailability, pH value, temperature, light and stability of individual ingredients, overall stability, colour and flavour of the finished beverage. As a beverage becomes fortified, the pH of the beverage rises and therefore the beverage becomes more alcaline. To counter this, an acidifying additive is typically added to the beverage in order to keep the pH of the beverage lower. As the beverage is more acidic, it becomes more resistant to microbial growth. In

most cases, the pH of fruit drinks and juices is below 4.5 and the heat treatment is required in the pasteurization. Some loss of the vitamins, thiamin, folic acid and ascorbic acids occurs as a result of the heating. The vitamin stability is affected most by the heat, the moisture, the pH and the light; but, given their chemical heterogeneity, vitamin losses in different foods vary considerably during both processing and storage of the final product. The most unstable vitamins are C, A, D, B_1 and B_{12} (http://hexagonnutrition.com/published-articles/ 03.06.2017). Since certain vitamins can be partially degraded during storage, their initial amount should be slightly higher than the declared, and therefore the risk of excessive intake must be taken into account (Bonner et al., 1999). The second phase is a continuous process, for homogenization, pasteurization, filling and finalization. Pasteurization is performed only if no preservatives are added, and can be performed before and after filling the beverages in a bottle. The temperature is in the range of 75-100 ºC and the heating time is determined for each product separately (Vereš, 2003). A combined preservation, by chemicals and pasteurization is also possible, and is performed before the filling: in a plate pasteurizer, if the beverage does not contain fruit particles, at a temperature of 85 to 90 ºC, for 30 to 60 seconds, or in a tubular pasteurizer if the beverage contains fruit particles. Preservatives are added to the soft drinks in a certain concentration immediately before filling, but only in the permissible concentrations, in accordance with national legislation (usually up to 120 mg/L). K-Sorbate and Na-benzoate are the most commonly used preservatives. The process of filling takes place in an aseptic block for filling (in sterile conditions) on a line with 3 processes: sterilization of bottles, filling and closing. (Levaj, 2013).

Material and methods

In this research were analyzed different non-carbonated soft drinks fortified with vitamins from domestic and foreign production, which were delivered for regular quality control during 2014, 2015 and 2016 years, at the Laboratory for food quality testing, Institute of Public Health, Skopje. A total of 75 samples were analyzed, which differ according to the composition of the basic raw materials and the technology of production. The analysis has been made of hydrosoluble vitamins (Thiamine B_1, Riboflavin B_2, Niacin B_3, Pantothenic acid B_5, Pyridoxine B_6 and vitamin C) and of vitamin E, as a liposoluble vitamin, by applying a liquid chromatography HPLC-DAD method, with prior appropriate methods of extraction for the vitamins from the samples, by organic solvents. For the hydrosoluble vitamins of group B, the extraction was performed with acetic acid (1 : 4), and detected by using HPLC method, column Supelcosil LC – 8DB, 250 x 4 mm, mobile phase: hexan-1-sulfonic acid-sodium salt, water, HPLC purity, 99-100 % acetic acid, methanol and triethylamine, pH = 3.2; flow: 2.0 mL/min.; T = 35°C; λ = 275 nm. For detection of the Pantothenic acid B_5, another mobile phase was used: a mix of two water solutions of KH_2PO_4, in different concentration, with pH value 2.5, by adding of concentrated phosphoric acid. The vitamin E was extracted with methanol, and determined by applying a gradient HPLC method, using a column Rp8, 250 mm x 5 µm, mobile phase: A methanol; B acetonitrile: methanol : water HPLC purity (63 : 33 : 4 v/v), by gradient: 0.5 min.: 60 % A : 40 % B; 0.5 min. 70 % A : 30 % B; 0.5 min. 80 % A : 20 % B; 0.5 min. 90 % A : 10 % B; 8 min. 100 % A. Vitamin C determination was performed by using a solution of 0.1 N I_2 and 1 % starch solution as an indicator. The statistical processing of the results was made according to the obtained values, in order to make a comparison between the years and during each year for those samples of products that were repeated. The obtained values for vitamins were compared with the values declared on each of the products.

Results and discussion

Based on performed analysis of total 75 samples, during the years of research, 20 different products of soft drinks were determined, according to the producer; the basic raw material from which they were produced; the type and the fruit share, individually or mixture; the production by using pasteurization or combination of pasteurization and preservatives. According to the production

origin of the product, it was determined that 80 % of the analysed samples were from domestic production, and 20 % were from abroad.

In the Table 1 are presented a list of products, where each product has been replaced with a mark, according to the trade names of the producer for the soft drinks, the fruit share in the product, the content of dry matter as an important parameter for the quality of the soft drinks and the manner of production, i.e. the application of the technological pasteurization or combination pasteurization and preservatives. Regarding the fruit share, it can be concluded that 15 % of the products were with 50 % fruit share; 5 % with a fruit share of 40 %; 10 % with a 30 % fruit share; 5 % with a fruit share of 20 %; 45 % with a fruit share of 12 %; 5 % with a fruit share of 11 % and 15 % with a 10 % fruit share. In terms of the content of soluble dry matter, it has been determined that 55 % of the examined soft drinks contain 12 %; 5 % contain 11 % soluble dry matter; 25 % contain 10 % and 15 % contain 7 % soluble dry matter.

According to the technological procedure of production, 35 % of the soft drinks were produced by a pasteurization procedure, while 65 % were produced by applying a pasteurization and preservatives, as Na-bezoate and K-sorbate, in concentrations in accordance with national regulations. After the performance of the analysis and determination of the content of hydrolytic vitamins from the group B (B_1, B_2, B_3, B_5, B_6), vitamin C, and vitamin E as liposoluble vitamins, the obtained results are presented graphically in Figures 1, 2, 3. According to results it can be concluded that the vitamin C was one of the most common vitamins that is present in most of the beverages, primarily as an antioxidant, but also to increase the biological value of the product itself. The presence of the vitamin C was established in 65 % of the products, of which in 8 % of the analysed samples the presence of the vitamin C was not confirmed. In the examined samples, the content of the vitamin C was in range from 9.06 ± 0.9 mg/100 g in the soft drink marked S, with fruit share of 10 %, to 16.41 ± 1.05 mg/100 g in the soft drink marked N, with fruit share of 12 %. It can be notice that the vitamin C was the only present vitamin in beverages with a fruit share of 50 %.

Table 1. Products of soft drinks, marked according to the producer, the fruit share, content of dry matters and the technology of production

Mark	Fruit share	Dry matters	Produced by
A	min. 50 %	min. 12 %	pasteurization
B	min. 50 %	min. 12 %	pasteurization
C	min. 50 %	min. 12 %	pasteurization
D	min. 40 %	min. 12 %	pasteurization
E	min. 30 %	min. 12 %	pasteurization
F	min. 30 %	min. 12 %	pasteurization
G	min. 20 %	min. 12 %	pasteurization
H	min. 12 %	min. 12 %	pasteurization and preservatives
I	min. 12 %	min. 10 %	pasteurization and preservatives
J	min. 12 %	min. 10 %	pasteurization and preservatives
K	min. 12 %	min. 10 %	pasteurization and preservatives
L	min. 12 %	min. 11 %	pasteurization and preservatives
M	min. 12 %	min. 10 %	pasteurization and preservatives
N	min. 12 %	min. 12 %	pasteurization and preservatives
O	min. 12 %	min. 12 %	pasteurization and preservatives
P	min. 12 %	min. 12 %	pasteurization and preservatives
Q	min. 11 %	min. 10 %	pasteurization and preservatives
R	min. 10 %	min. 7 %	pasteurization and preservatives
S	min. 10 %	min. 7 %	pasteurization and preservatives
T	min. 10 %	min. 7 %	pasteurization and preservatives

According to the performed analyzes, it can be concluded that in the samples of soft drinks, the most commonly used vitamins were from group B: B_1, B_2, B_3, B_5, B_6, and for each of them has been determined presence in different combinations and concentrations in the products. According to the

obtained results from the examined samples, the following concentrations were determined: vitamin B_1 was within from 0.18 ± 0,01 mg/100 g in the soft drink marked N, with 10 % fruit share, to 0.30 ± 0.05 mg/100 g in the sample of soft drink marked E, with 30 % fruit share; the values for vitamin B_2 were within the range from 0.23 ± 0.01 mg/100 g in the soft drink marked E with 30 % and H with 12 % fruit share, to 0.28 ± 0.02 mg/100 g in beverage marked M with 12 % fruit share; the lowest value of vitamin B_3, 2.40 ± 0.10 mg/100 g was determined in the soft drink sample, marked E with 30 % fruit share and the highest value 3.20 ± 0.10 mg/100 g was determined in sample marked F, with fruit share 30 %; for the vitamin B_5 has been determined the lowest concentration of 0.80 ± 0.05 mg/100 g in the sample N, with fruit share 12 %, and the highest value 1.04 ± 0.08 mg/100 g in the soft drink sample marked E, with 30 % fruit share; the obtained values for vitamin B_6 were within the range from 0.2 ± 0.01 mg/100 g in the sample R with fruit share 10 %, to 0.37 ± 0.04 mg/100 g, determined in the soft drink sample marked F, with fruit share 30 %. It has been determined that the vitamins from B group were present in 70 % of the analyzed products, of which, the most commonly present in all of the products was the vitamin B_6. It was estimated that the vitamins B_1, B_3 and B_5 were present in 71.42 % of products, of which, for the vitamin B_1 in 9.52 % and the vitamin B_5 in 5 % of the sample products, in certain years, was not detected their presence. The vitamin B_2 was determined in 35.71 % of the products where the vitamins from group B were present, from which for 36.36 % of the samples in certain years, its presence was not detected.

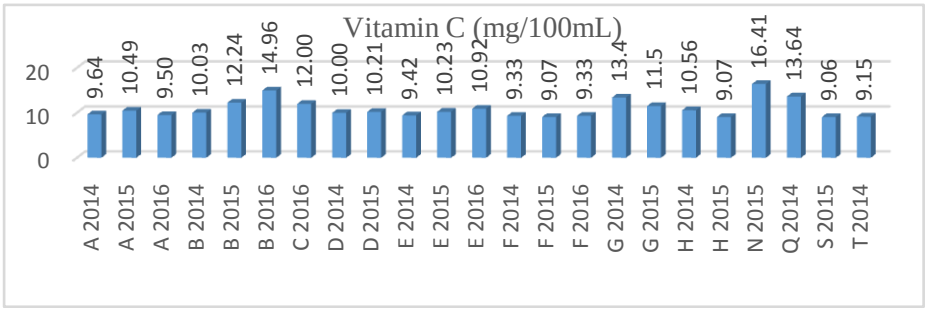

Figure 1. Content of the Vitamin C in the examined samples of the soft drinks

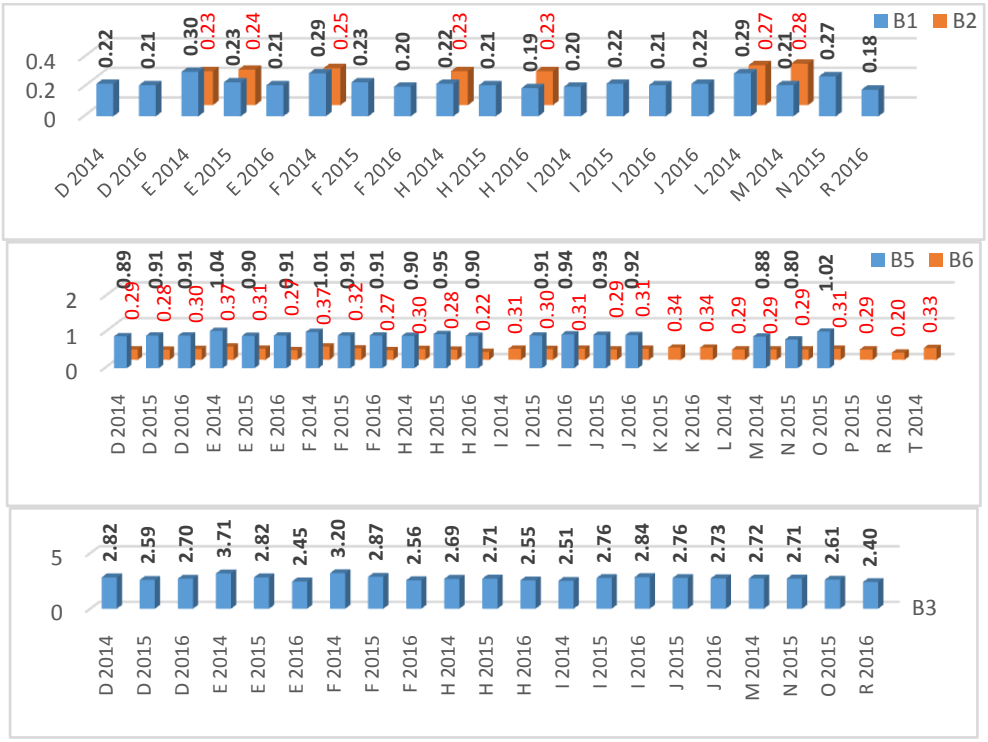

Figure 2. Vitamins B_1, B_2, B_3, B_5 and B_6 in the examined samples of soft drinks

Based on this research, the vitamin E has the lowest value of 0.7 ± 0.02 mg/100 g determined in the soft drink marked T, with 10 % fruit share, while the highest value of 1.33 ± 0.07 mg/100 g was determined in the soft drink sample marked N, with fruit share of 12 %. It was estimated that the vitamin E has been present in 40 % of the products, of which in 35.71 % of the samples its presence was not determined.

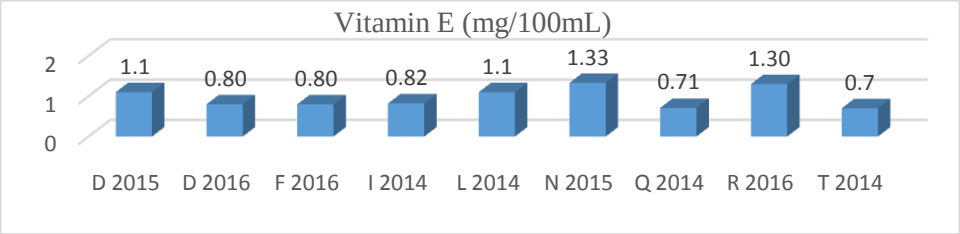

Figure 3. Vitamin E in the examined samples of soft drinks

The results for the vitamin content in the samples of various soft drink products, examined during years of research, were processed by using an ANOVA variance analysis, to determine the effect of the factor year on the quantity of vitamins in the products. Based on the statistical processing of the obtained results, it has been assesed that there were no statistically significant differences in the content of vitamins in the samples of the products examined several times during the year and in different years, but was noticed the absence of certain vitamins in certain years. A comparison was made for the obtained values with the declared values of the packaging of the products, where it is notable that in some products the presence of the vitamin A, the vitamin D, the folic acid, the biotin and the vitamin B_{12} was not determined, despite their declared value, what can be due to their low concentrations in the products or the possibility of interference during analysis with another component of the matrix or the laboratory capability to properly extracti and detecti them using the existing equipment. According to the obtained values for the vitamins, the comparison was made with those declared, and the greatest deviation of the presence of the vitamin E in the products was noticed, 40 % of the products declared its presence, but it was not detected during the analysis. For the other products, the values obtained for the vitamin E 57.14 % of products statistically significantly differ from the values on the declaration, which is due to several factors, but above all that this vitamin is liposoluble and during the technological process of fortifying the juices, it is necessary to add appropriate carriers and stabilizers. In terms of the other analysed hydrosoluble vitamins from group B and the vitamin C, it was assessed that the obtained values were not statistically significantly different from the declared values on the products.

Conclusions

Based on the research, we came to the following conclusions:

Of the total of 75 examined samples, over the three years, it was determined that there were 20 products of soft drinks, assessed according to the producer, the basic raw material, the type and the fruit share and of the technology of production. It was determined that 80 % of the analysed samples were from domestic production, and 20 % were from abroad. Regarding the fruit share, it can be concluded that 15 % of products were with 50 % fruit share; 5 % with a fruit share of 40 %; 10 % with a 30 % fruit share; 5 % with a fruit share of 20 %; 45 % with a fruit share of 12 %; 5 % with a fruit share of 11 % and 15 % with a 10 % fruit share. The vitamin C was present in 65 % of the products and is the only present vitamin in beverages with a fruit share of 50 %. It has been determined that the vitamins from B group were present in 70 % of the analysed products, of which, the most commonly present in all products was the vitamin B_6. It was estimated that the vitamin E has been present in 40 % of products, of which in 35.71 % of samples its presence was not detected. There were no statistically significant differences in the content of the vitamins in the samples of the products examined several times during the year and in different years, but the absence of certain

vitamins in certain years was noticed. For the vitamin E it was noticed that for 40 % of the products its presence was declared, but not detected during the analysis. For analysed hydrosoluble vitamins, group B and the vitamin C, the obtained values were not statistically significantly different from the declared values on the products. Factors that affect the stability of the vitamins in the product are: the technological procedure, the form in which the vitamins are added, their quantity, the presence of other additives and the possibility of interaction with them, but of particular importance are also the conditions of storage and the storage of the final products (temperature, light and shelf life). The vitamins need to be added in higher quantities because it is considered that the declared amount of the given vitamin should be sustained by the end of the prescribed shelf life for the given product.

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CHANGES OF NUTRITIONAL PROPERTIES OF THREE VARIANTS PEPPERS BY PROCESSING OF PICKLED RED PEPPERS

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Abstract
The pepper (*Capsicum annuum* L.) is an important vegetable crop due the multiple ways it can be used in the processing industry, as well as the presence of nutrients and bioactive matters that are important for health. The pepper fruits for processing is necessary to have a larger useful part, with expressed red color, a higher content of dry matter and to possess inherent sensory characteristics. The quantity and the inter-relationship of chemical components, including water, are responsible for their nutritional value. The aim of this investigation was to compare the nutritional composition of three varieties of peppers, fresh and processed, as well as pasteurized pickled peppers. As a raw material had been used pepper varieties *kurtovska kapija, palanechko chudo* and *horgosh*, harvested at their technological maturity. Blanching was done at 85-90 °C for 5 min. To the pasteurized pepper, acetic acid was added at a concentration of 1.5 % in terms of finished product, which belongs to the low marinated products. Pasteurization is carried out at T 92 °Cfor 30 minutes. The chemical properties were determined by analyzing the following parameters: total dry matter, carbohydrates, proteins, oils, total acids, vitamin C, β-carotene and ash, both in fresh and in pasteurized peppers. Based on the obtained results the quality and nutritional composition were determined. The variety *horgosh* shows the highest content of total dry matter (11.45 %), vitamin C (138.9 mg/100 g), β-carotene (22.7 mg/100 g), and the highest energy value (41.7 kcal). In the gourp of the pasteurized peppers, the variety of *palanechko chudo* has been characterized by the lowest average value of total dry matter (7.49 %) and lowest total energy value (24.6 kcal); the variety of *kurtovska kapija* had the lowest contents of vitamin C (52.1 mg/100 g) and β-carotene (6.09 mg/100 g).

Keywords: chemical composition, pasteurization, energy value.

Introduction

Pepper (*Capsicum annuum* L.) is an annual shrub belonging to the nightshade family *Solanaceae* and is one of the commercially important crops which is classified under vegetable fruits (Sethu et al., 1996). Growers have selected many pepper cultivars with different properties and characteristics. The result is a great number of very different cultivars showing a wide range of morphological and organoleptic characteristics, including color, which determine their use. At first all cultivars must meet a series of appropriate agronomic and industrial requirements (Hornero-Méndez et al., 2000). Peppers are becoming increasingly popular among consumers due to their broad variety of shapes (bell or tomato shaped), sizes, colours (starting from green, yellow or white for the unripe fruit, and turning to red, dark red, brown, and sometimes almost black in the ripe state) and its characteristic flavour, which mostly determines their application. They can be used to produce dehydrated products (such as paprika), in salads, pickled peppers, sliced or diced frozen peppers to be used in different products or as a food colorant (Castro et al., 2008). Peppers are very popular vegetables because of the nutritional value. They are a good source of numerous antioxidant compounds. The

chemical composition of pepper fruits vary, even within the same variety, depending on maturity, the location of production, and agricultural practices as well as on numerous environmental factors (Pérez-López, 2007). The beneficial effects of peppers have been related to the presence of vitamin C, vitamin A is present as provitamin – carotenoids, dietary fibre and other phytochemicals (polyphenols, carotenoids) in these plant food products. The intense red color of pepper fruits is due to carotenoid pigments that are synthesized mainly during fruit ripening (Guil-Guerrero et al., 2006). The red fraction contains the pigments exclusive to the *Capsicum* genus (capsanthin, capsanthin-5,6-epoxide, and capsorubin), and the yellow fraction comprises the rest of the pigments (zeaxanthin, violaxanthin, antheraxanthin, β-cryptoxanthin, β-carotene, and cucurbitaxanthin A), which act as precursors of the former (Hornero-Méndez et al., 2000). Some of them have provitamin A activity (β-carotene, α-carotene, β-cryptoxanthin), and some act as antioxidants. These pigments occur in non-esterified, mono- and di-esters with fatty acids (Vračar, 2007*).* They are more liposoluble and at the same time more stable to photo- and thermoxidative reactions and other processes (Pérez-Gálvez et al., 1999). Fresh peppers have a high content of vitamin C, and are also a good source of pro-vitamin A carotenoids. The importance of carotenoids in the diet has been recognised, as vitamin A and other retinoid precursors and antioxidants in cell protection, for the prevention of degenerative diseases and for human epithelial cell differentiation (Pérez-López, 2007). The safety and impact of industrial operations is necessary to be in balance with optimal process methods and applying the most suitable processing conditions for achieving a desired high quality products, in organoleptic and nutritional terms (Silva et al., 1993). Basically, the thermal processing of the products during preservation should be performed at the lowest possible temperature and for the shortest period of time, provided that a microbiologically stable product is obtained, which will not get spoiled during the intended shelf life (Vereš, 2004). For the success of the heating effect, the most important are: the temperature and the heating time; the type and number of microorganisms in the raw material; the chemical composition and physical properties of the product (content of: water, sugars, fats, preservatives; pH, packaging and pre-treatment of product). Pasteurization uses temperatures of up to 100 °C and heating times of up to 30 minutes. It is mainly used for vegetable products, which in the technological process of preservation have increased acidity (marinated, pickled, and fermented vegetables) (Niketić-Aleksić, 1994).

Material and methods

For this experiments we used ripe pepper fruits (*Capsicum annuum* L.,) of three varieties, *kurtovska kapija, palanechko chudo* and *horgosh*. They originated from different regions in R. Macedonia. The varieties *kurtovska kapija* and *palanechko chudo* came from Strumica region and the variety *horgosh* from Demir Kapija region. The laboratory analyses were made at the laboratories for food quality control in the Institute of Public Health in Skopje, at the laboratory of the Department for Processing Fruits and Vegetables at the Faculty of Agricultural Sciences and Food in Skopje and at the laboratory of Food Chemistry, Institute of Biochemistry, Graz University of Technology, Austria. The analyses were performed by applaying standard laboratory methods, equipment and standard chemical reagents, according to the laboratory procedures.

Technology of pasteurization of pickled red peppers

The production of pasteurized pickled red peppers from the three varieties was performed industrially, at the factory "Bonum", Kumanovo. Uniform raw fruits were used for production; that is, a batch of pepper fruits with similar color, size and ripeness. The peppers were washed with water to remove impurities, partly from the microorganisms and waste parts and other substances. Afterwards the fruits were inspected and selected manually, cut of the stalk and seeds removed, and then blanched. Blanching as a pre-treatment is used to inactivate deleterious enzymes which can have a series of detrimental effects such as hydrolysis of pectin. Simultaneously the required elasticity of the pericarp is achieved (Castro et al., 2008). Also, due the blanching a series of changes of the physical-microbiological nature occur, such as a decrease in the total number of

microorganisms and an undesirable change in the sensory properties, such as loss of colour, flavour, texture and nutrients. The changes are depending on the blanching conditions (Cruz et al., 2011; Каракашова, 2011). The process of pepper blanching is performed at a temperature of 85-90 °C for 5 min. After blanching, the raw material must be cooled in order to preserve the structure of the blanched product and to prevent the undesirable effects associated with high temperature. Only healthy blanched pepper fruits, equal in size, shape and color are using for further processing. Then the peppers are submerged in a hot solution for pickling, which contains water, salt, sugar, and acetic acid. In pasteurized pickled pepper, the acetic acid is added in a concentration of up to 1.5 %, in terms of the final product. Due to the low concentration of acetic acid this type of product is called "weakly marinated" (Каракашова, 2011). The filled jars closed and washed prior to pasteurization. The process of pasteurization of pickled peppers in jars is done in an autoclave according to the established heating regime. During heating the quality of the peppers changes to a typically cooked product meaning that texture, taste, flavour, and colour as well as nutritional quality attributes (e.g. reduction of ascorbic acid) are altered (Castro et al., 2008). The pasteurization is performed at a temperature of 92 °C for 30 minutes with a cooling cycle for 30 minutes in cold water.

Determination of quality and nutritional value

The determination of quality and nutritnional value were performed by analyzing the following parameters: total dry matter by drying the samples in an oven dryer at 105 ºC to obtaining constant mass (AOAC 925.10, 1995); total acidity by using titration solution 0.1 M of NaOH and 1 % solution of phenolphthalein as indicator (AOAC, 942.15, 1995); content of salt in final products by titration with 0.1 M AgCl and 1 % solution of K_2CrO_4 as indicator (AOAC, 971.27, 1995); pH value of the pickled solution of the final products, by pH meter (AOAC, 981.12, 1995); the total ash by incineration and burning of samples in a Muffle oven, at a temperature of 550 °C; ash insoluble in HCl, by incineration and burning in a Muffle oven at a temperature of 550 °C (AOAC, 941.12, 1995); determination of sugars by applying HPLC-method with RI-detector (ASU# 35 LMBG L00.00, 1984); fats determination, according to the Soxhlet method (AOAC 960.39, 1995); the content of proteins was analyzed with the Kjeldahl method (AOAC 978.04, 1995); determination of Vitamin C was performed by using a solution of 0.1 N I_2 and 1 % starch solution as an indicator (http://www.canterbury.ac.nz/media/documents/scienceoutreachvitaminc/ iodine.pdf -15.09.2017). β-Carotene was extracted by organic solvents and the analysis was performed by thin layer chromatography TLC. The quantification was performed by using Camag Scanner II.V.3.14/PC/Cats HPTLC densitometer, at 450 nm (Zeb et al., 2010). From the analysed values the energy was calculated according to (FAO, 2003). The sensory evaluation was done on basis of a linear scale with 20 points. This included the smell, taste, appearance and texture. The quality of the final products was determined in accordance with legal regulations of R. Macedonia (Official Gazette no. 69/2014). Statistics was done software supported by using SPSS (IBM Statistics 21). The obtained results were processed by statistical software package (SPSS).

Results and discussion

Determination of the quality of fresh and pasteurized pickled peppers

The term "pepper quality" as a raw material implies the affiliation to an appropriate variety of certain properties. For the fresh pepper fruits, the important quality characteristics are the different sensory properties appearance, color, taste, hardness, and texture, as well as changes that can occur under certain storage conditions before processing. The first sensorial assessment is the visual appearance. The sensory analysis of color is assessed by the intensity, tint, color uniformity, which is especially important (Каракашова, et al., 2013). The texture described as crunchiness, is also an important quality characteristic which is influenced by hardness, surface gloss, roughness/smoothness, dryness (Tijskens, 2000). The determination of the sensory properties of the pepper varieties, *kurtovska kapija, palanechko chudo* and *horgosh*, was done during the reception of

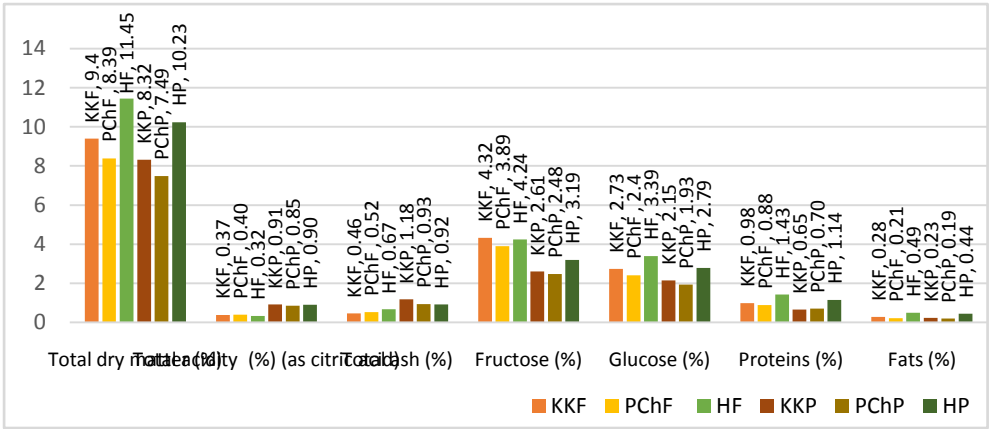
the raw material and immediately before the processing. This was necessary since the quality of the raw material had to be in accordance with the Law on the Quality of Agricultural Products (Official Gazette of RM no.140/2010) and the Law on Amending the Law on the Quality of Agricultural Products (Official Gazette of the Republic of Macedonia No. 53/2011 and 55/2012). The production of pasteurized pickled red peppers, as the processed vegetables, should be in accordance with the requirements of the Regulation on the requirements regarding the quality of processed products from fruit and vegetable, as well as mushrooms and their processed products (Official Gazette of the Republic of Macedonia No. 69/2014). These regulations reflect international norms. According to this Rulebook the term "pasteurized vegetables" is definedas a product obtained by processing the fruits of vegetables or parts thereof by pasteurization in hermetically closed packages. According to Jašić (2007), the color evaluation is often important, especially for final products, because food spoilage is regularly associated with a color change. The pickled solution in pasteurized pepper is mostly clear, with a beautiful light-yellow color, with a weak acid taste, a pleasant smell which is characteristic for pepper. The appearance of a slightly opalescent color of pickled solution in some samples is related to the degree of maturity (Марковић и Врачар, 1998).

The sensory assessments of pasteurized pickled pepper from the varieties *kurtovska kapija, palanechko chudo* and *horgosh*, were made for all sensory properties that are important for the quality. The significance of the individual properties that contributes to the total product quality can be determined by multiplying with a factor of significance (weighting factor) (Koprivnjak, 2014). The highest sensory score was given to pasteurized pickled pepper from the varieties *horgosh* and *palanechko chudo* for the best color (3.00 - average number of points); the best odour with 2.80 and best consistency 7.80 were given for the variety *kurtovska kapija*; best taste (5.80) had the products of the variety *palanechko chudo*. Regarding the overall sensory evaluation, it can be concluded that the pasteurized pickled pepper from the variety *kurtovska kapija* had the highest total score 18.6 (22.3 weighting factor). The lowest total score 16.8 (19.2 weighting factor) had the product from the variety *horgosh*. The analysis of variance (ANOVA) was made according to the obtained results for the total number of points from the sensory analysis on pasteurized pickled peppers. It showed that the interaction of factors variety/technology have a statistically significant influence on the total number of points.

Determination of nutritional composition of fresh and pasteurized pickled peppers

Марковић и Врачар (1998) indicate that the pepper has a specific and rich chemical composition. Water is the ingredient that is most present in the fruits of fresh pepper. From a technological point of view, an important parameter for the quality of pepper as a raw material is determining the content of total dry matter. The varieties of pepper with a better quality considered to be those that have a higher content of total dry matter, which causes also the higher content of individual ingredients leading to a higher nutritional value. The carbohydrates make up the bulk of dry matter. Together with acids they represent the basic components in the formation of flavor. Glucose and fructose are the dominating carbohydrates in pepper fruits (Niketić-Aleksić; 1994; Марковић и Врачар, 1998). Gvozdenović and co-workers (2004) point out that in comparison to the other vegetable crops, pepper is rich in nitrogen (proteins, amino acids, various peptides, etc.). The oils in peppers are present in the seeds and the pericarp. The pepper oils play an important role as a solvent of colored substances, primarily carotenoids. The content of vitamin C varies greatly and it depends on many factors. It mainly depends on the variety and the conditions of breeding, the maturity phase and the way of cultivation (Марковић и Врачар, 1998). The content of total mineral matter is actually expressed as total ash and the ash alkalinity (ash) is obtained after neutralization by hydrochloric acid. For pepper, the content of total ash is an important parameter for the quality control of the raw material as well as the appropriate technological procedure (Niketić-Aleksić, 1994). The acidity of the pepper originated from the presence of organic acids and their salts (Марковић и Врачар, 1998). For poasteurized pickled pepper a weakly acidic solution is used, to which acetic acid is added, and therefore the pH value of the pickled solution is important for the

quality and safety of the final product. By adding acetic acid, the pH of the medium decreases, thus inhibiting the growth of many microorganisms. Typically, acidic foods are pasteurized at up to 100 ° C (not below 75 °C) (Vereš, 2004). Determination of the salt content (sodium chloride) in pickled peppers is one of the most important parameters (max. 2 %). The sensitivity of microorganisms towards to the amount of added salt is in direct relation to the applied temperature. This means that with a combination of salt (3 - 6 %) and temperature, spores of some bacteria at lower temperatures can be destroyed (Vereš, 2004). The energy value was derived from the content of the individual nutrients, carbohydrates, fats (oils), proteins, and total acids. (Ѓорѓев и сор., 2008). The results are presented in Figure 1 and Figure 2. The variety *kurtovska kapija* had the highest value on: fructose (%) (4.32 ± 0.38) in fresh peppers and total acids (%) (as citric acid) (0.91 ± 0.06) in pasteurized pickled peppers. This variety had the lowest value for content of: total ash and β-carotene in fresh peppers; ash insoluble in HCl, salt, proteins, vitamin C and β-carotene in pasteurized pickled peppers. The variety *palanechko chudo* had the highest values for: water (%) (91.6 ± 0.13); total acids (%) (as citric acid) (0.40 ± 0.04) in fresh peppers and in pasteurized pickled pepers, for the content of: water (%) (92.5 ± 0.27); pH value (6.15 ± 0.19); salt (%) (1.10 ± 0.10). This variety had the lowest values for: total dry matter, fructose, glucose, proteins, fats, vitamin C, as well as energy value (133.3 ± 15.3 kJ or 31.8 ± 3.1 kcal) in fresh pepers; in pasteurized pickled pepers the lowest value were for: total dry matter, total acids, fructose, glucose, fats and energy value (103.3 ± 19.3 kJ or 24.6 ± 3.2 kcal). From the results presented in Figure 1 and Figure 2, it can be concluded that the variety *horgosh* had the highest values for: total dry matter (11.5 ± 0.15 %); total ash (0.67 ± 0.04 %); glucose (3.39 ± 0.49 %); proteins (1.43 ± 0.12 %); fats (0.49 ± 0.06 %); vitamin C (139 ± 13 mg/100 g); β-carotene (22.7 ± 2.4 µg/g), as well as energy value (174.8 ± 16.1 kJ) or (41.7 ± 5.41 kcal) in fresh peppers and total dry matter (10.2 ± 0.31 %); ash insoluble in HCl (0.08 ± 0.02 %); fructose (3.19 ± 0.25 %); glucose (2.79 ± 0.18 %); protenis (1.14 ± 0.17 %); fat (0.44 ± 0.08 %); vitamin C (69.6 ± 11.1 mg/100 g); β-carotene (19.5 ± 1.3 µg/g); as well as energy value (170.2 ± 15.9 kJ) or (40.65 ± 3.64 kcal) in pasteurized pickled pepers. The lowest values were found in the variety *horgosh* which included water and total acids for fresh peppers; water, total ash and pH value in pasteurized pickled pepers.

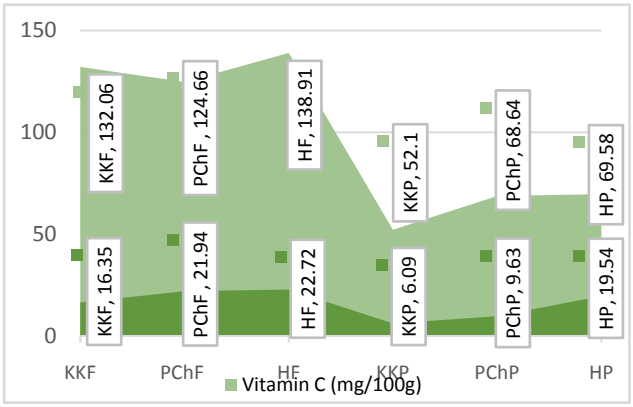


KK-kurtovska kapija; PCh-palanechko chudo; H-horgosh; F-fresh; P-pasterized pickled peppers

Figure 1. Comparison of quality and nutritional parameters of fresh and pasteurized pickled peppers from varieties: *kurtovska kapija*, *palanechko chudo* and *horgosh*

The results obtained from the analysis of the chemical properties and the total energy value, both for fresh and pasteurized pepper, were processed using the ANOVA in order to determine the effects of the variety and technological factors, as well as their interaction. It has been shown that "variety" as a factor has a statistically significant influence on the variability of the total energy value and the investigated chemical properties. Statistically significant differences were determined in interacting the factors "variety"with "technology", for folowing parameters: water content, total dry

matter, glucose, vitamin C and total energy value. Other chemical properties were not statistically significant different.



KK-kurtovska kapija; PCh-palanechko chudo; H-horgosh; F-fresh; P-pasterized pickled peppers

Figure 2. Comparison of vitamin C and β-carotene content in fresh and pasterized pickled peppers from varieties: *kurtovska kapija*, *palanechko chudo* and *horgosh*

Conclusions

The sensory characteristics of all fresh pepper fruits: whole fruits, healthy and fresh, in a stage of technological maturity with a characteristic red color, with a characteristic surface gloss, a normal external moisture of the surface of the pepper fruits and suitable for further processing. With the lowest sensory score, were the final product produced from the variety *horgosh.* Statistical significant differences in final products were found for the senxsory evaluation. In terms of examined chemical components, the variety *horgosh* had the highest content of total dry matter, as well on most constituents and therefore also the highest energy value, in fresh and in final products. Oppositely, the lowest content of dry matter, as well on most constituents and energy value, were found in fresh and in final products from the variety *palanechko chudo*. Among the examineted varieties of red peppers, there were statistically significant differences in relation to the average energy value. Also, between the energy values of fresh and pasteurized acid peppers for each of the varieties, were noticed reduction of energy value due to the passing of some of water-soluble nutrients into the pickled solution in which the pepper fruits are dipped. The pasteurized varieties *kurtovska kapija, palanechko chudo* and *horgosh* were in accordance with the quality prescribed by the national regulations.

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FATTY ACID PROFILE AND SENSORY PROPERTIES OF TRADITIONAL SHEEP KASHKAVAL

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Abstract

The purpose of this paper was to present the fatty acid profile of Galichki sheep kashkaval cheese from Bistra mountain and present its sensory characteristics. The tests were carried out in four iterations on the 180th day of the cheese production. The fatty acids were analyzed under the method AOAC 996.07. The organoleptic assessment was conducted under 20 points scale method, by examining four characteristics (flavor, aroma, texture and additional flavor in the mouth and appearance when cut). Based on the results obtained, it was concluded that the biggest percentage of the fatty acids belongs to palmitic acid (C16:0, hexadecanoic acid) with 30.57±4.17 g/100g, then the oleic (C18:1, z-octadec-11-enoic) with 24.89±1.05 g/100g and myristic acid (C14:0, tetradecanoic acid) with 11.01±0.69 g/100g. Based on the results of the sensory analysis we concluded that the Galichki kaskhaval cheese belongs to the category of very good quality cheese. The flavor was rated as distinct, characteristic for sheep cheese and moderately salty, while the aroma was characterized as pleasant, slightly sour, with no foreign odors. The cheese texture was evaluated as compact, homogeneous, and the firmness as characteristic for the product. The cheese was top-rated in terms of the appearance when cut. This property depends mostly on the skills of the master in the fast and successful shaping of the curd dough and the proper shaping of the cheese.

Keywords: quality, cheese, traditional production.

Introduction

Each traditional cheese originates from a complex system which results in unique organoleptic characteristics. The development of these unique characteristics is linked to several biodiversity factors: the environment, the climate, the natural pasture, the breed of the animals, the use of raw milk and its natural microflora, the cheesemaking technology with the unique role of human beings rather than automated technology, historical tools as well as the natural aging conditions (Licitra, 2010). Kashkaval is one of the most popular hard cheeses in many Mediterranean countries and its production is dating back to the eleventh and twelfth centuries (Kindstedt et al., 2004). One of the traditional cheeses with long history which is produced on mountain Bistra in the Republic of Macedonia is kaskaval from the region in Galichnik. The flora in this country is among the richest not only in the Balkans but also in the entire European continent (Niketić, 2014). The mountain Bistra belongs to the group of mountains which constitute the most interesting mountain-pasture area of the Balkan Peninsula, with the most beautiful hilly pastures (Srbinovska and Santa, 2014). According to Bertozzi and Panari (1993), the traditional character of the cheeses and the possession of the geographical indication are the two most important factors which influence the selection of cheese by the consumers. Cheese made using sheep milk is very popular among the consumers, primarily due to the specific sensory characteristics which have this cheese (Kalantzopoulos, 1993). Cheese flavor is one of the most important criteria determining consumer choice and acceptance. There is a lack of knowledge about the nature of the aroma compounds, but it is clear that the breakdown products of lactose and citric acid (lactic acid, diacetyl, CO2, etc.), of para casein (peptides and amino acids), and of lipids (free fatty acids) are essential for the flavor (Fatma et al., 2013). Free fatty acids are generated during cheese ripening as a result of lipolytic ensyme action. The extent of lipolysis has been used as indicator of the degree of ripening of some type of cheeses such as Italian and blue

cheeses (Martin Hernández, 1988). However, no fatty acid profile of kashkaval from Galichnik have been investigated so far. The purpose of this paper was to present the fatty acid profile of Galichki sheep kashkaval cheese from Bistra mountain and investigate its sensory characteristics.

Material and methods

Analyses for free fatty acids on 180th day of production of cheese were carried out in four itterations. FFA were detected by AOAC 996.07 method with GC 7890A with FID detector- Agilent. Nine months old kashkaval was used for the sensory analysis which was carried out by a panel of assessors and wider group of consumers. In the assessment, a scoring method was applied with a sum of 20 ponderable scores (Ritz et al., 1991; Mandic and Perl, 2006). In all samples, four characteristics (flavor, aroma, texture and additional flavor in the mouth and appearance when cut) were examined, with grades from 0 to 5, using the coefficient of significance. Regarding the amount of points scored, the category of quality where it belongs cheese was given. The table by category according to the number of ponderable points is given in Table 1.

Table 1. Clasification of kashkaval in quality category

Quality category	Ponderable scores
Excelent	17,6-20
Very good	15,2-17,5
Mediocre	13,2-15,1
Still acceptable	11,2-13,1
Not acceptable	<11,2

Results and discussion

Free Fatty Acid in kashkaval

Lipids in foods may undergo hydrolytic or oxidative degradation. Although some lipolysis occurs in most or all cheeses, it is most extensive in some hard Italian varieties and in blue cheese (Mc Sweeney, 2004). While short-chain fatty acids contribute directly to cheese flavor, free fatty acids (FFAs) also contribute indirectly to cheese flavor by acting as precursors for the production of volatile flavor compounds through a series of reactions known collectively as metabolism of fatty acids. Lipolysis in kashkaval cheese is not very intense. According Кожев (2006) kashkaval made from cow's milk has low fat hydrolysis, and thus not very intensive taste and aroma. More evident and intensive hydrolysis cheese is noticed in the kashkaval from sheep milk. The profile of free fatty acid of kashkaval is given in Table 2 and two representative chromatograms are presented in Figure 1. Based on our results on FFA shown in Table 2, we can conclude that the major acids present in the cheese are palmitic (C16:0), oleic (C18:1) and myristic acid (C14:0). Among them, palmitic acid was predominant, followed by oleic , myristic. This finding is in agreement with the results of Kindstedt et al.(2004) and Omar and El-Zayat (1986) on kashkaval cheese. The content of butiric acid (C4:0) corresponds to the results obtained by Gobbetti et al. (2002) in cheese Caciocavallo Pugliese. The results of our research are similar with the results of the research by Woo et al. (1984) on concentration of butiric acid (C4:0) in Parmesan and Provolone. A similar fatty acid profile was reported by Ergönül et al. (2011) on haloumi cheese. Dairy products have been identified as a good source of conugated lonoleic acid (CLA), consequently leading to greater research development in this area (Ha et.al, 1989, Chin et al., 1992). The authors found great variability in the concentration of CLA in dairy products and noted that ripening, heat treatment and protein contents were among the main factors affecting the concentration of this acid. When examining the different types of cheeses, the authors found that the highest concentration of conjugated linoleic acid is found in the blue cheese (7.96 mg/g), then in the Swiss cheese (5.45 mg/g) and edam cheese (5.38 mg/g). In our research, the conjugated linoleic acid ranged from 3.28 to 4.16 %.

Table 2. Free fatty acid profile of kashkaval (n=4), g·100g^{-1} of cheese

FFA g·100g^{-1}	X	FFA g·100g^{-1}	X
C4:0	2.09±1.98	C17:0	0.51±0.04
C6:0	/	C17:1	0.22±0.01
C8:0	0.83±0.18	C18:0	7.68±2.43
C10:0	6.46±0.60	C18:1 c	24.89±1.05
C11:0	/	C18:1 t	/
C12:0	5.20±1.28	C18:2 t	0.47±0.05
C13:0	/	C18:2 c	2.12±0.99
C14:0	11.01±0.69	C20:0	0.44±0.10
C14:1	0.26±0.10	C18:3 n-6	0.92±0.29
C15:0	1.35±0.13	CLA	4.13±0.74
C15:1	0.28	C18:3 n-3	0.85±0.22
C16:0	30.57±4.17	C21:0	1.66±0.08
C16:1	/	C20:4 n-6	0.38±0.01

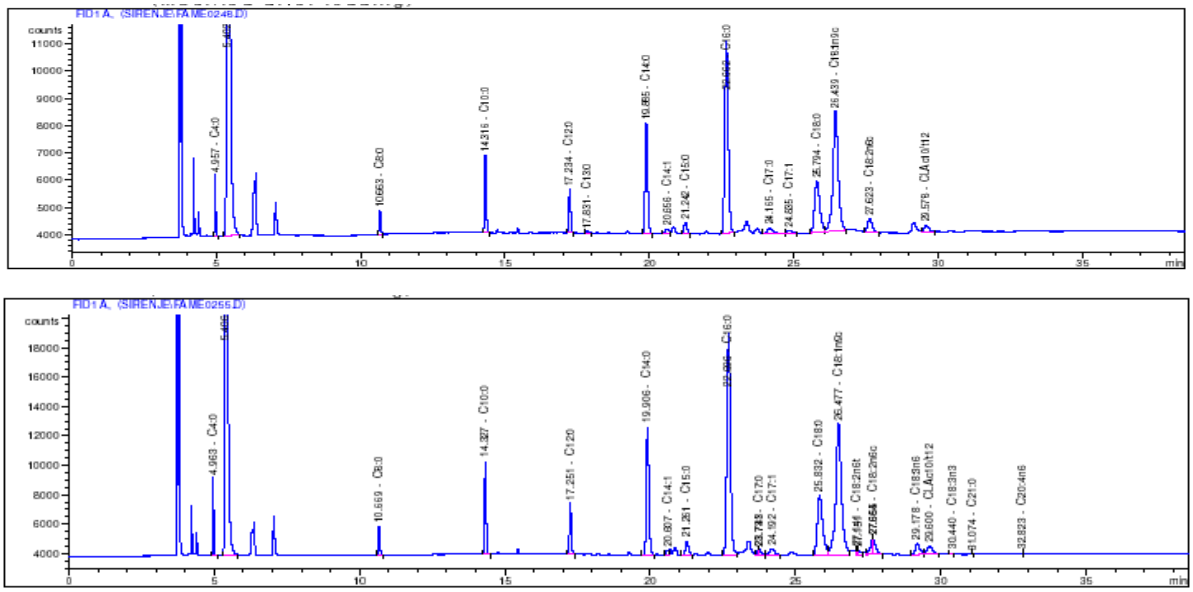

Figure 1. Representative chromatograms of the samples of cheese

Sensory properties

The assessors evaluated the taste as clearly expressed, characteristic for sheep kashkaval cheese and moderately salty, while the aroma was characterized as a pleasant, discreetly sourish, without external odors. These positive features of the sheep kashkaval are associated with its specific geographic area, considering that Bistra Mountain abounds with aromatic plants, whose alkaloids affect the sensory properties of milk, and hence the dairy products as well. Additional influence on the more intensive taste of this cheese has the very use of raw sheep milk as a raw material for the production of the traditional kashkaval. This is also confirmed by other authors (Mendia, 2000; Ballesteros, 2006) in the sensory evaluation of the sheep milk cheeses: Idiazabal and Manchego, who have shown that cheeses made from raw sheep milk receive higher scores than the ones made from pasteurized sheep milk. Namely, Ballesteros (2006) noted that higher scores are given to raw milk cheeses with regard to their intensity and duration of the aroma, the intensive aroma of the sheep milk and bitter taste. The authors associate the obtained higher sensory ratings with the presence of heterogeneous microflora, the complex enzyme system and the high concentration of nitrogen fractions in the cheeses made from raw milk.

Table 3. Results of the sensory properties of Galichki kashkaval (n=4)

Parameter	n	Score	□	SF	PMS	MNP
Flavour	1	4.16	4.05	1.50	6.071	7,5
	2	4.00				
	3	4.02				
	4	4.02				
Aroma	1	4.12	4.03	0.50	2.017	2,5
	2	3.67				
	3	4.36				
	4	3.99				
Texture and additional flavor in the mouth	1	4.27	4.04	1.00	4.044	5
	2	3.45				
	3	4.34				
	4	4.12				
Appearance when cut	1	4.31	4.50	1.00	4.499	5
	2	3.61				
	3	5.95				
	4	4.13				
Total:					16.631	20

n –itterations
SF – Significance factor
PMS - Ponderable mean scores
MNP – Maximum number of possible ponderable points

Thus, we come to the conclusion that the characteristic taste of the traditional sheep kashkaval results from many factors like the presence of the microflora in the raw milk, as well as from the proteolysis and lipolysis level and other factors, including the location itself and the sheep's nutrition and feeding. Generally, the texture of the kashkaval was rated as compact, homogeneous and its hardness characteristic for the product. During storage, formation of a mold was noted on the kashkaval wheel's surface. This phenomenon is frequent in the traditional kashkaval cheeses, primarily because of the manual formation of the wheels and removal of the ''navel'' where there is a possibility cracks to be formed in which the mold develops. In order to prevent further penetration of the mould inside the kashkaval cheese , the mould was regularly removed from the surface. After this, on the 100th day of the production, the kashkaval cheese wheels were protected by polymer emulsion, which, in the research period , such protection proved to be a good protective layer. Talevski (2013), in his research on cheese with different surface protection, concluded that the best sensory characteristics showed the kachkaval protected with polymer emulsion. The appearance when cut piece was evaluated as regular without unevenness, homogeneous and equal throughout the whole surface. This feature depends largely on the skills of the master who needs to quickly and successfully mold the cheese dough, appropriately remove the air and form regularly shaped wheels, to the contrary a horizontal layering will appear, which can often occur in traditional cheese. No such occurrence was noted in our cheese during ripening and storage. Based on the results of the sensory analysis we concluded that the Galichki kaskhaval cheese belongs to the category of very good quality cheese.

Conclusions

Each traditional cheese is a complex system, with defined flavors that represent the territory where it is produced. Galichki kashkaval from mountain Bistra is one of the famous traditional product in the country. The major acids present in the cheese are palmitic (C16:0), oleic (C18:1) and myristic

acid (C14:0). The concentration of conjugated linoleic acid (CLA) was 4.13±0.74%. Based on the results of the sensory analysis, Galichki kaskhaval cheese belongs to the category of very good quality cheese.

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FOOD COMPOSITION DATABASE IN MACEDONIA- NEED AND IMPORTANCE

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Abstract

Food Composition Databases (FCDBs) represent fundamental information resources for nutrition science. The information is used for wide spectrum of purposes i.e. for food labelling, product development and innovation, dietary treatment, consumer information and research. Thus, high quality food composition data are fundamental to most issues related to nutrition and health and their importance is increasingly being recognized for agriculture, trade and economics. INFOODS is the International Network of Food Data Systems. It is a worldwide network of food composition experts aiming to improve the quality, availability, reliability and use of food composition data. Twenty-Eight European countries has developed the Food Composition Databases. The development of Food composition databases depends on the combination of the expertise of a diversity of specialists with professional background from agronomy, analytical and food chemistry, food technology, dietetics and nutrition to database and quality management and information technology. To support this European Food Information Resource (EuroFIR) has developed various training tools for the production and use of food composition data. However, many countries in Balkan region do not have any form of national food composition data or have been using borrowed information. This is a problem because people need essential information on food composition as well as an access to the information in order they can influence their own dietary patterns. Republic of Macedonia needs to move forward and improve the contribution to development of the FCDB and share it with different stakeholders. There is a need to prepare and implement measures like: increasing capacity development in generating and compiling food composition data; strengthen collaboration with other national and international bodies, organizations and projects working on food composition issues; incorporation of food composition into formal education curricula of schools and universities in nutrition, food science, dietetics and grants to various training and capacity building activities.

Keywords: food composition data, capacity development.

Introduction

Food composition is the determination of what is in the foods we eat and is the critical bridge between nutrition, health promotion, disease prevention, and food production. (Pehrsson and Haytowitz, 2016). Food composition databases (FCDBs) play an important role in various professional domains including food labelling, dietary guidelines, assessment of nutrient intake, formation of specific diet models, epidemiological studies, consumer choices, identifying allergens in restaurants, etc. (Porubska, 2014). Thus, high quality food composition data are fundamental to most issues related to nutrition and health and their importance is increasingly being recognized for agriculture, trade and economics. In developing countries, there are still many professionals lacking the required knowledge and skills and who need to be trained in food composition in a more cost-effective manner. Such training could be offered through distance-learning courses and by incorporating food composition into the curricula of future professionals (Charrondiere R., 2010). Macedonia as many other countries in Balkan region do not have online national food composition data base (EuroFIR, web site). The people need essential information on food composition as well as an access to the information in order they can influence their own dietary patterns, therefore it

important the country to establish the database. The information is needed also for nutritional labelling, and for the monitoring of the quality, safety and authenticity of foods on the market. Users of data collected in food composition tables and databases are very diverse, and include researchers, clinical dieticians, public health officers, nutritional policy makers, the food industry, and educational facilities (Korošec, 2013). Republic of Macedonia needs to move forward and improve the contribution to development of the FCDB and share it with different stakeholders. This could be realized with cooperation with international organization, communication and collaboration with all stakeholders in the country.

International organizations

FAO is leading global food composition activities since its beginning and has published several regional food composition tables. Since 1999, FAO is operating its food composition activities through INFOODS, the International Network of Food Data Systems, aiming to improve the quality, availability, reliability and use of food composition data. As a result of this cooperation many instrumental standards, tools, databases and publications in the field of food composition, and more recently also on biodiversity. These public goods assist countries to generate, manage and use food composition data for different purposes. In collaboration with the Commission on Genetic Resources for Food and Agriculture (CGRFA) several documents on biodiversity and nutrition were elaborated. FAO and INFOODS decided in 2006 to develop a distance-learning tool because no comprehensive distance, e-learning or on-line training was publicly available covering all aspects of food composition, and because the demand for well-trained professionals in food composition by far exceeds the supply through the existing lecture-based postgraduate training courses. Within the European Food Information Resource project ‘‘Network of Excellence’’ (EuroFIR NOE, 2005–2010) complex set of rules, guidelines, and recommendations, such as requirements for FCDBs, a system of controlled vocabularies (thesauri) for FCD description and identification, standard operating procedures for data compilation, quality framework for analytical laboratories and FCDBs, communication tools and centralized web platforms for FCD, have already been created (Porubska, 2014). Books were published in the areas of nomenclature and food descriptions to facilitate data interchange among member countries. Since food composition as an academic discipline is not usually covered in detail, a number of training courses were held in the Netherlands and in other locations. Much of the information contained in these courses has been incorporated into an e-learning course available on the Internet.

Food composition database, examples

There are a number of different types of food composition tables FCT and FCDBs vailable. Perhaps, the most common is a national database, which is developed for a specific country. When developing an FCDB/FCT, it is important to use good quality data. For example, the USDA has developed a data quality evaluation system to evaluate analytic data. It looks at five key attributes: (1) sampling plan, (2) sample handling, (3) number of samples, (4) analytic method, and (5) analytic quality control. Each of these attributes is scored and summed to yield a quality index for the nutrient in each food. For an FCDB/FCT compiler to properly evaluate the data, it is critical that this information be included in journal articles and various reports. (Pehrsson and Haytowitz, 2016). The preliminary FCDB in Slovenia was prepared following international recommendations and the instructions of Central and Eastern European Food Data Systems (CEECFOODS) initiative. Among the main tasks of the initiative were provision of a network of national data centres, generation of a repository of national FCDBs and promotion of national food composition programmes (Holcikova, 2000). First prepared was the Slovenian food composition tables on meat and meat products and later tables on food of plant origin. Korošec et al., 2013 in her paper emphasize the importance and purposes for establishing the FCDB. According the author it enables insight into the nutritional value of foods of domestic origin, along with the monitoring of their quality; it provides information on the compositions of traditional foods and dishes and of foods that are typical to Slovenia; and it

promotes the development of quality standards for products that will wish to carry EU quality distinctiveness labels (e.g. protected designation of origin (PDO), protected geographical indication (PGI), and traditional specialities guaranteed (TSG). The data included are also important for the Slovenian food industry for product labelling, and for more accurate results of dietary studies carried out in Slovenia and internationally. The 1st electronic format of FCDB in Serbia was created 1995. Also the web application has been used for the development of Serbian FCDB. This specific system was developed according to EuroFIR technical standards which contains all relevant technical information. The database is online and username and password is needed for accessing the database. As coordinating centre for the region, Institute for Medical Research in Serbia gained partnerships support from EuroFIR and UN System Standing Committee on Nutrition and United Nations University to set up 'Network for Capacity Development in Nutrition in Central and Eastern Europe' (NCDNCEE-CAPNUTRA) where Macedonia is also a member country. FCDM software Version II was created in 2010, with the updates that enabled inputs of food data from other CE/B countries: Croatia, Slovenia, R. of Macedonia and FB&H, aiming at establishment of a regional FCDB (Gurinovic, 2016).

Capacity development

Capacity development includes human resource development, organizational, institutional and legal framework development with the aim of enhancing knowledge and skills. Capacity development in food and nutrition is therefore much more than formal training and takes often place in parallel with such training (Pavlovic et al., 2009a). According the author capacity development is a long-term, continuing process, with policies, plans, and activities and should have national priority. Special attention should be given to the capacity development dimensions generated by global and European strategies. Capacity development networks would be important tools in addressing present and future needs in various countries. Author recommends that the EuroFIR training and exchange program is a valuable tool in achieving that goal. Capacity development networks can be useful tools in addressing needs in various countries and in streamlining regional involvement and collaboration, and can increase capacity to address food, health and nutritional challenges, including FCDB development (Pavlovic et al., 2009b).

Conclusions

For establishing the FCDB the collaboration among data generators, compilers, and users is essential. Many results which are published in scientific literature like journals, dissertations and laboratory reports by the Universities and institutions that work in the field of food could be used for the establishing national FCDB. FCDB should be developed and realized mainly with the projects, grants and with the support by the national authorities and international institutions which deal with this subject as well as other institutions in the region. There is a need to prepare and implement measures like: increasing capacity development in generating and compiling food composition data; incorporation of food composition into formal education curricula of schools and universities in nutrition, food science, dietetics; and grants to various trainings.

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POLLEN VIABILITY IN QUINCE CULTIVARS

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Abstract
Pollen viability of eight quince cultivars ('Leskovacka', 'Vranjska', 'Morava', 'Pazardzijska', 'Hemus', 'Asenica', 'Portugal' and 'Triumph'), was studied in the two-year period (2011-2012). Testing of pollen viability was performed using two methods: the staining of pollen with acetocarmine (indirect method) and pollen germination *in vitro* with sucrose and agar-agar (direct method). Studied cultivars differed significantly in terms of pollen viability. The lowest percentage of stained pollen grains was detected in 'Leskovacka' cultivar (70.29%) and the highest in the cultivars 'Asenica', 'Hemus' and 'Triumph' (over 90%). Similarly to that, the lowest percentage of pollen germination was obtained in 'Leskovacka' cultivar (62.86%) and the highest in the cultivars 'Morava', 'Asenica', and 'Triumph' (over 80%). With the exception of 'Portugal' cultivar, the values of pollen viability determined by staining with acetocarmine were higher for 3-15% compared to the pollen germination *in vitro*. However, values obtained using these tho methods are highly positively correlated. On the basis of obtained results, the both methods can be recommended as reliable tests for pollen viability of quince, although priority should be given to the method of pollen germination *in vitro*, because it is more accurate. All tested cultivars are distinguished for high pollen viability, and can be successfully used as male parents in hybridization. In addition, they also can be recommended as a good pollenisers when are planting new quince orchards.

Key words: *Cydonia oblonga*, pollen staining, pollen germination *in vitro*, breeding.

Introduction

Achieving high yields and obtaining high quality fruits are main goals in production of each fruit species, including quince. In order to obtain high yields, successful pollination and fertilization are necessary. In these processes, one of the key factors is the pollen viability. Pollen viability was primarily determined by genotype. It varies between different cultivars of the same species of fruit trees (Stösser et al., 1996), and depends on rootstock on which the cultivar is grafted (Kidman et al., 2014). In addition to genetic factors, some environmental factors may also influence pollen germination (Sorkheh et al., 2011). The external factors influencing pollen germination are temperature (Chagas et al., 2008; Radović et al., 2016a), boric acid (Liu et al., 2013), plant hormones (Sotomayor et al., 2012; Radović et al., 2016b), fungicides (Kargar and Imani, 2011) and the presence of heavy metals (Gür and Topdemir, 2008). Two methods are most often used to test the pollen viability: pollen staining (indirect method) and pollen germination *in vitro* with sucrose and agar-agar (direct method). Pollen staining is a quick and cheap method in assessing pollen viability. However, Pearson (1984) states that pollen germination *in vitro* is a better indicator to evaluate pollen viability than the pollen staining. Knowledge of pollen viability is very important for breeding and production of quince. For breeders it is very important to know the functional ability of pollen before crossing. For producers it is important for the choice of appropriate pollenisers when planting new quince orchards. The aim of this study was to examine pollen viability of eight quince cultivars in order to determine the cultivars with the highest pollen viability. They will be recommended as a potentially good pollenisers when planting of quince orchards and as parents for breeding work.

Material and methods

Investigations were carried out at the Experimental field "Radmilovac" of the Faculty of Agriculture, University of Belgrade (Serbia) during the two-year period (2011-2012). The subject of this research was eight quince cultivars: 'Leskovacka', 'Vranjska', 'Morava', 'Pazardzijska', 'Hemus', 'Asenica', 'Portugal' and 'Triumph'. The orchard was established in the spring of 1999, with the planting space of 4.5 m × 3 m. The examined cultivars were grafted on the rootstock 'Quince MA'. For examination of pollen viability branches with flower buds in the 'balloon' phase were taken and carried to the laboratory. In order to collect pollen anthers were isolated from the flower buds in Petri dishes. They are stored at room temperature (20ºC) for 24-48 h to dry and to release the pollen. Testing of pollen viability was performed using two methods: the staining of pollen with acetocarmine (indirect method) and pollen germination *in vitro* with sucrose and agar agar (direct method). Pollen viability was tested immediately after the anthers drying and pollen release. For the pollen staining assay, 1-2% acetocarmine solution was used. One to two drops of acetocarmine were placed on the microscope slide, and the pollen was added using special needles. Immediately after placing, pollen was covered with a cover slip and observed under light microscope 'Leica DM LS' (Leica Microsystems, Wetzlar, Germany). The experiment was done in three repetitions. In each repetition at least 300 pollen grains were analyzed. Pollen grains that were colored in pink were considered vital, while pollen grains that lack vitality were not colored at all. From the ratio of the number of colored and uncolored pollen grains, the pollen vitality was calculated. For testing of pollen germination *in vitro*, the pollen of each cultivar was sown with fine brushes in Petri dishes (9 cm diameter) on the previously prepared nutrient medium consisting of 15% sucrose and 0.7% agar-agar. After 24 hours at 20ºC, Petri dishes with a sowed pollen were observed under the light microscope 'Leica DM LS', for counting of germinated pollen grains. The experiment was done in three repetitions, each including at least 300 pollen grains. Pollen is considered as germinated if the length of pollen tube was larger than the diameter of the pollen grain. The obtained results were processed statistically using the two-factorial analysis of variance. Percentage data were subjected to arcsin square root transformation before the statistical analysis. Tukey's test (5%) was performed for means comparison. Correlations among the parameters were determined by correlation-regresion analysis and Pearson's correlation coefficients. Data analysis was performed using the statistical software package 'Statistica' (StatSoft, Inc., Tulsa, Oklahoma, USA).

Results and discussion

Pollen staining is a quick method in assessing its viability. Vital pollen grains are pink, unlike non-functional pollen grains which do not colored at all (Figure 1). The number of stained pollen grains was quite high and significantly varied between the investigated quince cultivars. It was in the range from 70.29% in the 'Leskovacka' cultivar to 94.04% in the 'Asenica' cultivar (Table 1). In addition to the 'Asenica' cultivar, high levels of pollen staining (over 85%) were also found in the cultivars 'Morava', 'Pazardzijska', 'Hemus' and 'Triumph'. The high values of pollen staining (over 90%) were also found in some quince cultivars in Turkey (Dalkiliç and Mestav, 2011).

Table 1. Pollen staining with acetocarmine of quince cultivars (%).

Cultivar/Year	2011	2012	Mx
Leskovacka	62.81	77.78	70.29 e
Vranjska	75.57	82.43	79.00 d
Morava	86.11	87.90	87.00 bc
Pazardzijska	81.10	83.47	82.29 cd
Hemus	95.20	89.28	92.24 ab
Asenica	94.40	93.67	94.04 a
Portugal	74.73	78.94	76.83 de
Triumph	88.83	92.20	90.52 abc
Mx	82.34 b	85.71 a	-

In addition to the cultivar, the pollen staining was varied considerably between the years studied. It was slightly higher in 2012. The 'Leskovacka' cultivar had the lowest value of pollen staining in both years (62.81% - 2011 and 77.78% - 2012), while the highest values in 2011 were determined for the 'Hemus' cultivar (95.20%), and in 2012 in the 'Asenica' cultivar (93.67%).

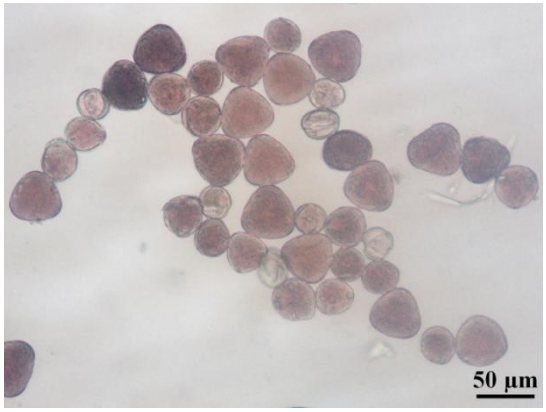

Figure 1. Pollen staining with acetocarmine of Portugal cultivar.

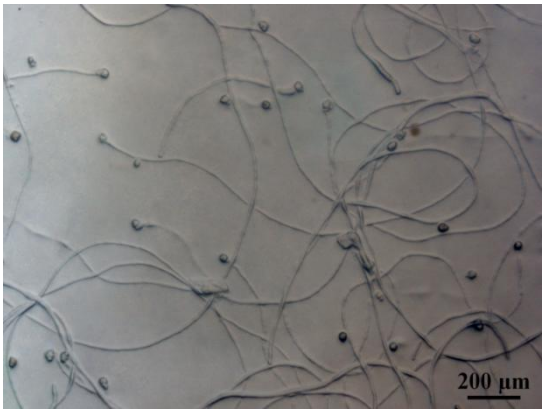

Figure 2. Pollen germination *in vitro* of Morava cultivar.

Test of pollen germination *in vitro* is one of the main indicators of pollen viability (Figure 2). Pollen germination was significantly different among the quince cultivars. In accordance with the staining of pollen, the highest pollen germination was found in 'Morava' and 'Asenica' cultivars (84.34% and 84.19%). In addition to the 'Morava' and 'Asenica' cultivars, high pollen germination had 'Triumph' cultivar (81.82%) (Table 2). On the other hand, as with the staining of pollen, pollen germination rate was the lowest in the 'Leskovacka' cultivar (62.86%). The variability of pollen germination in our work was in the range determined by Dalkiliç and Mestav (2011) in the quince cultivars in Turkey.

Table 2. Pollen germination *in vitro* of quince cultivars (%).

Cultivar/Year	2011	2012	Mx
Leskovacka	75.33	50.39	62.86 d
Vranjska	83.78	53.36	68.57 cd
Morava	90.22	78.46	84.34 a
Pazardzijska	89.89	61.61	75.75 bc
Hemus	88.81	64.70	76.75 abc
Asenica	86.03	82.35	84.19 a
Portugal	80.66	75.08	77.87 ab
Triumph	91.65	71.99	81.82 ab
Mx	85.80 a	67.24 b	-

Pollen germination significantly differed between years. Depending on the cultivar, it was for 4-30% lower in 2012. Different meteorological conditions during the development of pollen between the years studied were most likely contributed to this. Of the meterological factors, the greatest influence on pollen germination has the temperature (Pirlak, 2002; Milatović and Nikolić, 2014). In both examined years, the smallest pollen germination was determined in the 'Leskovacka' cultivar (75.33% - 2011 and 50.39% - 2012), and the highest in the cultivars 'Triumph' (91.65% - 2011) and 'Asenica' (82.35% - 2012). Pollen staining and pollen germination *in vitro* were in a strong positive correlation (r = 0.80). That is in line with previous studies (Khatun and Flowers, 1995). Therefore, both these methods can be recommended as reliable in testing of pollen viability. However, the pollen germination *in vitro* should be preferred because it is stricter and more reliable.

Conclusions

The staining of pollen with acetocarmine and pollen germination *in vitro* are very important methods in assessing pollen viability in quince. These two methods were highly positively correlated. Pollen viability significantly varied among the studied quince cultivars. It was the lowest in 'Leskovacka' cultivar, and the highest in the cultivars 'Morava', 'Asenica', and 'Hemus'. Generally, all tested cultivars are characterized by high pollen viability, and can be successfully used as male parents in hybridization and as a good pollenisers when planting new quince orchards.

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CONTAMINATION OF CULTIVATED VEGETABLES BY HEAVY ELEMENTS FROM FLOODED ARABLE SOIL: HUMAN EXPOSURE

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Abstract

The consumption of vegetables is one of the most important pathways for heavy elements to harm human health. Direct deposition of contaminants from the atmosphere onto plant surfaces and accumulation of heavy elements in flooded arable soil are of great concern because of the potential health risk to the local population. Thus, the present study was carried out to analyse distribution and soil-plant transfer of Pb, Cd, As, Co, Cr, Ni, Cu, Mn and Fe in potato, carrot, celery, parsnip and onion in order to evaluate their potential effects on human health. Total content of heavy elements in 26 vegetable samples collected from different flooded fields was analysed by atomic absorption spectrometry with a graphite furnace (AASGF) after microwave digestion of the analysed samples. Average concentrations of lead (Pb) and cadmium (Cd) in some of the investigated vegetable samples were higher than maximum allowable concentrations set by EC/Serbian regulation. On the other hand, arsenic (As) was not detected in any of the analysed samples. Accumulation and translocation of analysed elements were varied from element to element as well as among selected vegetable crops. The results showed that the parsnip had highest uptake for the most analysed elements (Fe (107 mg/kg) > Mn (6.98 mg/kg)> Cu (1.94 mg/kg)>Ni (0.34 mg/kg)> Pb (0.13 mg/kg) compared with the other investigated crops. The bioaccumulation factor (BA) for analysed elements in different vegetables was found in order of Cd (0.08)>Fe(0.07)>Cu (0.06)>Pb (0.01)=Ni>Mn (0.001), indicating that analysed crops are categorized as excluder (BA<1). The total health risk associated with the consumption of investigated vegetables grown in studied flooded arable soil was assessed using target hazard quotient (THQ). The THQ values estimated for investigated crops were notably below the safe limit of 1, except for Mn (2.10) and Fe (11.65).

Keywords: Soil contamination**,** plant uptake, risk assessment, bioaccumulation factor.

Introduction

Heavy elements are of great concern because of their persistence in the environment and toxicity to humans and other organisms. Sources of heavy elements in the environment include natural occurrence and contamination by anthropogenic sources (Xu et al. 2013). In the 20th century, agricultural activity led to an apparent increase in environmental pollution, especially in the concentration of toxic elements and agrochemicals in arable topsoil (Orlowski et al. 2014). Thus, the contamination of heavy elements in agricultural soils can also contribute to human health particularly taking into consideration the capacity to accumulate in edible parts of the plants. On this way, the heavy elements might be found in food chain and cause adverse toxicological effects for the consumers (Beccaloni et al. 2013). Also, the ability of plants to accumulate heavy elements into their organs could be used to monitor soil pollution, in particular the amount of heavy elements (Malizia et al. 2012). Therefore, plants can take up elements from soil by their root, transport them upwards to their shoots, and finally accumulate them inside their tissues, although there are large variations among different plant species in terms of metal accumulation ability (Luo et al. 2006). Oral ingestion of contaminated food has been proved to be an important pathway for the transfer of heavy elements from the environment to human bodies. Thus, there is the need to investigate the possible risk for the population due to the chronic exposure to heavy elements present in vegetables and fruits. The European Union has also published (EC, 2006) the regulation in which maximum

levels have been derived for Cd and Pb in foodstuffs such as vegetables. Many studies have been conducted to investigate the heavy element contamination of soil and plants (Luo et al. 2011; Tiwari et al. 2011; Beccaloni et al. 2013; Inácio et al. 2014; Orlowski et al. 2014; Galal and Shehata, 2015). However, there is very little information from Serbia for heavy element contents in flooded arable soil and their accumulation and translocation to crop plants such as vegetables. In this context, the risk associated with consumption of contaminated vegetables grown in flooded region may be a potential health concern. Therefore, the presented study was carried out in region of intensive agricultural production - heavily flooded in May 2014, located in the northern Serbian province of Vojvodina in order to establish direct relationship of level of elements in flooded arable soil and the vegetable crops grown there in. Thus, the main objective of this investigation is to quantify the elements concentration in soil and their translocation in vegetables to evaluate health hazards which may be helpful in making policies for growing safe vegetables in flooded areas.

Material and methods

Reagents and solutions

All chemical was of analytical reagent grade. Ultra-pure water was produced by Milli-Q purification system (Simplicity, Millipore, Molsheim, France) and used for preparation of standards and sample solutions. Concentrated 69% nitric acid (ccHNO3) ("for trace metals analysis" grade) and 30% hydrogen peroxide (H_2O_2) were purchased from J.T. Baker (Deventer, Netherlands). All the plastic and glassware were cleaned by soaking in a 20% hydrochloric solution (J.T. Baker, Deventer, Netherlands) overnight then in 20% nitric acid overnight and finally rinsed with Milli-Q water. The As, Cd, Co, Cr, Cu, Pb, Fe, Mn and Ni stock standard solutions (1 g/L) were supplied by J.T. Baker (Deventer, Netherlands). The working standard solutions of 1 µg/mL for each element were obtained by diluting stock solutions in 3% nitric acid. The calibration curve was prepared using the so-called bulk solution prepared by mixing the standard solutions and the subsequent dilution. Automix option of the GFAAS was applied enabling automatic preparation of the calibration standards.

Sampling and sample preparation

Sampling was performed in autumn 2016. A total of 21 topsoil samples (0-30 cm) were collected from selected locations. Each sample was a composite of 10 subsamples collected from a 100 m x 100 m grid using a plastic hand trowel and transported to the laboratory. Subsamples were thoroughly mixed to provide a composite sample of 3 kg of soil. Soil samples were air-dried at room temperature (25 ^{O}C), then passed through a 2 mm polyethylene sieve and finally ground into fine powder with a pestle. The ground samples stored (at 4 ^{O}C) in hermetically sealed polyethylene bags for further analysis. Furthermore, available vegetables (potato, carrot, celery, parsnip and onion) were collected from selected sampling points. Vegetables (n=26) were washed with distilled water to remove residues of soil and then the samples were wiped. After that, only edible part of crops was analysed. Microwave (Ethos One, Milestone, Bergamo, Italy) with segmented rotor of high pressure (HPR-1000/10S) and internal temperature sensor was used for digestion of the samples. The method applied for heavy elements determination is previously used by Škrbić et al. (2013).

Instrumental analysis

A Varian AA240/GTA120 (Mulgrave, Australia) model atomic absorption spectrometer (AAS) with deuterium background correction, equipped with a graphite furnace (GF) for electrothermal atomization and an automatic sampler was used in this study. The assembly was operated from an interfaced computer running SpectrAA software. Varian hollow cathode lamps (Mulgrave, Australia) were used as line sources for all analytes. Argon (99.999%, Messer Tehnogas A.D., Belgrade, Serbia) was used as the inert gas. The wavelengths used for determination of the elements in analyzed samples were as follows: 193.7 nm for As; 228.8 nm for Cd; 283.3 nm for Pb; 240.7 nm for Co; 357.9 nm for Cr; 232 nm for Ni; 324.8 nm Cu; 372 nm for Fe; and 279.5 nm for Mn.

Quality control

The developed method was validated by in-house quality control procedure. The correlation coefficients obtained for calibration curves were all greater than 0.9950. The limit of detection (LOD) and limit of quantification (LOQ) were calculated as the mean signal of five blanks plus three and ten times the standard deviation, respectively. LODs/LOQs (mg/kg) were as follows: 0.025/0.075 for As; 0.012/0.025 for Cd; 0.013/0.026 for Pb; 0.003/0.003 for Co; 0.043/0.076 for Cr; 0.005/0.005 for Ni; 0.010/0.026 for Cu; 0.565/1.182 for Fe; and 0.002/0.006 for Mn. Recoveries for analyzed elements were determined by spiking soil and plant sampleswith mixture of analytes of interest and ranged from 74 to 110%. The repeatability expressed as relative standard deviation of spiked samples ranged from 0.1 to 11%.

Data analysis

Bioaccumulation factor

The bioaccumulation factor (BF), an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil substrate (Ghosh and Singh, 2005), was calculated as follows: $BF=C_{plant\ root}/C_{soil}$, where $C_{plant\ root}$ and C_{soil} represent the heavy element concentrations in the plant root and soil, respectively.

Estimated daily intake of analysed elements from vegetables

The estimated daily intake (EDI) of selected elements through vegetables was depended on both the element concentrations in vegetables ($C_{plant\ root}$, mg/kg) and the amount of daily intake of vegetables ($W_{vegetable\ intake,}$ kg/day), which was calculated as follows:

$EDI= C_{plant\ root} \times W_{vegetable\ intake}$

Average daily intake of vegetables for adult consumers was adopted according to the Serbian market basket (Statistical Office of the Republic of Serbia, 2015) as: 18 g/day for carrot, 31 g/day for onion and 144 g/day for potato. Consumption rate of celery and parsnip is not included in the Serbian market basket, but it is estimated as daily intake of carrot, considering the common habits of Serbian population to use the same quantity of both of these vegetables with carrot for meal preparation.

Target hazard quotient

The target hazard quotient (THQ) was conducted by considering the parameters according to Wu et al. (2016). THQ was determined by the following equation:

$$THQ = \frac{\mathrm{E_f E_d F_{ir} C}}{R_{fd} W_{ab} T_a} x10^{-3}$$

where E_f is the exposure frequency (365 days/years); E_d is the exposure duration (70 years); F_{ir} is the food ingestion rate (g/person/day); C is the metal concentration in vegetable samples (mg/kg); R_{fd} is the oral reference dose (mg/kg/day); W_{ab} is the average body weight (60 kg for adults); and T_a is the average exposure time (365 days/year x 70 years). The oral reference doses for selected elements are 0.004 mg/kg/day, 0.001 mg/kg/day, 0.04 mg/kg day, 0.02 mg/kg/day, 0.005 mg/kg/day and 0.7 mg/kg/day for Pb, Cd, Cu, Ni, Mn and Fe (EPA, 2016). A THQ less than 1 indicates no obvious health risk to adults through vegetables consumption. If the THQ is greater than 1, it means there is a potential health risk to humans.

Results and discussion

Heavy element contamination in soil

Table 1 summarizes the total concentration of analysed heavy elements in the top 0-30 cm of the flooded arable soil. According to the Serbian national target limits for heavy elements in soil (OG RS 80/10, 2010) only the average level of Cd in the flooded soil samples exceeded the maximum permissible value. The remediation values were not exceeded for any of the studied elements in any of the studied locations.

Table 1. Average content of heavy elements analysed in flooded arable soil

Elements mg/kg	As	Cd	Co	Cu	Cr	Pb	Ni	Mn	Fe
Mean	5	1.4	3.1	19	37	18	18	1015	1281
Target values[a]	29	0.8	9	36	100	85	35	-	-
Intervention values[a]	55	12	240	190	380	530	100	-	-

[a] Serbian standard target values for soil (OG RS 80/10, 2010)

Heavy element contamination in vegetable crops

Contents of heavy elements in the edible part of analysed vegetables are shown in Figure 1. The highest average concentrations of Cu, Ni, Mn and Fe were found in parsnip, while the highest average levels of Pb and Cd were detected in carrot and celery, respectively. Generally, the lowest average concentrations of detected elements were in onion. To ensure food safety, the European Commission Regulation (EC,1881/2006) has set maximum levels for some contaminants, including also heavy elements like Pb (0.1 mg/kg) and Cd (0.05 mg/kg) in vegetables, whereas for As the maximum allowed concentration has not yet been proposed. Similarly, the latest Official Bulletin of the Republic of Serbia No 29/14 (The Serbian regulation, 2014) has established the maximum levels (ML) for Pb and Cd in foodstuffs in line with the EC regulation, but it regulates wider spectrum of food commodities and it also set the maximum level of As (0.3 mg/kg in vegetables). The Pb was found in carrot samples at the level of 0.54 mg/kg, which was almost 5 times higher than the maximum residue level of 0.1 mg/kg sets by EC/Serbian regulation. In other studied vegetables the determined concentrations were at the level of maximum allowable concentrations or below. The Cd detected above ML in samples of celery, parsnip and carrot. The lower concentrations of Cd were found in samples of potato (0.05 mg/kg) and onion (0.02 mg/kg).

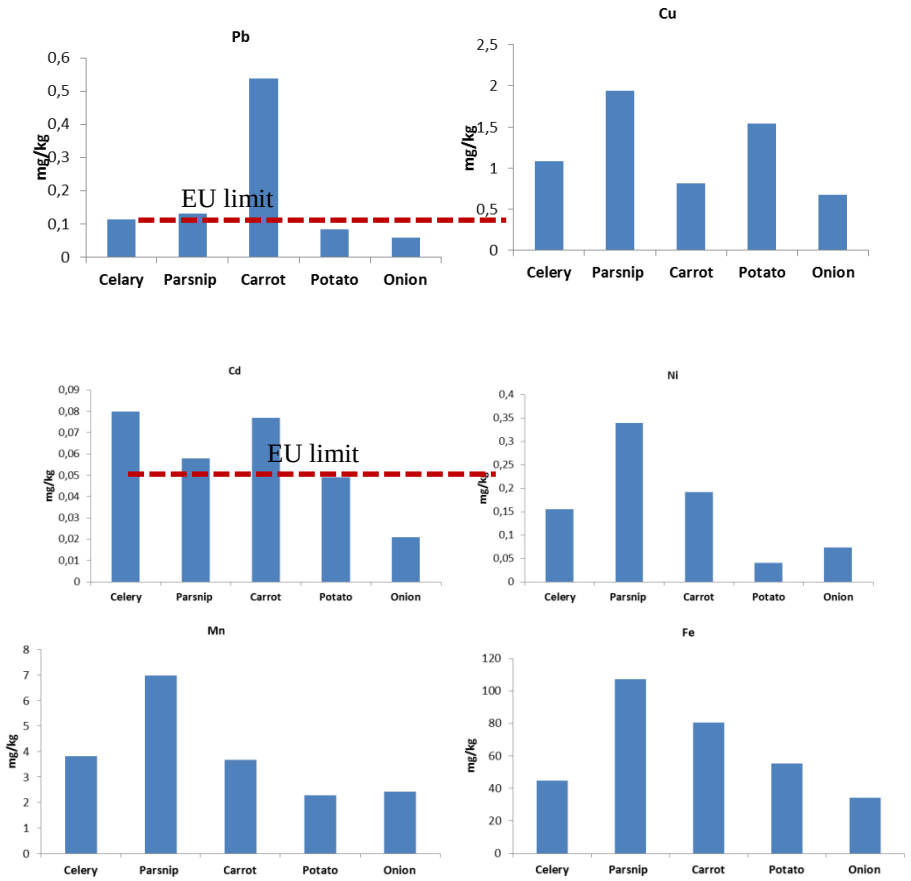

Figure 1. Average concentration of analysed heavy elements in different vegetables collected from analysed flooded arable soil

Table 2 presents the heavy element transfer from soil to vegetable crops i.e. bioaccumulation factors (BF). Elements with greater BF are more easily transferred from soil to the edible parts of plants than the ones with lower BF. The greatest accumulation of Fe was observed in parsnip followed by carrot and potato. The most dominant accumulation of Cd was found in potato and celery. The BF value of Cu was the greatest in parsnip. The presented results indicate low BF for other studied heavy elements in vegetables analysed. As can be seen from Table 2 there are difference in BF values among the analysed vegetables because heavy element uptake by plants depends on the plant type and their physiological character.

Table 2. Bioaccumulation factor, a ratio of concentration in edible parts of vegetables to that of the corresponding soil

Bioaccumulation factor	Pb	Cd	Cu	Ni	Mn	Fe
Potato	0.005	0.107	0.073	0.002	0.002	0.065
Carrot	0.032	0.079	0.039	0.010	0.034	0.097
Onion	0.004	0.015	0.031	0.004	0.003	0.036
Celery	0.006	0.108	0.046	0.011	0.004	0.024
Parsnip	0.003	0.067	0.100	0.029	0.005	0.131

Table 3. Estimated daily intakes (EDIs, mg/day) of selected elements and target health quotients (THQs) through consumption of analysed vegetables for Serbian adult consumers

Vegetables	Pb		Cd		Cu		Ni		Mn		Fe	
	EDI*	THQ	EDI*	THQ	EDI*	THQ	EDI*	THQ	EDI*	THQ	EDI*	THQ
Potato	0.012	0.05	0.006	0.12	0.222	0.09	0.006	0.001	0.327	1.09	7.939	0.19
Carrot	0.009	0.04	0.001	0.02	0.014	0.01	0.003	0.003	0.062	0.22	1.371	0.03
Onion	0.002	0.01	0.002	0.001	0.021	0.01	0.002	0.001	0.075	0.25	1.068	0.03
Celery	0.002	0.01	0.001	0.01	0.018	0.08	0.003	0.01	0.065	0.01	0.762	3.36
Parsnip	0.002	0.01	0.001	0.004	0.033	0.15	0.006	0.03	0.119	0.52	1.822	8.04

*The recommended safe limits (mg/day) are: 48, 3, 11, 0.1-0.3, 0.058 and 0.044-0.105 for Fe, Cu, Mn, Ni, Cd and Pb respectively, (JECFA, 1999, 2011, National Research Council, 1989, WHO, 1994, EFSA, 2010).

The EDI and THQ of the analysed elements calculated through the consumption of investigated crops in this study by Serbian adult consumers are shown in Table 3. The calculated EDIs of heavy elements (Pb, Cd, Cu, Ni, Mn and Fe) through consumption of vegetables were lower than the recommended safe limits reported by EU. The greatest daily intake was estimated for Fe through consumption of potato which is in consequence of its highest concentration found, and therefore highest consumption rate among the analysed crops. The THQ of each element through consumption of analysed vegetables decreased in the order of Fe>Mn>Cu>Cd>Pb>Ni. The THQ values for most analysed elements were below 1, (except for Mn and Fe), indicating that intake of a single element through consumption of vegetables does not pose a significant potential health hazard. The Mn and Fe exhibited higher THQ (2.10 and 11.56, respectively) compared to all other elements in the investigation region, and these elements are major components contributing to the potential health risk. The total element THQ value (sum of individual element THQ for vegetables) was 14.40 (>1) and it might be associated with potential health risk which cannot be ignored.

Conclusions

This study presents the first insight into the concentrations of investigated 9 heavy elements in flooded arable soil, their translocation and accumulation in edible parts of selected crop plants as well as potential health risk. The study highlights the fact that levels of Pb and Cd were above MLs in some plant crops, although, only the average content of Cd in flooded arable soil exceeded the maximum permissible value. The BF was less than 1 for all elements, indicating that edible parts of plants did not show great capacity to absorb element from the investigated soil samples. Although, the THQ values for most elements were below 1, total element THQ was above 1. The potential health risks of analysed elements presented in investigated vegetables are therefore of some

concern. Furthermore, Fe and Mn are the elements with the highest contribution to the health risk. However, health risk can be increased with consumption of other contaminated crop plants that was not analysed in this study. Thus, a long-term risk assessment needs to be carried out in order to determine the migration potential of the studied elements in different and the most consumable crop plants which grown in this region.

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RAPID RESOLUTION LIQUID CHROMATOGRAPHY METHOD FOR DETERMINATION OF CHLOROGENIC ACID IN ECHINACEA EXTRACTS

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Abstract

This study presents a development and validation of a new, fast, efficient and cost effective reversed-phase rapid resolution liquid chromatography (RP-RRLC) method for determination of chlorogenic acid in echinacea extracts. The optimum separation with symmetrical peak shape and good index purity of the analyte was achieved on a Poroshell 120 EC-18 (50 mm x 3 mm; 2.7 µm) analytical column, mobile phase consisted of acetonitrile/(water with 1 % phosphoric acid), (10/90, *V*/*V*)in isocratic elution with flow rate of 1 mL/min and UV diode-array detection (UV-DAD) at 325 nm. The developed method was validated by testing specificity, selectivity, linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ). The calibration curve of chlorogenic acid followed Beer's law within the range 28.97 ng - 362.19 ng (R^2 = 0.9994). The LOD was 0.29 pg, while LOQ was 0.96pg. The intra-day precisions was evaluated for the retention time, peak area and peak height and the calculated values for relative standard deviations (RSD) were 0.21 %, 0.11 % and 0.22 %, respectively. The mean recoveries ranged from 98.75 to 104.63 % and RSD was less than 0.23 %. The developed method was successfully applied for identification and quantification of chlorogenic acid in three samples of echinacea extracts, taken from local pharmacies.

Keywords: RP-RRLC, UV-DAD, method development, validation.

Introduction

Echinacea species are perennial plants which originate from North America. There are nine *Echinacea* species, three of which are used for medical purposes: *Echinacea angustifolia* DC, *Echinacea purpurea* (L.) *Moench* and *Echinacea pallida* (Nutt.). About 80 % of commercial products such as tinctures, alcoholic and non-alcoholic extracts, capsules, tablets, syrups, teas, beverages and the like are produced by *Echinacea purpurea*. The latest research shows that *Echinacea* species have anti-inflammatory, wound-healing and immune system stimulating effects against bacterial and viral infections (Ma et al., 2011). *Echinacea* speciescontain polysaccharides, flavonoids, caffeic acid derivatives,essential oils, polyacetylenes, alkylamides and miscellaneous chemicals (Cozzolinoet al., 2006). According to the United States Pharmacopoeial Convention,caffeic acid, cichoric acid, chlorogenic acid, dicaffeoylquinic acids, echinacoside and dodecatetraenoic acid isobutylamides are found in *Echinacea* species (Members of the USP, 2009).

Chlorogenic acid is an ester of caffeic acid and quinic acid (Fig. 1) (dos Santos et al., 2006).

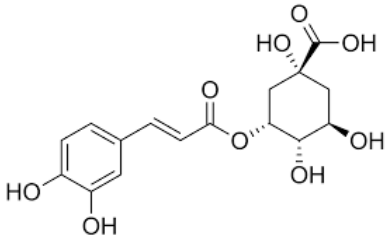

Figure 1. Chemical structure of chlorogenic acid

Although in the name it has the word "chloro", the chlorogenic acid does not contain chlorine. Its name comes from the Greek word which means light green, because of the green colour it receives in oxidation (Kremret al., 2016). Chlorogenic acid has many beneficial properties such as antioxidant activity, anticarcinogenic potential and may also slow the release of glucose into the bloodstream after a meal (Bakuradzeet al., 2011; Tunnicliffeet al., 2011). Additionally, chlorogenic acid has anti-inflammatory, antibacterial and anti-obesity properties (Chagas-Paula et al., 2011). Standardized methods with more analytical techniques were developed for the quantification of chlorogenic acid, but high-performance liquid chromatography (HPLC) remains one of the techniques that have the widest use and give accurate results (Craig, et al., 2016; Naegele, 2016; Brown et al., 2011;Pellati et al., 2004). The aim of this paper was to develop a fast, simple and accurate analytical method, for qualitative and quantitative determination of chlorogenic acid as one of the most active components in echinaceaextracts using Rapid Resolution Liquid Chromatography (RRLC) and ultraviolet diode array detection (UV-DAD).

Material and methods

Reagents and Chemicals

HPLC-grade methanol, acetonitrile and water, as well as pure analytical standards of chlorogenic acid (95 %) and phosphoric acid (85.5 %) were purchased by Sigma-Aldrich (Germany). Three different preparations obtained by extraction of echinacea were used as test material: non-alcoholic extract, herbal drops of echinacea (aqueous-alcoholic extract) and capsules produced in Italy and Germany that were procured from local pharmacies.

Equipment

The chromatographic analysis was performed on an Agilent 1260 Infinity Rapid Resolution Liquid Chromatography (RRLC) system equipped with: vacuum degasser (G1322A), binary pump (G1312B), autosampler (G1329B), a column compartment (G1316A), UV-VIS diode array detector (G1316B) and ChemStation software. For the better dissolving of the stock solutions an ultrasonic bath “Elma” was used. The investigations were carried out using Poroshell EC 120-C18 (50 mm x 3 mm; 2.7 µm) analytical column produced by Agilent Technologies (USA).

Preparation of Standard Solutions

The stock solution was prepared by dissolving 0.0061 g of the pure analytical standard of chlorogenic acid with a mixture of methanol/water (80/20, *V/V*) in a 10 mL volumetric flask. The prepared standard solution was ultrasonized in an ultrasonic bath of type "Elma" for 15 minutes, in order to better dissolve of the analytical standard.

For determination of the linearity a series of working standard solutions was prepared by taking 100, 250, 500, 750, 1000, and 1250 µL of the standard solution in the measured flasks of 10 mL. The flasks were filled-up with a mixture of methanol/water in a volume ratio of 80/20. Each working standard solution was injected three times with a volume of 5 µL.

Preparation of Sample Solutions

Sample solution 1 was prepared by measuring 5.0393 g of the non-alcoholic extract of echinacea in a 10 mL volumetric flask, filled-up to the mark with the mixture of methanol/water (80/20, *V/V*). The sample was degassed for 15 min in an ultrasonic bath, and after cooling, 2 mL of this solution,was transferred in a 10 mL volumetric flask and filled-up to the mark with the mixture of methanol/water (80/20, *V/V*). Sample solution 2 was a pure aqueous-alcoholic extract (plant drops of echinacea). Sample 3 was prepared by measuring 1.14 g of echinacea capsules in a 10 mL volumetric flask, filled-up to the mark with the mixture of methanol/water (80/20, *V/V*). The prepared sample was dissolved by ultrasonification for 15 min. Before HPLC analysis, all samples were filtered through 0.45 µm Iso-Disc PTFE syringe filters (Supelco, Sigma-Aldrich, Germany).From all sample solutions, three injections were performed with 5 µL each.

Sample preparation for recovery determination

The recovery of the method was determinated by dissolving 5.0393 g of sample 1 in 10 mL volumetric flask and filled-up to the mark with methanol/water (80/20, *V/V*) and ultrasonificated for 15 min. Into three flasks (10 mL), 2 mL from this solution were transferred, and known concentration (7.24, 14.49 and 28.97 µg/mL) of the analytical standard was added into each flask, than filled-up to the mark with methanol and water in the volume ratio 80/20. All the sample solutions were filtered through 0.45 µm syringe filters and the three injections were performed with 5 µL each for all cases.

Results and discussion

For the preparation of the standard solution, working solutions and test samples, a mixture of organic solvent methanol and water in a volume ratio of 80/20 was used, taking into account the research of Pellati et al. (2004) in which, among other things, they made selection of the best solvent for the extraction of phenolic compounds from the *Echinacea* root. In doing so, they examined the various concentrations of aqueous solutions of methanol and ethanol (50%, 60%, 70%, 80%, 90% and 100%), so that they found that higher water concentrations lead to hydrolysis of phenolic compounds and the best results were obtained by using 80% methanol in water. Preliminary chromatographic studies made on the Agilent Poroshell 120 EC-C18 column (50 mm x 3 mm; 2.7 µm) showed that optimal conditions for the separation of chlorogenic acid using reversed-phase rapid resolution liquid chromatography (RP-RRLC) were achieved with an isotactic elution with a mobile phase consisted of a mixture of acetonitrile and 1 % phosphoric acid in water with a volume ratio of 10/90, a flow rate of 1 mL/min and column temperature at 25 °C (Jakimoski, 2015). Therefore, the same chromatographic conditions were applied in the research. The chromatographic process was followed at a wavelength of 325 nm. In Fig. 2 is presented a chromatogram obtained from the analytical standard of chlorogenic acid under the defined experimental conditions. As can be seen from this figure, a narrow, high and symmetrical chromatographic peak was achieved.

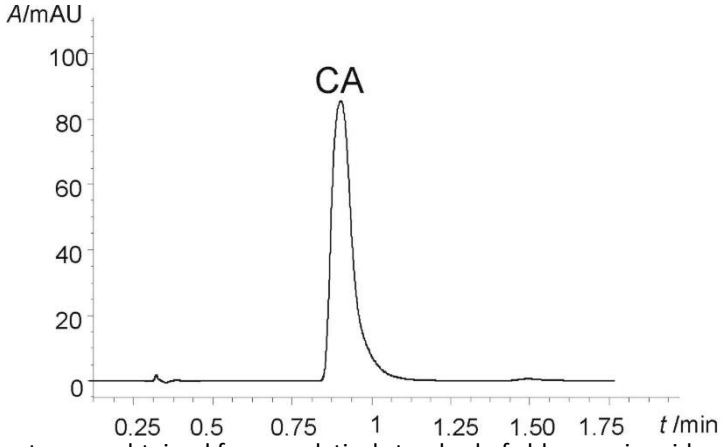

Figure 2. Chromatogram obtained from analytical standard of chlorogenic acid on the Agilent Poroshell 120 EC-C18 (50 mm x 3mm; 2.7 µm) column with acetonitrile/1 % phosphoric acid (10/90, *V/V*) as mobile phase, flow rate of 1 mL/min, column temperature at 25 ºC, and UV-detection at 325 nm

The proposed reversed-phase rapid resolution liquid chromatography method was successfully applied for the determination of the chlorogenic acid in echinacea extract samples. In Fig. 3 are shown the chromatograms obtained from the analyzed samples using the proposed method. The figure shows that the chromatographic peak of chlorogenic acid (CA) has symmetrical peaks shape and it is well separated from other components in the samples. The choice of the wavelength to monitor the chromatographic process was made based on the UV spectrum of chlorogenic acid recorded in a solution of acetonitrile and 1% phosphoric acid dissolved in water, with a volume ratio of 10/90 (Fig. 4). Thus, from the recorded UV spectra it can be noticed that the chlorogenic acid has two maxima, one at a wavelength of about 325 nm and the other smaller at a wavelength of 220 nm.

Because chlorogenic acid exhibits maximum absorption at 325 nm, the chromatographic process was followed at a wavelength of 325 nm.

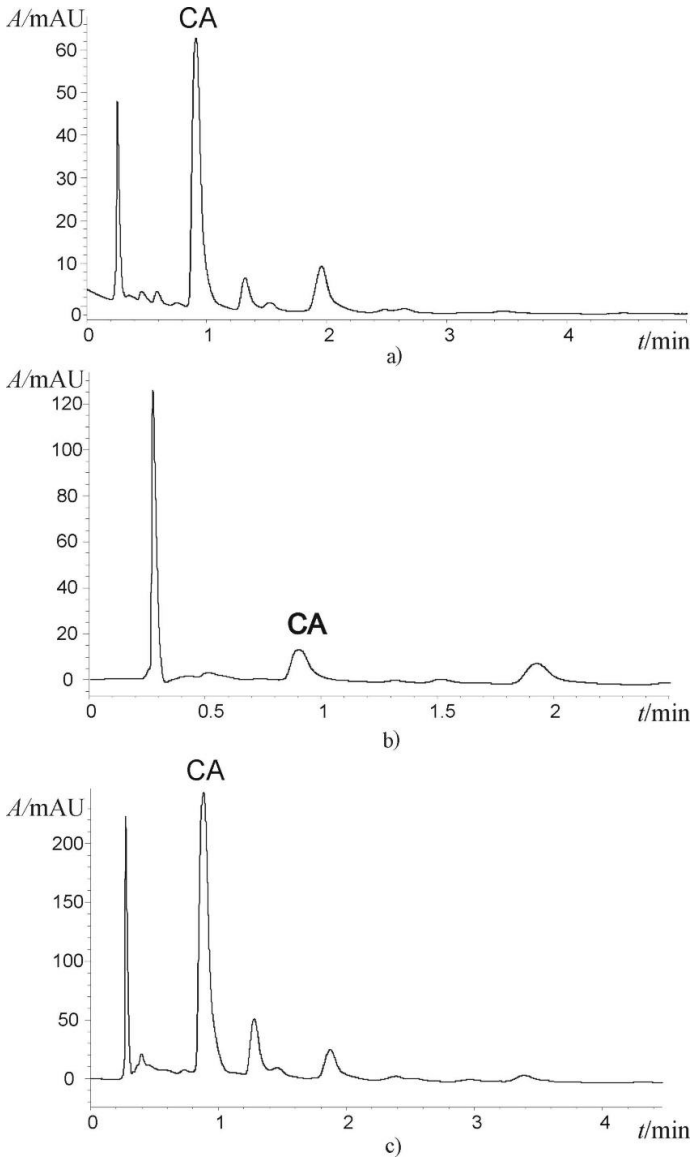

Figure 3. Chromatograms obtained from analyzed samples 1 (a), 2 (b) and 3 (c) using the developed method

For the method validation were determined: specificity, selectivity, linearity, precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ). The specificity and selectivity of the method were estimated by identifying the peak of interest and the value obtained for the purity index. The identification of the analyte was performed by comparing the retention time of the analytical standard of chlorogenic acid with the retention time of the same component in the analyzedechinacea samples. The identification of the analyte was also confirmed by overlapping the absorption spectra of the pure analytical standard of the chlorogenic acid and the absorption spectrum of the same substance in the samples. The high value of the match factors (> 999) were evidence that the chromatographic peak was of the same substance. Under the stipulated chromatographic conditions the obtained value for column dead time was 0.26 min, and the mean value for the retention time of the analyte was 0.96 min. Hence, the calculated value for retention factor (*k'*) was 2.69 which is the optimum value for this parameter (Dong, 2006). For the estimation of the linearity of the method, calibration curves in a concentration range of 28.97 ng to 362.19 ng were constructed. The obtained results show that the curves followed Beer's law in the investigated range. The obtained values for multiple correlation coefficients (R^2 = 0.9994) revealed good linearity

of the developed method (Table 1). The limits of detection (LOD) was defined as the amount of analyte for which the signal-to-noise ratio (*S*/*N*) was 3, whereas the limits of quantification (LOQ) was defined as the amount of analyte for which *S*/*N* =10. The LOD and LOQ are listed in Table 1.

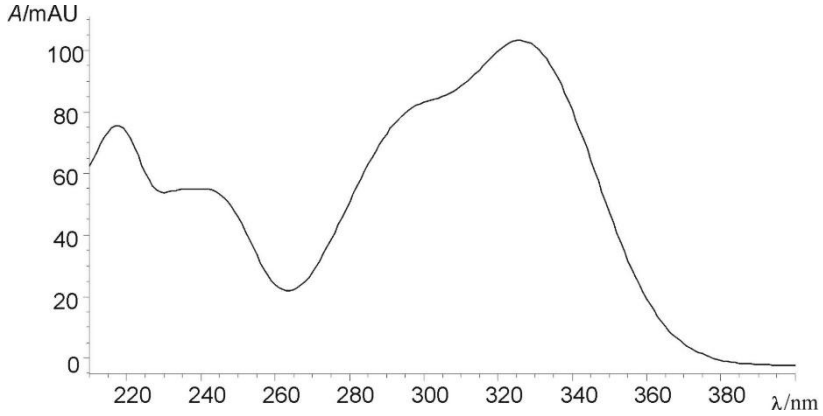

Figure 4. UV spectrum of chlorogenic acid in acetonitrile/1 % phosphoric acid (10/90, *V*/*V*) solution

Table 1. Linearity and sensitivity data for chlorogenic acid

	Linearity range (ng)	Regression equation	R^2	*LOD* (pg)	*LOQ* (pg)
Area	28.97 – 362.19	y = 3.8571x + 12.064	0.9994	0.29	0.96
Height		y = 0.6741x + 2.7508	0.9994		

In order to determine the precision of the elaborated method, expressed as intraday precision, 10 successive injections of the analytical standard with a mass concentration of 28.97 µg/mL were made. The calculated values for the relative standard deviation (RSD) for the peak area was 0.11 %, for the retention time 0.21 %, and for the peak height was 0.22 %. The obtained results show that the method has an excellent intraday precision. The accuracy of the method was confirmed by standard additions (Snyder, 1997). According to this method, a known amount of the analytical standard was added to sample 1, which already contains a certain amount of the analyte. Accuracy of the method was expressed as the deviation between the calculated mean value obtained by examination and the true value of the spiked amounts of the analyte into a sample 1 (Table 2). As it is shown in Table 2, the obtained values for recovery are within the following ranges 98.75 - 104.63 % (Table 2). Consequently, it can be concluded that the developed method is accurate enough and can be used for the quantitative determination of chlorogenic acid in echinacea extracts.

Table 2. Results from the recovery experiments (*n*=3)

m(analyte) before the ddition (ng)	*m*(analyte) added (ng)	*m*(analyte) after the ddition (ng)	***Recovery*** (%)	***RSD*** (%)
83.72	36.22	125.49 ± 0.31	104.63	0.23
	72.44	154.22 ± 0.21	98.75	0.08
	144.87	229.04 ± 0.21	100.22	0.06

Table 3. The content of chlorogenic acid in the analyzed samples

Sample	*m* (ng) ± SD (in V_{inj} = 5µL)	*RSD* (%)	Content of chlorogenic acid (ppm)
1	83.73 ± 0.23	0.27	166
2	19.50 ± 1.30	6.69	4.21
3	350.07 ± 34.38	9.82	638.44

The proposed method was successfully applied for the quantitative determination of chlorogenic acid in three commercial products designated as test 1, 2 and 3. The obtained mean values for the content of chlorogenic acid in the analyzed samples are shown in Table 3. As can be seen from the obtained results, the content of chlorogenic acid in the test samples, which are actually echinacea extracts, is very different and ranges from 4.21 to 638.44 ppm.

Conclusions

A new, fast, efficient and cost-effective reversed-phase RRLC method for the determination of chlorogenic acid in echinacea extracts has been developed. According to the experimentally obtained results, the optimal conditions for the qualitative and quantitative determination of the analyte in the analyzed samples were obtained using the Poroshell 120 EC-C18 (50 mm x 3 mm; 2.7 µm) analytical column, in isocratic elution mode with a mobile phase consisting of acetonitrile/1% phosphoric acid (10/90, *V/V*), 1 mL/min flow rate, column temperature at 25 ºC and UV detection at 325 nm. The obtained results revealed that the method is characterized by excellent linearity, precision and accuracy and it could be used for routine analysis of chlorogenic acid in echinacea extracts.

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MECHANICAL COMPOSITION AND CHEMICAL PROPERTIES OF CALCOMELANOSOLS AND CALCOCAMBISOLS ON THE JABLANICA MOUNTAIN

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Abstract

Differences in mechanical composition of two soil types on hard limestone and its subtypes were investigated in order to get closer look on variability and dynamics of this soil property over long period of pedogenesis of these soils. Soils on hard limestones on Jablanica Mountain were select as a case study. During the field survey campaign on different locations 18 basic soil profiles of calcomelanosols were excavated, with the following subtypes: organogenic (5) organomineral (12) and brownized (1) and 4 soil profiles calcocambisols with a typical subtype. Field survey and laboraotry testing was implemented according standard methods adopted in our country and the Former Yugoslavia. Clay content is generally high and increases from organogenic subtype towards brownized calcomelanosols and calcocambisols subtypes. During the process of evolution of the calcomelanosols into brownized calcomelanosols and calcocambisols, the content of soil organic matter decreases. The fine earth of examined soil samples usually is non-carbonate, but in a limited number of soil samples a very small quantities of $CaCO_3$ were detected. In terms of its soil reaction, the examined soils can be classified into three categories (very strongly acid, moderately acid and neutral). The content of total nitrogen in the examined soils is high and vary in the ranges of 0.21-1.95% in calcomelanosols and 0.31-0.56% for hor. A and 0.18-0.27 for hor. (B)rz of the calcocambisols. The examined soil samples are with low content of easy available phosphorus and optimal content of potassium.

Keywords: soil**,** clay, humus, pH, total nitrogen.

Introduction

In this article, data related to the mechanical composition and some chemical properties of calcomelanosols and calcocambisols on Jablanica Mountain are presented. During the field survey campaign on different locations 18 basic soil profiles of calcomelanosols were excavated, with the following subtypes: organogenic (5) organomineral (12) and brownized (1) and 4 soil profiles calcocambisols with a typical subtype. On the highest parts of the Mountain the organogenic calcomaelanosols has been excavated. In the lower parts of the mountain the organomineral and brownized calcomelanosols are spread, while calcocambisols are identified as a comlex with the former ones on more falt relief forms. A detailed data for the soil forming factors, genesis, evolution and classification of the investigated soils are presented in our previous reports (Andreevski et al. 2013). Data related to the mechanical composition and some chemical properties of the calcocambisols and calcomelanosols of Jablanica Mountain are presented in the works of Петковски et al. (1995), Андреевски (1996) and Мукаетов (1996). Data related to the mechanical composition and chemical properties of soils on hard limestones on Jablanica Mountain and other locations can be find in the previous works of Маркоски (2013) and Markoski et al. (2015). Filipovski sums up all existing results from previous investigation of these soil types in his Monograph's (Филиповски, 1996, 1997). The mechanical content and chemical properties of the investigated soils are of particular importance for its production potential. With these investigations we will contribute to gaining a better understanding of these fairly prevalent soils in our country.

Material and methods

Field examinations have been performed according to accepted methods in Former Yugoslavia (Filipovski ed. 1967). The laboratory analyses have been done according to the standard adopted methods in Former Yugoslavia and Republic of Macedonia, as follows:

- Mechanical composition of soil has been determined according to the international A-method, and the peptization has been carried out with 0,1M sodium pyrophosphate (Resulović ed. 1971). The separation of the mechanical elements in fractions has been done by the international classification.
- pH (reaction) of the soil solution has been determined with glass electrode in water suspension and in NKCl suspension (Bogdanović ed. 1966)
- Easy available forms of P_2O_5 and K_2O were determinate by Al method (Bogdanović ed. 1966)
- Content of calcium-carbonate was determined using Scheibler's calcimeter (Bogdanović ed. 1966)
- The content of humus has been determinate at the base of total carbon by the method of Tjurin modified by Simakov (Орлов and Гришина 1981)
- The total N has been determinate by Kjeldahl micro method (Bogdanović ed. 1966).

Results and discussion

Mechanical composition

Data for the mechanical composition of the investigates soils are presented in Tab. 1. The mechanical composition of the tested soils, depends on the residue released by the dissolution of the limestone, the characteristics of the rock itself (limestone, dolomite, silicate limestone), the deposition of other materials from the higher terrains, the processes that cause the texture differentiation, the eolian deposition of tiny particles and selective erosion of clay from the hor. A. The average content of skeleton in calcomelanosols is 6.62% (4.30% in organogenic and 8.51% in organomineral). The hor. A of the calcocambisols contains an average of 7.81%, while the contents of skeleton in the cambic horizon, decreases and account for 6.78%. It can be concluded that the skeleton content is low. From the data in Tab. 1, it can be seen that the fine earth content, dominates the skeleton in all profiles. The most common fraction in the calcomelanosols and hor. A of the calcocambisols is clay. Then follows, the fractions of fine sand, silt, and the coarse sand with lowest content. Similarly, in the cambic horizon of the calcocambisols, the dominant fraction is clay, but in this case silt is on a second place, followed by fine and coarse sand. In all examined soil samples, physical clay (<0.02mm) is more common than physical sand. The content of physical sand in profiles 2, 10 and 21 (calcocambisols formed on plain limestone with horn is higher compared to prof. 1 (calcocambisol formed on dolomitized limestone) and is due to the presence of horn material. The content of clay in the calcomelanosols is in average 38.63%. Going from the organogenic to the organomineral and browned calcomelanosols and the calcocambisols, the clay content increases. The average content of clay in organogenic calcomelanosols is 31.1%, in organomineral 39.65% (hor A. of browned calcomelanosol contains 50.8%) and in hor. A of calcocambisols the clay content yields 41.20%. The clay content in the cambic horizon of brownized calcomelanosol and calcocambisols is increasing. In calcocombisols it is in average 51.93% and in brownized calcomelanosol 58.6%. The increase in the clay content in the cambic horizon can be explained by clay formation processes, with selective erosion of the fine particles from the hor. A as well as the eolic application of dust particles. According to Маркоски (2013), the average clay content in the organogenic calcomelanosols from the Republic of Macedonia is 22.27%, in the organomineral 32.68%, in the hor. A of the brownized 36.60% and in the hor of A. the calcocambisols 38.19%. According to the same author, the cambic horizon of brownized calcomelanosols contains an average 41.03% clay, while the calcocambisols contains 46.00% of clay. Examined soils, especially calcomelanosols and hor. A of the calcocambisols are rich in humus. From the literature data it is known that without the combustion of organic matter, full peptization cannot be achieved, and therefore lower values for the clay content are obtained.

Table 1 Mechanical composition of soil

			In % of fineearth						
Profile No	Horizon and depth cm	Skeleton > 2mm	Coarse sand 0.2 - 2mm	Fine sand 0.02 - 0.2mm	Coarse + fine sand 0.02 - 2mm	Silt 0.002 - 0.02mm	Clay* <0.002mm	Silt + clay <0.02mm	Clay** <0.002mm
Calcomelanosol organic									
9	A 0-25	0,23	0,5	40,5	41	11,7	47,3	59	16,5
38	O 4-24	7,05	3,1	47,4	50,5	15,8	33,7	49,5	2,1
8a	A 0-22	0,07	0,3	42	42,3	23,5	34,2	57,7	3,8
60	A 0-10	12,27	19,1	48,2	67,3	14,7	18	32,7	2
61	O 0-12	1,90	12	54,7	66,7	11	22,3	33,3	4,4
Organomineral									
3	A 3-28	14,60	19,2	28	47,2	18,4	34,4	52,8	29,3
4	A 4-40	20,11	12,6	34,6	47,2	21,1	31,7	52,8	21,7
5	A 3-26	20,40	17,5	40	57,5	16,6	25,9	42,5	21
7	A 0-21	1,87	7	28,8	35,8	25,8	38,4	64,2	28,5
11	A 0-27	2,56	0,9	23,2	24,1	23,5	52,4	75,9	33,2
20	A 0-18	7,25	1,6	26,8	28,4	15,1	56,5	71,6	37,4
22	A 0-30	9,41	19,7	31,2	50,9	20,8	28,3	49,1	13,9
30	A 0-29	4,17	10,3	39,4	49,7	19,2	31,1	50,3	23,6
33	A 0-18	3,19	0,7	26,3	27	22,7	50,3	73	37,2
	A 18-36	0,10	1	22	23	25,2	51,8	77	21,7
35	A 0-27	13,79	5,7	21,7	27,4	32	40,6	72,6	4,4
4a	A 0-25	0,60	0,7	26,6	27,3	23,5	49,2	72,7	7,5
62	A 0-18	12,53	24,8	37,2	62	13,1	24,9	38	2,7
Brownized									
8	A 0-8	0,12	1	36,5	37,5	18,2	44,3	62,5	31,9
	A 8-36	0,20	2,3	21,9	24,2	18,5	57,3	75,8	40,7
	(B)rz 36-52	0,35	3,3	20,3	23,6	17,8	58,6	76,4	50
Calcocambisol									
1	A 2-21	3,76	6,40	23,60	30,00	13,00	57,00	70,00	38,7
	(B)rz 21-55	0,85	2,10	13,60	15,70	19,10	65,20	84,30	54,7
2	A 3-20	15,73	13,80	29,40	43,20	31,00	25,80	56,80	23,3
	(B)rz 20-35	11,97	18,00	17,60	35,60	23,10	41,30	64,40	38,9
	(B)rz 35-70	13,19	17,00	14,60	31,60	20,40	48,00	68,40	46,1
10	A 0-24	8,65	5,30	28,50	33,80	23,80	42,40	66,20	30,5
	(B)rz 24-58	6,58	7,60	19,50	27,10	22,00	50,90	72,90	49
	(B)rz 58-70	7,11	7,30	14,10	21,40	13,10	65,50	78,60	63,3
21	A 2-21	3,12	8,10	24,50	32,60	27,80	39,60	67,40	28,4
	(B)rz 21-73	1,00	6,50	21,90	28,40	30,90	40,70	71,60	40,4

*SOM combusted with H_2O_2

**SOM is not combusted

For comparison, we performed mechanical analysis without combustion and by combustion of SOM with H_2O_2. From the data presented in tab.1 it can be seen that the clay content is higher when the organic matter was previously combusted. For the same soil samples, the content of clay is 8.39 times higher in organogenic subtype when organic matter is combusted, in organomineral 3.10 times higher, while in hor. A of calcocambisols 1.34 times. In the cambic horizons (low humus content) of the brownized subtype of calcomelanosols and calcocambisols, the differences in the clay content according to the applied methods are negligible. According to Павићевић, cited by

Филиповски (1996) for 18 soil samples of calcomelanosols from Montenegro, the content of clay is 2.5 times higher than those where the organic matter has not been combusted.

Chemical properties

The data for the chemical properties of the examined soils are presented in Tab. 2

Table 2 Chemical properties of soil

Profile No.	Horizon and depth in cm	$CaCO_3$ %	Humus %	Total N %	C/N	pH		Easyavailable mg/100g soil	
						H_2O	MKCl	P_2O_5	K_2O
Calcomelanosol organic									
9	A 0-25	0,00	18,74	1,14	9,55	6,25	5,50	3,00	17,40
38	O 4-24	0,23	27,36	1,21	13,08	6,75	6,25	7,00	26,70
8a	A 0-22	0,12	17,38	1,07	9,46	6,65	5,70	1,80	18,70
60	A 0-10	0,00	15,15	0,91	9,67	5,85	5,10	5,60	23,40
61	O 0-12	0,00	36,61	1,95	10,89	6,10	5,60	16,20	33,30
Organomineral									
3	A 3-28	0,00	7,11	0,45	9,28	5,55	4,60	2,50	16,10
4	A 4-40	0,00	11,15	0,63	10,22	6,40	5,65	<1	19,20
5	A 3-26	0,00	5,49	0,37	8,58	6,50	5,80	<1	12,20
7	A 0-21	0,00	10,5	0,55	10,99	6,30	5,65	4,20	34,20
11	A 0-27	0,22	7,75	0,44	10,23	7,10	6,15	1,80	29,60
20	A 0-18	0,46	8,45	0,45	10,92	7,15	6,30	5,40	30,80
22	A 0-30	0,11	12,34	0,73	9,81	7,05	6,20	4,00	22,50
30	A 0-29	0,00	4,5	0,21	12,4	6,15	4,85	1,00	10,00
33	A 0-18	0,34	7,1	0,42	9,81	7,10	6,25	1,50	26,70
	A 18-36	0,45	4,79	0,31	8,93	7,30	6,15	1,00	17,00
35	A 0-27	0,00	6,27	0,42	8,77	6,20	5,30	2,40	15,20
4a	A 0-25	0,34	10,09	0,64	9,21	6,95	6,10	1,80	31,70
62	A 0-18	0,34	14,55	1,01	8,38	7,00	6,20	2,30	11,70
Brownized									
8	A 0-8	0,00	20,41	0,98	12,12	5,95	5,20	7,20	39,20
	A 8-36	0,00	7,69	0,46	9,61	6,30	5,30	1,20	13,90
	(B)rz 36-52	0,00	3,36	0,27	7,19	6,95	6,20	1,00	17,00
Calcocambisol									
1	A 2-21	0,00	9,51	0,56	9,83	6,75	6,05	2,00	31,70
	(B)rz 21-55	0,00	2,91	0,27	6,37	6,90	6,10	1,30	20,80
2	A 3-20	0,00	4,26	0,31	8,08	5,50	4,40	<1	6,2
	(B)rz 20-35	0,00	2,42	0,23	6,2	5,60	4,45	1,30	12,20
	(B)rz 35-70	0,00	1,64	0,19	5,03	5,65	4,50	15,00	13,00
10	A 0-24	0,00	8,9	0,43	12,06	4,95	4,00	3,00	16,10
	(B)rz 24-58	0,00	3,67	0,23	9,38	5,00	4,05	<1	15,20
	(B)rz 58-70	0,00	2,61	0,2	7,73	5,05	4,05	<1	12,20
21	A 2-21	0,11	7,48	0,42	10,45	6,70	5,80	3,60	17,00
	(B)rz 21-73	0,11	1,93	0,18	6,14	7,00	5,85	12,70	19,60

The chemical properties of the soils tested depend on the residue released by dissolving of the solid lime rock (limestone, dolomite, silicate limestone), the relief conditions especially altitude, vegetation and the degree of degradation, cultivation, the depth of the soil profile, deposition of materials from the higher parts, erosion, the previous stage and evolution. The investigated soils are non-carbonate. Only some profiles contains minimal amounts of carbonates, which is due to the physical decomposition of the rock. These are
actually sandblasted particles from the limestone cork. Therefore, they do not have a major impact on the soil reaction. The humus content in the calcomelanosols averages 12, 6%. The average humus quantity in hor. A on 134 profiles from the republic is 11, 19% (Филиповски, 1996). The humus content in the organogenic calcomelanosols is highest and averages 23,04%, organominerals 8, 46%, brownized 14, 05% and hor. A of the calcocambisols 7, 53%. As a result of reduced flow of organic waste and improved conditions of mineralisation, the humus content reduces in the cambic horizon of the brownized calcomelanosols (3, 36%) and calcocambisols (average 2, 53%). According to Маркоски (2013), organogenic calcomelanosols of the Republic of Macedonia on average contain 19, 47% humus, organominerals 13, 17% and brownized hor.A 12, 44% and hor. (B)rz 6, 66%. According to the same author, the humus content in hor.A of calcocambisols averages 8, 50%, and cambic horizon 5, 18%. The humus content in calcomelanosols under оак vegetation (average of 5 profiles) is 8, 19%, and under beech vegetation (average of 8 profiles) 13, 69%. Equally in calcocambisols under beech vegetation the humus content is higher (average of two profiles under оак and beech vegetation for hor.A) and is 8, 19%, and under оак vegetation 6, 88%. The production of plant waste is higher in beech phytocenoses, and plant waste is more difficult to decompose. On the other hand, beech phytocenoses are prevalent at higher altitudes (lower temperatures, more freezing days), where mineralization is difficult. For these reasons, the content of humus in beech phytocenoses is higher. The average content of total nitrogen in the calcomelanosols is 0,71%. Organogenic calcomelanosols contain 1,25% and organominerals 0,51% total nitrogen. Horizon A of brownized calcomelanosol contain 0,72% and cambic horizon 0,27% total nitrogen. Calcocambisols (hor. A) contain an average 0, 43%, and cambic horizon 0, 21% total nitrogen. The C/N ratio is an important indicator of the conditions in which the organic matter is being transformed. In adverse climate conditions (low temperatures, big number of icy days, extremely dry conditions), cold expositions, organic waste with wide ratio of C/N and poor in ash substances the transformation of organic waste is hindered, and the C/N ratio is wider. This ratio in organogenic calcomelanosols is 10,53 and in organominerals 9,81. The C/N ratio in hor.A of the brownized calcomelanosol is 10,86 and in hor.A of calcocambisol 10,10. This ratio in the cambic horizon of the brownized calcomelanosols is 7,19 and in the caclocambisols is on average 6, 80. The C/N ratio narrows in the depth of the profile as a result of the advanced degree of decomposition of organic matter. According to Филиповски (1997) the C/N ratio in hor.A of the calcocambisols is 10, 05 and in the cambic horizon 8, 23. The C/N ratio in the calcomelanosols under beech vegetation is 10, 66 and under oak 9, 54. Calcocambisoles (hor.A) developed under a beech vegetation are characterised with wider ratio (11,25) in relation to the ones under oak vegetation (8,95). This relates to the hindered conditions of transformation of the organic waste in beech phytocenoses. However, under all plant communities humus is of the mull-humus type. Soil reaction in water in the calcomelanosols on average is 6,53. The average pH value in the organogenic calcomelanosols is 6,32, in organominerals 6,67, hor.A of the brownized 6,13 and hor.A of the calcomelanosols 5,98. The reaction on the soil increases in the cambic horizon of the brownized calcomenalosol (6,95) and calcocambisols (on average 6,14).The upper layer of the profile is absolutely older and was exposed longer time on acidification , and on the other side the impact of the lime base is expressed stronger on the cambic horizons, hence hor.A has lower pH values. According to Маркоски (2013), the soil reaction in water in organogenic calcomelanosols is 6.99 on average, organomineral 6.93, brownized hor.A 6.12 and hor. (B) rz 6.68. According to the same author the reaction of the soil into water in the hor.A and a hor. (B) rz of calcocambisols is 6.63. With the evolution of the calcomelanosols to the calcocambisols, debasification and acidification progress, resulting in acidification of the soil

solution. The reaction of soil in water in calcomelanosols under beech vegetation is on average 6.36, and under oak 6.84. The same tendency is present in the calcocambisols, under beech vegetation 5.83 and under oak 6.13. The organic wastes of beech vegetation are poorer with basic substances, while on the other hand the soils are prevalent at higher altitudes and colder exposures (more humid climate) that causes stronger acidification. An insufficient supply of easyavailable phosphorus is striking. From the data in the table it can be seen that only one soil sample of the calcomelanosols and two of the calcocambisols are with medium content of easy available phosphorus. The rest are poorly supplied with easy available phosphorus. This is not a worrying fact, since most of the soils are under forest vegetation, and trees have the ability to consume even more difficult phosphorous compounds. Phosphorus fertilizing can be considered for lawns and small surface areas that are cultivated. The content of easy available potassium is on satisfactory level. One soil sample is poorly provided, 18 soil samples have a medium content of easy available potassium and the rest of 12 soil samples, are well provided with this element.

Conclusion

Fine earth dominates the skeleton in all the examined profiles. The most common fraction in the calcomelanosols and hor. A of the calcocambisols is the clay fraction, followed by the fine sand, silt and clay. In the cambic horizon of the calcocambisols, the clay fraction is the most present fraction of the fine earth, followed by silt, fine sand and coarse sand. In the examined soils, physical clay (<0.02mm) has higher content than the physical sand. The clay content is several times higher when preliminary combustion of the organic matter was carried out. Therefore, the international A method is recommended for these soils. The investigated soils are non-carbonate. Only a small number of soil samples contains a minimal amounts of carbonates. Humus content is higher in calcomelanosols and calcocambisols under beech vegetation compared to soils under oak vegetation. The ratio C/N in soils formed under beech vegetation is wider compared to soils under oak vegetation and is in accordance with the difficult conditions for transformation of organic matter. In the examined soils, the humus is of the type mull-humus. pH values in water are lower in soils formed under beech plant communities compared to soils under oak communities. With the evolution of the calcomelanosols to the calcocambisols, debasification and acidification progress, because the soil solution is acidic. The examined soils are insufficiently supplied with easily available phosphorus, while with easily available potassium are sufficiently supplied.

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CONTENT OF HEAVY METALS IN RIGOSOLS FROM THE AREA OF VELES

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Abstract
The scope of the investigations was to determine the quantity of total forms (Cu, Mn and Zn) and available forms of heavy metals (Cu, Fe, Mn and Zn) in rigosols from the area of Veles. Digestion of soil samples was performed with concentrated HCl and HNO_3 in a 3:1 ratio. The available forms of heavy metals were extracted with the DTPA method. Determination of the content was performed on atomic absorption spectrophotometer Agilent 55. The results of the investigation showed that the total zinc contents in all soil samples were lower than the reference value. Total copper content in 3 soil samples is lower than the reference values, while in 5 soil samples had higher contents than reference value, but much lower than intervention value. Total manganese content is lower than the maximum allowed concentration for agricultural soils. The quantities of available copper are in the ranges of low to very high, of iron and zinc is between very low to medium, while of manganese between low to high.

Keywords: soils, zinc, copper, iron, manganese.

Introduction

This paper presents data on the mechanical composition, some chemical properties and the content of the total (Cu, Mn and Zn) and available forms of copper, iron, manganese and zinc in rigosols from the Veles region. The rigosols are formed by with deep plowing (80-100 cm) of chromic luvisol on saprolite. The investigated site is located about 5 km southeast of Veles in the vicinity of the village Karaslari. Data on the content of total and available forms of copper, iron, manganese and zinc in the chromic luvisol on saprolite and other soils from the Veles region are encountered in the works of Стојковска and Спировски (1963), Јекиќ and Савиќ (1972), Џекова et al. (1988), Андреевски et al. (2006), Stafilov et al. (2008) и Mitkova and Markoski (2008). For the content of total and available forms of these heavy metals in the chromic luvisol on saprolite from other parts of the Republic, we find data in the works of Стојковска and Спировски (1963), Spirovski and Georgiev (1971), Јекиќ and Савиќ (1972), Митрикески (1995), Митрикески et al. (1997), Митрикески and Миткова (2000), Петковски et al. (2001) и Petkovski and Melovski (2006). According to the proposed classification of the soils of the Republic of Macedonia (Филиповски, 2006), we classified the surveyed profiles in the large group of soils Anthroposols, soil type rigosol, subtype chromocambsolic, variety nonterraced. Based on this, it can be concluded that the soils are formed by deep plowing of the chromic luvisol on saprolite. Out of the field research we conclude that the plowing was performed to a great depth (somewhere and deeper than 1 m), so that part of the carbonate lake sediment was also involved and mixed in the soil profile. The examined area was a fallow, which perversely was a vineyard uprooted more than ten years before our investigations. In fact, on the examined parcel, the vineyard was grown over a long period of time. The parent material is Neogene lake sediment. The examined site is located at an altitude of about 160-190 m. The relief is wavy with a slope of 3-8% and is characterized by good external drainage. In the profiles there were no signs of gleyic processes (oxidation-reduction processes) which means that the drainage is good. The aim of this paper is to investigate the content of the total (Cu, Mn and Zn) and available forms of copper, iron, manganese and zinc in rigosols from Veles area, which will

contribute to gaining a better understanding of the content of these heavy metals in the soils of the Republic of Macedonia. One of the goals of this paper is to examine the influence of soil properties and agrochemicals on the distribution of heavy metals in the profile.

Material and methods

Field examinations have been performed according to accepted methods in Former Yugoslavia (Filipovski ed. 1967). The laboratory analyses have been done according to the standard adopted methods in Former Yugoslavia and Republic of Macedonia, as follows: Mechanical composition of soil has been determined by the pipet method (Resulović ed. 1971); 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. pH (reaction) of the soil solution has been determined with glass electrode in water suspension and in NKCl suspension (Bogdanović ed. 1966). Easy available forms of P_2O_5 and K_2O were determinate by Al method (Džamić et al. 1996). Content of calcium-carbonate was determined using Scheibler's calcimeter (Bogdanović ed. 1966). Active lime has been determinate by the method of Galet (Bogdanović ed. 1966). The content of humus has been determinate at the base of total carbon by the method of Tjurin modified by Simakov (Орлов and Гришина 1981).Dissolution of soil samples was performed by concentrated. HCl and HNO_3 in a ratio 3:1 (Džamić et al. 1996). The available forms of heavy metals are extracted with the DTPA method (Page ed. 1982).Determination of the content is perfumed with AA spectrophotometer Agilent 55 and Agilent graphite furnace 240 Z.

Results and discussion

Mechanical composition and chemical properties

Data on the mechanical composition and some chemical properties of the tested soils are presented in Tables 1 and 2.

Table 1. Mechanical composition of rigosols from the area of Veles

			In % of fine earth					
Profile No	Horizon and depth cm	Skeleton > 2mm	Coarse sand 0.2 - 2mm	Fine sand 0.02 - 0.2mm	Coarse + fine sand 0.02 - 2mm	Silt 0.002 - 0.02mm	Clay <0.002mm	Silt + clay <0.02mm
1	P 0-30	4,6	10,0	50,9	60,9	15,4	23,7	39,1
1	P 30-51	5,6	11,5	46,9	58,4	11,4	30,2	41,6
1	P 51-73	15,2	10,0	52,5	62,5	10,1	27,4	37,5
1	C 73-105	12,1	5,5	44,7	50,2	21,4	28,4	49,8
2	P 0-31	10,2	12,7	56,9	69,6	9,3	21,1	30,4
2	P 31-69	5,2	11,0	58,9	69,9	8,0	22,1	30,1
2	P 69-105	4,2	12,9	61,9	74,8	7,9	17,3	25,2
2	C 105-140	18,2	20,0	50,6	70,6	16,4	13,0	29,4

Table 2. Chemical properties of rigosols from the area of Veles

Profile No.	Horizon and depth in cm	$CaCO_3$ %	Active $CaCO_3$ %	Humus %	pH		Easyavailable mg/100g soil	
					H_2O	nKCl	P_2O_5	K_2O
1	P 0-30	3,71		2,00	8,30	7,00	12,22	21,30
1	P 30-51	2,89		1,88	7,50	6,40	10,90	14,80
1	P 51-73	1,65		1,74	7,50	6,40	0,56	11,91
1	C 73-105	45,40	19,00	1,50	8,50	7,20	7,89	5,42
2	P 0-31	2,09		1,85	7,90	6,60	6,95	16,25
2	P 31-69	2,51		1,14	7,70	6,50	1,50	11,55
2	P 69-105	2,92		1,01	7,90	6,40	0,75	8,30
2	C 105-140	25,47	9,00	0,81	8,50	7,60	9,77	3,61

Content of total forms heavy metals Cu, Mn and Zn

In Table 3 are shown the data related to the content of total forms of Zn, Mn and Cu in examined soils. For comparison of the results the Dutch reference standards will be used (Ministerie van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer 2010).

Table 3. Content of total forms heavy metals in rigosols from the area of Veles

Profile No.	Horizon and depth in cm	Total content in $mg \cdot kg^{-1}$		
		Cu	Mn	Zn
1	P 0-30	80,52	693,76	111,21
1	P 30-51	55,41	806,43	78,74
1	P 51-73	36,82	565,93	66,85
1	C 73-105	18,27	139,92	30,11
2	P 0-31	51,40	661,14	83,11
2	P 31-69	54,65	649,23	69,85
2	P 69-105	30,04	509,81	58,38
2	C 105-140	16,86	241,30	35,55
Referent value		36		140
Intervent value		190		720

From Table 3 it can be seen that the total copper content in the tested soil samples is higher than the reference values (except for hor. P 69-105 cm and hor.C of prof. 1 and 2), but lower than the intervention values, which means that there is no danger of contamination of the soil and plants with this metal. An increased content at the top of the profile is notable. Bearing in mind that the surface area has been under vineyards for many years, it can be assumed that the increased content originates from the use of chemicals (pesticides) containing copper. Increased content of total copper in the upper part of the profile in rigosols under vineyards was noted by Андреевски et al. (2011). Андреевски et al. (2012) found an increased content of total copper in the soils from Kochani, on which rice was grown for many years and copper containing chemicals were used. In the chromic luvisol on saprolite from various parts of the Republic, Јекиќ and Савиќ (1972) found a total copper content of 12.0 to 30.4 mg kg^{-1} soil. Митрикески and Миткова (2000) reports data for 11.16 to 36.03 mg kg^{-1} of total copper content in chromic luvisol on saprolite from the Prilep and Kumanovo regions. The content of total copper of 12.3 mg kg^{-1} soil in chromic luvisol on saprolite

from Kamnik-Skopje (0-20 cm) was confirmed by Петковски et al. (2001). Petkovski and Melovski (2006) reported data of 7.4 and 9.4 mg kg^{-1} soil for two surface soil samples of the chromic luvisol on saprolite from the Skopje region (Morani). The total copper content of Veles and its surroundings based on 201 surface soil samples (0-5 cm) ranges from 11 to 1700 mg kg^{-1} (Stafilov et al. 2008). It should be noted that the high values are in the immediate vicinity of the lead and zinc smelter. Stafilov and Šajn (2016) conducted soil analysis (0-30 cm) for 60 heavy metals from 1025 locations in Republic of Macedonia. According to these authors the content of total copper ranges from 1.6-270 mg kg^{-1} soil, mean value 27 mg kg^{-1} soil and median 28 mg kg^{-1} soil. The total manganese content in the examined soils ranges from 139.92 to 806.43 mg kg^{-1}. According to Pendias-Kabata and Pendias (2001), the global soils average is 437 mg kg^{-1}. Maximum allowed concentrations for Mn in agricultural soils are 1500 mg kg^{-1} (Pendias-Kabata and Pendias 2001). The examined soils contain significantly less total manganese and no danger of toxicity. Стојковска and Спировски (1963) concluded that the total Mn content in chromic luvisol on saprolite from Republic of Macedonia is 540-1200 mg kg^{-1}. Јекиќ and Савиќ (1972) reported data of 340-900 mg kg^{-1} for the chromic luvisol on saprolite of the R of Macedonia. The content of total manganese of 322.2 mg kg^{-1} soil in chromic luvisol on saprolite from Kamnik-Skopje (0-20 cm) was confirmed by Петковски et al. (2001), and for two surface soil samples of chromic luvisol on saprolite from the Skopje region (Morani), Petkovski and Melovski (2006) reported data of 235.9 and 286,5 mg kg^{-1} soil. The total manganese content of Veles and its surroundings of 159 surface soil samples (0-5 cm) ranges from 160 to 8300 mg kg^{-1} (Stafilov et al. 2008). According to Stafilov and Šajn (2016), the content of total manganese in surface soil samples from the Republic of Macedonia (0-30 cm depth, 1025 locations) ranges from 17 -> 10 000 mg kg^{-1}, mean value 880 mg kg^{-1} and median 900 mg kg^{-1}. The content of total zinc varies within the range of 30.11 to 111.21 mg kg^{-1} soil and is lower than the reference values. This means that there is no danger of contamination of the soil and plants with zinc. According to Lindsay 1982 in: Kastori ed. (1997) the total zinc content of the soil ranges between 10 and 300 mg kg^{-1}, an average of 50 mg kg^{-1}. Chromic luvisol on saprolite from different parts of the Republic (0-20 and 20-40 cm) contains from 10.8 to 56.0 mg kg^{-1} soil total zinc (Јекиќ and Савиќ 1972). For the chromic luvisol on saprolite from the Prilep and Kumanovo regions Митрикески and Миткова (2000), report data from 12,21-52,04 mg kg^{-1} soil. The content of total zinc (two soil samples of chromic luvisol on saprolite from 0-20 and 20-40 cm from the site of village Izvor, Veles) of 74.64 and 98.76 mg kg^{-1} are reported by Андреевски et al. (2006). One surface soil sample of chromic luvisol on saprolite from Kamnik-Skopje location had a content of 75.6 mg kg^{-1} soil (Петковски et al. 2001), and two soil samples from the cultivated surface layer of chromic luvisol on saprolite from the location of Morani-Skopje had a content of 40.17 and 41.99 mg kg^{-1} soil (Petkovski and Melovski 2006). The total zinc content of Veles and its surroundings in 201 surface soil samples (0-5 cm) ranges from 22 to 27 000 mg kg^{-1} (Stafilov et al. 2008). High values were detected in the immediate vicinity of the lead and zinc smelter. According to Stafilov and Šajn (2016), the content of total zinc from 1025 sites from the Republic (surface soil samples from 0-30 cm depth) ranges from 8 to> 10 000 mg kg^{-1} soil, the average value is 82 mg kg^{-1} soil and median 83 mg kg^{-1} soil.

Content of available forms heavy metals Cu, Fe, Mn and Zn

Table 4 shows the content of available forms of heavy metals Cu, Fe, Mn and Zn in the studied soils. These heavy metals are essential trace elements in plant nutrition and their deficiency can cause growth failure while the greater scarcity can cause extinction of plants. On the other hand, if these trace elements are present in high concentrations can lead to phytotocsicity. From the data in the table it can be concluded that the tested soils have low (2), medium (1), high (1) and very high (4) content of available copper. This raised values in the upper part of the profiles are probably as a result of the use of copper containing chemicals over many years, which are used to protect the vineyards. Out of the 12 soil samples from the chromic luvisol on saprolite from different parts of the country (0-20 and 20-40 cm) 10 are with rich and 2 with very high content (Јекиќ and Савиќ 1972). Chromic luvisol on saprolite from the Prilep and Kumanovo regions have poor, medium to rich

content of available copper (Митрикески et al. 1997). Spirovski and Georgiev (1971) reported data from 15 soil profiles (76 soil samples) on the chromic luvisol on saprolite from various parts of the country. They concluded that all soil samples have good content, except two that have poor and one with insufficient content. From the obtained results, they conclude that the appearance of carbonates leads to a reduction in the available copper. In our research, we also found that soil samples with the highest content of carbonates (hor. C in prof. 1 and 2) contains least available copper.

The tested soils have very low (1), low (5) and (2) intermediate content of available iron. Due to the presence of $CaCO_3$, the content of available iron is unsatisfactory, with a risk of appearance of iron chlorosis in some crops. Unsatisfactory content of available iron is found in the carbonate rigosols from Kavadarci region (Андреевски et al. 2008). The condition with available manganese is satisfactory. The tested soil samples have low (2), medium (1) and high (5) content of available manganese. It is especially important that soil samples in the upper part of the profile have high content. Reduction of the available manganese for different soil types (including the chromic luvisol on saprolite) in depth of the profile was determined in the work of Стојковска and Спировски (1963). Same conclusion can be find in the work of Spirovski and Georgiev (1971) for the chromic luvisol on saprolite from various parts of the country. Furthermore, same authors pointed out that these soils, with rare exceptions, are well supplied with active manganese. All 12 soil samples of chromic luvisol on saprolite from various parts of the country (0-20 and 20-40 cm) are very richly supplied with available manganese (Јекиќ and Савиќ 1972). The most unfavorable condition is with the content of available zinc. The investigated soils have very low (4), low (1) and intermediate (3) content of available zinc (Table 4), due to the high pH values and the content of $CaCO_3$. The solubility of zinc is especially low in soils with high pH value, as well as in the presence of $CaCO_3$ (Kastori, 1990). According to Јекиќ and Савиќ (1972), the chromic luvisol on saprolite from various parts of the country (0-20 and 20-40 cm) have very poor, poor to medium content of available zinc. For two soil samples of chromic luvisol on saprolite from the location of Izvor-Veles, high content of available zinc was detected (Андреевски et al. 2006). Chromic luvisol on saprolite from the Prilep and Kumanovo regions have very poor, poor, medium to rich content of available zinc (Митрикески et al. 1997). As a general conclusion, it can be said that the content of available micronutrient forms is larger in the upper part of the profile and is a result of the long-term use of agrochemicals that accumulates mainly in the upper part of the profile and the favorable conditions for their mobilization. The lowest is the availability in the parent material (the highest pH values and the highest content of $CaCO_3$).

Tab. 4 Content of available forms heavy metals in rigosols from the area of Veles

Profile No.	Horizon and depth in cm	Available forms in $mg \cdot kg^{-1}$			
		Cu	Fe	Mn	Zn
1	P 0-30	10,30	9,88	21,68	2,93
1	P 30-51	3,39	14,00	23,16	1,14
1	P 51-73	1,47	7,68	16,98	0,41
1	C 73-105	0,71	5,62	4,05	0,23
2	P 0-31	6,21	12,22	29,24	2,59
2	P 31-69	2,93	10,16	22,04	0,96
2	P 69-105	1,03	6,74	10,71	0,27
2	C 105-140	0,44	2,64	4,34	0,16
very low		<0,3	0-5	0-4	<0.5
low		0.3-0.8	5-10	4-8	0.5-1.0
medium		0.9-1,2	11-16	9-12	1.1-3.0
high		1.3-2,5	17-25	13-30	3.1-6.0
very high		>2,5	>25	>30	>6.0

Conclusions

Based on the conducted research the following conclusions can be drawn:

Total zinc contents in all soil samples are lower than the reference value. Total copper content in 3 soil samples is lower than the reference values, while in 5 soil samples had higher contents than reference value, but much lower than intervention value. The increase content of total coper in the upper part of the soil profile originates out of the prolonged use of agrochemicals containing coper in vineyards. Total manganese content is lower than the maximum allowed concentration for agricultural soils. Out of the obtained results we can conclude that there is no danger of contamination of the soil and plants with these heavy metals. The content of available copper and manganese is satisfactory, while the content of available zinc and iron unsatisfactory.The availability of micronutrients is higher in the upper part of the soil profile and is a result of long-term use of agrochemicals that accumulate mainly in the upper part of the profile and the more favorable conditions for their mobilization.

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CONSERVATION AGRICULTURE ON UKRAINIAN CHERNOZEMS

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Abstract

As the granary of Europe, Ukraine has seen a significant rise in productivity and efficiency of its agriculture during the past decades. Chernozems are the primary soils in Ukraine. In order to maintain continued increases in grain production, Ukraine needs to implement conservation tillage and other sustainable land management practices to reduce soil degradation. The paper reviews the problem of Chernozems degradation and summarizes the influences of conservation tillage, cropping systems, fertilization, crop residue management, strip-cropping and contour farming on physical, chemical, and biological properties of Ukrainian Chernozems for the past 50 years. The conversion from plow-tillage to minimum tillage and no-till improves infiltration rate, pH and soil organic matter (SOM) in 0- to 10-cm layer and lead to carbon accumulation in fulvic acids and humins. No significant differences in SOM storage were observed among tillage systems in the 0- to 100-cm layer. The five crop rotation increased 0.8, 0.71, 0.94 t ha^{-1} yield of cereals and 5.8, 1.0, 4.2 t ha^{-1} sugar beet under conventional tillage, deep minimum tillage and reduced minimum tillage, respectively. Recommended application of manure in conservation agriculture in the humid zone - 12 t ha^{-1}, in semi-humid zone - 10-12 t ha^{-1} and 8-10 t ha^{-1} in semi-arid zone. Green manure, cover crops and inter crops increased crop yield by 2-10% on Forest-Steppe and Steppe Chernozems. The combination of strip cropping, contour farming, contour bunds, and terracing are particularly recommended for the sloping farmland in order to reduce soil erosion. Land-related policies and relevant legislation in Ukraine are also outlined with an aim to contribute to the creation of guidelines and strategies for further implementation of appropriate practices for sustainable agriculture.

Keywords: Chernozems, conservation agriculture, tillage; cropping systems, fertilization.

Introduction

Worldwide use of soil resources was intensified dramatically ever since the beginning of agricultural civilization and domestication of plants and animals (Dudal, 2002). Land has been cultivated for 2500-3000 years in North and South American continents, 2000-4000 years in Western European and Mediterranean countries, 5000 years in the Middle East (Sandor, 1998), and 4000-6000 years in Ukraine (Lisetskii, 2010). The transformation of natural grassland into crop or pasture land with improper anthropogenic activities such as lack of cover, less input, and overgrazing in particular resulted in an accelerated soil degradation, and is of great concern in every agricultural region of the world (Liu et al., 2010). Ukraine, the granary of Europe, witnessed a significant rise in agricultural? Productivity and efficiency during the first decade of the new millennium due to the intensity of its land use. Ukrainian grain export of wheat, corn and barley was in the eighth, third and first place of the world grain market in 2011 (Potiha, 2011). However, the country has to deal with soil degradation problems in maintaining its grain production momentum. The impact of Ukrainian agricultural production system on the environment contributed to 35-40 % of the total environmental degradation (Sutton et al., 2007). It is estimated that over 8-10 million ha of farmland or 24.6-30.8% of arable lands is degraded and 4.5 million ha of farmland are in the moderate and severely eroded stages. The agricultural lands subjected to water erosion are estimated at approximately 13.3 million ha (including 10.6 million ha of arable lands). Over 1.9 million ha of these soils have been identified as wet or poorly drained. Irrigated land area decreased by 15% over the

past 15 years (Anisimova et al., 2009). More than 500,000 gullies are spread out on 140,000 ha of Ukrainian terrain. The soils with a lost surface horizon of humus accumulation occupy 68,000 ha. Approximately 600,000 ha of arable soils have been covered by eolian medium and coarse textured clastic sediments. Annually, 6 million ha of lands are affected by wind erosion and increased up to 20 million ha in the dust storm periods (Zubets et al., 2010).

Chernozems are the primary soils in the Ukraine, and account for 62% of all agricultural lands and approximately 78% of these soils have been cultivated (Kravchenko et al., 2011). Agricultural management strongly influences soil properties and the assessment of agricultural management on Ukrainian Chernozems may identify the degree these soils are degraded and thus provide priorities for policy-makers and stakeholders in adopting more appropriate practices and guidelines. This review paper summarizes the influences of conservation agriculture on physical, chemical, and biological soil properties on Ukrainian Chernozems. The policies and relevant legislation in Ukraine were also outlined.

Conservation tillage

The moldboard plow inverts the furrow at least 135^{o}, mixes and incorporates the residues and fertilizers within the tilled zone, displaces and shatters the soil aggregates and plant residues. Soil inversion is highly effective in burying crop residues and killing annual and perennial weeds as well as volunteer crops (Kurdyukova and Konoplya, 2011). Other studies have found that tillage systems disturb the weed seeds in different ways (Clements et al., 1996). The conventional tillage buries surface weed seeds in the lower 10-15 cm plow layer, whereas chisel plowing leaves the seeds closer to the surface, and with no-till, 90% of seeds remains in the 0-5 cm top layer. Continual tillage, however, can in some situations lead to soil degradation processes, such as organic matter decline, loss of soil structure and compaction, leaching of calcium and other soil nutrients (Gilley and Doran, 1997). Posing a high risk for crops, conventional tillage (moldboard plow) is seen as a major factor for increased erosion risks (Prager, 2010). The numerous studies (Liu, 2011; FAO, 2002; Gallaher and Maglene, 1987) have shown preferences for conservation tillage for the effects on soil fertility improvement and soil erosion control. The modern concept of conservation tillage defines it as a non-inversion tillage based on: no-tillage, strip tillage, stubble mulching, zonal tillage, reduced or minimum tillage (FAO, 1993), direct drilling, and/or ridge-tillage with retention in all systems of at least 30% of ground cover by residues, and technologies that conserve time, fuel, money, labor, soil structure, nutrients, soil biomass, and soil water (Baker et al., 2007) and reduction in the number of passes over the field (Flower and Rockstrom, 2001). The adoption of minimum tillage under winter crops in the South and South-East regions of Ukraine was successful, and about 50% of arable land area is now under no-till, minimum till, and disking, though conventional tillage practices are prevalent in the humid and semi-humid regions of Ukraine on Spodosols and Alfisols, respectively (Sayko, 2007). Our study, has been conducted on a Typical Chernozem over a 7-yr period from 2006 to 2013 in the Forest-Steppe zone of Ukraine, showed increasing bulk density, compaction, infiltration rate, pH, soil organic carbon (SOC) concentration, carbon of humic acids (HA), carbon of fulvic acids (FA), molecular weight fractions humic acids (HA Mw) with minimization tillage. However, reduced tillage systems had a higher proportion of labile organic carbon , a lower ratio of C in humic acids/C in fulvic acids in comparison with conventional tillage as well as heavier molecular masses of humic acids ranged from 110 to 2000 kDa (Kravchenko et al., 2012).

Cropping systems

The monitoring and control of the crop rotations in Ukraine is, according to the Cabinet of Ministers Directive № 164, exercised by both local authorities and state agencies. Traditionally in Ukraine a crop rotation is made up of eight – ten fields (Tarariko et al., 2012). The crop sequence in a typical forest-steppe rotation is: (1) green manure fallow or grain legume; (2) winter wheat (*Triticum aestivum L.*); (3) sugar beet (*Beta vulgaris L.*); (4) spring barley (*Hordeum vulgare L.*) with perennial grasses; (5) perennial grasses; (6) winter wheat (*Triticum aestivum L.*); (7) sugar beet or corn (*Zea*

mays L.); (8) pea (*Pisum sativum L.*); (9) winter wheat, and (10) sunflower (*Helianthus annuus L.*). Unfortunately, at the current time, the rotation cycle length has been reduced to three or four crops, owing to economic considerations. Crop rotation is very much dependent on region, farm specialization, and available agricultural machinery. Crop rotations are classified into field, fodder, vegetable, and special types. In compliance with Directive № 188/98-BP of Ukrainian Parliament (VVR, 1998), there was adopted a "Contour – ameliorative" system of farming which determined the frequency and diversity of row crops in depending on slope gradient and severity of erosion. The guiding principles of this system were to reduce the number of row-crops (maize, sugar beets, sunflowers) and increase the number of cereals and leguminous grasses in rotations on the slopes. Clean fallows were substituted by green manure fallows. Degraded soils in field crop rotations were replaced by grasslands and forests. The greater parts of plant residues are now left in the fields. The system also introduces strip cropping across the slope, contour farming, shelter belts of forest trees and terraces on plowed land. Tsilyurik (2009) found that crop species impacted soil properties differently. Percentage of water stable aggregates in the size of 0.25-7 mm from the Steppe Chernozems cultivated with alfalfa (*Medicago sativa L.*) was 92.6%, and was decreased to 88.1% in pea, 87.7% in winter wheat, 85.8% in barley, 76.2% in corn, 77.4% in sugar beet, and 74.2% in sunflower. Crop rotation can affect soil properties and increase crop production. A long-term experiment by Gangu et al. (2011) showed that yields of continuous sugar beet were 8.9-10.7 tha^{-1} less than that of the rotation system. The data Demidenko (2012) showed, that a 5-yr crop rotation had an increased yield of cereals on 0.8, 0.71, 0.94 t ha^{-1} and sugar beet on 5.8, 1.0, 4.2 t ha^{-1} than 3-yr crop rotation under conventional tillage, deep minimum tillage and reduced minimum tillage, respectively. Still, 932,000 hectares of arable lands in Ukraine are in monoculture (FAO, 2013). The frequency with which crops are grown affects crop yields.

Fertilization

Though Chernozems are fertile and productive soils, organic and mineral fertilizers are required to maintain their fertility. The increased yield from fertilizers was 50% in Forest Chernozems and 40% in Steppe Chernozems, and additional 20-15% and 40% yield for these soils with irrigation (Nosko, 1991). The commonly used fertilizer rates (kg ha^{-1}) in typical Chernozems are: $N_{80}P_{70}K_{60}$ for winter wheat, $N_{80\text{-}120}P_{90\text{-}120}K_{120}$ + manure 30 t ha^{-1} for corn, $N_{60}P_{60}K_{40}$ for barley, $N_{90\text{-}120}P_{70\text{-}90}K_{40\text{-}60}$ + manure 20-40 t ha^{-1} for rice, $N_{60\text{-}80}P_{60\text{-}80}K_{40\text{-}60}$ for buckwheat, $N_{30\text{-}45}P_{45}K_{45}$ for soybean, $N_{160}P_{170}K_{150}$ + manure 30-50 t ha^{-1} for sugar beet, $N_{60}P_{60}K_{60}$ for sunflower, $N_{45\text{-}90}P_{60}K_{60\text{-}120}$ + manure 30 t ha^{-1} for potato (*Solanum tuberosum*), and $N_{90\text{-}120}P_{40\text{-}60}K_{80\text{-}120}$ + manure 20-30 t ha^{-1} for rape (Marchuk et al., 2011). The way the soils are managed can improve or degrade their natural quality. Applying mineral fertilizers may increase soil acidity (all forms) and enhance the leaching of exchangeable bases. Soil organic matter loss is another of the major characteristics of soil degradations in the Ukraine. Soil organic matter (SOM) content declines steadily during the first 60-year of the virgin Chernozems cultivation of, and maintaines at a relatively stable level afteryears (Degtyarov, 2011). SOC content can be recovered or even increased by applying cattle/pig/poultry manure, compost, peat, sapropel, green manure, plant residues, and cover crops. According the data of Hospodarenko et al. (2012), 45-year cattle manure application of increasing annual rates, noticeably increased percent base saturation, and available nutrient content, improving soil aggregation. Increasing annual average solid manure rate from 9 to 13.5 t ha^{-1} allowed to increase SOM content from 32.4 to 34.3 g kg^{-1}, whereas further increase of manure application annual rate to 18 t ha^{-1} resulted in a less pronounced SOM content increase (to 34.9 g kg^{-1}). Greatest value of total porosity (0.58 m^3 m^{-3}) and infiltration rate (23.85 mm hr^{-1}) was observed by Nazeer and Malik (2011) in the treatment of farm manure (FM) at the rate of 40 t ha^{-1}, followed by FM rate of 20 t ha^{-1} (0.41 m^3 m^{-3}, 15.00 mm hr^{-1}) and without FM (0.36 m^3 m^{-3}, 12.00 mm hr^{-1}). Over the past several decades intensive cropping practices have led to the increasing demand for trace elements to the level higher than the soil can supply. A fundamental difference from traditionally used salt (ionic) form of fertilizers is a nanoform of mineral nutrients. A nanofraction, which is a result of melting and evaporation, followed by

condensation of the vapor phase with an average sizes in the range of 10–150 nm and the corresponding structural phase composition of the solid phase, has signs of a biological functionality and can be used in plant growing. The use of pre-treatment of wheat seeds with colloidal solutions of metals, obtained by electrical discharge treatment, at an application rate of 2 liters per 1 ton of seeds and 2–3 times processing of plants during the growing season enhances winter wheat productivity by 15–20% on Typical Chernozem (Lopatko et al., 2013). The colloidal forms of metals make a positive nutrient effect on the crops grown in calcareous and saline soils. The use of metal colloids normalizes the osmotic properties of cells, since the use of colloidal forms of metal for seed germination under saline conditions, stimulate the swelling and seed germination. Our data (Kravchenko et al., 2015) demonstrate that under the salt stress conditions colloidal forms of manganese, zinc, cuprum, iron, unlike their salts increase the availability of this element for plants, facilitating its admission and participation in biochemical processes.

Mulching, Strip Cropping, Contour farming, Terracing

Cover crops are usually grown together with winter or spring crops in sequence or planted after harvesting. They are grown as autumn/winter annuals and ploughed in to form a green manure prior to sowing the main crop. Total incorporation of 40 t ha^{-1} lupine biomass into soil brings in 180 kg N/ha^{-1}, 40 kg P/ha^{-1}, 68 kg K/ha^{-1}, which is equivalent to 15-30 t ha^{-1} of manure (Makarova et al., 2008). Cover crops not only influence soil properties but also increase crop production. Results obtained by Datsko & Stcherbatenko (2006) demonstrated that the use of cover crops increased yield by 0.17-0.43 t ha^{-1} in winter wheat, 5-9 t ha^{-1} in potato, 5-14 t ha^{-1} in sugar beet, 7-13 t ha^{-1} in silage corn, 0.9-1.3 t ha^{-1} in grain corn, and 0.6-1.0 t ha^{-1} in buckwheat. Eroded Ukrainian Chernozems require the application of mulches at rates depending on soil texture: 1.3 t ha^{-1} for sandy loams, 1.9 t ha^{-1} for sands, and 1.1 t ha^{-1} for silt loams (Zubets, 2010). Tsvey et al. (2009) reported that increasing application rates of cereal straw from no mulch, 2.5 t ha^{-1}, 5 t ha^{-1} and 5 t ha^{-1} + N_{30} resulted in additional sugar beet seed yields of 1.31 t ha^{-1}, 1.41 t ha^{-1}, 1.54 t ha^{-1}, and 1.67 t ha^{-1} respectively. The effect of incorporated mulch depends upon the material used. Univer et al. (2009) found higher strawberry yields were obtained from mulches of white clover *(Trifolium repens L.)*, timothy *(Phleum pratense L.)*, Kentucky bluegrass *(Poa pratensis L.)* and red fescue *(Festuca rubra L.)*. While implementing mulches, it is important to consider the carbon to nitrogen ratio of the organic residues as the organisms consume the soil N and immobilize it if the C:N ratio is above 25. In order to overcome nitrogen deficiency in Ukraine 10-15 kg N/ha^{-1} and 8 kg P/ha^{-1} of chemical fertilizers are applied for each metric t of straw (Grechkosiy, 2008). The effectiveness of mulching in reducing erosion was demonstrated in the field experiments in typical Chernozems (Kravchenko et al., 2010). Minimal tillage with 2.5 t ha^{-1} mulch in the eroded Chernozems saved greater amount of available water for the plants, reduced runoff up to 3.8 m^{-3} ha^{-1}, and increased spring barley grain yield by 1.6 t ha^{-1}. Strip cropping is a method of growing row crops in alternating strips following the contour of the land, in order to minimize erosion (NRCS, NHCP, 2008). Strip cropping is often applied in slopes exceeding 2° steepness or/and 150-200 m length field. Deep heavy-rooted plants in this arrangement should alternate with loosely-rooted plants. The strip widths on 3° slopes are usually about 60-70 m for corn/rape/sunflower, and 70-150 m for spring-winter cereals. The widths of buffer strips made up of grasses and legumes should be no less than 4-6 m on 3° slope and 8-10 m on 3-7° slopes (Tarariko, 2006). Contour farming is not recommended for areas where the slope is less than 1° and the slope is not long (Tarariko and Lobas, 1998). For the field with 1-3° slopes, common practices carried out are: plowing along the lines of the contours, 1-3 m buffer strips comprised of buckwheat, phacelia *(Phacelia)*, oats, annual legumes planted at intervals of 60-80 m; 6-8 m wide forest belts along the field margins and perpendicularly to the wind direction; contour ridges or channels established at 160 m intervals; mulching with cover no less than 65 per cent of the soil surface; establishment of permanent vegetation barriers; growing multiple crops for use in rotations, and application of an additional 10-15% chemical fertilizers. For the fields with 3-7° slopes, recommended practices are: alternative strips across the slope parallel to each other in breadth of

60-80 m under annual grasses mixed with cereals or 20-40 m under corn-legumes mixtures; crop rotations with 40% cereals and 60% legumes; forest belts mixed with bushes 8-10 m wide at an intervals of 200 m; mulching cover no less than 75% of the soil surface; additional 15-20% fertilizers applied. For steeper slopes with very erodible Chernozems, the top priority practices are: growing perennial forage and pasture crops. The benefits of contour farming can be enhanced by combination with the other relevant conservation practices suitable to local soil, relief/geology and climate conditions (Balyuk and Tovazhnyanskiy, 2010). Contour bunds, suitable for slopes 1-7°, are 1.5-2 m wide, 0.25-0.4 m high, spaced at 18-50 m intervals, which were built across the slope to form a water storage area on their upslope side and frequently used in a strip-cropping systems covered by vineyards, gardens, and shrubs. Ivanytska (2010) found the earth bunds increased the effective volume of plum roots to 515 m^3, as compared to 347 m^3 on the slopes without the bunds. Soil total porosity increased to 55%, compared with 49% on the slopes without the bunds, and soil bulk density improved to 1.23 g cm^{-3}, as compared to 1.33 g cm^{-3} on the slopes without bunds. Terracing across the slope intercepts surface runoff and minimizes soil erosion. Three classes of terraces are employed in Ukraine: diversion, retention and bench. The common ground terraces are normally used on slopes less than 7°, with the embankment up to 1 m high and 3-12 m wide (Dzhamal and Shelyakin, 1986). Terracing and slope steepness affect the Chernozems morphological features. Svitlichniy and Chorniy (2007) reported that the soil on the terraced slopes lost less nitrogen, phosphorus, potassium and calcium as compared to non-terraced slopes. Zuza (2011) reports a significant improvement in snow-trapping and available water storage by 12-26 mm with terraces. The same phenomena were earlier reported by Gichka and Timchenko (2007) in spring on terraced slopes.

Policy and legislation in Ukraine

Being a member of Council of Europe since November 1995, and an active participant in the "Environment for Europe" process, Ukraine inherits numerous European obligations and has its own legislation with corresponding measures in soil protection. More than 400 policy measures were developed by EU Member States (Kutter et al., 2011). The soil-relevant policies, addressed to soil degradation, can be outlined in four categories: mandatory measures, voluntary incentive-based measures and awareness-increasing measures and private initiatives (Baumol and Oates, 1979). The European Commission Directive COM (2002) 179 final "Towards a Thematic Strategy for Soil Protection" is one of the most relevant to soil conservation. This Directive describes the multiple functions of soils, identifies the main threats to soils (erosion, decline in soil organic matter and biodiversity, soil contamination, soil sealing, soil compaction, salinization, floods and landslides), and changes in soil characteristics relevant to policy development (EC, 2002). The Sixth Environment Action Program of the European Community entitled "Environment 2010: Our Future, Our Choice" defines the priorities and objectives of European environment policy up to 2012 dealing with a coherent approach to soil protection with legislation, integrating environmental concerns, partnership with business, empowering citizens and changing their behavior, and taking account of the environment in the land-use planning and management (Montanarella, 2005). There are a number of directives regulating soil quality, such as the "Nitrates Directive" 91/676/EEC and the "Water Framework Directive" 2000/60/EC, combined with the "Groundwater Directives" 80/68/EEC and new directive 2006/118/EC. Holistic approach in soil protection and sustainable land use was also targeted in "Soil Framework Directive" COM (2006) 232 and "Global Environment Outlook". The National Ukrainian legislation takes into account interrelationships between soil friendly practices to decrease soil degradation and direct policy measures. The Land Code of Ukraine (effective from January 1, 2002) is the most advanced and closest to European legislative norms. It defines legislative codification and summarizes the rules, regulating land relations into a coherent system, built upon unified principles, taking into account the world experience and requirements regarding harmonization of Ukraine's legislation with legislation of the European Union. Some norms of this act contain direct guidance for land protection, use, reclamation, recovering of contaminated and

damaged soils, restoration of soil fertility, standards in land protection, and state oversight of land use and conservation. The legislative authorities, responsible for budget initiation and regulation in land/soil conservation, are the Ministry of Agrarian Policy and Food, Ministry of Ecology and Natural Resources, The State Agency of Land Resources, State Forest Resources Agency, State Water Resources Agency, and the Statute of the National Environmental Investment Agency of the Ukraine. All environmental principles of land protection are embodied in the Ukrainian Constitution. All agricultural lands in Ukraine, according to the President Decree № 1118/95 and Directive №536, must possess an agrochemical passport. The certificate includes common soil parameters (soil organic matter content and its distribution downwards soil profile, soil texture, storage capacity of available for plants water, acidity, salinity, soil nutrients and microelements content) as well as the concentrations of the soil contaminants determined by the regulations № 4433-87 "Sanitary code of MPC (maximum permissible concentration) of chemical substances in soils". The sanitary condition of the Ukrainian soils is also determined by the State Standard №17.4.2.01-81 "Nature Protection. Soils. Nomenclature of sanitary condition indices". According to the law "On State Control over Use and Protections of Lands", the control of land use and protection is carried out by the authorized body of The State Agency of Land Resources. The control of the observance of laws for soil protection is fulfilled by the authorized body of Ministry of Ecology and Natural Resources, and the monitoring of soil fertility is fulfilled by the authorized body of Ministry of Agrarian Policy and Food. Some older legislative acts contain direct operating instructions for soil management: Directive №320 from 16.05.1967, "Immediate measures of the soil protection against of wind and water erosion", Directive №407 from 02.06.1976, "On land reclamation, conservation and rational use after open-pit mining", but now they are not widely used because of the adoption of new scientific approaches, technology, and standards of soil conservation. The principles of ecological policy in management of land resources are governed by the law "On Environmental Protection". This law enacts norms for environmental state inspection, assessment, standardization and liability of infringement.

Conclusions

According to our findings, the practices of no-till and minimum tillage with the application of 2.5 t ha^{-1} of shredded cereal straw, resulted in 1.31-1.67 t ha^{-1} added yield of sugar beet, surface runoff reduction up to 3.8 m^{-3} ha^{-1}, increases in SOM concentration, infiltration rate, pH, and the amount of available N, P and K. This effect is enhanced by the use of an eight-ten field crop rotation, enriched by small grain crops and leguminous forages. The recommended fertilizers in conservation agriculture include full NPK rates plus manure of 12 t ha^{-1} in the humid zone, 10-12 t ha^{1} manure in semi-humid zone and 8-10 t ha^{-1} manure the in semi-arid zone of the Chernozem region (Marchuk et al., 2011). Green manure, cover crops and inter crops increased yield by 2-10% on Forest-Steppe and Steppe Chernozems. The combination of strip cropping, contour farming, contour bunds, and terracing are particularly recommended for sloping in order to minimize soil erosion, water losses and provide sustainable management practices on sloped farmland. The Ukrainian government is keen to address all recognized soil degradation processes through legislation. However, few policies are relevant to soil conservation or do not address soil degradation, and even if they do, are not oriented towards specific results of improved soil quality with appropriate farm management.

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GASTROINTESTINAL PARASITES OF SHEPERD DOGS FROM TETOVO REGION MACEDONIA

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Abstract
A total of 78 faecal samples from owned shepherd dogs were collected in Tetovo, Macedonia and were examined for the presence of intestinal parasites by centrifugation - flotation method that is more sensitive. The overall prevalence of parasitism in the tested samples was 0,71%. The species found and the infective percentage were: *Ancylostoma caninum* 41%, *Trichuris vulpis* 39.2% and *Toxocara canis* 17,8. The parasite *Angiostrongylus vasorum* was found in only 1 sample. There was not observed any significant difference in prevalence between dogs genders according the species of parasite found. Young animals were found to more frequently shed nematode eggs in faeces than adult animals. The presence of zoonotic species of parasites in dogs in the studied region, associated with the elevated degree of misinformation of the owners, indicates that the risk of zoonotic infection by canine intestinal parasite may be high.

Keywords: Dogs, intestinal parasites, flotation methods, prevalence.

Introduction

Intestinal parasites of dogs are diffused worldwide and intestinal parasitic infections in dogs are commonly recognized as a cause of gastrointestinal disorders with a high prevalence in developing countries (David ÉB, 2015). The veterinarian concern for these parasites is still a living matter due to their zoonotic potential. Among intestinal helminthes of dogs, *Toxocara canis* represents the major concern as it can cause severe infection in humans (Nijsse R, 2015). Other zoonotic helminthes like *Ancylostoma caninum* are primary causes of cutaneous, visceral, and ocular larva migrants and eosinophilic enteritis (McKenzie E, 2010). Environmental faecal contamination by infected dogs represents a source of infection for humans. In fact, parasitic elements, like eggs, larvae, cysts, and oocysts excreted via canine faecal route can survive over a long time and be infective in the environment at different condition. For that reason is necessary to make epidemiological studies to obtain data from dogs which can undoubtedly contribute to preventing direct zoonotic transmission from dogs to humans via the control of infectious animals (Lee AC, 2010). Most intestinal parasites do not show symptoms until the infestation has become severe. This is why preventative care and regular faecal exams are important to catch the infection in its early stages this is performed by collecting stool samples and check frequently for any parasites. Hookworms will attach to the wall of the stomach and puncture blood vessels to feed on the blood also they are transmitted to humans. Roundworms are the most common intestinal parasites in dogs almost all dogs will have an infestation of roundworms at some point in their lifetime and they can be also transmitted to humans also tapeworms can be transmitted to humans. Major aim of this survey was to determine the prevalence of intestinal parasites in shepherd dogs in Tetovo region, Macedonia from May to June 2017. Samples are taken from shepherd dogs of various ages and sex and parasite prevalence was calculated. Influence on prevalence of individual pet features (age, gender, size, and presence/absence of clinical signs) were also considered. Due to regional variations in parasite prevalence, such information is often of limited value outside the specific areas evaluated.

Material and methods

The survey was carried out in Tetovo region, located in the northwestern part of Macedonia. During May to June 2017 a total of 78 fresh faecal samples were collected from shepherd dogs. Data about age, sex, and presence/absence of clinical signs of animals were recorded. The group of tested animals consisted in 48 dogs ≤12 months and 30 dogs >12 months, from them 43 males and 35 females. A minimum of 2 g of faeces was collected from each animal, immediately placed into a plastic container, labelled and stored at 4°C until they were examined within 48 hours. Faeces (2 to 3 g) were mixed thoroughly with 15 mL zinc sulphate solution (ZnSO4, specific gravity 1.18) and transferred to a 15-mL conical tube. The faeces solution was centrifuged in maximal speed for 5 min. Additional ZnSO4 was added to bring the volume up to 15 mL to form a positive meniscus, onto which a cover slip was placed and left for 5 – 10 min. The cover slip was removed, placed on a glass slide and examined by light microscopy. The entire cover slip area was examined using a 10× and 40× objective.

Results and discussion

A total of 78 fecal samples were collected from dogs of Tetovo region of Macedonia. The dogs taken in study didn't show any visible clinical signs. After performing the flotation-centrifugation method in all feces resulted that 56 of them (71%) were positive. Precisely the parasite *A.caninum* was found in 41% of positive samples; *T.vulpis* in 39,2%; *T.canis* in 17,8% and the parasite *A. vasorum* was found in only 1 sample (1,7%).

Table 1. Parasites found, percentage and Confidence Interval of the samples

Variable	Infectedn (%)	95% CL
Ancylostoma caninum	23 (41%)	0,411
Trichuris vulpis	22 (39,2%)	0,293
Toxocara canis	10 (17,8%)	0,179
Angiostrongylus vasorum	1 (1,7%)	0,18
Total Examined (78)	56 (71%)	0,718

The Confidence Interval – Likelihood ratio was used to capture the magnitude of abnormality of test results. A likelihood ratio is defined as the probability of a given level of a test result in those with disease divided by the probability of that same result in those without the disease (Fletcher RH,1996). From the dog taken in survey 43 of them were male and 35 female. The results showed that the sex didn't play any significant role in parasite infection. The male dogs infected were 29 (51,7%) and the infected females 27 (48,2%).

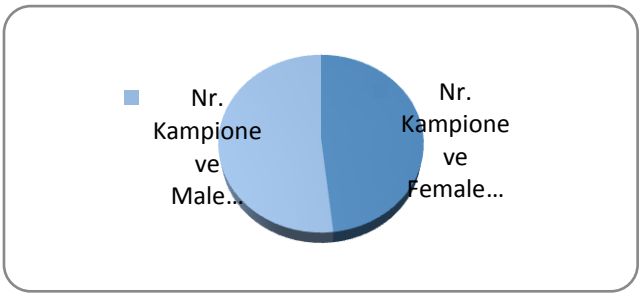

Figure 1 The infection differences between male and females dogs

Risk factors reported to be associated with endoparasitism in dogs include age, sex, reproductive status, median household income, breed size, population, and geographic location (Little SE, 2009). Considering the analysis of the results obtained in dogs, age was the strongest predictor of intestinal parasite infection, being younger than 12 months is a risk factor for infection. Our study showed that

from 48 dogs ≤12 months and 30 dogs >12 months the most infected dogs belonged to the first group, exactly 33 dogs were ≤12 months and 23 >12 months year old.

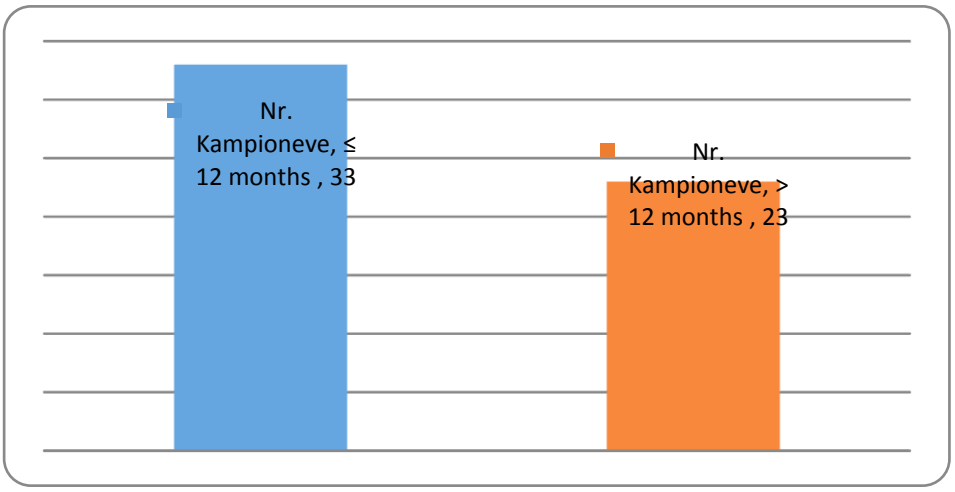

Figure 2. The infection differences between dog ages.

It is a known fact that dog domestication has played an active role for the spreading of different zoonotic parasites, especially in developing countries (Salb AL, 2008). The dogs taken in study live near people so they represent a risk for spreading zoonotic diseases. Among the recovered parasites species *A. caninum* which accounted the most frequent, in 41% of the infected dogs is considered with public health significance as important zoonose (C.N.L. Macpherson,2013). Other parasite diffusing zoonose of minor importance such as *T. vulpis* was found in 39,2% of positive samples. The concurrent infection with two or more parasite species was very common in some samples investigated during this study. The coinfection was seen between *A.caninum* and *T.vulpis* in 8 samples. Ascarid as *Toxocara spp*., was one of the most prevalent parasite. The presence of multiple infected pets brings the environmental contamination with infective stages of these taxa. With very interest was the finding of the metastrongyloid nematode *Angiostrongylus vasorum* in one the tested samples. This is a parasite of the heart and pulmonary circulation of dogs and foxes. Infection can cause a wide range of disease outcomes, which are most often characterized by respiratory dysfunction, but can also manifest as bleeding, neurological, cardiovascular or gastrointestinal disorders, with or without respiratory involvement (E. Morgan, 2010).

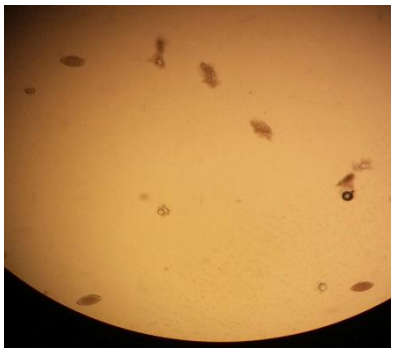

Figure. 3 Trichuris vulpis (40X)

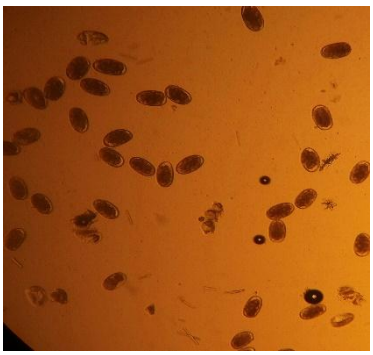

Figure 4. Ancylostoma caninum (40X)

Figure 5. Coinfection *Trichuris vulpis* and *Ancylostoma caninum (40X)*

In conclusion, this study showed a high overall prevalence of intestinal parasites, precisely 0,71% of the dogs resulted positive. The high percentage of infection is probably due the movements of the sheepherd dogs that are always on their walks and not regularly treatment with anthelmetic medication. Another risk factor for infection is the colonies of stray dogs that exist in this area that serve as source of infection. According the literature the cohabitation with other dogs is one of the most important risk factors associated to endoparasitism (S. Katagiri, 2008). The presence of important zoonotic endoparasites should raise the attention of taking preventive and therapeutic measures routinely all year round for the sheepherd dogs.

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AGRI-ECOLOGICAL ZONING OF MUNICIPALITIES IN THE KYUSTENDIL REGION

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Abstract

It has been done agroecological zoning of nine municipalities in Kyustendil area - Bobovdol, Boboshevo, Kocherinovo, Kyustendil, Nevestino, Rila, Sapareva bania, Dupnitza and Treklyano. Agro-ecological zoning is done based on certain environmental characteristics, basic of which are: prevailing soil type; an extreme manifestation of certain climatic factors; altitude; relief features and other. In the presented paper were analyzed and described: weather conditions; soil resources; agronomic suitability of land for organic growing of suitable for the region crops. There were considered the potential threats of soil erosion, acidification and compaction. It was prepared land evaluation regarding the potential for growing grape and fruit, vegetable crops, meadows and pastures. It was found that the areas with altitude above 800 m are unsuitable for cultivation with fruit crops, which can be planted in areas with an altitude of 1800 m. Vegetable crops are not suitable to be cultivated in areas with altitude above 800 m are with the exception of late potatoes which may be planted in places with an altitude of 1800 m. It was found that the areas with altitude above 800 m are unsuitable for growing vines. The territories with altitude above 2400m are unusable for grassland.

Keywords: weather conditions; soil resources, agronomic suitability, land evaluation.

Intrduction

Agricultural practices are certainly one of the most important factors in the evolution of mankind. Since ancient times, for centuries and millennia, they have defined its social status (political, demographic, economic, etc.). There are no known civilizations without agricultural land, and those that have destroyed its productivity - have died.The natural conditions and resources of the Republic of Bulgaria (soil, climate, water, biota, relief, etc.) give it the advantage of being a developed country. For a long time, this advantage has been used actively, experience has been gaining, our country has even been a center of agricultural know-how. The two main conditions for successful agricultural development are scientific high-level service and a good agricultural policy. Kyustendil region has a centuries-old tradition in agricultural production. Much of the agricultural land in the area is occupied by orchards (mainly cherry and apple plantations) (Annual Report on the Status and Development of Agriculture, 2016). The area is located in Southwestern Bulgaria, with an area of 2.7% of the country's territory. It borders the regions of Sofia, Pernik and Blagoevgrad, and to the west - with the Republic of Macedonia and Serbia. The administrative, business and cultural center is the district of Kyustendil. Kyustendil district comprises 9 municipalities - Kyustendil, Dupnitsa, Bobov dol, Sapareva Banya, Rila, Kocherinovo, Nevestino, Boboshevo and Treklyano with total number of settlements 182. This report presents the geographical distribution of major agro-ecological characteristics (soil, climatic and their combinations) within the boundaries of Kyustendil region. They are the result of a large set of data resulting from years of observation and research.

Material and methods

Agroecological zoning in the municipalities of Kyustendil - Bobov Dol, Boboshevo, Kocherinovo, Kyustendil, Nevestino, Rila, Sapareva banya, Dupnitsa and Treklyano was made based on certain environmental characteristics, basic of which are: prevailing soil type; availability of moisture and

warmth in the area (vegetation period); extreme manifestation of certain climatic factors; altitude; relief features. For the purpose of the project the card for agri-environmental areas in Bulgaria was used, which is compiled from aggregated soil and climate information and is 1: 600,000 (Jolevski et al., 1980). Across the country there are 50 separated agri-environment area, of which 40 cover the agricultural fund, and 10 the forest one. Map of agri-environmental areas is digitized using advanced software programs and it allows the use of available digital information, adding new and visualization through the map material. The electronic map of Agri-environment areas (georeferenced) is imposed on municipal boundaries. The soil, climate and agro-climatic data was processed by GIS in order to spatially delineate the most homogeneous regions. In each municipality there are formed from 2 to 3 and more areas. As noted above they are separated based on differences in soil and agro-climatic conditions. Information on soils is represented by "soil map of Bulgaria" in scale 1: 200 000. Climatic conditions are described, taking into account the most important indicators-air temperatures and rainfall. Data is geathered from weather and rain gauges from the study area. The mentioned municfipalities are part of the transitional continental climatic sub-region (Sabev, L. 1959). In this sub-region, which lies entirely south of the Balkan Mountains, winter is markedly softer than in temperate continental sub-region, and the summer is generally hot. The rainfall regime also has continental character - with summer maximum and winter minimum. However, the difference between summer and winter rainfall is very low - averaging about 6-8% of their annual amount as in the southern parts of the subregion practically disappears. Moreover many local minima of precipitation already moved from the winter months - to August or September. All these features, together with a relatively mild winter and low snowfalls shows that the climate of this subregion as a transition between moderate continental North Bulgaria and mild climate of our most southern areas. For the differentiation of the climatic conditions in these areas play a special role primarily physiographic conditions in different parts of the transitional continental climatic zone.

Table 1. Average monthly and annual air temperature in ^{0}C

Station	Months												Av. annual t
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
Donitz	-0,9	1,3	4,7	10,6	15,3	18,8	21,0	20,5	16,7	11,4	6,5	1,5	10,6
Kiustendil	-0,8	1,7	5,7	11,4	16,0	19,5	21,8	21,5	17,4	11,8	6,7	1,7	11,2
Nikolichevtzi	-1,3	1,1	5,1	10,7	15,1	18,7	20,9	20,5	16,3	10,8	6,0	1,1	10,4
Rila	0,1	2,4	6,1	11,6	16,2	19,7	22,2	21,9	18,1	12,4	7,3	2,3	11,7

Table 1 shows average monthly and annual air temperature in certain places in the Kyustendil region. In the plain parts the lowest winter temperatures are in January in the range of 1,3 to 0,1 ^{0}C ^{0}C, and the highest summer temperatures are in the range of 20.5 to 22.2 ^{0}C. In the higher mountain areas, average temperatures in January reach from minus 110 to minus 7 ^{0}C (Kyuchukova, M. et al. 1983).

Table 2. Average monthly minimum air temperature in ^{0}C

Station	Months											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Dupnitza	-14,4	-11,8	-7,4	-2,0	3,3	7,2	8,9	7,5	3,2	-1,1	-4,8	-11,3
Kiustendil	-13,8	-11,7	-7,5	-1,0	3,9	7,9	9,6	9,0	4,3	-0,6	-5,1	-10,9
Nikolichevtzi	-15,8	-13,2	-8,3	-2,9	2,6	6,4	7,8	6,6	2,2	-2,5	-6,1	-11,4
Rila	-12,7	-10,7	-7,0	-1,2	4,1	8,2	9,8	9,1	4,7	-0,1	-4,5	-10,3
Rila monastery	-15,9	-14,3	-11,8	-5,3	-0,5	3,0	4,5	3,8	0,2	-3,1	-7,3	-11,8
Osogovo rest house	-16,3	-15,3	-13,6	-8,0	-1,2	2,0	4,5	4,1	0,9	-4,3	-9,0	-13,7

Minimum air temperature occurs most frequently in the hours before sunrise, when the radiation cooling of the underling surface is strongest (Table 2). The average monthly minimum temperature gives an indication of the average range (in a month), between which oscillates the air temperature during the day.

Table 3. Average monthly maximum air temperature in ^{0}C

Station	Months											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Dupnitza	3,1	6,2	10,5	16,7	21,5	24,9	27,6	27,8	24,1	18,1	11,3	5,3
Kiustendil	3,4	6,6	11,0	17,5	22,2	25,4	28,5	28,1	24,9	18,5	11,3	5,4
Nikolichevtzi	3,4	6,5	10,8	17,2	21,9	25,6	28,2	27,8	24,6	18,4	11,5	5,6
Rila	4,3	7,3	11,7	17,7	22,5	26,0	28,7	28,8	25,3	19,1	12,3	6,5
Rila monastery	1,4	3,3	7,3	12,7	17,2	20,5	23,1	23,8	19,9	14,8	9,2	3,2
Osogovo rest house	-1,0	0,4	2,6	7,0	12,1	15,5	18,0	18,6	15,1	10,6	6,7	1,5

The data on the average monthly maximum air temperature were obtained from the hour maximum temperatures averaged over a sufficiently long period of time. The maximum temperature of the air is influenced to a lesser extent on the shape of the relief than the average hour temperature as it normally occurs in the afternoon (13-15 hours) when turbulent air exchange is the highest (Table 3) (Koleva, E., Peneva, R. 1990).

Table 4. Average monthly and annual rainfall (mm/m^2)

Station	Months												Annual amount
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
Dupnitza	45	38	37	61	69	79	52	40	38	56	61	51	627
Kiustendil	48	45	42	52	68	65	54	36	38	59	62	55	624
Rila	43	38	38	56	62	69	41	38	36	55	62	56	594
Vaksevo	52	47	45	58	69	72	50	39	38	62	68	62	662
Kocherinovo	45	38	36	52	56	59	41	35	33	55	62	52	563
Osogovo rest house	74	65	57	68	92	83	58	45	48	81	90	87	848

Rainfall in Kyustendil region during the year are characterized by a pronounced spring and autumn peak (May and June, November and December) and winter and summer minimum. The total annual rainfall is smallest in Kocherinovo (563 mm/m^2), and the highest one is for the mountainous parts of the regions (848 mm/m^2). The amount of rainfall by seasons is presented in Table 4. In the lower, plains areas, soil moisture is inadequate, especially during the critical summer period (July and August), which adversely affects the crop. Drought is observed in the month September.

Table 5. Average monthly and annual relative humidity (%)

Station	Months												Annual amount
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
Dupnitza	79	75	69	61	64	63	57	57	59	69	77	81	68
Kiustendil	82	78	70	63	64	63	58	56	63	72	80	83	69
Rila	80	75	69	63	63	62	57	56	60	69	77	81	68
Nikolichevtzi	82	76	69	64	67	67	62	60	68	75	79	82	71
Osogovo rest house	83	80	80	75	74	75	71	67	71	74	82	83	76

On the table are presented on a monthly and annual average daily relative humidity. The relative humidity is characterized by high stability of the monthly average values. This feature is more stable in winter compared to summer (Table 5) (Kyuchukova, M. et al. 1979).

Table 6. Duration (days) and temperature sum of the periods of detention sustainable temperatures > 0 ^{0}C, > 5 ^{0}C,>1 0 ^{0}C, >15 ^{0}C

Station	Duration (days)				Temperature sum (C x days)			
	0 ^{0}C	5 ^{0}C	10 ^{0}C	15 ^{0}C	0 ^{0}C	5 ^{0}C	10 ^{0}C	15 ^{0}C
Dupnitza	340	251	194	133	3905	3710	3285	2520
Kiustendil	346	259	201	140	4110	3930	3500	2730
Nikolichevtzi	333	252	191	129	3815	3705	3200	2385
Rila	365	266	216	146	4270	4070	3710	2880
Rila monastery	286	213	141	39	2630	2455	1925	615

Temperature amounts and periods indicated are derived by collecting temperature amounts for the respective months. The indicators are used in the agro-climatic assessment of certain territory and to what extend they can ensure the ripening of the crops. As the 10 ^{0}C spring is an indication of the occurrence of the favorable moment of sowing of the thermophilic spring crops (maize, etc.). During this time begins the development of the vine. Lasting shift in temperature over 15 ^{0}C spring is usually taken as the end of spring and early summer. Temperature sums (50, 100 and 15 ^{0}C) serve to evaluate the conditions for the ripening of various crops. Zoning in temperature conditions lands of municipalities located in Kyustendilska and Dupnitsa Plain fall in moderately warm sub-belt. In hilly parts to semi temperature amounts above 10 ^{0}C are 1925 ^{0}C and divided by temperature conditions they fall to moderately warm to moderately cool sub-belt. In such thermal conditions have capabilities for raising a thermophilic cultures (mostly early). In high temperature areas amounts above 10 ^{0}C are 1235 ^{0}C and divided by temperature conditions they fall into the cool climate region (Kyuchukova, M. et al. 1983). Zoning in terms of land humidity municipalities located in Kyustendil and Dupnitsa Plain fall into the "dry zone", the difference between money and precipitation (mm) for the period from June to August showed a deficit of minus 200-300 humidity and less what allows up to 90% for the cultivation and maturation of crops, but also the need for irrigation. In the hilly parts and semi hilly the humidity conditions are defined as "weak drought" as the difference between the vapor and precipitation (mm) for the period from June to August showed a deficit of humidity minus 100-200 mm. In alpine regions the conditions of humidity are defined as "wet" as the difference between the vapor and precipitation for the period from June to August showed a deficit of humidity less than 100 mm and less (Hershkovich, E.1970, 1984).

Results and discussion

Agri-ecological zoning of municipalities in the Kyustendil region

1. Agroecological zoning in municipality Bobov dol (Table 8)

The municipality is divided into two distinct agro-ecological regions - Sofia-Pernik (IV_1) and Kyustendil (IV_2). In Sofia-Pernishki area (IV_1) most widely spread are Hromic Luvisols, Haplic Vertisols, Fluvisols and Leptic Hromic Cambisols + Rendzic Leptosols. Hromic Luvisols which have weakly potent /20-25 cm/ humus layer, a powerful profile /100-220 cm/, medium to heavy sandy clay mechanical composition and weakly acidic media reaction /pH in H_2O ranges from 5.4 to 6.6/. Some of these soils are affected by erosion. Haplic Vertisols are distinguished by a large capacity of the humus layer /70-75 cm/ and profile /180 cm/, heavy mechanical composition. The content of organic matter in the surface horizon is about 3%. The soils are characterized with neutral to weakly alkaline media reaction /pH 7.0-7.5 in H_2O/. Fluvisols are distributed mainly along the Struma River and its tributaries. It is characterized by a fluctuation in the range of their individual performance, but are mainly characterized by the average power of the humus layer /30-40 cm/ and the power of the profile - 80 cm, average sandy clay mechanical composition, slight to moderate availability of organic matter /1.0 - 3.0% of humus/ and a weakly acidic to alkaline media reaction /pH 5.5-8.0 in

H_2O/. In the region there are also Leptic Hromic Cambisols + Rendzic Leptosols, formed on the non-carbonate material. They are characterized by low power humus layer / 20-22 cm / and soil profile /40-50 cm/, mild to moderate sandy clay mechanical composition and low availability of organic matter /1.0-1.5% humus/. The reaction media reaction is slightly acid /pH 5.5-6.5 in H_2O/. These soils are not suitable for growing organic production. Leptic Cambisol, distributed in the area, have a low power humus layer /about 20 cm/ and profile in the range of 60-70 cm. They are characterized by a soft, skeletal mechanical composition, low humus content /1-2% humus/ and acidic media reaction /pH at about 5.0 in H_2O/. In Kyustendil region (IV_2), the soil cover is composed of different soil types, which are analogous to those described for the Sofia-Pernishki area. It is worth noting here that Haplic Vertisol occupy a smaller area while Hromic Luvisols are more prevalent. Haplic Vertisol are distinguished by a small power of the humus layer, lighter skeletal mechanical composition, a low humus content and a larger area occupied by eroded lands. Rendzic Leptosols are poor in organic matter and in skeletal.

2. Agroecological zoning Municipality Boboshevo (Table 8)
There are three distinct regions in the municipality - Kyustendil (IV_2), Rila-Pirin (VI_6) and High Rila-Pirin (VII_2). Region Kyustendil (IV_2), has already been described above. Rila-Pirin region (VI_6) covers the higher parts of the Rila and Pirin mountains - from 800 to 2000 m altitude. The relief is with alpine character. Most common in the region are Leptic Cambisol. They are characterized by low power humus layer /10-15 cm/ and profile /50-60 cm/, slight mechanical composition, low to medium availability of humus /2.0-3.0% humus/ and acidic media reaction /pH in H_2O - 5.3/. Most of these soils are subject to erosion. High Rila-Pirin region (VII_2) comprises the highest treeless parts of Rila and Pirin mountains - over 1700-1800 m altitude. The relief is alpine. In specific environmental conditions here are formed Umric Leptosols or Leptic-humic Umrisols. They are characterized by powerful /40-50 cm/ humus horizon and moderately profile /55-90 cm/, light, rock mechanical composition, very rich in organic matter /12-23% humus/ and acidic media reaction /pH in H_2O - 5.5/. There is also erosion.

3. Agroecological zoning in the municipality of Kocherinovo (Table 8 and Figure 2)
The municipality is divided into two separate areas - Kyustendil (IV_2), Rila-Pirin (VI_6), already described.

4. Agroecological zoning in the municipality of Kyustendil (Table 8)
The municipality is divided into four regions - Sofia-Pernik (IV_1), Kyustendil (IV_2), Tran-Osogovski (VI_3) and High-Osogovski (VII_3)._Sofia-Pernik region covers the territory between Milevska, Rila and Malashevska mountains. The landscape is as mountainous as well as valleys and determines the manifestation of erosion. The soil is comprised of similar differences properties as previously described neighboring Agroecological region IV_1. However, unlike here Haplic Vertisol occupies a smaller area while Hromic Luvisols are more prevalent. The latter are characterized by a smaller power of the individual horizons, lighter and skeletal mechanical composition, a low humus content and a larger area of the eroded land. Tran-Osogovski area covers the highlands of Western mountain suburbs: Ossogovo Vlahina, Malashevska. The terrain is rugged, soil forming materials are presented mainly by crystalline schists and very few granites, sandstones and others. The soil is occupied by Leptic Cambisol. They are characterized by low power humus layer /10-15 cm/ and profile /50-60 cm/, lighter mechanical composition, slight to moderate humus content /2-3%/ and acidic media reaction /pH in H_2O - 5.3/. High-Osogovski area takes the treeless alpine areas in Kyustendil over 1700-1800 m. The relief is alpine. In this specific environmental conditions here are formed Umric Leptosols or Leptic-humic Umrisols. They are characterized by powerful /40-50 cm/ humus horizon and moderately profile /55-90 cm/, light, rock mechanical composition, very rich in organic matter /12-23% humus/ and acidic media reaction /pH in H_2O - 5.5/. There is erosion processes.

5. Agroecological zoning Municipality Nevestino (Table 8)
The municipality is divided into two distinct agro-ecological regions: Kyustendil (IV_2) and Tran-Osogovski (VI_3) - already described.

6. Agroecological zoning in the municipality of Rila (Table 8)
The municipality is divided into three agro-ecological regions: Kyustendil (IV_2), Rila-Pirin (VI_6) and High Rila-Pirin (VII_2) - already described.

7. Agroecological zoning in Sapareva bathroom (Table 8)
The municipality is divided into three agro-ecological regions: Kyustendil (IV_2), Samokovsko-Srednogorski (VI_5) and Rila-Pirin (VII_2). Soils in Kyustendil region and Rila-Pirin region are already described above. The relief of the territory of Samokovsko-Srednogorski area is mountainous. The altitude is from 630 to 1400 m. The most widely spread are Leptic Cambisol. They are characterized by low power humus layer /10-15 cm/ and profile /50-60 cm/, lighter mechanical composition, slight to moderate availability of humus /2.0-3.0%/ and acidic media reaction /pH in H_2O - 5.3/. Most of these soils are subject to erosion.mAt the foot of the slopes, on the talus cone, there are wide areas occupied by Dystric-sceletic + Eutric-scletic Fluvisols. They are characterized by moderately humus layer /50 cm/, deep profile /120 cm/, stoned mechanical composition, low humus content /1-2%/ and a weakly acidic to neutral media reaction /pH is from 6.5 to 7.5/. In this region widely spread Leptic-cromic Luvisols.

8. Agroecological zoning in the municipality of Dupnitsa (Table 8 and)
The municipality has five agro-ecological regions: Sofia-Pernik (IV_1), Kyustendil (IV_2), Samokovsko-Srednogorski (VI_5), Rila-Pirin (VII_2) and High Rila-Pirin (VII_2) - already described.

9. Agroecological zoning in ObshtinaTreklyano (Table 8 and)
The municipality has three agro-ecological regions: Sofia-Pernik (IV_1), Kyustendil (IV_2) and Tran-Osogovski (VI_3) - already described.

Potential threat for soil erosion
The predominant relief of Kyustendil region is mountainous and valleys, heavily indented in the northern and western parts. More than 1/5 of the lands in the area (22%) are located at more than 1200 m above sea level. Most of those lands are in the municipalities of Rila and Sapareva bathroom - respectively 69 and 47% of their area and in Treklyano, Dupnitsa and Kyustendil occupy 15 to 23 percent. Most of the remaining land in Kyustendil have a slope above 150. Their involvement has been greatest in municipalities Kyustendil Kocherinovo Boboshevo Nevestino and Treklyano (37, 39, 41, 47 and 51% of the surface), 17-20% in Bobovdol, Rila and Sapareva bath and 8% Dupnitsa Municipality. The proportion of land with a slope of 3-6 and 6-90, which are the main part of agricultural land is only 17% (at 28% of the country). Their involvement is greater in municipalities Dupnitza Bobovdol (31 and 51%), from 16 to 22% in Kyustendil Treklyano, Nevestino Kocherinovo and Boboshevo and 4-9% in Rila and Sapareva bath. The total area of arable land with varying degrees of erosion risk in Kyustendil 90 995.1 ha. 19 out of these 101.4 ha (21%) are arable land with very low erosion risk and land at an altitude of more than 1 200m which are not going to have anty erosion treatment. On the remaining 79% (71 893.6 ha) of arable land to permanent use of land - fields, permanent crops (orchards and vineyards) and pastures are recommended basic protivoerozinni practices depending on the degree of erosion risk, which should limit the prevention of erosion to acceptable limits. This can be achieved by applying the soil conservation crop rotations (crop rotation by a fused surface and row crops), establishment of grass buffer strips (in the fields of row crops and areas with perennials) and others. Terracing as erosion control practices recommended for arable land with high erosion risk. Paving run-off holding furrows in pastures and implementation for improvement activities such as cleaning of stones and shrubs, planting, fertilizing, controlled grazing, etc. (Ruseva Sv. L. Lozanova, D. Nekova and others. 2010).

Table 8. Agroecological zoning in municipalities from Kyustendilska area

Municipality	Agro-ecological area	№ /index/ of AER
Bobov dol	Sofia-Pernik Kyustendil	IV_1 IV_2
BOBOSHEVO	Sofia-Pernik Kyustendil	IV_1 IV_2
Kocherinovo	Kyustendil Rila-Pirin	IV_2 VI_6
Kyustendil	Sofia-Pernik Kyustendil Tran-Osogovski High-Osogovski	IV_1 IV_2 VI_3 VII_3
Nevestino	Kyustendil Tran-Osogovski	IV_2 VI_3
Rila	Kyustendil Rila-Pirin High-Rila-Pirin	IV_2 VI_6 VII_2
Sapareva Banya	Kyustendil Samokovsko-Srednogorski Rila-Pirin	IV_2 VI_5 VI_6
Dupnitsa	Kyustendil Samokovsko-Srednogorski Rila-Prin High-Rila-Pirin	IV_2 VI_5 VI_6 VII_2
Treklyano	Sofia-Pernik Kyustendil Tran-Osogovski	IV_1 IV_2 VI_3

Potential threat from acidification

Acidic soils are referred to soil with a chemical imbalance because they contain excessive amounts of exchangeable hydrogen, aluminum, iron and manganese which are toxic to plants and are one of the reasons for the low structural stability of these soils. Particularly vulnerable are Leptic-albic Luvisols, Leptic Cambisols, Leptic Cambisols or Humic Leptosols, Umbric Leptosols or Leptic-humic Umbrisosls, distributed in Kocherinovo. The possible measure to prevent such processes are: introduction of finely ground lime materials (lime) for neutralizing toxic to plants acidity.

Potential threat from soil compaction

Under the conditions of modern intensive farming soil density is exposed to intense impact and change under the influence of agricultural machinery and vehicles who repeatedly go on the soil surface in the growing and harvesting of crops. To secondary soil compaction leads especially when working at high soil moisture, especially if the soil has heavier mechanical composition. To prevent these processes there is needed a proper selection of agricultural machinery and the use of new technologies, in which the number of operations decreases (minimum tillage) or to the soil generally not processed (zero tillage); applying a profiling autumn plowing depth 30-35 cm reverse layers and similar. Such soils in the region are: Haplic Vertisols, Hromic Luvisols and Leptic-albic Luvisols (Atanas Atanasov at al., 2014).

Conclusions

It was prepared spatial distribution of groups of crops by municipalities in Kyustendil according to the procedure of FAO. The attached map material shows the suitability for organic farming of vineyards and fruit orchards, vegetable crops and grassland /Figure 1/. It was found that the areas with altitude above 800 m are unsuitable for cultivation with fruit crops, which can be planted in areas with an altitude of 1800 m. Vegetable crops are not suitable to be cultivated in areas with

altitude above 800 m are with the exception of late potatoes which may be planted in places with an altitude of 1800 m. It was found that the areas with altitude above 800 m are unsuitable for growing vines. The territories with altitude above 2400m are unusable for grassland.

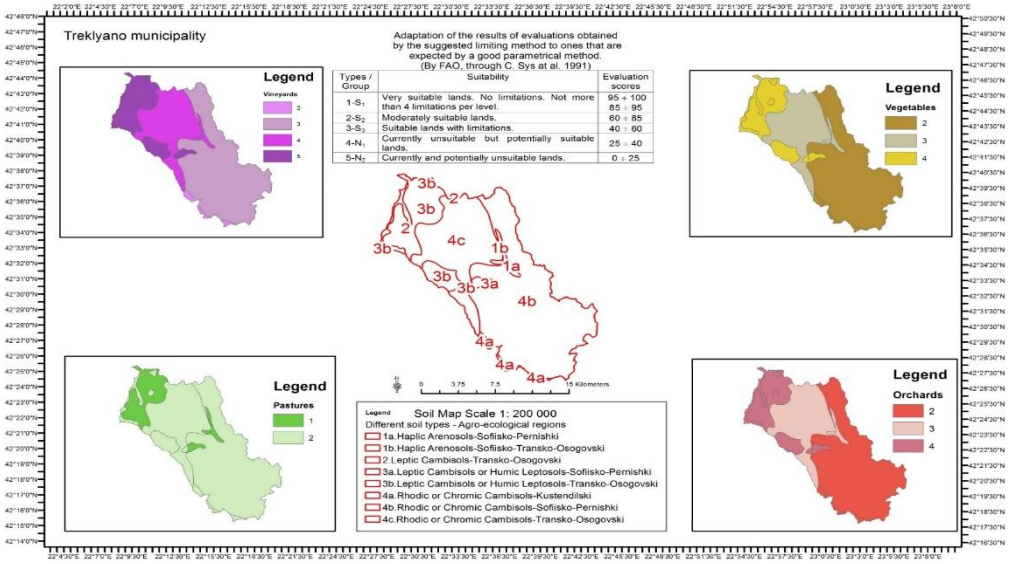

Figure 1. Soil map and suitability by crop groups in Treklyano municipality

In the consideration municipalities, the suitability of land for cultivation of wheat are in a wide range - land evaluation are in the range 30-75 marks, corn - 25-55 marks, tomato - 20-75 marks, pepper - 40-80 marks, cabbage - 30-80 marks, cucumber - 40-80 marks, apples - 30-80 marks, cherries - 40-80 marks, cherries - 40-80 marks, potato - 60-90 marke, grassland and pastures - 65-80 marks, hazelnuts - 40-80 marks, raspberries - 60-80 marks and vineyards - 0-80 marks). Lower quality assessment ratings are the result of the presence of shallow and eroded soils, acid or gravel which acts negatively on the crop. Soil from the high part, are suitable, but for a limited number of crops (hazelnuts, potatoes cultivated blueberry, strawberry, raspberry, herbal plants cultivated rose hip, etc.) (Petrov E., at al. 1988; Maps of agro-climatic areas in Bulgaria and coefficients of suitability for different crops, M 1: 400000, 1986). Soil resources in the municipalities of Bobov dol, Boboshevo, Kocherinovo, Kyustendil, Nevestino, Rila, Sapareva bathroom, Dupnitsa and Treklyano are varied. In the regions there is a deep, fertile soils and shallow-land unsuitable for agriculture. The great wealth in these communities have thermal resources, water resources (irrigation possibilities) and purity of nature. In municipalities of Kyustendil, Nevestino, Boboshevo, Kocherinovo are present light alluvial soils, which are very suitable for vegetables and in particular for fruit trees. In these regions there is a specific microclimate that allows the development of fruit (apples, cherries, pears, plums, raspberries) and obtaining products with unique taste. In secured irrigation, even small areas can be very effective for the owners. Environmental conditions in the region are suitable for organic farming, as there is no pollution and large industrial enterprises.

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INFLUENCE OF PRECIPITATION UPON DRAINAGE DISCHARGE IN TWO DIFFERENT CLIMATIC REGIONS

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Abstract
The goal of three-year investigations was to determine the influence of precipitation upon the drainage discharge in two different climatic regions (Croatia and Lithuania) at two different pipe drainage spacing in each region (first region with 15 m and 20 m drainage spacing and second region with 12 m and 24 m drainage spacing), to calculate the soil water balance according Thornthwaite's method and compare the measured drainage discharge and the calculated surplus of water in soil. Investigations were carried out at the experimental amelioration sites in the central Sava River Valley (Croatia) on hydroameliorated Gleyic Podzoluvisol soil and in Middle part of Lithuania on hydroameliorated Hypogleyic Luvisol soil in the period 2009 - 2011. The research results showed that the drainage discharge and its duration depended on the amount and distribution of the precipitation during the study period. There isn't difference in the total drainage discharge between the tested drainpipe spacing in each investigation year, but there are differences in the duration of the drainage discharge both on an annual scale and depending on drainpipe spacing. In each year, the duration of drainage discharge was smaller at the 12-15 m drainpipe spacing than at the 18-20 m drainpipe spacing. The calculated surplus of water followed the monthly amounts of precipitation, but in all years was higher than the drainage discharge. The 12 to 15 m pipe spacing is more efficient for draining the surplus water from drained soils, since the surplus of water from soil is drained in a shorter period of time and better water-air relationships in soil are created faster, which is a prerequisite for timely application of agricultural management practices on hydroameliorated arable areas.

Keywords: experimental amelioration site, water balance, Thornthwaite's method, surplus soil water.

Introduction

The effects of climate change have become increasingly apparent over the past decades (Patt and Schröter, 2008). The average temperature was increased by 1.1-1.3 °C in 100 years in Central Europe (Kutilek, Nielsen, 2010). The climate change projections suggest a more variable climate with higher vulnerabilities in the lower income countries (Easterling et al., 2000). Global increase of precipitation is forecasted under changing climatic conditions; however, its extremes will also increase **(**Climate Change, 2007). The changes of climatic elements, influencing the discharge – temperature and precipitation - have already been recorded in Lithuania (Bukantis and Rimkus 2005). In case of low temperature and low moisture, assimilation of nutrients goes on much worse; therefore, they are leached from the soil with the drainage discharge more intensely (Soussana, Luscher, 2006). The drainage discharge has the tendency to increase in winter and to decrease in spring in Lithuania (Miseckaite, 2010). Global climate change and the associated impacts on water resources are the most urgent challenges facing mankind today and will have enduring societal implications for generations to come. Potential impacts may include the changes in watershed hydrologic processes including timing and magnitude of surface discharge, stream discharge, evapotranspiration, and flood events, all of which would influence other environmental variables such as nutrient and

sediment flux on water sources (Simonovic and Li, 2004; Zhang et al., 2005). Agricultural production is very risky on such developed/undeveloped agricultural areas, especially when surplus and/or deficit of precipitation occurs before or during the growing season. Such conditions make production planning very difficult and/or almost impossible, because production, and there by yield, depends on the weather conditions, making yields of field crops and their quality highly variable. If soil water surplus persist during a longer period and is in the zone of plant roots in a part of the growing period, then the hydroamelioration measure of drainage should be applied. Drainage of surplus water is an ameliorative procedure that involves collection and removal of surplus water from soils intended for cropping or some other activity (Šimunić, 2016). The goal of three-year investigations was:

- determine the influence of precipitation upon drainage discharge in two different climatic regions at different pipe drainage spacing;
- calculate soil water balance according Thornthwaite's method;
- compare measured drainage discharge and calculated surplus of water in soil.

Material and methods

Investigations were conducted during three years (2009 - 2011) at the experimental amelioration site in the central Sava River Valley, Croatia (45°34'6 N, 16°37'17 E) on hydroameliorated Gleyic Podzoluvisol soil (Object No. 1) and the site under study is located in the Middle part of Lithuania (54°52'46 N, 23°51'30 E, Object No. 2 (Table 1).

Table 1. Main technical characteristics of experimental amelioration sites

	Area of plots	Pipe spacings	Average depth	Average slope	Pipe length	Pipe diameter
Croatia (Object No. 1)	1425 m^2 1900 m^2	15 m, 20 m	1.0 m	3 $^0/_{00}$	95 m	65 mm
Lithuania (Object No. 2)	4400 m^2 4400 m^2	12 m, 18 m	1.1 m	8 $^0/_{00}$	75 m 80 m	65 mm

On Object No.1, in the drain ditch above the drainage pipes is installed hydraulic material gravel up to plough layer and drain directly into an open detailed canal. Climatic data used were provided by the weather station at Sisak, at 15 km distance from the experimental amelioration site. As per textural composition, hydroameliorated Gleyic Podzoluvisol soil is silty clay to 75 cm depth, silty clay loam at 75 cm to 115 cm depth, and silty clay from 115 cm to 130 cm. It belongs to the class of porous soils (average porosity 49 vol.%), on the borderline between medium and high water retention capacity (average water capacity 45 vol.%) and of very low air retention capacity (average air capacity 4 vol.%), as well as of very low water permeability (average water permeability 0.011 m/day). According to the MKCl (Molar potassium chloride) reaction to 75 cm depth, the soil has acid reaction; according to humus content to 35 cm depth, it is fairly humus-rich; according to the supply of available phosphorus and potassium (to 35 cm depth), it is in the poor availability class. Object No. 2. The test site soil sod podzolic (the experimental according to FAO: calcar - Hypogleyic Luvisol), texture - light loam, dripping down on medium loam. Topsoil layer thickness is 0.2 to 0.25 m. Arable layer (0-20-25 cm) of filtration rate - 1.0 to 2.0 m / day, the lower layers of soil - from 0.01 to 0.004 m / day. Drainage discharge in each pipe spacing variant was continuously measured with the aid of a limnigraph, installed at the pipe outlet to the canal. Soil water balance is calculated according Thornthwaite's method, software USGS Thornthwaite Water Balance Model, Version 1.1.0 - April 26, 2010 (USA). The Thornthwaite method is based on the fact that water infoltation into soil, water loss from soil and water storage in soil are dependent on soil characteristics (Šimunić, 2016). The following data was used to calculate soil water balance: monthly evapotranspiration potential (mm), monthly amount of precipitation (mm) and presumption that water storage in soil (root zone depth) is 100 mm at the beginning of the year (January). To facilitate interpretation of research results, site factors (soil) and climate (precipitation and temperature) were taken into consideration.

Results and discussion

According to its general climatic characteristics, the Sisak region (Object No. 1) belongs to the central-European temperate climate, warm climate zone, moderately rainy climate with expressly continental traits. In the twenty-year period 1986 - 2005, an average of 925 mm of precipitation fell in the Sisak region, which fluctuated from 614.8 mm to 1086.9 mm (Fig.1). The growing seasons had 523.4 mm or 56.6 %, which is a characteristic of the continental precipitation regime. Monthly precipitation maxima were recorded in late spring and late summer parts of the year (June and September). The multi-year average and distribution of precipitation over the year allow the conclusion that the precipitation regime is favourable for agricultural production. Based on the multi-year precipitation average, the Sisak region is on the borderline between semi-humid and humid climate (K_f = 81.9, K_f - Rain factor: the ratio between total annual rainfall (mm) and average annual air temperature (°C)), pursuant to Lang's rain factor, whereas pursuant to the monthly heat index, the region belongs to moderately warm climate (t = 11.3°C) (Šimunić et al, 2013). In the twenty-year period 1986 - 2016, an average of 646.3 mm of precipitation fell in the Kaunas region (object No. 2), which fluctuated from 437.2 mm to847.0 mm (Fig.1). The growing seasons had about 374.5 mm or 57.9 %, monthly precipitation maxima were recorded in June and August.

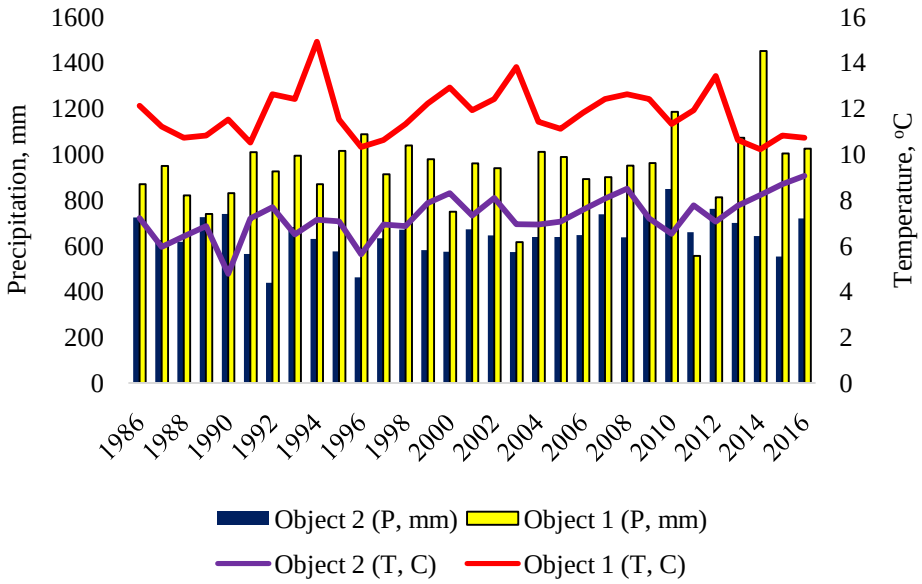


Figure. 1. Variation of the yearly precipitation (mm) and temperature (°C) in the Objects

In the investigation period an average of 933.5 mm of precipitation fell, which fluctuated from 554.9 mm to 1285.3 mm in Object No. 1 and average of 749.4 mm of precipitation fell, which fluctuated from 657.0 mm to 847.2 mm in Object No. 2 (Table 2). In two years recorded extremes of precipitation, i.e. less and more precipitation than in the period 1986-2005. In the three investigation years, the average data of temperature was 11.9 °C (Object No. 1, Table 2) and temperature has increased with 0.6 °C in relation to the period 1986-2005. The average temperature was 7.4 °C in Object No. 2, and comparing with Climate Normals (CN, 1981-2010), has increased with 0.4°C, the average annual precipitation quantity was about 749 mm, or 18% higher than the CN. Basing on the increase of these climatic elements, it is possible to speak about climatic changes, what was stated by other authors (Bukantis and Rimkus 2005; Kutilek, Nielsen, 2010).

The climatic and hydrological characteristics of the studied region are some of the indispensable indicators for the planning and designing of drainage systems, since multi-year precipitation, its monthly or seasonal distribution or maximum daily precipitation and its intensity define the key characteristic of climate and determine the type of agriculture and management on ameliorated areas. Precipitation amount, distribution and time of precipitation occurrence during the

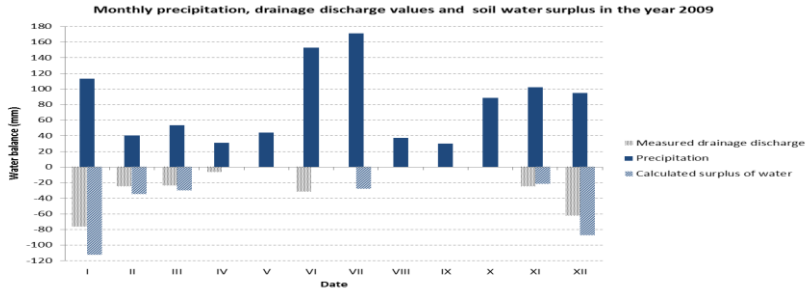
investigation period influenced the drainage discharge and its duration (Table 2), both at the annual and monthly levels, as well as the differences between the tested pipe spacing's.

Table 2. Total drainage discharge (mm), precipitation (%) and duration of drainage discharge (days) for different drainpipe spacing's (m)

Year	Average air temperature (t, °C)	Drainpipe spacing (m)	Total precipitation (P, mm)	Total drainage discharge (mm)	Precipitation (%)	Duration of drainage discharge (days)
2009	7.3	12	742.1	40.6	6.0	138
	12.4	15	960.2	253.0	26.3	88
	7.3	18	742.1	41.9	6.2	131
	12.4	20	960.2	250.0	26.0	96
2010	8.1	12	847.2	135.3	21.0	176
	11.3	15	1285.3	460.0	35.8	138
	8.1	18	847.2	128.4	6.0	179
	11.3	20	1285.3	462.0	35.9	148
2011	6.9	12	659.0	24.0	4.2	112
	11.9	15	554.9	21.0	3.8	8
	6.9	18	659.0	18.3	3.2	109
	11.9	20	554.9	21.0	3.8	10

The highest amount of the drainage discharge and its duration (Tables 2) was in 2010 at the highest amount of precipitation and the smallest in 2011 at the smallest amount of precipitation. There is not difference in total drainage discharge between the tested drainpipe spacing in each investigation year. Data were approximately equal. Therefore, there are differences in the duration of the drainage discharge both at the annual and at the drainpipe spacing. In each year, the duration of drainage discharge was smaller at 15 m drainpipe spacing than at 20 m drainpipe spacing from. in Object No. 1, and the duration of drainage discharge was smaller at 12 m drainpipe spacing than at 24 m drainpipe spacing from each year in Object No. 2. Wallace and Batchelor (1997) suggest that combined discharge and drainage losses are often in the range 40–50 % of rainfall. In this study was found, that drainage discharge varied from 3.2 % to 35.9 % of total precipitation. Fig. 2 (a, b, c) shows flowchart monthly precipitation values, monthly drainage discharge values and calculated surplus of water, for 15 m drainpipe spacing (the same is valid for the 20 m drainpipe spacing) and Fig. 3 (a, b, c) for 12 m drainpipe spacing. Generally, in the winter/spring period and autumn/winter period, the monthly drainage discharge followed the monthly amounts of precipitation, that is, the higher the monthly amount of precipitation, the higher was the drainage discharge, and vice versa (especially in the first two years). The calculated surplus of water followed the monthly amounts of precipitation, but the data were higher in all years. In the late spring and summer months, due to intensified growth and development of plants, the amount of drainage discharge was not proportional to precipitation. Namely, evapotranspiration was increased at later plant development stages due to higher spring and summer air temperatures, so that lower drainage discharge was recorded in both pipe spacing variants. Autumn drainage discharge wasn't recorded in 2011 , neither surplus of water was calculated because soil water supply wasn't filled up with precipitation from the preceding months. As regards drainage discharge duration, Table 2 shows that the shorter duration was determined at the narrower pipe spacing (12, 15 m) than at wider pipe spacing (18, 20 m), which may be attributed to the drainage system efficiency. According to the investigations conducted by Petošić et al. (1998), Tomić et al. (2002) and Šimunić et al. (2011; 2013), narrower pipe spacing is more efficient for draining surplus water from drained soils, since larger amounts of water

are drained in a shorter period of time and better water-air relationships in soil are created faster, which is a prerequisite for timely application of agricultural management practices on hydroameliorated arable areas. Surplus soil water calculated according Thornthwaite's method was in all years higher than measured drainage discharge in both objects. In Object No. 1, the difference was approximately 65 mm (first year), 190 mm (second year) and 12 mm (third year), while in the Object No. 2 the difference was about 93 mm in the first year, 133 mm in the second year, and 266 mm in the third year. This can be explained by the fact that this method includes only a climatic parameter (temperature) but on the water loss from soil can influenced other climatic parameters too, such as air humidity, wind speed, insolation, as well as different types of crops, etc. Drainage systems are especially important in spring, during the snow melting period, because the excess of water is removed quickly from the arable layer of the ground. Therefore, the conditions to start spring field works for about two weeks earlier are guaranteed. It is also very important to remove the excess of water which forms in the fields during summer season after abundant precipitation (Lukianas and Ruminaite, 2009). Pipe spacing from 12-15 m is more efficient for draining surplus water from drained soils, since surplus of water from soil is drained in a shorter period and better water/air relationships in soil are created faster.



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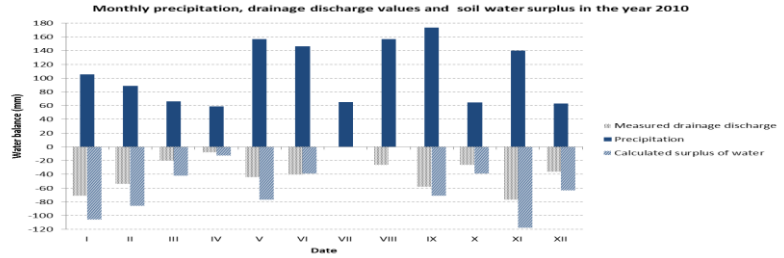

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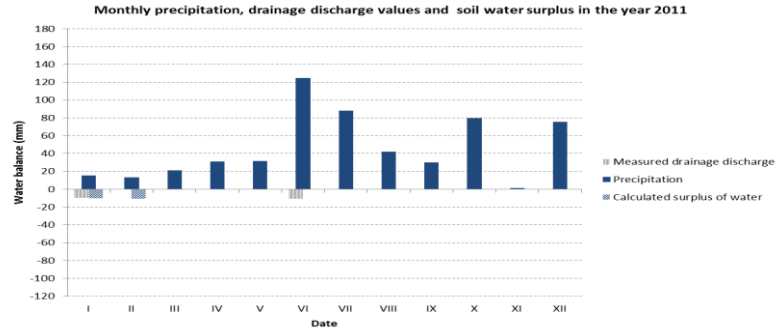
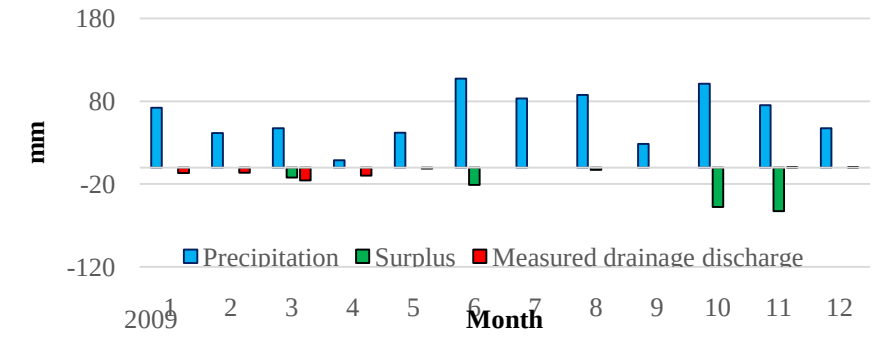


c)

Figure 2. Monthly precipitation values, monthly drainage discharge values and calculated surplus of water, for the drainpipe spacing of 15 m (a) – 2009, b) – 2010, c) – 2011)



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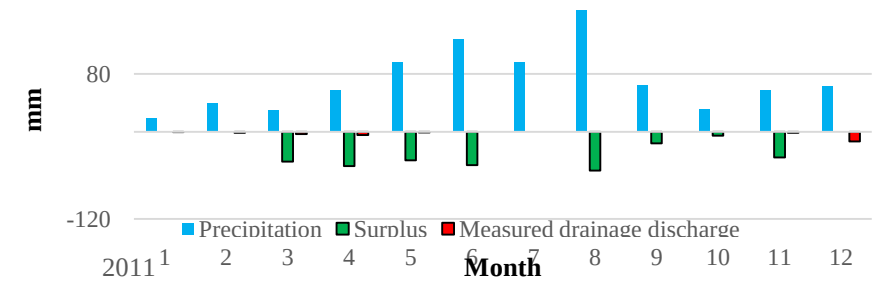

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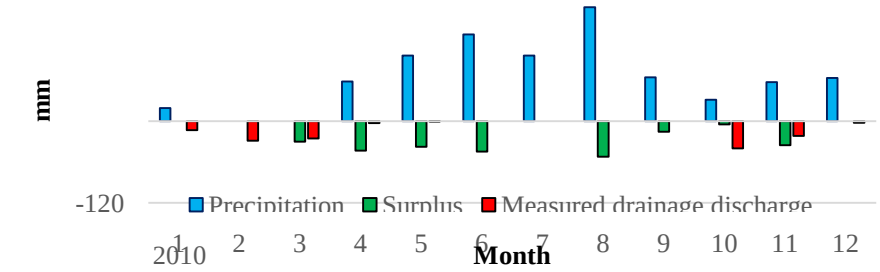

c)

Figure 3. Monthly precipitation values, monthly drainage discharge values and calculated surplus of water, for the drainpipe spacing of 12 m (a) – 2009, b) – 2010, c) – 2011)

Conclusions

Monthly drainage discharge followed the monthly amounts of precipitation in two different climatic regions, that is, the higher the monthly amount of precipitation was, the higher was the drainage discharge, and vice versa. There is no difference in the total drainage discharge between the tested drainpipe spacing in each investigation year. There are differences in duration of the drainage discharge both at the annual and at drainpipe spacing. In each year, the duration of the drainage discharge was smaller at drainpipe spacing from 12/15 m than at drainpipe spacing from 18/20 m. The calculated surplus of water followed the monthly amount of precipitation, but in all years the calculated data were higher than the drainage discharge. Pipe spacing from 12/15 m is more efficient for draining surplus water from drained soils, since the surplus of water from soil is drained in a shorter period of time and better water-air relationships in soil are created faster, which is a prerequisite for timely application of agricultural management practices on hydroameliorated arable areas.

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APPLICATION OF METHODS BASED ON SYNCHROTRON RADIATION FOR SPECIATION OF HEAVY METAL IN SOIL

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Abstract
The study is aimed at analyze atomic and electronic structure of Cu (II) and Pb (II) ions in the artificially contaminated soil and its mineral components using X-ray absorption spectroscopy methods based on synchrotron radiation. Soil sampling was taken in the 0-20 cm topsoil of the Haplic Chernozem of the South Russia. In a model experiment the samples taken were artificially contaminated with higher portions (2000 and 10000 mg/kg) of nitrates and oxides of Cu and Pb. The experimental X-ray absorption (XANES and EXAFS) spectra at the K-edge of Cu and L_{III}-edge of Pb were performed at the Structural Materials Science beamline of the Kurchatov Center for Synchrotron Radiation (Moscow) in the fluorescence regime. The features of XANES spectra indicate different orbital transitions in the electron shells of Pb (II) and Cu (II) ions for monoxide and soluble salt, which affect the ion properties and determine the individual structure of the coordination sphere. Analysis of the EXAFS revealed that Cu (II) ions are incorporated in the octahedral and tetrahedral sites of minerals and bonded with humic materials at the expense of covalent bond and the formation of coordination humate copper complexes. Lead ions in soil are incorporated in the positions of the inner-sphere complex replacing some aluminum ions in the octahedral sites. This results in changes the Pb–O distances in Pb-bearing octahedrons. We may suggest that Pb (II) is also sorbed by dimer (Pb–Pb) silicate and/or aluminum groups.

Keywords: XANES, EXAFS, Cu (II), Pb (II), contamination.

Introduction

The bioavailability of metals is closely related to their forms of occurrence. Therefore, studies of soil contamination aimed at obtaining objective information about metal speciation become of special importance (Minkina et al., 2010, 2013). The complexity of the occurrence forms of metals and metalloids is most manifested in highly dynamic, physically and chemically heterogeneous ecological systems like soils, bottom sediments, and sewage sludge (Hesterberg et al., 2011). Analytical methods used to assess metal compounds are usually suitable for the study of limited combinations of metals and metalloids in environmental objects. The mechanical transference of extraction systems developed for background soils introduces additional uncertainties and errors in the study of contaminated soils. The proportions of phosphates, sulfides, and arsenates, for which there are no adequate extractants, increase in industrially contaminated soils (Orlov et al., 2005). Heavy metals in the soils are traditionally analyzed by the indirect method of chemical fractionation; however, direct methods for the determination of heavy metals in soils based on X-ray absorption spectroscopy rapidly developed in the last decades. X-ray spectral analysis methods provide information on the dispersion of elements and contribute to the identification of carrier minerals in the soil, the determination of the mechanisms of cation sorption at the molecular level, and the specification of local atomic structure (Lombi and Susini, 2009). They ensure the selectivity and sensitivity to the local structure of a wide range of elements, low detection limits, high spatial resolution, and simple procedure of sample preparation, which makes these methods the most universal and accuracy in the study of the composition of trace elements and contaminants. X-ray

absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) are the most commonly used spectroscopic techniques. They provided essential information about the forms of heavy metals in soils (Manceau et al., 2002). The aim of this work was to analyze atomic and electronic structure of Cu (II) and Pb (II) ions in the artificially contaminated soil and its mineral components using X-ray absorption spectroscopy methods based on synchrotron radiation.

Material and methods

Soil for model laboratory experiments collected from the humus-accumulative A1 horizon (from the top layer, 0–20 cm) of Haplic Chernozem (IUSS, 2015) in the "Persianovskaya Step", Specially Protected Natural Territory, Rostov oblast, Russia. The A1 horizon (0-20 cm layer) has the following properties: C_{org} 3.7%, $CaCO_3$ 0.4%, pH_{H2O} 7.6; exchangeable bases (cmol+/kg): Ca^{2+} 31.0, Mg^{2+} 6.0, Na^{+}, 0.06; particle size analysis: sand fractions (particles 1.0-0.05 mm) – 42.8%, silt fractions (particles 0.01-0.001 mm) – 29.9% and clay particles (particles <0.001 mm) –28.1%. The content of total Cu and Pb in the samples was determined by synchrotron radiation X-ray fluorescence analysis (SR XRF). Chemical compositions of the mineral component of Haplic Chernozem and the phases of layered silicates samples are given in Table 1. The analyses intended to broaden information about chemical compositions of the sampling sites while improving the knowledge about the presence of possible interferences for synchrotron X-ray methods (for example Fe). The analysis of data obtained with X-ray fluorescence method XRF has allowed to establish quantitative differences of oxides composition for phases of layered silicates samples. The mineralogy of the clay and fine silt fractions from the humus-accumulative horizon of Haplic Chernozem is characterized by the following phase composition of layered silicates: the contents of illite, labile silicates, and kaolinite are 51–54 and 51–60, 23–27 and 12–27, and 22–23 and 22–28% in the clay and fine silt fractions, respectively. The fine silt fraction also contains micas, amorphous silica, and crystallized iron and aluminum oxides and hydroxides (Kryshchenko and Kuznetsov, 2003; Nevidomskaya et al., 2016; Sokolova, 1985).

Table 1. Chemical compositions of the mineral component of Calcic Chernozem and the phases of layered silicates, wt %

Sample	SiO_2	Al_2O_3	Fe_2O_3	CaO	MgO	K_2O	P_2O_5
Haplic Chernozem	63.95	12.08	5.26	2.35	1.35	2.16	0.16
Kaolinite	55.24	33.17	0.42	0.13	0.05	0.41	0.09
Montmorillonite	75.60	7.68	1.17	1.22	1.17	0.39	0.05
Hydromuscovite	60.69	18.97	4.66	2.22	1.45	4.54	0.17
Gibbsite	4.28	56.99	0.19	0.08	0.02	0.10	0.13

To study transformation of Cu (II) and Pb (II) ions absorbed by soil, the samples taken in uncontaminated soil (Haplic Chernozem) contaminated with compounds of Zn and Cu oxides and nitrates. The model laboratory experiment has been established under controlled conditions. The soil selected for the experiment was air-dried, triturated using a pestle with a rubber head, and sieved through a 1-mm sieve. Dry compounds of Cu ($Cu(NO_3)_2$ and CuO) and Pb ($Pb(NO_3)_2$ and PbO) were added to the soil at a rate of 2000 and 10000 Cu and Pb mg/kg. The soil was thoroughly mixed, wetted, and incubated for 3 years at 60% of the maximum water capacity. Experiments were performed in triplicates. Analogous procedures but without addition of metal were performed with the control sample. After the end of incubation (3 years layer), an average sample was taken from each pot for analysis. The soil was brought to the dry state. Samples of separate mineral phases (montmorillonite, kaolinite, hydromuscovite, and gibbsite) were saturated with Cu^{2+} and Pb^{2+} ions. For this purpose, the studied samples were put into a saturated $Cu(NO_3)_2$ and $Pb(NO_3)_2$ solution. The solution was changed twice a day for a week. After a week, the preparation was removed from the solution, dried, and grounded. The experimental Cu K-edge X-ray absorption near edge structure (XANES) spectra (~899–8995 eV) and L_{III}-edge of Pb (13040 eV) were performed at the Structural Materials Science beamline (Chernyshov et al. 2009) of the Kurchatov Center for Synchrotron Radiation (NRC “Kurchatov Institute”, Moscow) at room temperature in fluorescence

mode. A two-crystal Si(111) monochromator with the energy resolution ΔE/E ~ $2 \cdot 10^{-4}$ was used to monochromate the X-ray radiation. To obtain the data for statistical method the exposition time of 60 sec. was taken for each point in the spectrum. 10 spectra were statistically averaged to determine a final spectrum for every sample. The obtained spectra were processed using standard procedures for noise discrimination and normalization by the K-edge jump. First-derivative XANES spectra were analyzed to specify information about the state of Cu^{2+} and Pb^{2+} ions and reveal the differences in the analyzed samples that escaped detection during the analysis of XANES spectra. Along with the experimental XANES spectra (the channel-cut monochromator with routinely switchable Si(111)), experimental spectra of the original Cu-containing and Pb-containing compound were also studied. The gonimeter head of the monochromator constructed at the Institute of Crystallography of RAS (Moscow, Russia, 2004) driven by a step motor provides a rotational scanning step of 1 arcsec. Quick and reliable photon energy tuning is crucial for the X-ray absorption spectroscopy measurements. EXAFS spectra of the Cu K-edge and Pb L_{III}-edge were measured at the same synchrotron beamline in the fluorescence yield mode using a Si avalanche photodiode to count fluorescence photons and an ionization chamber to monitor the incident intensity (Chernyshov et al. 2009). The spectra were processed with the help of the Ifeffit software package (Newville, 2001).

Results and discussion

The morphology, size, and peculiarities of edge and near-edge areas on XANES spectra of soil samples contaminated by CuO and $Cu(NO_3)_2$ have clear differences mainly controlled by the differences in their local atomic structure around the central Cu ion (Fig. 1). The intensity of the α peak is controlled by the degree of bond covalence and characterizes the coordination environment and chemical bonds of the absorbed metal ion with its closest surroundings. With a decrease in the α peak energy, Cu complexes with soil components have predominantly the covalent character of the bond. The intensities of the α and β peaks in the experimental spectra of the initial copper-bearing compounds are close (Fig. 1b). Analysis of the EXAFS revealed that lead to ion exchange in the tetragonal plain of water molecules with ligands. The interaction between copper ions and humic acid may result in the formation of multilateral 6-coordinated spatial structure of humate complex (Minkina et al., 2017). Application of this method demonstrated that the state of copper introduced in Haplic Chernozem as CuO did not change after three year of incubation (Fig. 1). Copper is absorbed after being introduced as $Cu(NO_3)_2$, and Cu ions are incorporated in the octahedral and tetrahedral sites of minerals and bonded with humic materials at the expense of covalent bond and the formation of coordination humate copper complexes.

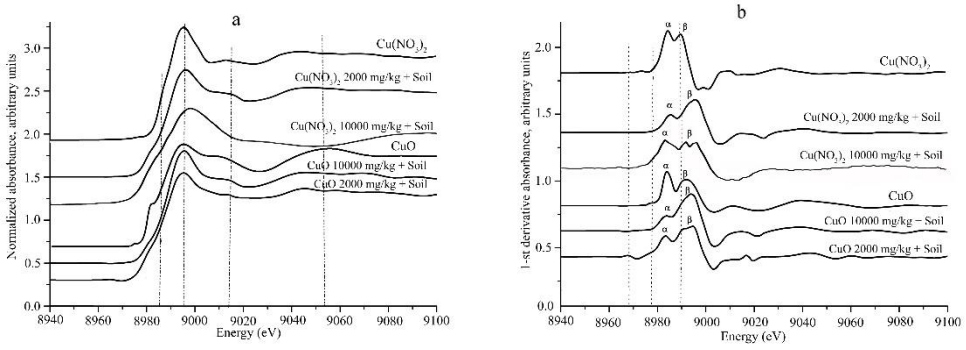

Figure 1. The experimental Cu K-edge X-ray absorption spectra (a) and their first derivatives (b) for the reference compounds and the Cu-treated soil samples

Parameters of the experimental XANES spectra obtained for the studied soil samples saturated with high rates of Pb compounds, as well as the spectra of the initial PbO and $Pb(NO_3)_2$ are given in Fig. 2. The spectra are characterized by an energy region of ~13030–13058 eV related to the presence of lead ions, from which the molecular-structural state of the metal is assessed. The highest absorption intensity is recorded in the energy region of ~13038–13040 eV for the samples saturated with PbO

and at ~13042 eV for the samples saturated with $Pb(NO_3)_2$, which characterizes the $2p_{3/2}\rightarrow 6d$ electron transition (Fig. 2b). The modulations of $2p_{3/2}$ electrons in the first derivative spectra of PbO and PbO-saturated soil samples are appreciably different (Fig. 2b), which is due to the different shoulder amplitudes in the energy region of ~13032 eV, especially for the initial PbO. This X-ray absorption peak is manifested only for the spectra of PbO and PbO-saturated soil samples and is related to the $2p_{3/2}\rightarrow 6s$ electron transition, indicating the 6s and 6p hybridization for Pb and $2p_{x,y}$ for oxygen; therefore, Pb^{2+} participates in the formation of numerous distorted complexes, because the adsorbed Pb ions can have different O–Pb–O valent angles.

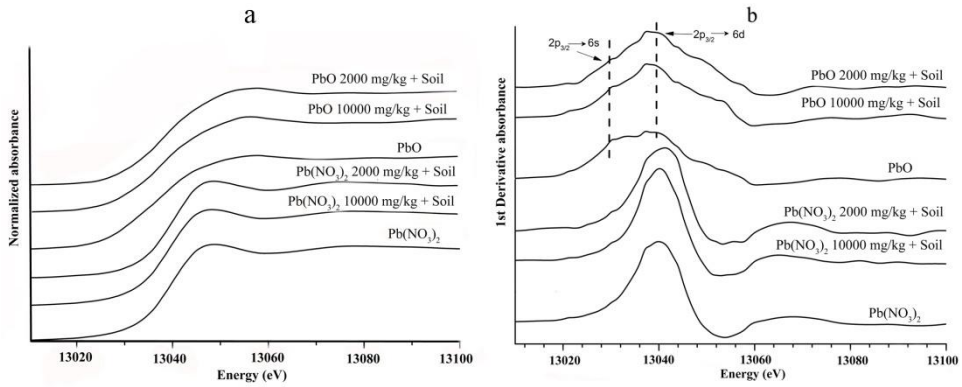

Figure 2. The experimental Pb L_{III}-edge XANES spectra (a) and their first derivatives (b) for the reference compounds (PbO and $Pb(NO_3)_2$) and the Pb-treated soil samples

Using results of EXAFS method was determined that lead ions incorporated in the phyllosilicate minerals structure favoring a decrease in the bond distances between Pb^{2+} ions and O atoms in equatorial and axial coordination positions in Pb-bearing octahedrons. Divalent Pb has the $6s^2$ electronic configuration of the outer shell. This lone electron pair is frequently stereochemically active and causes a strong deformation of divalent Pb in polyhedrons. Thus, it can be concluded that Pb is sorbed as a bidentate inner-sphere complex at the edges of the octahedrally coordinated aluminum ions. XANES data for the studied soil samples and mineral phases of layered minerals artificially contaminated with $Cu(NO_3)_2$ are shown in Fig. 3. Comparison of the first-derivative Cu K-edge XANES spectra for all samples with the spectrum of the $Cu(NO_3)_2$ standard showed sensitivity of the method for changes in the immediate surrounding of Cu (II) ions in these structures. The samples are characterized by the existence of peak A in the middle part of the spectrum (~899–8995 eV) due to the presence of Cu^{2+} ions. The absence of chemical shift of the main absorption edge in contrast to the initial spectrum of the compound indicates that the charge of Cu^{2+} ion in the soil does not change. The spectral features of the central peak and low-amplitude lateral maximums of layered silicates, their shapes, and shifts against the original copper-containing compound indicate a shortening of interatomic distances between the adsorbed Cu^{2+} ions and the oxygen surrounding in accordance with the Natoli rule (Natoli, 1984). This agrees with data of X-ray diffraction analysis and earlier molecular dynamic simulation (Minkina et al., 2013).

Parameters of the experimental XANES spectra obtained for the studied soil phases are given in Fig. 4a; their first derivatives are given in Fig 4b. The spectra of these phases include the energy region of ~13035–13055 eV. Absorption spectra of soil phases saturated with Pb nitrate together with those of $Pb(NO_3)_2$ are shown at the Fig. 4a and their first derivatives are shown at the Fig. 4b. Comparison the first derivative of the Pb L_{III}-edge XANES of all the soil samples to the reference spectrum of $Pb(NO_3)_2$ show similarity of the main features: a central peak and low amplitude left- and right-hand shoulder features.

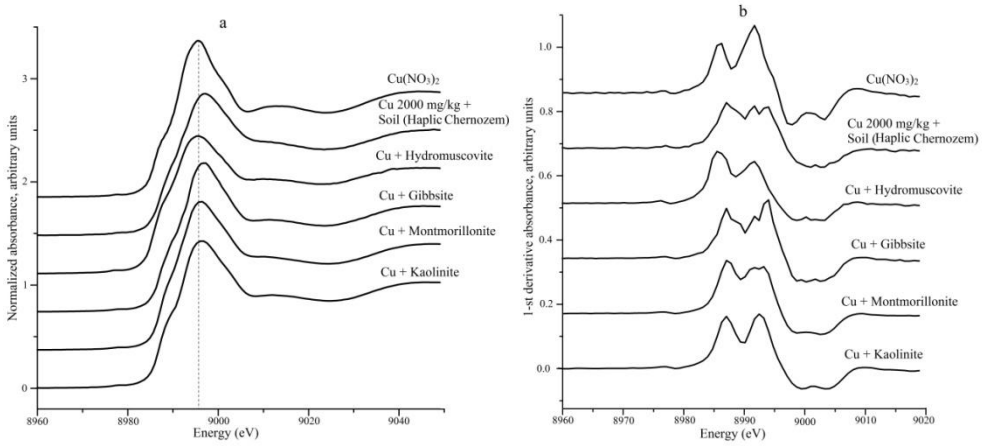

Figure 3. (a) Experimental Cu K-edge XANES spectra and (b) 1-st-derivative X-ray absorption spectra for soil, layered silicate phases, and $Cu(NO_3)_2$ standard

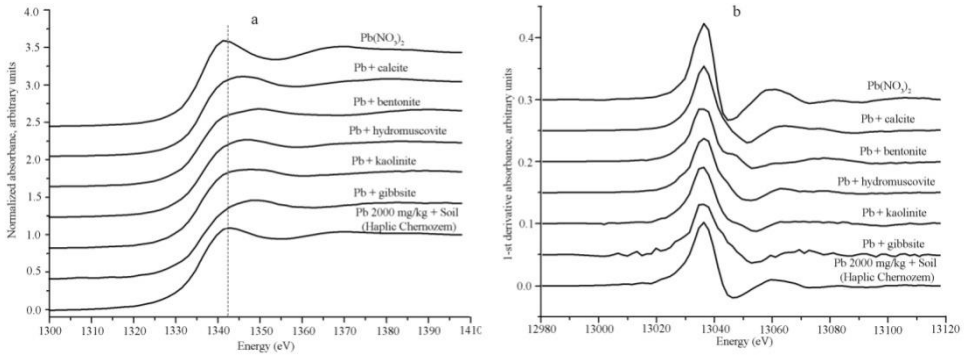

Figure 4. (a) Experimental Pb L_{III}-edge XANES spectra and (b) 1-st-derivative X-ray absorption spectra for soil, layered silicate phases, and $Pb(NO_3)_2$ standard

Analysis of the EXAFS revealed that Pb^{2+} ions in soil are incorporated in the positions of the inner-sphere complex replacing some aluminum ions in the octahedral sites. Lead oxides show a particularly wide variety of nearest Pb-O distances. According to the structural database (ICSD database) the shortest Pb-O distance ranges from 0.216 nm in PbO up to 0.275 nm in $Pb(NO_3)_2$. Thus the distribution of the oxygen atoms around Pb can serve as a sensitive probe for structural changes in the samples. This results in changes the Pb–O distances in Pb-bearing octahedrons. We may suggest that Pb (II) is also sorbed by dimer (Pb–Pb) silicate and/or aluminum groups.

Conclusions

Synchrotron radiation's methods are an effective approach for the study of bonds between metals and soil components. Application of these methods demonstrated different orbital transitions in the electron shells of Cu^{2+} and Pb^{2+} ions for monoxide and soluble salt, which affect the ion properties and determine the individual structure of the coordination sphere. Copper is absorbed after being introduced as Cu $(NO_3)_2$, and Cu^{2+} ions are incorporated in the octahedral and tetrahedral sites of minerals and bonded with humic materials at the expense of covalent bond and the formation of coordination humate copper complexes. Lead ions are sorbed as a bidentate inner-sphere complex at the edges of the octahedrally coordinated aluminum ions. This results in changes the Pb–O distances in Pb-bearing octahedrons. We may suggest that Pb (II) is also sorbed by dimer (Pb–Pb) silicate and/or aluminum groups.

Acknowledgments
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MODELLING THE ADAPTATION CAPABILITIES OF SUNFLOWER AND WINTER WHEAT TO CROP ROTATION AND POSSIBLE CLIMATIC CHANGE IN THRACE

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Abstract
In agriculture, most changes in atmospheric conditions are uncontrolled factors caused by climate change or variability that could have effects on different sectors. Globally, major important sector is agriculture by means of supplying food on Earth. Determination of the positive and negative effects of climate change to make provision for possible future conditions could be executed using Crop-Climate models. Developed by the Food and Agriculture Organization (FAO), the AquaCrop is an explanatory Crop-Climate model, which could be used to analyze such relationships between climate change and food productivity. In this research, variations in the growth and yield parameters associated with rotation between the sunflower and winter wheat were investigated using AquaCrop between the periods of 2014 and 2016 in Kırklareli and Edirne cities which have specific climates and soil types in the Thrace Region. Moreover, main yield-related outputs of the model will been validated after model AquaCrop will simulate sunflower and winter wheat crop rotation until 2099 for Thrace Region to expand our future perspective.

Keywords: AquaCrop, simulation, crop rotation, Turkey.

Introduction

Atmospheric conditions are the uncontrollable processes in nature and have positive or negative impacts on most sectors. Agriculture is one of these sectors. Climate is a primary environmental factor that effect life of the living beings. Researches about different environmental and other factors on crop growth and yield are not only cheap but also long process (Şaylan, 1994). For this reason, answers about any crops whether or grow in our country conditions are found in many years of study. AquaCrop, crop growth simulation model, has become popular in recent years, which is developed by Food and Agriculture Organization of the United Nations (FAO). Researchers in different countries have intensively tested the AquaCrop model and wanted to expand their perspective how effects crop development, yield and etc. by agricultural drought in different climates, irrigation schedules, agricultural practices (Andarzian et al., 2011; Araya et al., 2010; Vanuytrecht et al., 2014; Voloudakis et al., 2015). According to Intergovernmental Panel on Climate Change (IPCC) Fifth Assessment Report (AR5), Mediterranean Basin is negatively affected by climate change. Rain accumulation will decrease and air temperature will significantly increase. Turkey is located in Mediterranean Basin and it is expected that different region in Turkey will be affected differently by climate change. The Northwestern part of Turkey which includes Thrace Region plays an important role on sunflower and winter wheat cultivation. Percentage of sunflower and winter wheat production are 75% and 35%, respectively. This research was pursued at Atatürk Soil Water and Agricultural Meteorology Research Institute to analyze between modelled and observed sunflower and winter wheat yield.

Material and methods

This research is conducted at two different cities, which are Kırklareli (40°43'42.43"N latitude, 26°26'42.69"E longitude) and Edirne (41°41'55.34"N latitude, 27°12'38.71"E longitude), in Thrace

Region. Beeline distance between these two locations is 126.2 km (Figure 1). Soil types of Kırklareli and Edirne are sandy loam and clay, respectively. These two locations represent Kırklareli and Edirne.

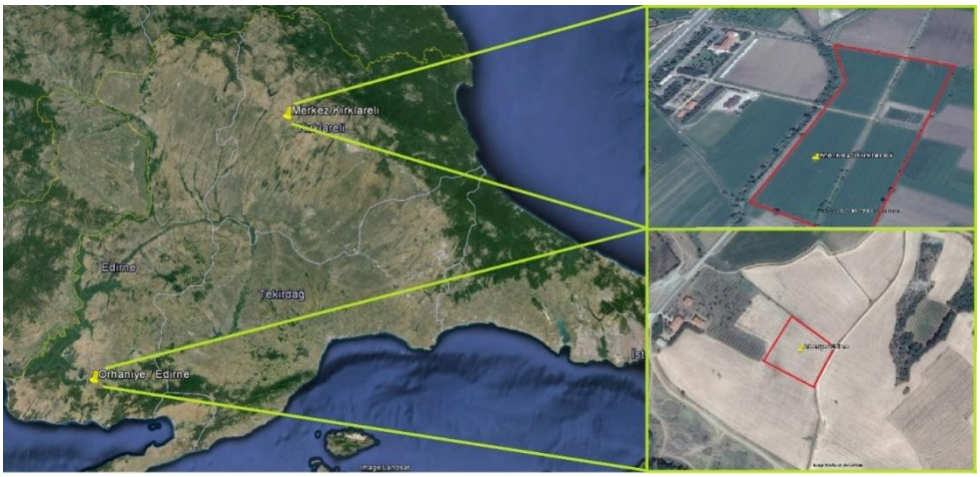

Figure 1. Location of Study Area

Two Meteorological stations were installed at research fields. Each station includes data logger (CR1000, Campbell Sci.), pyranometer (CMP6, KippZonen), temperature and relative humidity (Rotronic and Vaisala), wind speed and direction (NRG), soil water content (CS616, Campbell Sci.) at 3 different (0-30, 30-60, 60-90 cm) depths. These sensors measure 30 sec. time interval and log data 10, 30, 60 min and 24 hour. In order to estimate sunflower and winter wheat crop yield and other crop parameters, AquaCrop developed by FAO were performed. The flowchart of AquaCrop can be found in Figure 2.

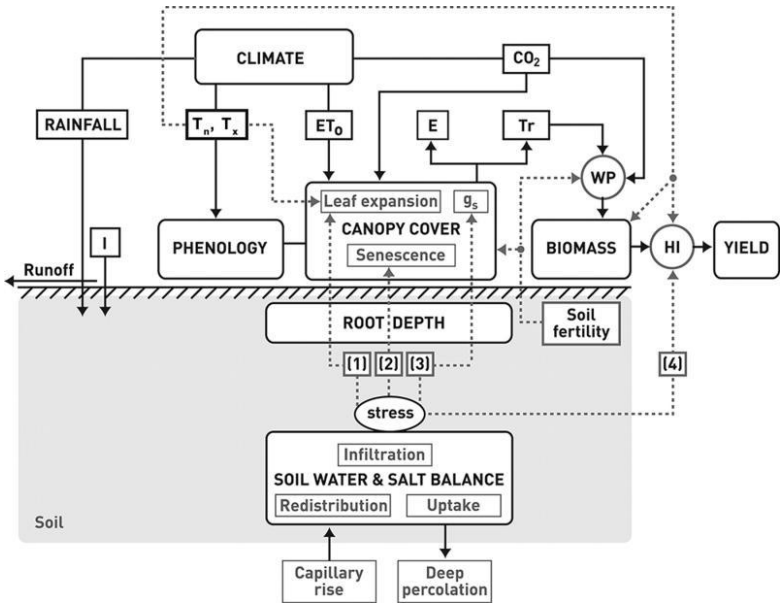

Figure 2. The Flowchart of AquaCrop Model (Raes et al., 2009)

General calculation of the AquaCrop model depends on the soil-water balance method with a daily time step. Partitioning of biomass into yield given the simulated above ground biomass (B), crop yield is obtained with the help of the Harvest Index (HI). In response to water and/or temperature stresses, HI is continuously adjusted during yield formation. Reference Evapotranspiration (ET_0) is calculated by FAO Penman-Monteith method (Eq. 1) with measured meteorological factors at two different research locations.

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

where; ET_0 reference evapotranspiration (mm/day); Δ, slope vapour pressure curve (kPa/°C); R_n, net radiation (MJ/m^{2}day); G, soil heat flux density (MJ/m^{2}day); γ, psychrometric constant (kPa/°C); T, air temperature at 2 m height (°C); u_2, wind speed at 2 m height (m/s); e_s, saturation vapour pressure (kPa); e_a, actual vapour pressure.

Results and discussion

Meteorological variables and crop biomass of two locations were simultaneously measured. Sunflower and winter wheat simulations of the AquaCrop model were performed and can be seen in Figure 3, 4, and 5. Sunflower simulations for Edirne and Kırklareli, measurements from meteorological stations and crop phenological observations became input values for AquaCrop model. Calculation of soil water content in AquaCrop was done from initial soil water content and using soil water balance method. For sunflower simulations in Kırklareli, 2014 and 2015 growing season predictions were overestimated in comparison with in-situ measurements. Sunflower yields in 2014 and 2015 growing season are 250 kg/da and 202 kg/da, respectively. On the other hand, AquaCrop simulations for these growing seasons were 443 kg/da and 279 kg/da, respectively (Figure 3).

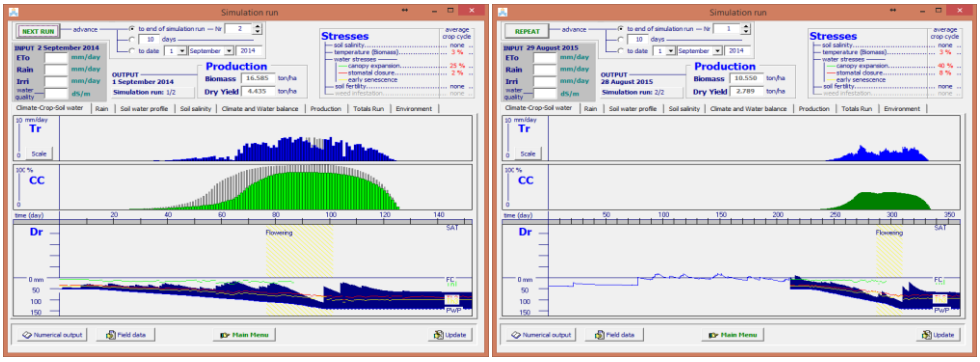

Figure 3. Sunflower Simulations for 2014 and 2015 Growing Season for Kırklareli

For sunflower simulations in Edirne, 2014 and 2015 growing season predictions were again overestimated. Sunflower yields in 2014 and 2015 growing season are 300 kg/da and 164 kg/da, respectively. On the other hand, AquaCrop simulations for these growing seasons were 314 kg/da and 288 kg/da, respectively (Figure 4).

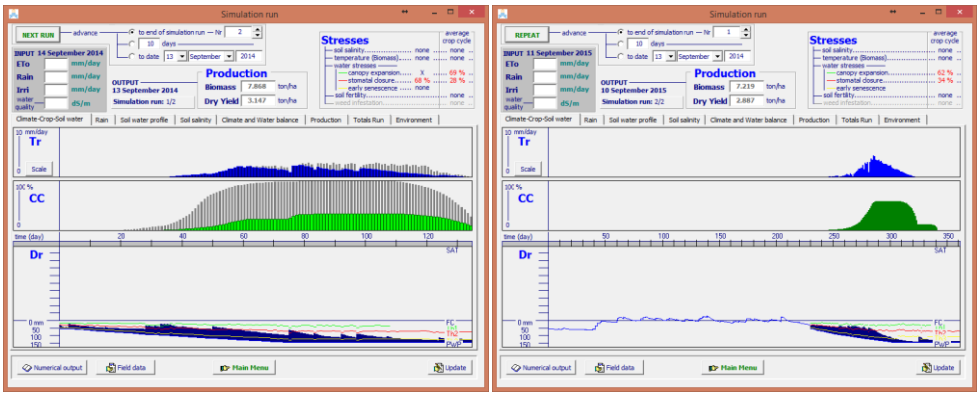

Figure 4. Sunflower Simulations for 2014 and 2015 Growing Season for Edirne

Moreover, winter wheat simulations for Kırklareli and Edirne were performed for 2015-2016 growing season. While winter wheat simulation for Kırklareli was underestimated, simulation for Edirne was overestimated for yield. This difference could come from soil texture and the capacity of

available water in root zone. But, locations, estimation and measurement biomass values are not considerably different. Simulations for winter wheat yield in 2015-2016 growing season in Edirne and Kırklareli 417 kg/da and 532 kg/da, respectively (Figure 5). In-situ measurements of winter wheat yield 444 kg/da in Kırklareli and 478 kg/da in Edirne.

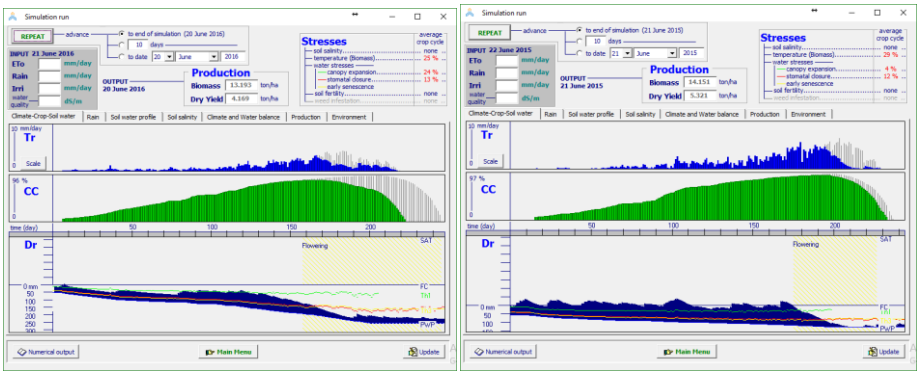

Figure 5. Winter Wheat Simulations for 2015-2016 Growing Season for Kırklareli and Edirne locations

Measurements and modelled biomass time series can be found in Figure 6. According to sunflower simulations, modelled biomass values are underestimated for two locations. However, yield performance of AquaCrop was overestimated. In winter wheat simulations, AquaCrop performance is relatively higher than sunflower simulations.

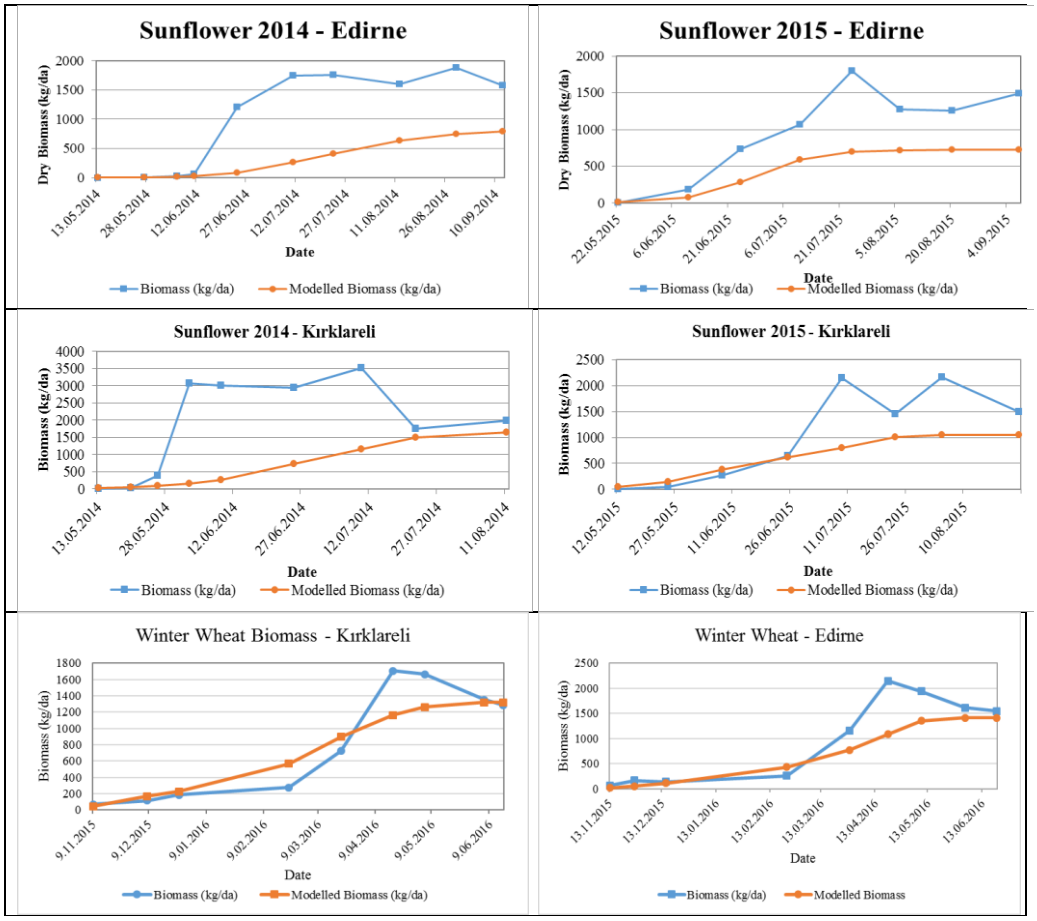

Figure 6. Measurements and Modelled Biomass of Simulations

AquaCrop simulates crop development, soil water content, crop water requirements, soil water profile, evapotranspiration etc. according to the soil water balance method. It is also user-friendly

and preferred by among researchers. Simulations for different crops and locations help us to understand how climate affect crop development, biomass, and yield etc. Our simulations cover two consecutive sunflower growing periods and a winter wheat growing period in two locations. These locations do not have the same soil type and climate features. Therefore, water content in different soil type affect crop development. In addition, sunflower is more sensitive to soil water content than winter wheat. Especially in flowering phenological stage for sunflower is considerably important for yield formation. Because, this phenological stage period is in the middle of the summer. In drought years, crop development may be critical circumstances. In next years, sensitivity analysis of sunflower and winter wheat will be performed. Moreover, climate projections data until 2099 for these two locations will be obtained. After model calibration, AquaCrop will simulate sunflower and winter wheat crop rotation until 2099 for Thrace Region to expand our future perspective.

Acknowledgments
We would like to thank General Directorate of Agricultural Research and Policies (TAGEM) for support of this project. Additionally, we also thank Department of Meteorology, Faculty of Aeronautics and Astronautics, Istanbul Technical University for their cooperation. Special thanks for technical persons who help us during the field studies at Atatürk Soil Water and Agricultural Research Institute Directorate in Kırklareli.

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EVALUATION OF CROP ALBEDO OF DIFFERENT SUNFLOWER CROP ROTATION CULTIVARS AND ITS EFFECT ON LATENT HEAT FLUX

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Abstract
Surface albedo is expressed as the fraction of incoming shortwave solar radiation reflected by the surface. Surface albedo is one of the primary factors influencing regional and global climates as well as ecological and biophysical processes especially in the study of water and energy fluxes of ecosystems. In these research areas, accurate determination of net radiation plays a vital role for determination of the fluxes. In this study, it is aimed to establish the crop albedo values of three sunflower cultivars in the northwestern part of Turkey where sunflower production is represented as country's production. It can be seen that crop albedo values are related to the crop and meteorological factors like crop phenology, soil moisture etc. Crop albedo also varies by each cultivars of sunflower. Moderate Resolution Imaging Spectroradiometer (MODIS) albedo values and in-situ measurements were compared within this study. In the light of this perspective, albedo is considerably sensitive parameter and has influence on the latent heat flux. Also, comparison between calculated latent heat flux from MODIS data and in-situ measurements were investigated.

Key words: Albedo, sunflower, latent heat flux, MODIS.

Introduction

Land surface albedo is an important variable which controls the energy budget between land-atmosphere interactions. Albedo measures the reflectivity of the surface. It gives information about the absorption of incoming shortwave radiation by the surface. It varies in time and space and mostly depends on land color, soil moisture, crop types, development period of crops and sky conditions. Some of the albedo studies in the literature are listed below. Changing in albedo is affected the micro and regional climate (Zhang et al., 2013). Doughty et al. (2011) investigated that increasing agricultural albedo can cause a 0.25 °C cooling regional climate in a small changes (0.01) of albedo. In order to calculate actual evapotranspiration (ET_a), net radiation (R_n), which controls the evapotranspiration mechanism, of over interested area must be properly determined.Crop albedo diurnal, seasonally or annually varies relation to earth-atmosphere energy balance. Crop albedo also has an important role in actual evapotranspiration with related to R_n. In order to determine the crop albedo is a complex process by atmospheric and surface conditions: diurnal solar position, cloudiness, soil type and soil water content (Giambelluca et al, 1999; Iziomon and Mayer, 2002). In developing countries, actual evapotranspiration is calculated by using net radiation which is calculated by using global solar radiation values from meteorological station. Therefore, it is important to well-estimate the net radiation which has component of surface albedo and global radiation. Thrace region locates northwestern part of Turkey in the European Continent, has the 75% total sunflower production of Turkey. Sunflower is planted in an area of 578 000 ha and produced 843 000 tons per year in Turkey. The purpose of this research, determining the variation of the albedo for three sunflower cultivars during the growing period of 2016. By the way, the influence of calculated and measured albedo on the estimated and measured net radiation and actual evapotranspiration of sunflower were also investigated.

Material and methods

The research field is located in the Kırklareli city, Northwestern part of Turkey. Meteorological data was collected from 6th April to 27th September 2016 during the growing season of sunflower at Atatürk Soil Water and Agricultural Meteorology Research Institute Directorate (40°43'42.43"N latitude, 26°26'42.69"E longitude) (Fig. 1).In this period, phenological development of sunflower varieties were observed and their leaf area index (LAI) values were biweekly measured by using LAI-2200C (LiCor, Nebraska). Incoming, outgoing short and longwave radiations, global solar and reflected radiations (CMP6 and CNR4, KippZonen, Delft), air temperature and relative humidity (Rotronic and Vaisala), soil water content at 3 different depths (CS616, Campbell Sci.), wind speed and direction (NRG Systems) values were continuously and simultaneously measured and the data were recorded by the datalogger (CR1000, Campbell Sci.) with an interval of 10, 30, 60 min. and daily time steps for three different parcels.

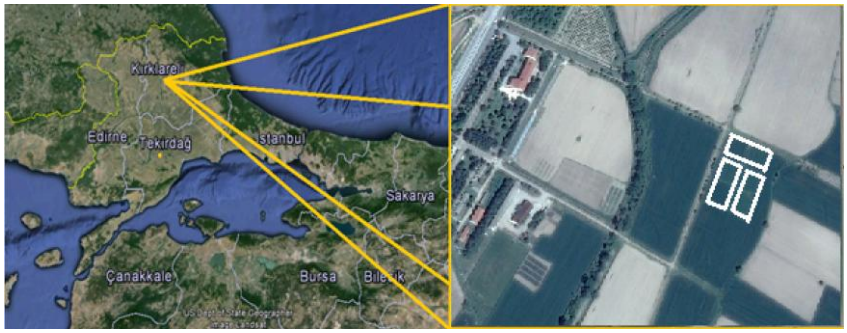

Figure 1. Atatürk Soil Water and Agricultural Meteorology Research Institute Directorate

Three different sunflower cultivars which are mostly planted in Thrace part of Turkey, were cultivated to determine albedo values. These cultivars' names are Tunca, Sanay, and Poinner and they represent Thrace Region by 99%. In order to calculate net radiation, global solar radiation measurement and albedo valuesare required (Eq. 1) (Allen et al., 1998):

$$R_{ns} = (1 - \alpha)R_s \quad (1)$$

where R_{ns}, net shortwave radiation (MJ m^{-2} day^{-1}); α, surface (here is sunflower) albedo; R_s, global radiation measurement (MJ m^{-2} day^{-1}).

Net longwave radiation (R_{nl}) and net radiation (R_n) can be calculated using equation 2 and 3:

$$R_{nl} = \sigma\left[\frac{T_{max,K}^4 + T_{min,K}^4}{2}\right](0.34 - 0.14\sqrt{e_a})(1.35\frac{R_s}{R_{so}} - 0.35) \quad (2)$$

$$R_n = R_{ns} + R_{nl} \quad (3)$$

$$R_{so} = (0.75 + 2x10^{-5}z)R_a \quad (4)$$

where e_a actual vapor pressure; R_{so} (MJ m^{-2} day^{-1}), clear sky radiation; R_a, extraterrestrial radiation (MJ m^{-2} day^{-1}).

Evapotranspiration calculation from Penman Monteith equation,

$$\lambda ET = \frac{\Delta(R_n - G) + \rho_a c_p \frac{(e_s - e_a)}{r_a}}{\Delta + \gamma(1 + \frac{r_s}{r_a})} \quad (5)$$

where G, soil heat flux; $e_s - e_a$, vapour pressure deficit of the air; ρ_a, air density at constant pressure; c_p, specific heat of the air; Δ, slope of the saturation vapour pressure temperature relationship; γ, psychrometric constant; r_s and r_a are the (bulk) surface and aerodynamic resistances, respectively. Evapotranspiration can also be determined by measuring the various components of the soil water balance (Eq. 5). The method consists of assessing the incoming and outgoing water flux into the crop root zone. Irrigation (I) and rainfall (P) add water to the root zone. Part of I and P might be lost by surface runoff (RO) and by deep percolation (DP) that will eventually recharge the water table. Water might also be transported upward by capillary rise (CR) from a shallow water table towards the root zone or even transferred horizontally by subsurface flow in root zone

$$ET = I + P + RO - DP + CR \quad (6)$$

This study in Turkey is the only project about determination of vegetative surface albedo and its effect on evapotranspiration. In this study, it is emphasized that surface albedo is one of the most important parameter to estimate evapotranspiration.

Results and discussion

Diurnal profile of sunflower albedo values are asymmetrical changed (Ogundunte and Giesen, 2004; Bsaibes et al., 2009) in daily hours (Figure 2). The reason for this is incoming midday radiation is relatively higher than the other periods. Three different sunflower cultivars have considerably the same attitude and do not show significant changes from one another. Tunca cultivar is different in morning because this cultivar was sown on 31.05.2016 while other sunflower cultivars were sown on 06.04.2016.

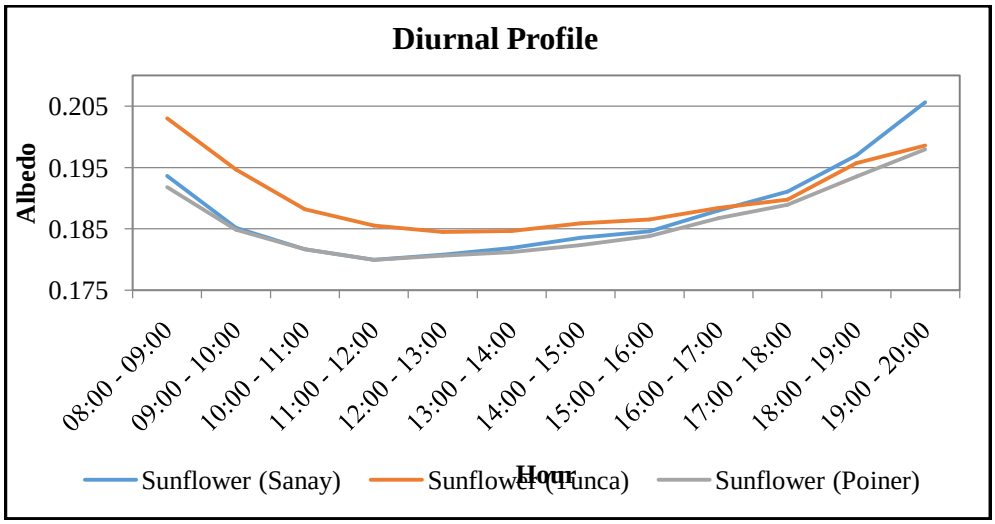

Figure 2. Diurnal Profile of Sunflower Albedo

Time series of the MODIS and measured albedo values can be found in Figure 3. According to this figure, MODIS data is not enough to represent the comparison with in-situ measurements.

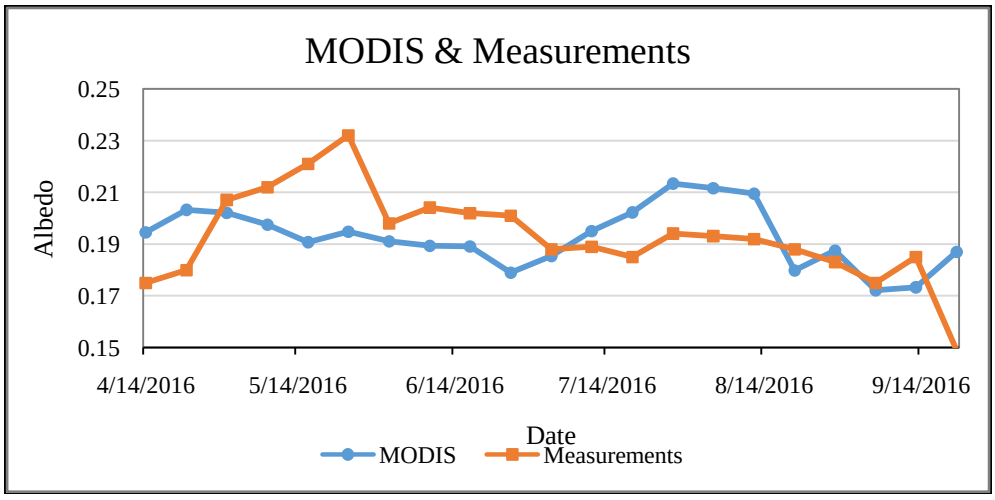

Figure 3. Time Series of MODIS and In-Situ Measurements

Time series of the daily mean albedo and precipitation data were given in Figure 4. Not only surface but also meteorological variables affect the surface albedo. Albedo values decrease in rainy days due to lack of global radiation and increasing soil moisture (Gascoin et al., 2009; Xiaodan et al., 2009). Because, precipitation from June to September is about 10 mm, summer 2016 was a drought period.

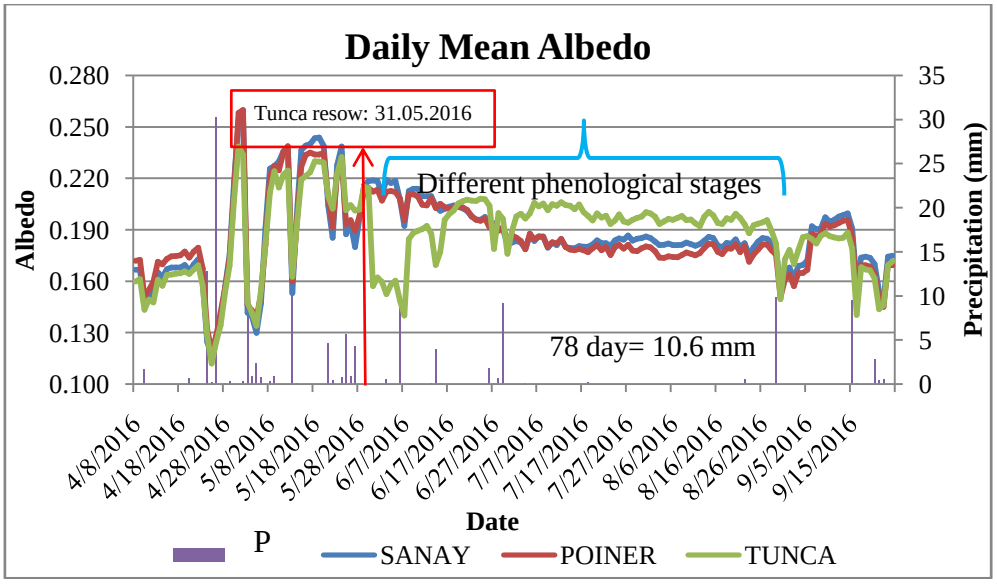

Figure 4. Daily mean albedo and precipitation time series

Albedo values of sunflowers were determined according to the phenological stages as shown in Figure 5. Soil was bare when crops were cultivated. From sowing to emergence period soil surface albedo was represented. After emergence, crop albedo has been increasing according to cultivars. The highest albedo values of sunflowers are in the leafing phenological stage. On the other hand albedo difference in this period is significant. Sunflower in leafing stages covers whole field and soil effect is not dominant. While sunflower albedo values are different in phenological stages, there is a not significant change between them.

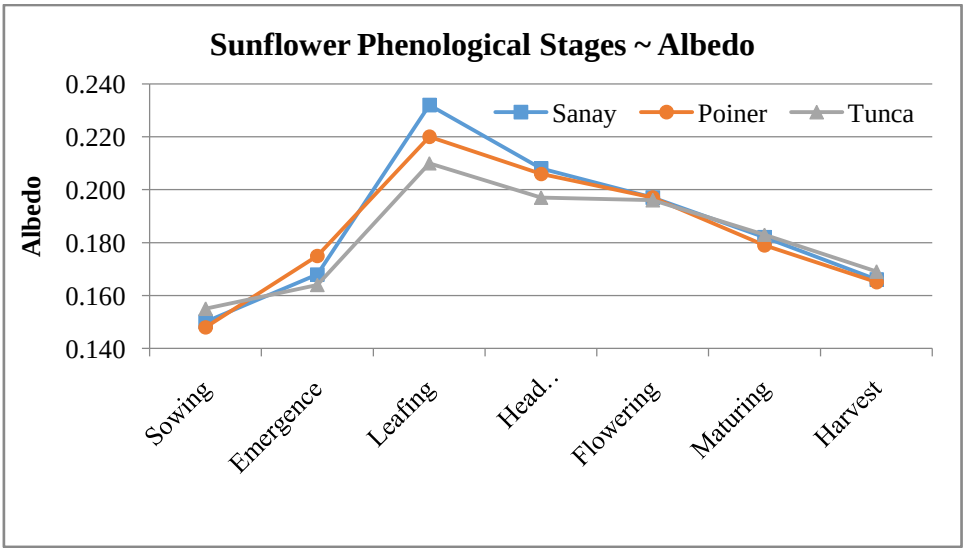

Figure 5. Crop Albedo Changes in Phenological Stages

Monthly evapotranspiration estimation with Soil-Water Balance method and FAO Penman Monteith can be found in Figure 6. Evapotranspiration values of Tunca cannot be found in April and May months because the sowing date is 31.05.2016. In July and August months, precipitation amount is 10.6 mm in 78 days. Therefore, crop water requirements estimation from Soil-Water Balance is decreased. Penman Monteith approachment depends on meteorological variables, that's why evapotranspiration values is considerably higher than the Soil-Water Balance method results in July and August. Evapotranspiration calculations of other months are close to each other. Total evapotranspiration of Sanay, Poiner, and Tunca cultivars were calculated as 524.2, 526.8, and 424.4

mm, respectively. Lower evapotranspiration of Tunca is caused by different sowing date. Total evapotranspiration in this period with Soil-Water Balance was calculated as 341.1 mm.

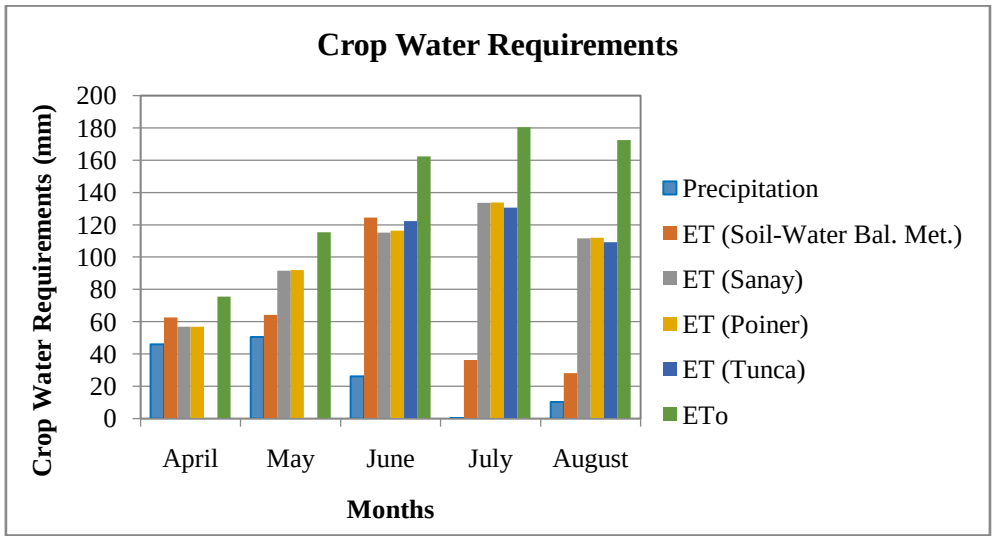

Figure 6. Monthly Crop Water Requirements

This study is the first throughout analysis of crop albedo in Turkey. Results of this research may provide reliable albedo values that are needed for determination of the actual evapotranspiration of winter wheat as well as calibration of remote sensing data. Additionally, daily mean albedo values were used for calculation of net radiation values. We recommend the usage of different albedo values for different phenological stages (e.g. while obtaining crop coefficients) instead of using a single albedo value for whole growing period.

Acknowledgments

We would like to thank General Directorate of Agricultural Research and Policies (TAGEM) for support of this project. Additionally, we also thank Department of Meteorology, Faculty of Aeronautics and Astronautics, Istanbul Technical University for their cooperation. Special thanks for technical persons who help us during the field studies at Atatürk Soil Water and Agricultural Research Institute Directorate in Kırklareli.

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WATER HOLDING POLYMERS OF THEIR USE IN AGRICULTURAL IRRIGATION

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Abstract

World population is increasing at an alarming rate and expected to the major challenges of the near future. Population growth and the water demand is expected to be 50% higher than today. Using of Water Holding Polymers in agriculture has provided solutions to the problems of the present day agriculture increasing of the soils capacity to store water. Water holding polymers potentially influence soil evaporation and infiltration rates of water through the soils. Particularly, the polymers reduce irrigation frequency and compaction tendency, stop erosion and water run off. This study has been put together the possible effecs water holding polymers in water scarcity on yield and water using of the crops during critical periods of water. Polimers store rainwater in the soil in dry conditions. As a result, this review aimed to given information about the benefits of water holding polymers in the arid and semi-arid regions of the world.

Keywords: Agricultural irrigation, water holding polymers, water scarcity.

Introduction

The optimization of the use of water resources is strategic for the long-term competitiveness of the agricultural industry. As we know, the population of the world and our country is rapidly increasing day by day, while the water resources are seriously decreasing, which makes effective use of water compulsory. Water management is considered one of the major challenges of the near future (Saguy et al, 2013); At this point, all sectors and areas related to water develop new and effective water management strategies. As can be guessed, agriculture is at the forefront of the most used areas of water. Almost 70% of the world's available water is spent for agriculture. In such a context, it is apparent the importance to develop innovative agricultural systems and to promote technologies that could optimize the exploitation of water resources; nonetheless, it is crucial to guarantee that the appropriate amount of water is timely and efficiently delivered to the plants. Water holding polymers associated quickly with irrigation water to form gels resulting in an increase of the soils capacity to store water. The water stored in this way is available to plants for some considerable time. The polymers are present in the soil together with the water they receive in their constructions; fertilizers, nutrients and mineral salts.In this way, irrigated water is efficiently supplied to the plant roots when needed. The pores in the ground are filled with water after rain or full watering and become available for plants. The filled water in the pores is drawn by gravity. Therefore, plant roots can’t benefit from the whole of this water and nutrients and lose a significant part. . SAPs can absorb and retain extremely large amounts of a liquid (water or an organic liquid) relative to their own mass (Horie et al, 2004) In agricultural applications, SAP granules are mixed with the soil in given amounts. After watering, the granules absorb the water by swelling, and then release it slowly through a diffusive mechanism, as the soil gets dry. Water absorbant polymers reduce the evapotranspiration and protect the plants from problems related to the lack of water and plant nutrients. Furthermore, SAP granules increase their size upon swelling, thus enhacing soil porosity and providing a better oxygenation to the roots. Since water absorbant polymers provide excellent moisture to the root zone,the chance of survival of newly planted plants increases significantly. As SAP increases the soil water storage capacity, irrigation applications should be made at times when the moisture content of the soil falls to a minimum level (Fonteno ve Bilderback,

1993). SAP enhance the development of root and stem. Moreover, the germination process, the plant growth, the nutrients uptake, the yield and both the water and fertilizer use efficiency were beneficially increased by mixing the plant pits in sandy soil with SAP (Ouchi et al. 1990; El-Hady et al. 2002). They are also responsible for the reduction of root fungi. This effect is associated with the crystallizing high pH value. (Johnson ve Hummel, 1985). It has been reported that when SAP are added in very dry soil, water conductivity is increased and channels are formed that allow the saline to drain through the soil. Hydrophilic polymers can also reduce salinity, especially in highly dry and swollen sodic soils. (Malik et al., 1991). Several researchers in this area have stated that certain SAP in the soil can absorb water 200-300 times their own weight. They also said that since SAP give the absorbed water to the soil in a controlled manner, so the loss of water from the root region is prevented. It is known that SAP, which can be used in many areas, are used in agriculture more than 40 years around the world. The SAP in the market have less than one hour of water absorption with 100% capacity. At least 99% of the absorbed water can be used by plants. SAP provide effective irrigation support by showing swelling and recrystallization periods in the soil and by repeatedly performing this property. It is claimed that this situation lasts 3-5 years and it is saved from water at least 30-60% depending on soil and climatic conditions. SAP have different efficiencies according to the place of use and their chemical structures. Today, there are three types of water-holding polymers that are widely used; natural polymers, semi-synthetic polymers and synthetic polymers. Natural polymers are based on starch and derived from grains such as corn and wheat. Natural polymers have a common usage as a thickener in the food industry. Firstly, semi-synthetic polymers are obtained from cellulose and then mixed with petrochemicals. These polymers differ in cation or anion. Synthetic polymers are mainly used in agriculture. Synthetic hydrophilic polymers generally come from polyvinylalcohol and polyacrylamides (Mikkelsen, 1994). The chemical structure of polymers acting as a kind of microsponge in the soil is potassium based water absorbers consisting of cross-linked acrylamide, acrylic acid, potassium salt and ammonium salt. The cross-linking molecules in the structure of SAP form a three-dimensional network and render the materials insoluble in water. When these materials interact with water, they swell rapidly, and form a gel structure by absorbing water and water-soluble nutrients. Acrylamide increases long-run stability and maintains this property for about 4-5 years. According to the researches, these materials have been disintegrated into soil by microorganisms in the soil. However, the rate of disintegration in the soil is between 10-15% per year on average and it takes a long period about 5-7 years, which means pollution of the environment by SAP and it is considered as a disadvantage. Ben-Hur-M (1994) reported that in a study with 20 kg polyacrylamide (PAM) and 40 kg polysaccharide (PS) application to the soil surface before the irrigation resulted in significant decreases in surface flux and erosion as well as potato and cotton yield increases. The same researcher stated that the PS application is more suitable than PAM because of its low viscosity and high water solubility. Wallace (1987) obtained high output and development in tomato and lettuce in thin textured soil with PAM. In studies with applied PAM on lettuce, radish and wheat, the plants have benefited more effectively the water absorbed in gel form compared to normal water. Furthermore, during the temporary drought period, gel water is used as a buffer and reduces the risk of product destruction at the germination period (Jhonson, 1990).

Impact of SAP's on the environment

According to recent ecotoxicological researches it has not been found that water-absorbant polymers have adverse effects on existing organisms in water or soil (Ohkawa ve ark., 1999). According to research results on acute toxicity, it is harmless after oral ingestion. It has also shown that transient intense skin contact has no irritating effect on the skin or eyes and has no potential for allergies. Mutagenic properties that could cause genetic alterations have not been observed (Madakbas et al, 2014). It is known that the roots of the plants are able to absorb acrylamide from the soil and carry it to other parts of the plants. Various investigations have been carried out to determine the level of pollution of the plants with acrylamide. In a study conducted in greenhouse

conditions, 1% SAP application was made at seed stage in lettuce and carrot cultivation. These seeds are planted in sandy soil.Vegetables were harvested at different intervals and tried to determine the amount of polymer. The amount detected in the lettuce was only 0.12% of the maximum amount of applied polymer. The polymer value found for carrots is much lower than the lettuce. Free acrylamide was not detected in both types of vegetables (Madakbas et al, 2014). The consumption of cultivated plants using SAP has no risk for humans in terms of toxicology (Sojka et al., 2007; Viero and Little, 2006).

Benefits

Studies (Flannery and Busscher, 1982; Johnson, 1984) have shown that hydrogel application to soil does not affect the amount of water used by the plant. The applications show that the beneficial water in the plant root zone has increased and that the plants have less damage than water stress by extending the time between irrigations. El-Sayed and Kirkwood (1992) reported that addition of soil hydrogel leads to a decrease in salt ions in stationary corn pollen. According to this, it can be estimated that the hydrogels changes the level of salt ions in the plant tissues and decrease the negative effects of saltiness (Chen et al., 2004).

Hydrophilic polymers potentially influence infiltration rates, density, soil structure, compaction, soil texture, aggregate stability, crust hardness (Helalia and Letey, 1989), and evaporation rates (Teyel and El-Hady, 1981).

- Water loss due to evaporation or infiltration to the soil is prevented, thereby the water holding capacity of the soil increases and the irrigation range is extended.
- Decreases plant nutrient loss due to washing, increases plant growth.
- Water is used more efficiently, excessive and unnecessary consumption of water is avoided
- It provides durability against drought and water stress.
- Provides easier and faster germination
- Because the seed is wrapped with moisture layers, it increases the germination rate and gives uniform germination.
- It improves the physical properties of impermeable soils by correcting the aeration capacity and drainage of the soil.
- Prevents soil erosion.
- Protect the environment against underground water pollution.
- It provides economical benefit by reducing the cost of irrigation.
- Reduce production costs without reducing product yield.
- Activity to fight against root nematodes and fungi (Madakbas et al, 2014).
- It is ensured that the soil which is not suitable for harvesting.

Areas Used In Agriculture

- Plant growing pots

Water and plant nutrients are taken directly into the gelatin by thin fibrous roots, or they are slowly released by the osmotic process to the surrounding soil. Regular moisture supply leads to improved plant growth. Due to rapid and intensive root growth, the chance of survival of newly planted plants is significantly increased.

- Heather and tree planting

SAP is applied in the solid form with fertilizers or liquid form into soil depth of 20-120 cm via injectors. Injection is more advantageous than other application methods. In this method, the injector is applied very easily in hard soil without damaging the roots. Air, food and water are infiltrated by bringing a network between the gaps in the soil. In addition to increased plant yield, the quality also increases at a high rate. The application of SAP in the soil via injector is a low cost and very useful method

- Grass and grass areas

The use of water absorbant polymers in grass areas meets the general water requirements of plant species and affects plant performance. It allows for the necessary work to restore a green appearance of arid areas.

- Extensive spreading and surface application

It is applied hand application or with the fertilizer to the soil before the planting. After spreading, the soil is mixed about 15-25 cm. In this application form, 3-5 kg SAP is used per decare.

- Layerig and planting

This method is used to prevent root dryness during planting and to transport bare roots to long distances without being damaged. It is possible to add nematicides and fungicides to this mixture to protect the roots against nematode and fungi. The density of the SAP mixture is adjusted to best adhere to the roots (1 kg SAP to 100 - 300 lt water). SAP is slowly poured and mixed into water. By waiting for a while (15 minutes) the viscosity of the gel increases and the roots become better attached.

- Soilless cultivations

In soilless cultivations, plants are grown using mineral nutrient solutions in water, without soil (Di Lorenzo et al, 2013). In open soilless systems, there is a massive waste of water and nutrients, which is responsible for an increase in running costs and in contamination of ground and surface water (Vox, 2010). The adoption of the SAP to ration the delivery of nutrients to the plants would improve the overall environmental sustainability of these systems. Also for closed systems (in which water recirculates), the use of SAP may help hindering water retention of the plants;

- Semination with water

For coating with water, 1-2 kg of SAP is added per m3 of water. In addition to the seed, cellulose and fertilizer can be added to this mixture. Depending on the field moister capacity, type of soil and salt in the water, 10 to 20 kg of polymer per hectare are needed for SAP that covered water.

- Seed coating

In this method, the SAP is homogeneously mixed with the seeds. The SAP in the form of dust is attached to the surface of the seeds with a static charge. Better germination occurs in the soil because the seed will be covered with SAP. After the crop is harvested, the frozen SAP is a suitable regulator for storing cold air. This basic feature is valid as long as the SAP crystals remain solid. However when the crystals are soft, this basic feature disappears. SAP is placed in hermetic containers made of various materials according to the intended purpose. The polymers contained in these containers are frozen. It is placed in the middle of the harvested crop to be cooled. Frozen SAPs, which are active for 72 hours, are the most economical application method in terms of ease of use, reliability, no chemical contamination and friendliness to nature. It is also a great advantage to use again.

Results and discussion

This review was prepared considering the possible effecs water holding polyacrylamide (PAM) polymers in dry agriculture on yield and water use of the crops during critical periods of water. Water holding polymers were described in terms of their positive impacts on the soil and plants. We aimed to given information about the benefits of water holding PAMs in areas where there was not sufficient rainwater and irrigation water. SAP, which provide optimum conditions for plant growth, provide crop production profitability related to yield, yield and quality of the crop. They increase the living rate of the plants. They enable the sale of products with higher quality plant production and a higher market price. They allow plants to grow more comfortably in very hot and dry climatic conditions. We do not have the chance to increase water resources today, so we must protect what is available. We must use SAP to prevent evaporation and infiltration of the water which is insufficient in arid regions. The results suggest that hydrogels can improve sandy soil properties for plant growth by absorbing and keeping water longer in the soil matrix thus re-ducing watering frequency. The results show that hydrogels can be better utilized than sandy soils for plant growth with longer water holding in the soil matrix. The intelligent, economical and efficient use of water

has also become a necessity in our country. In order to reduce irrigation and fertilization costs, SAP that can hold water with different properties are used as alternatives to excess water consumption. Because SAP polymers can also hold fertilizer, and mineral salts available in the soil. Thus, plants are protected from problems related to water stress and lack of nutrients.

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POSSIBILITIES OF APPLYING BIOMASS FOR THE PURPOSES OF ENERGY PRODUCTION AND ENVIRONMENTAL PROTECTION

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Abstract
The aim of the paper is to raise, direct and encourage awareness of the importance of biomass in energy production, as well as to present various possibilities of its use. Increasing demands for energy and growing environmental issues impose the need for energy production from renewable resources. Fossil fuel reserves are finite and their deficit is projected for the coming period. Biogas is one of the renewable resources. The said gas consists of a large amount of methane gas produced by fermenting organic substances from biomass, manure or any other biodegradable material in anaerobic conditions. Electricity production from renewable resources on farms needs to meet numerous conditions, such as environmental protection, bio-safety and animal welfare, as well as a series of technical, organisational, construction, manufacturing and economic requirements imposed by this kind of production. The Lazar Company Ltd. from the town of Blace owns a farm with 600 dairy cows, a dairy which processes 60 000 litres of milk per day, as well as an energy production plant based on biogas, with the capacity of 1mW/hour. In addition, special attention is paid to organic farming, where biomass is said to have multiple applications in energy production in the context of a long-term development trends of certain industries.

Keywords: biomass, energy, agriculture, environment.

Introduction

Depending on the time necessary to produce energy, one can distinguish two types of energy resources: renewable and non-renewable. Non-renewable resources comprise resources whose formation time is incomparably longer than the time of their exploitation. These resources include coal, oil and oil gas, oil shale and nuclear energy. The energy generated from such resources has a very high coefficient of energy utilisation. As a result, the aforesaid resources are still among the most prevalent ones and world economies are directly dependent on them. However, the manners of their exploitation have caused the presence of large amounts of toxic chemical substances in the air, water and soil, which exerts a range of negative effects on human health, as well as on the entire biosphere. In addition, an accelerated industrial development increases the demand for energy which, by and large, imposes the need to attend to additional, alternative and renewable energy resources (Owusu and Asumadu-Sarkodie, 2016). Renewable or everlasting energy resources are those used for the purposes of producing electricity or heat, as well as those the reserves of which are uniformly or continually renewed. The name itself derives from the fact that energy is consumed in the amount which does not exceed the speed in which it is produced in nature. Among the renewable energy resources one can assort the resources which are claimed to have the reserves so considerable that they can be exploited for millions of years. Renewable energy resources, hydropower excluded, provide less than 1% of the total energy required on a global level.

Biomass as a renewable energy resource

Biomass is the oldest renewable energy resource that has ever been used. The term biomass implies biodegradable substances of either plant or animal origin, by-products of lumber industry, agricultural waste and residues of biological origin, as well as industrial and communal waste. The

use of biomass as an energy resource is rather diverse. It is used in combustion processes or converted in the systems which produce either heat or electricity or both. Likewise, it is used in the production of liquid fuels (bio-ethanol, bio-methane and biodiesel) and gas fuels (biogas, the gas from waste disposal sites). The main advantage of biomass in comparison with fossil fuels is a reduced emission of harmful gasses, as well as reduced discharge of wastewaters. Additional advantages include the disposal and utilisation of waste and residues produced by agriculture, forestry and timber industry, a reduction in energy import, investment in agriculture and underdeveloped areas. It is anticipated that the share of biomass in energy consumption shall be between 30% and 40% by the middle of the century (Kriz et al.2016).

The status of biomass in Serbia

Depending on the source, biomass can be divided into forest biomass (wood waste biomass), energy plantations, farm animal biomass, urban waste (waste biomass). A considerable potential for biomass production in the Republic of Serbia lies in agricultural residues and wood biomass, amounting to 2.7 million tons in total (1.7 million tons in agricultural residue and around 1 million tons in wood biomass). Furthermore, the livestock production residue can also be identified as a significant biomass source. The second group of biomass sources includes plantations of energy plants (for example, Miscanthus, fast-growing poplar, etc.), as well as plants which are used as a raw material for biodiesel, bio-ethanol (rapeseed, sunflower, corn, etc.) (http:// www.mre.gov.rs). As a country with large agricultural and forest areas, Serbia possesses a considerable potential for biomass production. Biomass comprises around 63% of the total renewable energy resources. Forests cover around 30% of the territory and around 55% of the territory is covered by arable land. In addition to crop residues intended for food production, there are enormous opportunities for dedicated biomass cultivation which will not trammel food production. Biomass can be used for obtaining bio-fuel, bio-ethanol, biodiesel and biogas. Biogas is produced via anaerobic digestion whereby organic carbon is translated into the highest level of oxidation (CO_2) and the highest degree of reduction (CH_4) by resorting to oxidation-reduction processes. The process takes place without the presence of oxygen under the influence of a large number of microorganisms. The result of this process is a mixture of gasses: methane 55%-75%, carbon dioxide 25%-45%, hydrogen sulphide 0%-1%, nitrogen 0%-2%, hydrogen 0%-1%, aqueous vapour 0%-2%, ammonia 0%-2%, oxygen 0%-0.5%. The composition and yield vary depending on the used raw materials as well as on technological conditions of the process (Manyi-Loh et al. 2013, Neshat et al., 2017). The process of anaerobic digestion is an efficient method of treating biological waste and it offers significant advantages in comparison with other forms of treatment: less sludge is produced in comparison to other techniques which resort to aerobic processes and the waste containing less than 40% of dry matter is successfully treated. Likewise, this process is more efficient in removing pathogens, the emission of unpleasant odours is at the minimum because 99% of volatile components are decomposed through oxidation in the process of combustion (for example, H_2S forms SO_2) and the obtained residue (sludge) is used for the purposes of improving soil fertility (Milosavljević et al, 2010, Babic et al.2010). Manure is present in significant amounts as a by-product on the farm which currently has more than 1000 heads of cattle, which comprise 600 milking cows in the production cycle, and if the aforementioned manure is not properly handled it can become a significant pollutant of the environment. It is necessary to apply a series of methods which would make liquid manure ready for storing and removing. The methods include a mechanical treatment which implies the following phases: separation and homogenisation and biological treatment. Liquid manure is an example of suitable raw materials intended for biogas production, which can further be used as an energy source, i.e. for obtaining electricity from renewable resources. The utilisation of liquid manure in this fashion contributes to the reduction of dangers of polluting the environment and groundwater, which affects the conservation of biodiversity and the environment (Šarić and Obradović, 2012, Neshat et al., 2017).

Material and methods

In recent years, the biogas production on the "Lazar" dairy farm located in the town of Blace (http://www.lazar.co.rs/) occupies the primary place with respect to the assessment of secondary products on livestock farms with the main aim of preserving the environment and producing energy from renewable resources. After the process of fermentation in a digester, a mechanical separator of fertilisers separates the residual waste into solid and liquid parts. The solid part is used as a cowshed bedding while the liquid part is discharged into a storage located below the energy plant and it is later used as a fertiliser in the fields. In addition, on this farm they also use dairy by-products (whey). Dairy is a part of the farm complex and it processes 60.000 litres of milk per day. An anaerobic partially buried mixed-flow digestion system, with the input of beef manure, whey and biomass, produces 482 Nm3/h of biogas of the lower thermal power between 5,5 and 6,5 kW/Nm3, a solid bio-fertiliser phase with 30% of DM, a liquid fertiliser phase with 5% of DM, whereby the JENBACHER JMS 320 GSB/LC co-generation plant with a biogas engine, which consists of a gas engine, generator, interchanger and noise suppressor on exhaust gasses, achieves a power output of 1000 kW, a heat output of 1060 kW and an annual electricity production of over 7.000.000 kW. Table 1 presents the composition of biomass as an input raw material which is inserted daily into an anaerobic digester. The preparation of mixture is carried out in the reception pit by mixing pure water with the liquid phase from the lagoon due to the DM correction which is necessary for the digestion. The digester is filled with 160 m^3 of mixture per day in two-hour intervals. The mixture passes through a chopper for the purposes of shredding the silage. The digester has got three chambers and each chamber has got its own heating and mixing systems. This kind of production does not require a reservoir, i.e. gas reserves. The digester is provided with a safety system against the excess of gas and increased pressure. The material is kept in the digester for 21 days. After that period the material is transferred to the separator for the purposes of separating the solid phase.

Table 1. The structure of a daily input of raw materials in the digester

MANURE	30% (12.045 t/year)	(33 t/day)
WHEY	40% (16.060 t/year)	(40 t/day)
SILAGE	30% (12.045 t/year)	(33 t/day)

Results and discussion

Figure 1 presents the data on the annual production of biogas and electricity for the period between 2011 and 2015. During the first year of the use of the digester the amount of produced biogas was 2.104.985 m^3, while in 2015 this amount was 3.151.775 m^3, which indicates the growth of 49.73%. The increase of 56.23% in electricity production is also visible, i.e. the increase went from 4,740.428 kWh to 7.406.070 kWh. This growth is a result of an efficient performance of the digester, of a competent process management, as well as of an increase in the total annual amount of biomass necessary for the production of biogas. The latter is also the result of an increased number of heads on the farm, as well as of an increase in the milk production and processing in the dairy. Table 2 presents one with the energy balance of the digester for the year 2015, where one can see that the Electric Power Industry of Serbia was delivered with 6.813.584 kWh of electricity, while 592.486 kWh were spent for personal needs and losses. Generally speaking, the realisation of processes in this plant is the best possible manner of utilising all outputs from the "Lazar Ltd". which comprises three production sectors:

- farm – milk production - manure
- dairy – milk processing – whey
- husbandry – cow nutrition – silage

Furthermore, all advantages of anaerobic digestion which have a positive effect on the environment are gained and these comprise the following: reducing the vaporisation of methane and ammonia, lowering the amount of nitrate in groundwater, limiting the emission of carbon dioxide, reducing

unpleasant odours, viscosity and the amount of pathogenic bacteria as well as improving conditions for storing manure, etc.

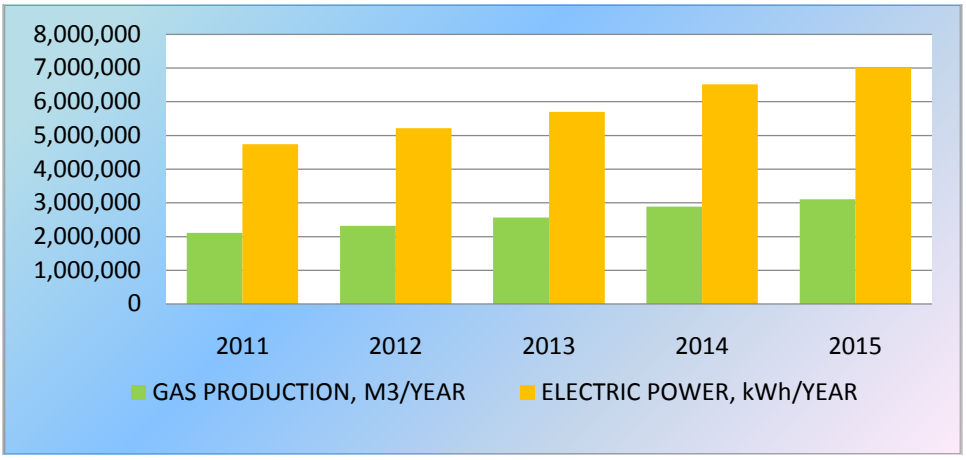

Figure 1. The total production of biogas and electricty in the period between 2011 and 2015

Table 2. Energy balance of the digester for 2015

	Manure	Whey	Silage	Total
Material input t/year	12.045	16.060	12.045	40.150
Gas production m^3/t	45	35	170	/
Gas production m^3/year	542.025	562.100	2.047.650	3.151.775
Methane %	55	53	55	/
Energy production kWh	2.981.137	2.979.130	11.262.075	17.222.342
Electric power production kWh	Personal consumption and losses 8%			7.406.070
Electric Power in the Serbian Power Network kWh	17,3%	13,3%	65,4%	6.813.584

Conclusions

Biomass is a biodegradable part of products, waste and residues of biological origin which comes from agriculture (including plant and animal substances), forestry and related industries, as well as a biodegradable part of industrial and communal waste. Although there is a significant potential of renewable energy resources, the Republic of Serbia has a small number of manure collection systems – open lagoons, manure is rarely used as a fertiliser, there is a lack of mechanisation for handling manure, there are no modern plants for biogas production, nor is there sufficient knowledge of and information on modern technologies. However, one can notice a growing interest among the owners of agricultural farmsteads in using new methods and technologies. The process of biogas production is at the same time a smart and efficient manner of waste management and it prevents the release of harmful and toxic compounds which greatly affect the environment. In relation to that, the project of the biogas plant of the "Lazar Ltd." is extremely significant in the field of renewable energy sources as it significantly contributes to sustainable development since this biogas power plant is one of the first on the territory of the Republic of Serbia.

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EVALUATION OF WATER DELIVERY EFFICIENCY IN IRRIGATION CANAL UNDER EXISTING MANAGEMENT STRATEGY USING HYDRAULIC MODEL

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Abstract

Managing water distribution in irrigation canals aims timely and in needed quantity implementation of supplies of water to irrigated area without excessive spillage i.e. at reduced conveyance loss and adequate water use for irrigation of the crops. This article analyzed existing management practice of water distribution in irrigation canal in view of efficiency. The study was carried out for an existing main irrigation canal. The operational water loss was evaluated by indirect quantitative approach. Hydraulic simulation model of the irrigation canal was created using hydraulic software HEC-RAS. Changes in hydraulic conditions due to existing structures along the canal course were taken into account in the model. The water operational loss was determined by simulation with HEC-RAS model in steady state conditions. The model was used to study the influence of the operating conditions on the size of loss. Results obtained show the influence of the management practice of the water distribution and operation conditions and maintenance of the canal on the magnitude of operational loss.

Keywords: water distribution, conveyance loss, steady state, simulation, HEC-RAS.

Introduction

Water distribution is the main technological process in the irrigation system and represents the transport of water from the water source to the irrigation area where the water volumes are distributed among the users in quantity and time. Managing water distribution in irrigation canals aims timely and in needed quantity implementation of supplies of water to irrigated area without excessive spillage or excess energy i.e. at reduced conveyance loss and adequate water use for irrigation of the crops. In many irrigation systems with manually operated open canals the existing practice of water allocation is the so-called planned water use, where pre-contracting between the water supplier and the water user is introduced for the time of delivery of the water volumes for irrigation. The water is delivered according to a weekly/decade water allocation plan, based on water requirements of the users within the limits of the seasonal contracts. The water allocation plan for the irrigation canal is based on the water balance for each canal section, taking into account the limited flow of the water source and the capacity of the canal itself. (Trifonov and Patamanska, 2006). Even when the water delivery schedule for the irrigation canal is properly drawn up and precise executed, there are large operational water loss in planned water use, the main reasons for which are:

- Delayed start of water supply in a remote section from the beginning of the irrigation canal. Because of water wave's propagation observed in the canals during changing discharges, one has to wait a long period of time, sometimes many hours, to arrive the needed water discharge and then to start water supply. By this reason, a certain volume of water is accumulated in the canal which, after the irrigation is stopped, escapes unused thus generating significant non-productive operational water loss (Ankum, 1993).
- Daily irrigation. Since it is watered only during a certain period of the day, and the flow in the main canal is continuous, much of the water supplied expires unused after the end of the irrigation.

The highest water loss is in the main irrigation canal, since there the largest water volumes are being allocated. The water loss should be reduced and thus loss management is needed. The aim of this

study is to analyze and evaluate the behavior of the main irrigation canal in steady state flow conditions, to evaluate the operational loss in existing management practice of water allocation and to study the influence of the operation conditions and the canal maintenance on the extent of operational water loss.

Material and methods

Description of the study canal

The study canal is a part of an existing irrigation canal - the main canal M-1 of „Sredna Tundja“ irrigation scheme from a distribution shaft in the region of village Gavrailovo to Sotirya inverted siphon 18.5 km in length (fig. 1.) The canal has trapezoidal cross-section with 2.5 m bottom width, a side slope of 1.5, the average bottom slope of the 0.00006. The canal is designed for a discharge of 20.5 m^3/s and is separated into four canal sections with by gated cross-structures, situated in the transitional sections with a rectangular cross section. The canal is lined with concrete. Along the canal course there are three laterals, located in the end of the each of the first three canal reaches: an open canal and a pipeline designed for a discharge of 1.5 m^3/s and another open canal designed for a discharge of 2 m^3/s.

Figure 1. Map of Sliven region showing main canal of „Sredna Tundja“ irrigation scheme
Source: www.topomaps.info

Operational water loss in main irrigation canal can be quantified by:

- Direct measurements of the water discharges entering and exiting from the canal sections. In this case periodic simultaneous measurements of two water discharges are required, which is difficult to obtain in-situ conditions.
- Indirect method for estimating operational water loss using a model. Operational water loss is determined after conducting analysis of the steady state flow as a difference between the water volume in the canal under operating mode of feeding a water user and this one after switching it off.

The irrigation canals are divided into sections with gated cross-structures, which causing the back water profiles in the canal. Accurate assessment of the water volume accumulated in the canal when changing from one steady state of flow to another can be obtained by hydraulic simulation model capable of modeling the hydraulic structures along its course.

Description of software used

In this study, the freeware software HEC-RAS, Version 4.1 (Hydrologic Engineering Center - River Analysis System) developed by U.S. Army Corps of Engineers, is selected to create a simulation model of the study canal. Using this software, one-dimensional hydraulic calculations are performed in a branched network of natural and / or artificial channels. The software system includes a user interface, steady flow model, unsteady flow model and modules that provide graphical and tabular presentation of the results. It can simulate steady and unsteady flows in open channel. For the steady state conditions, water surface profile can be simulated in critical, supercritical and mixed

flow regimes (US Army Corps of Engineers, 2010). For conducting hydraulic modeling and simulation of the water surface profile in irrigation canal data are required for its geometry, the boundary conditions, the water discharge, the canal roughness, geometric description of the hydraulic structures long the canal course, such as gates, culverts, weirs. Introducing the geometry of the canal includes defining the profile of the canal bed of the study reach by setting series of cross-sections that longitudinally define its shape. For the calculation of the longitudinal water surface profile at steady flow, the one-dimensional equation of energy (Bernoulli equation) is integrated by the standard step method. In order to be able to start the calculation, a discharge upstream of the canal and a stage downstream are set as boundary conditions. For the interior points the stage is estimated keeping the water discharge constant. As results of canal flow simulation the following hydraulic parameters: depth/ water surface elevation, energy grade line elevation, friction slope, flow velocity, critical depth/critical depths line elevation, water volume in the canal and others can be determined.

Results and discussion

Hydraulic simulation model of the study canal was created using hydraulic software HEC-RAS (Figure 2). It was built on the basis of the design parameters of main irrigation canal M-1-2. When creating the simulation model, a realistic representation of the existing situation was sought. To reproduce the real geometry of the canal, five cross sections are set - at the canal inlet and out let and at the end of each canal reach (Figure 3).

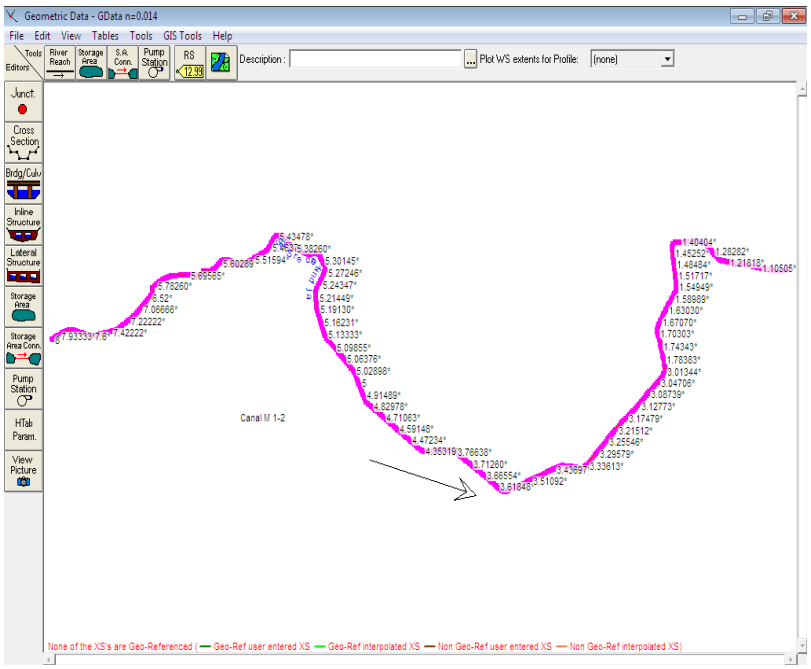

Figure 2. Lay-out of study canal

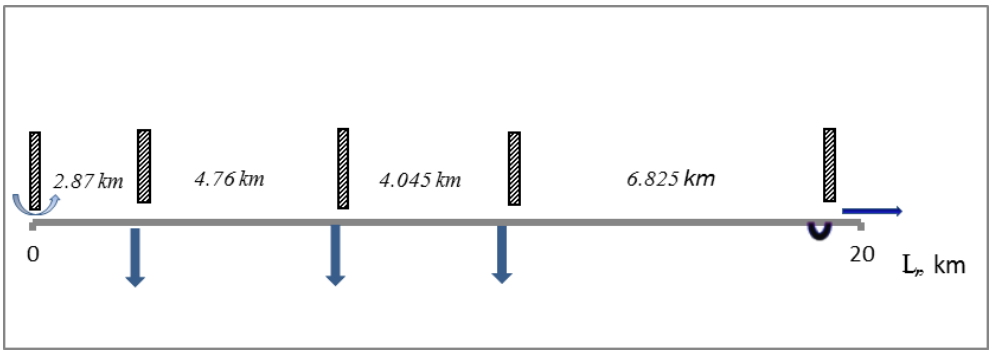

Figure 3. Calculation model

Changes in hydraulic conditions due to existing structures along the canal course were also taken into account in the model. Geometric descriptions of three gated hydraulic structures situated along the canal course (Figure 4b) and cross sections with water users were introduced. Since the canal is not completely lined, changes in the lining are recorded by entering two values of the roughness coefficient for the lined and unlined part of the bank (Figure 4a).

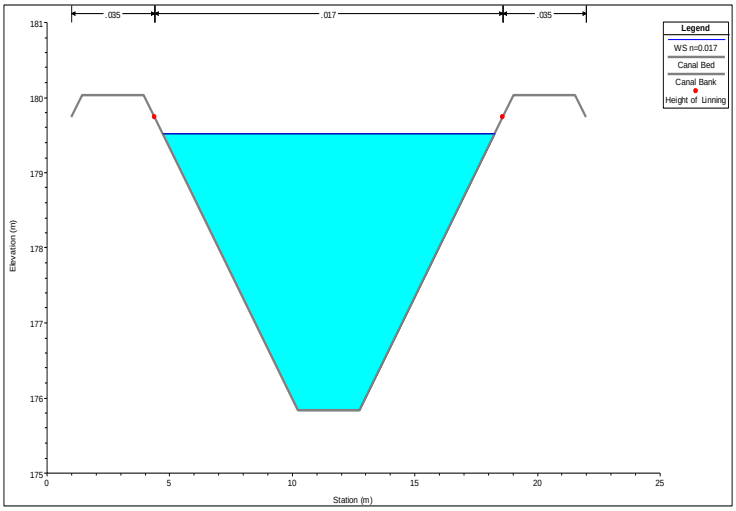

Figure 4a. Canal cross section plot

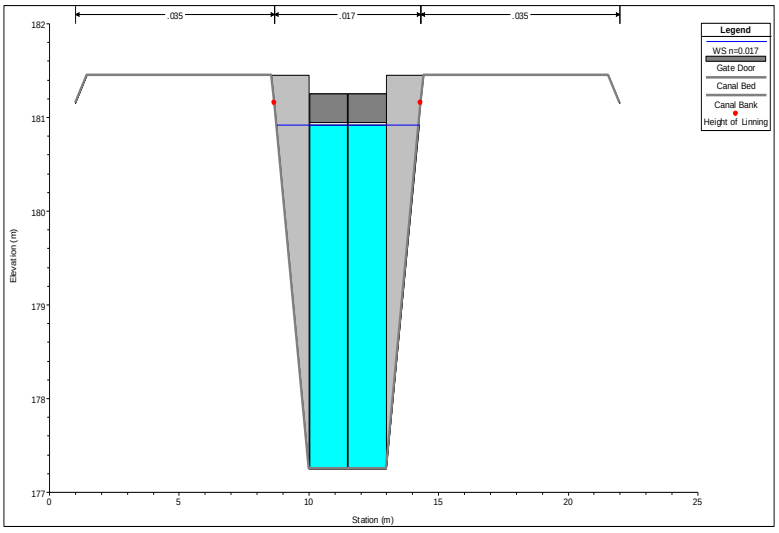

Figure 4b. Canal cross section plot

The water operational loss in existing management practice of planned water use was determined by simulation with HEC-RAS model of the study canal in steady state conditions. Steady flow simulations in the canal were performed in the following two cases:

- The canal gates are fully open and the canal flows at the maximum water discharge of 20.5 m^3/s supplying water to only one of lateral canals.
- The canal flows at decreased discharge after stopping water supply to a corresponding lateral canal. The case of simultaneously stopping the water to the lateral canal and correspondingly decreasing the water discharge at the study canal inlet was considered.

To investigate the influence of canal maintenance on the magnitude of operational water loss, the numerical experiments were conducted for several values of the roughness coefficient of the lined part of the canal cross section: 0.014, 0.017, 0.020, 0.025 and the roughness coefficient equal to 0.035 for the unlined part. The values of the roughness for concrete lined canal and grassed surface of unlined part of the banks were selected in tables published in (Chow, 1959). An exception is the

maximum value of the coefficient of roughness of 0.025, the choice of which aims at simulating a severe deterioration of the operational condition of the canal. A total of 12 experiments were conducted.

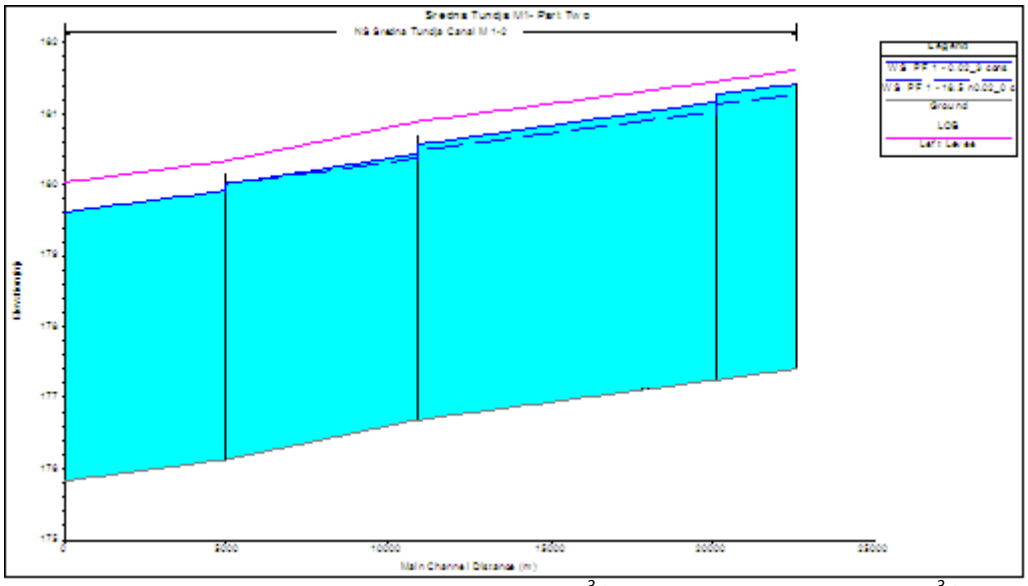

Figure 5. Water surface profiles at an inlet discharge of 20.5 m^3/s and a water discharge of 2 m^3/s supplied to a lateral canal and at an inlet discharge of 18.5 m3/s and n = 0.02 .

Fig. 5 shows the water surface profile obtained at an inlet discharge of 20.5 m^3/s and a discharge of 2 m^3/s supplied to the lateral canal at the end of the third canal reach and the water surface profile at an inlet discharge of 18.5 m^3/s and for roughness coefficient n = 0.02. The water surface profiles are backwater curves among which the accumulated water volume in the canal is located. This volume is determined as a difference of the results obtained for the canal volume under steady state flow conditions with maximum inlet discharge and working lateral canal and the canal volume after stopping the supply of water to it. The accumulated volume in the canal forms the operational water loss. The results for the accumulated water volume in the canal reaches for the different roughness coefficients are presented in Table. 1. and Fig. 6. It can be seen that if the user is located farther from the canal inlet, operating water loss is increased significantly. Poor operating conditions and canal maintenance also result in increased operational water loss.It is important to reduce operational water loss for increasing efficiency. Various principles and methods of canal management aimed at reducing water loss and increasing efficiency of water allocation were developed. Steady-state analysis of an irrigation canal flow using simulation model can be useful for choosing of an appropriate procedure for efficient canal management at any particular case.

Table 1. Accumulated water volume in the canal reaches

n	Canal reach lenght in km		
	2.87	7.63	11.675
	Wacc, m^3	Wacc, m^3	Wacc, m^3
0.014	160	4310	18780
0.017	200	4750	20330
0.02	230	5080	21730
0.025	380	6510	22760

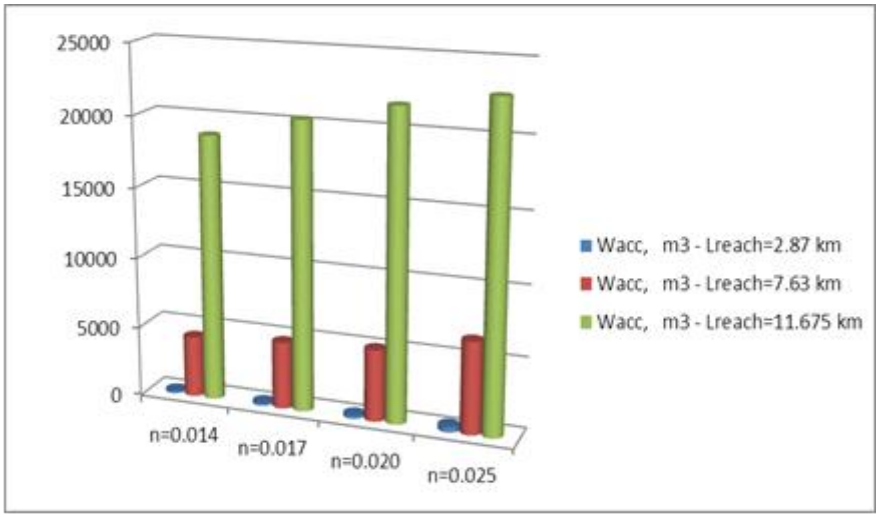

Figure 6. Accumulated water volume in the canal reaches

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EFFECTS OF DIFFERENT CHEMICAL PRETREATMENTS ON CELL WALL COMPOSITION AND ASH CONCENTRATION OF SWEET SORGHUM BAGASSE FOR BIOETHANOL PRODUCTION

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Abstract
Pretreatment is one of the key processes in lignocellulosic bioethanol production, which is needed to improve accessibility of enzymes to cellulose. This study was conducted to investigate the effects of different chemical pretreatments on cell wall composition and ash concentration of sweet sorghum bagasse. 9 different pretreatment methods used in the study can be categorized into 3 different methods such as dilute sulphuric acid (1, 1.5 and 2 % H_2SO_4 w/v), dilute sodium hydroxide (1, 1.5 and 2 % NAOH w/v) and sequential dilute sulphuric acid and sodium hydroxide (1 % H_2SO_4 w/v + 0.5 M NAOH, 1.5 % H_2SO_4 w/v + 0.5 M NAOH and 2 % H_2SO_4 w/v + 0.5 M NAOH). According to results, while 2 % H_2SO_4 w/v + 0.5 M NAOH gave the highest cellulose (91.51 %) and lowest lignin (1.7 %) concentrations, the lowest cellulose (65.11 %), hemicellulose (0.4 %), and highest lignin concentrations (23.42 %) were provided by 1.5 % H_2SO_4 w/v among pretreatments. Cellulose, hemicellulose and lignin contents of sweet sorghum bagasse after sodium hydroxide pretreatments ranged from 76.72 to 79.88, 11.75 to 14.62, and 2.05 to 4.11 %, respectively. The most appropriate cell wall composition for enzymatic hydrolysis was derived from sequential dilute sulphuric acid and sodium hydroxide pretreatments due to the fact that they provided the highest cellulose (90.68 – 91.51 %), lowest lignin (1.7 – 3.41 %) and desirable hemicellulose (1.10 – 1.82 %) contents. However, enzymatic hydrolysis must be done to learn which method enables the highest fermentable sugar production.

Keywords: Lignin, cellulose, hemicellulose, biomass.

Introduction

The inevitable depletion of fossil fuel sources and their adverse effects on environment, particularly greenhouse gas emissions has strengthened the interest in renewable energy sources (Hahn-Hagerdal et al. 2006; Chen et al. 2012; Dogaris et al. 2012). Among renewable energy sources, advanced biofuels derived from lignocellulosic biomass such as agricultural residues, forest products, and energy crops are the potential resources for the production of second generation ethanol reducing substantially carbon emissions (Liu et al. 2008; Arora et al. 2010; Aita et al. 2011). The main components of lignocellulosic biomass are two structural carbohydrates (cellulose and hemicellulose) and lignin (Sipos et al. 2009). Cellulose and hemicellulose can be hydrolyzed to fermentable sugars by enzymes prior to microbial fermentation but lignin is highly resistant to deconstruction and restricts enzymatic hydrolysis because of its intricate structure (Aita et al. 2012; Cao et al. 2012). Hemicellulose and lignin form a physical barrier which avoids enzymes to access cellulose (Qing and Wyman et al. 2011). Therefore, lignocellulosic biomass must be pretreated before enzymatic hydrolysis to remove lignin and/or hemicellulose thereby increase enzyme accessibility and cellulose degradation (Hendricks and Zeeman 2009; Zhang et al. 2010). For the sustainable lignocellulosic bioethanol production, pretreatment must be carry out in maximum efficiency because it covers approximately 30 – 40 % of the total processing cost (Eggeman and Elander 2005; Zhang et al. 2009; Alvira et al. 2010). Numerous pretreatment methods have been

developed for improving hydrolysis of lignocellulosic biomass and categorized as mechanical (e.g., milling, grinding), thermal (e.g., steam explosion), chemical (e.g., acid, alkaline) and biological (e.g., fungi) processes or combinations of these methods (Aita et al. 2011; Cao et al. 2012; Chen et al. 2012). Among these, chemical pretreatments, usually performed by dilute acids (e.g., sulphuric acid, hydrochloric acid) and alkalines (e.g., sodium hydroxide, lime), have been found to be the most cost effective (Pandey et al. 2000; Barcelos et al. 2013). Dilute sulphuric acid (H_2SO_4) pretreatment enables conversion of hemicellulose to monomeric sugars and thereby disrupt the lignocellulosic composite material linked by covalent bonds, hydrogen bonds and van der Waals forces (Li et al. 2010; Shatalov and Pererira 2012). However, it can result in the formation of polysaccharide degradation products that are often inhibitory to downstream fermentation organisms and lower the overall sugar yields (Fengel and Wegener 1984; Ramos, 2003; Li et al. 2010). Dilute sodium hydroxide (NAOH) pretreatment increases internal surface of cellulose and decreases the degree of polymerization and crystallinity, which provokes lignin disruption (Taherzadeh and Karimi 2008; Gao et al. 2013). In comparison with the dilute acid, it does not cause corrosion and is more effective in solubilizing the lignin but have a limited effect on solubility of hemicellulose (Carvalheiro et al. 2008; Gao et al. 2013; Menezes et al. 2014). Apart from these, a combined process using sequential dilute acid and alkali pretreatment steps have received increasing attention as a promising strategy because it can remove largely of lignin and hemicellulose fractions (Weerasai et al., 2014). In this process, hemicellulose is eliminated by dilute acid pretreatment in the first stage, while second stage is carried out by dilute alkali pretreatment primarily for delignification (Gao et al. 2012). Sweet sorghum is an annual C_4 crop which can be adapted to warm and dry areas thanks to its high drought tolerance. Its juicy stalk has high concentrations of fermentable sugars, mainly sucrose, making it one of the most promising energy crops for first generation bioethanol production (Cao et al. 2012). Besides, sweet sorghum bagasse is a valuable feedstock for lignocellulosic bioethanol production due to its high concentrations of structural carbohydrates, which can be hydrolyzed to fermentable sugars. This study was carried out to investigate the effects of different chemical pretreatments on cell wall composition and ash concentration of sweet sorghum bagasse for bioethanol production.

Material and methods

Sweet sorghum was harvested at research and experimental area of Field Crops Department of Cukurova University, Adana, Turkey when grains were at a hard dough stage. Leaves, roots and panicles were removed by hand then stalks were crushed five times to extract the juice through a roller press. 1 kg bagasse sample was washed with distilled water at least three times to remove remaining soluble sugars in the stalk. Finally, it was dried in an oven at 65 °C until a constant weight was achieved then ground to pass through a 1 mm sieve. 9 different pretreatment methods used in the study can be categorized into 3 different groups such as dilute sulphuric acid (1, 1.5 and 2 % H_2SO_4, w/v), dilute sodium hydroxide (1, 1.5 and 2 % NAOH, w/v) and sequential dilute sulphuric acid and sodium hydroxide (1 % H_2SO_4, w/v + 0.5 M NAOH, 1.5 % H_2SO_4, w/v + 0.5 M NAOH and 2 % H_2SO_4, w/v + 0.5 M NAOH). Untreated bagasse was used as a control in the study. The experiment was arranged according to complete randomized plot design with 4 replications. In dilute sulphuric acid and sodium hydroxide pretreatments, 10 gr of dry bagasse samples were slurried with 100 ml 1, 1.5 and 2 % H_2SO_4 (w/v) and NAOH solutions in a 250 ml flasks and heated in an autoclave at 121 °C for 30 min. After treatments, each sample were washed three times with distilled water and dried at 65 °C until a constant weight was achieved. Sequential dilute sulphuric acid and sodium hydroxide pretreatments were carried out as two-stages, differently from the other pretreatments. In the first stage, 10 gr of dry bagasse samples were slurried with 100 ml 1, 1.5 and 2 % H_2SO_4 (w/v) solutions in in a 250 ml flasks, then samples were washed with distilled water and dried at 65 °C until a constant weight was achieved. In the second stage, dried samples were slurried in 0.5 M NAOH solutions with solid: liquid ratio of 1:20 g/ml (Barcelos et al., 2013), then heated in an autoclave at 121 °C for 30 min. After treatments, each sample were washed with distilled water and dried at 65 °C until a constant weight was achieved. Cell wall compositions of samples were determined by Van Soest

(1963) method. In addition, ash concentrations of samples were determined by Kutlu, (2008) method in the study. Variance analysis of experimental results were carried out using JMP 7.0 (SAS Institute, 1994) statistical software and least significant differences (LSD) test was used to test the differences among means.

Results and discussion

As shown in Table 1, DM (Dry matter) loss ranged from 41.99 to 76.54 %. The pretreatments significantly differed in terms of DM loss, with 1.5 % H_2SO_4 (w/v) + NAOH leading the highest DM loss (76.54 %), followed by 2 % H_2SO_4 (w/v) + NAOH (76.28 %) and 1 % H_2SO_4 (w/v) + NAOH (75.74 %). On the other hand, dilute H_2SO_4 pretreatments led to significantly higher DM loss (43.15 – 52.31 %) compared to dilute NAOH (41.99 – 48.73 %) pretreatments. These results were in accordance with findings of Lee et al. (2015) and E Silva et al. (2015). Lee et al. (2015) reported that while dilute H_2SO_4 pretreatments led to DM losses between 42.2 – 58.1 %, DM loss was increased by sequential dilute H_2SO_4 and NAOH pretreatment up to 71.5 % in corn stover. In addition, E Silva et al. (2015) reported that sequential dilute H_2SO_4 and NAOH pretreatment lead to significantly higher DM loss with of 35.3 % than dilute H_2SO_4 pretreatment with of 28.6 %.

Table 1. Effects of different pretreatment methods on DM loss, cell wall composition and ash concentration of sweet sorghum bagasse

Pretreatments	DM Loss (%)	Cellulose (%)	Hemi-cellulose (%)	Lignin (%)	Ash (%)
Untreated	-	44.98	24.81	12.98	1.94
1 % H_2SO_4 (w/v)	43.15 h	65.21 g	0.90 f	20.96 c	1.19 c
1.5 % H_2SO_4 (w/v)	49.82 h	65.11 h	0.40 g	23.42 a	1.63 b
2 % H_2SO_4 (w/v)	52.31 d	65.13 h	0.44 g	22.91 b	1.74 a
1 % NAOH (w/v)	41.99 ı	76.72 f	11.75 c	4.10 d	0.83 d
1.5 % NAOH (w/v)	46.61 g	77.89 e	14.63 a	2.07 g	0.79 d
2 % NAOH (w/v)	48.73 f	79.88 d	13.28 b	2.05 g	0.59 g
1 % H_2SO_4 + 0.5M NAOH (w/v)	75.74 c	91.21 b	1.82 d	2.49 e	0.70 e
1.5 % H_2SO_4 + 0.5M NAOH (w/v)	76.54 a	90.68 c	1.72 e	2.21 f	0.65 f
2 % H_2SO_4 + 0.5M NAOH (w/v)	76.28 b	91.51 a	1.80 d	1.70 h	0.67 ef
Mean	56.80	78.15	5.19	9.10	0.98

Significant differences were observed in cellulose concentration among pretreatments, ranging from 65.11 to 91.51 %. All pretreatments tested in the study increased cellulose concentration of sweet sorghum bagasse. The highest value was observed in 2 % H_2SO_4 (w/v) + NAOH, followed by other dilute H_2SO_4 and NAOH pretreatments. Differently from the DM loss, dilute NAOH pretreatments provided significantly higher cellulose concentrations than dilute H_2SO_4 pretreatments. Similar results also observed in previous comparative studies (Lee et al. 2015; E Silva et al. 2015). Lee et al. (2015) reported that cellulose concentration of corn stover achieved by sequential dilute H_2SO_4 and NAOH pretreatments was found between 80.4 – 81.5 % whereas H_2SO_4 pretreatments led the cellulose concentration between 43.1 – 53.0 %. In addition, E Silva et al. (2015) stated that sequential dilute 1.1% H_2SO_4 (w/v) and 0.5 M NAOH pretreatments increased the cellulose concentration of giant reed from 30.7 to up to 81.5 % whereas highest cellulose concentration derived by dilute H_2SO_4 pretreatments was found as 53.0 %. The hemicellulose concentrations after pretreatments ranged from 0.40 to 14.63 % in the present study. The highest value was achieved by 1.5 % NAOH (w/v) whereas the lowest was in 1.5 % H_2SO_4 (w/v). Dilute H_2SO_4 pretreatments

decreased hemicellulose content of sweet sorghum bagasse between 96 – 98 %. Higher efficiency of dilute H_2SO_4 in removal of hemicellulose was also reported by previous authors in sugarcane (Barcelos et al. 2013; Jiang et al. 2013), sweet sorghum (Zhang et al. 2011) and bulbous canary grass (Pappas et al. 2014). Dilute H_2SO_4 pretreatments caused the significantly lower hemicellulose concentrations, when compared to the other pretreatments, indicating that they are more effective in hemicellulose solubilisation than the other pretreatments. This result is also supported by different authors (Weerasai et al. 2014; Lee et al. 2015; E Silva et al. 2015). Lee et al. (2015) reported that Dilute 1 % H_2SO_4 (w/v) pretreatment reduced hemicellulose concentration of switchgrass by 1.2 %, whereas sequential dilute 1 % H_2SO_4 (w/v) + 2 % NAOH (w/v) pretreatment reduced the hemicellulose concentration up to 5.5 %. NAOH pretreatments tested in the study provided the hemicellulose removal between approximately 41 – 53 %, which is comparable to reported by Cao et al. (2012) (45 %) in sweet sorghum and Wang et al. (2010) (41 %) coastal bermuda grass. The pretreatments were significantly differed in terms of lignin concentration, ranging from 1.70 - 23.42 %. While the highest lignin concentration was achieved by 1.5 % H_2SO_4 (w/v), the lowest was in 2 % H_2SO_4 (w/v) + NAOH. All dilute H_2SO_4 pretreatments significantly increased the lignin concentrations, differently from the dilute NAOH and sequential dilute H_2SO_4 and NAOH pretreatments. In spite of the fact that dilute acid pretreatments are generally more effective in extracting the cellulose and hemicellulose fractions than lignin, but only limited amount of lignin could be hydrolyzed compared to cellulose and hemicellulose because the lignin concentration was stabilized by a condensation reaction under acidic conditions (Ramos, 2003; Kim and Kim 2013; Lee et al. 2015). Similar to our results, previous authors also indicated that dilute H_2SO_4 pretreatment remarkably increased the lignin concentration of sugarcane (Barcelos et al. 2013), switchgrass (Li et al. 2010), corn stover (Lee et al. 2015) and sorghum (Zhang et al.2011; Wang et al. 2013). On the other hand, dilute NAOH and sequential H_2SO_4 and NAOH pretreatments led to considerable lignin removal in the study. Our results are associated with those of Xu et al. (2010), Cao et al. (2012), Kim and Kim (2013), Wang et al. (2013), Weerasai et al. (2014), Lee et al. (2015) and E Silva et al. (2015). Xu et al. (2010) reported that 0.5, 1 and 2 % (w/v) dilute NAOH pretreatments provided lignin reduction between 62.9 – 85.8 % in switchgrass, Cao et al. (2012) reported that 2 % dilute NAOH (w/v) pretreatments reduced the lignin from 10.8 to 1.68 % in sweet sorghum, Kim and Kim (2013) declared that 4 % H_2SO_4 (w/v) + 10 N NAOH pretreatment enabled the lignin reduction with the ratio of 70 % in empty palm fruit bunch fiber, Wang et al. (2010) stated that 3 % dilute NAOH (w/v) pretreatment decreased the lignin concentration of coastal bermuda grass from 19.33 to 2.82 %, Weerasai et al. (2014) reported that lignin concentration of rice straw was eliminated between 72 – 93 % by sequential dilute H_2SO_4 and NAOH pretreatments. Lee et al. (2015) reported that 12 different dilute H_2SO_4 pretreatments led to increase in lignin concentration of switchgrass from 14.2 to between 21.6 and 32.1 % whereas 2 % dilute NAOH (w/v) pretreatment after dilute H_2SO_4 pretreatment led to decrease lignin concentration up to 4 %. E Silva et al. (2015) reported that sequential dilute H_2SO_4 and NAOH pretreatment (1.1 % H_2SO_4 w/v + 0.5 M NAOH) reduced lignin concentration of giant reed from 18.49 to 10.05 % whereas 1.1 % H_2SO_4 (w/v) pretreatment increased lignin concentration up to 24.75 %. Lower ash concentration may be considered as an advantage, because biomass containing salts solubilize in the hemicellulose and cellulose hydrolysates during pretreatment. This increase in the concentration of ions leads to an increase in the osmotic pressure in the medium, hindering the fermentability of the generated hydrolysates (E Silva et al. 2015). Ash content of sweet sorghum bagasse ranged from 0.59 to 1.74 %. The pretreatments were significantly differed in terms of ash concentration, with 2 % H_2SO_4 (w/v) pretreatment producing the highest lignin concentration whereas the lowest was in 2 % NAOH (w/v). All pretreatments tested in the study decreased the lignin concentration of sweet sorghum bagasse. Our findings are in accordance with those of Jiang et al. (2013) in which dilute H_2SO_4 pretreatment reduced the ash concentration of sugarcane from 5.7 to 5.3 % and Weerasai et al. (2014) in which sequential dilute H_2SO_4 and NAOH pretreatment considerably decreased the lignin concentration of rice straw. Furthermore, our findings coincide

with those of Wang et al. (2013) in which 0.5 % H_2SO_4 (w/v) pretreatment increased the ash concentration of sorghum from 2 to 4.6 %.

Conclusions

Sequential dilute H_2SO_4 and NAOH pretreatments provided the most appropriate cell wall composition for enzymatic hydrolysis among all pretreatments tested in the study, due to the substantially increased cellulose, and reduced lignin and hemicellulose concentrations. However, considerably higher DM loss (90.68 – 91.51 %) in these pretreatments may be a challenge for satisfactory fermentable sugar production from sweet sorghum bagasse during enzymatic hydrolysis. Therefore, enzymatic hydrolysis must be done to learn which method enables to the highest fermentable sugar production.

Acknowledgments

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SOCIAL DIMENSIONS OF ENERGY DEVELOPMENT IN RURAL AREA

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Abstract
Any and all human interference will have effects on the environment in a way. The experts working in development are working hard to have interventions to improve the quality in life of their local community. It could be a city or a town or even a village, this depends on the existing natural conditions, the geographical location, the economic environment that was created and the local community that lives there which actually can be considered the most decisive. The lives of communities, the direction of developments at local levels are largely influenced by local governments, and their decisions often determine the situation of the given settlement for years. In the development of local governments, the protection of the environment and the management of the resources available play a decisive role in economic development. Their interventions primarily and directly serve to strengthen the environmental dimension of sustainability while contributing substantively to the promotion of economic growth. Within the local society, the most open layer of environmental awareness is youth, which in almost all segments of its life strives to take this very important principle into account. The goal of the research is to compile a comparative study on the use of renewable energy sources for a local government and for the population, for young people. In our primary research we examined the attitude of the city and population towards the use of green energy and the impact of local energy development on the local community.

Keywords: government system, local government, renewable energy, environment awareness, young generation.

Introduction

Renewable energies play an increasingly important role in our lives. Besides worldwide, also in our country, we can hear more about of the renewable energy, alternative energy sources and environmental protection (Hahnel, 2010). Many people are aware of the importance of environmental awareness and renewable energy sources, and by the help of some guidance, most of us know what we could or should do to protect our environment (Kovács, 2010). Much of the energy production is based today on exhaustible and non-renewable energy sources, for example in the production of heat for electricity generation and heating itself (Kovács and Mezei, 2013). The Hungarian local government system could not afford to ignore alternative energy sources during its operation. It is a fundamental feature of the system that it is characterized by responsibilities defined and delegated by the state, and on the other hand, the performance of tasks aimed at voluntarily improving the quality of life of the population. If not directly, but economically more efficient operation of these volunteered tasks are contributed by the increasingly widespread renewable energy systems in municipal institutions. Why can this be considered as a "volunteered" task? We can say in good faith that the municipalities are increasingly "getting green", but in reality they have much more economic and rational reasons, given the ever-decreasing normative support system. The application of certain "green systems" in the long run, despite the initial shocking investments, are cheaper and more economical, not to mention their environmentally-friendly operation. Obviously, the positive effects are not only enjoyed by the local governments but also by all those who make up the community of the given settlement. During our research, we wanted to compare the knowledge and methods of the Local Government of Gyöngyös and the inhabitants of the city with renewable energy sources. In order to obtain detailed results, we had to examine how

well the population knows about the types of renewable energy sources in general, such as solar energy, wind energy, hydropower, geothermal energy and biomass (Sembery and Tóth, 2004).

The local government system

The tasks of the local governments in Hungary are essentially broken down to two parts, on the one hand, mandated by the state and supported by the financial framework and on the other hand by voluntary tasks. The principle of voluntary service is the law that everything is able to work that is not forbidden, which means the local government can perform any task that is not prohibited by law. The law also stipulates that voluntary commitments cannot endanger the obligation of the obliged (Jókay et al, 2004). The assets of local governments have had two functions in the last fifteen years. The decisive part of the assets transferred to the local government by the state ensured the material conditions of local public services. The success stories of the local government system include drinking water, landline telephone network and gas pipeline developments. However, these success stories have generated a lot of tension. The development of the drinking water network was unable to follow sewage treatment and drainage capacity, the public utility was growing. The system of central government contributions and subsidies thus made the local governments into 'poor riches' and constantly do it. The most important system inconsistency of the Hungarian model is that local governments were not forced or encouraged to deal adequately with the management of this wealth (Vigyári, 2008). Over the years, with the decreasing central support, the number of tasks are constantly increasing, although over the last ten years, there is no longer just the volume of subsidies but also the quantitative reduction of the tasks to be carried out. The financing of the tasks are achieved by the local governments, not only through the subsidies received and by the increase in the number of taxpayers, but also by contributing to saving from the economical and energy efficient institutional operation. The former central, often prolific, source-user system and thinking was replaced by a new approach of the leaders of the town, which has stimulated responsibility and thoughts about the future. This new attitude, however, is not only observed in the local government, but also among the people living in the settlement and typically the young population, which is most likely to open up to new and alternative solutions and to try to protect their environment and consciously live their everyday lives.

Introduction of Gyöngyös

Gyöngyös is the second largest settlement in Heves County. It is especially famous for its vine growing, winery, commerce and, last but not least, its tourism. However, since the second half of the twentieth century, the industry has become the dominant sector, especially in the fields of microelectronics, machinery manufacturing, railway equipment manufacturing, boiler production and food industry. The industrial park in Gyöngyös won the industrial park title in 2000. The infrastructural advantages of the industrial park include the M3 motorway, so now it is only 70 km away from Budapest, the Csepel public port is 90 km away and the Danube port is 80 km away. The industrial park has an industrial railroad, the Budapest-Miskolc line is 13 km away, the Ferihegy Airport is 1 hour away, and Pipis-mountain Airport is 3 km away (TeIR, 2017).

Gyöngyös has an advanced education and training network. The Károly Róbert College aims to serve the quantitative and qualitative needs of the region's knowledge market, in particular the economics, agribusiness and tourism sectors of the North Hungarian region, with its advisory, logistic, research, training and advanced studies, event organization and information services in the field of training in environmental and rural development. The three-star Opál Hotel, which was inaugurated in 2001 by the Faculty of Business and Agriculture, and the Matra-Tan Research and Training Centre, which was established earlier, began to be built in 2001 by a successful Phare tender. The combined workforce, built infrastructure and the proximity of Budapest could have caused a drastic drop in the number of settlements in 2011 (Figure 1). The Károly Róbert College is also engaged in the development of biomass utilization methods too since 2012 (Boros and Takácsné, 2011). The income levels of the population are constantly exceeding, both the national,

county and district averages. In analysing incomes, we cannot ignore the fact that Budapest is close and presumably high proportion of people travel back and forth. In our opinion, the significant increase in the number of vehicles is related to that the employees have to travel further away to work. It is likely that the income gap is related to a gradual decline in gas consumption, which is due to the transition to alternative energy sources.

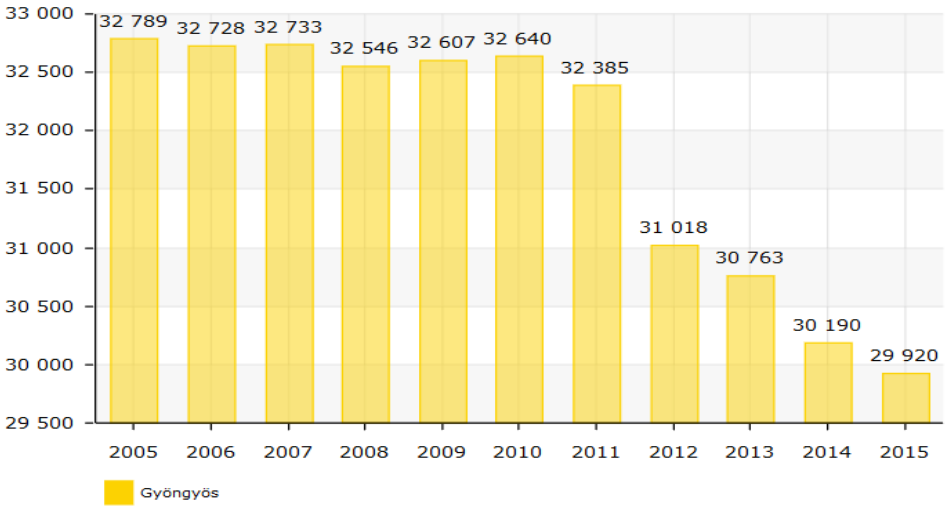

Figure 1. Population of Gyöngyös, 2005-2015 (person)
Source: TeIR, 2017

Mátravidék is fascinated by the unique natural values, natural habitats, unique flora and fauna, geological formations, historical and cultural history. The highest point in Hungary, 1014 meters high Kékes, Matrahaza, Galyatető, Mátrafüred, Mátraháza, Sástó and the 50 meters high lookout tower offers a wide range of sports, hiking and excursion opportunities throughout the year. The Farkasmályi cellar can be developed as a prominent place for wine tourism, with an especially valuable natural (platen and chestnut trees, a forest bridge with stone bridge) and its built environment (16 nationally protected cellars and wine houses). The area is famous for vine growing and its wines. The settlements in the area belong to the Mátraaljai Wine Route. The grapes and wine and the agriculture as a whole play a dominant role in the life of the region (Gyöngyös City Information Portal, 2017). The Local Government of Gyöngyös, in order to protect all these features, puts great emphasis on shaping the environmentally conscious attitude of the population, as well as the attention of local resources (Ritter, 2008). The town's website contains the environmental program of the settlement, composting information and climate change strategy. Among its developments so far, investments such as window-door replacement and optimized thermal insulation have been implemented to protect the environment (Own Research, 2017). The results of the survey reveal that the most active layer within the local society is the adults in the 30s and 40s. We assume this is the layer that has already heard about renewable energy sources and already has the financial resources from which to build these new systems. Longer and deliberately planning, taking into account the future of their children at all stages of their life (Own research, 2017). Concerning its future developments, it has issued a tender for the drainage of rainwater, for the creation of an electric charging station, for the purpose of increasing the urban green area and for the green parking lots (Gyöngyös City website, 2017).

Material and methods

As a first step in our research we became acquainted with the current situation of the use of renewable energies in Hungary and the current literature describing the development of such local government developments. We considered the study of the region's descriptive documents important (Heves County Regional Development Concept and Strategy, Gyöngyös City

Environmental Protection Program, 2012). For a more detailed analysis, we became acquainted with the TEIR Helyzet-Tér-Kép application and the interface of it, and the data within about the City of Gyöngyös (Káposzta and Nagy, 2013). One of the most commonly used methods in social science research is to take surveys based on sample and interviews. The standardized questionnaire guarantees that the same observation procedure is used for each respondent. We also used this method in our research for the total number of 408 respondents. With the questionnaire we tried to examine the willingness of the population and the local government to use renewable energies. The questionnaire was available electronically for locals and we consulted the local government beforehand.

Results and discussion

The aggregate results of the answers to our "What kinds of renewable energies do you know?" question in our questionnaire are shown in Table 1. The most, around 48.53% of respondents know solar energy as a renewable energy source (Table 1). Wind energy was known to almost the same percentage, which was 47.06% in value. This is followed by the third best known energy source: hydropower (44.36%), fourth geothermal energy (36.76%), fifth biofuel (35.78%) and least known renewable energy for the population is biomass (33.33%). 3.19% of respondents were informed of other types of energies.

Table 1. The distribution of the popularity of renewable energies among respondents (%)

No.	Renewable energy sources	The percentage of people asked know the given energy
1.	Biomass (combustible biomass, gasifiable biomass, firewood chips)	33,33%
2.	Biofuel (bioethanol, biodiesel)	35,78%
3.	Geothermal energy	36,76%
4.	Hydropower	44,36%
5.	Wind energy	47,06%
6.	Solar energy	48,53%
7.	Other	3,19%

Note: multiple responses were available. n = 408
Source: Own research and editing, 2017

Based on the results of our primary research, we were able to investigate the proportion of resident population in Gyöngyös who use some renewable energy sources. Based on the results obtained (Figure 2), nearly half of the respondents, about 44.6% of them, do not use any renewable energy sources. This result was surprising, among other things, because current EU tenders are increasingly allowing the number of subsidies to be used for renewable energy developments (MacKay, 2011). Regarding the city of Gyöngyös, solar energy is the most widely used renewable energy source, in the questionnaire we can see it is currently used by 18.4% of the respondents. As regards the location of Gyöngyös, this number may continue to increase in the future, as the utilization rate of solar collectors is significant compared to the number of sunny hours. The use of biofuels (12.3%) and hydropower (8.1%) and biomass (7.6%) can also be highlighted, which are moderately present at the level of household consumption compared to other renewable energy sources. The use of wind power (3.2%) and geothermal energy (2.7%) is negligible, and their utilization can be classified as non-recoverable energy sources for the city, one of which is mainly due to the location of the settlement.

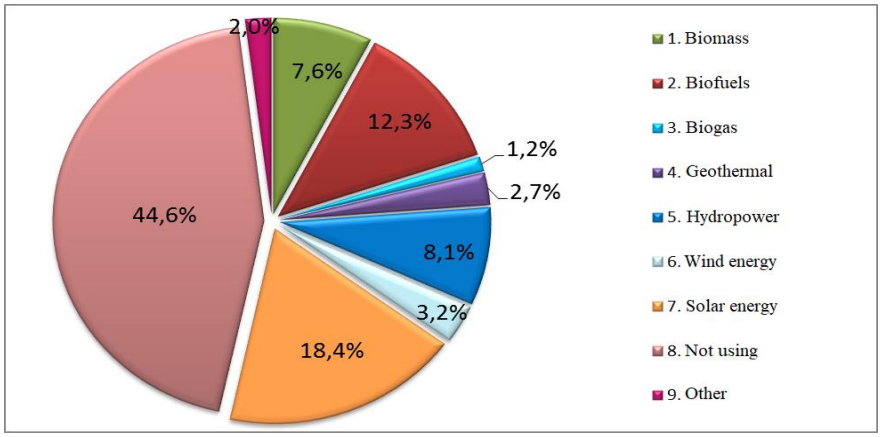

Figure 2. Use of renewable energy sources among the population of Gyöngyös
Source: Own research and editing, 2017 n = 408

In our comparative analysis, we also examined the renewable energy sources used by the local government (Figure 3). Since the local government does not use wind power in any form, therefore, it is not included in the distribution value in Figure 5 although the "not used" aspect is not relevant in this case. In our analysis, we also aimed to examine our assumption that the population and the local government use similar renewable energies. Gyöngyös can successfully develop the use of renewable energy sources through several EU tenders. The amount of investments made so far exceeds 15 million HUF, which can be concluded that this is a major development. For the time period 2016-2020, further projects or tenders will be planned to strengthen the presence and more efficient use of these energy sources (Own Research, 2017).

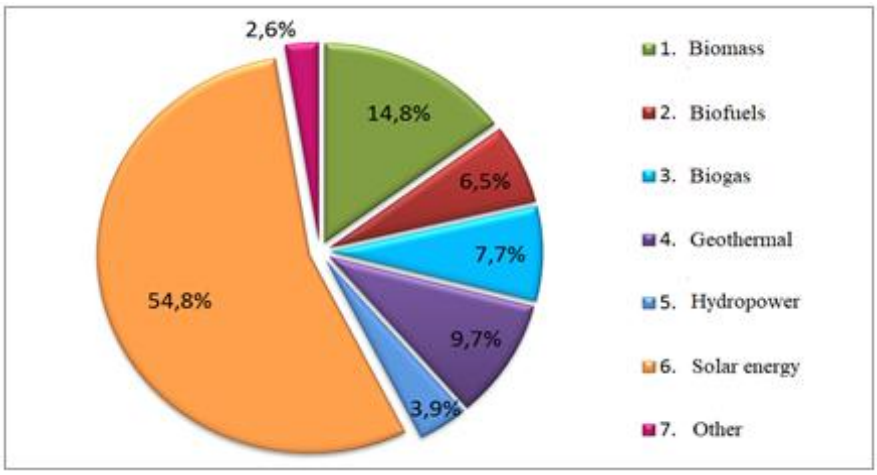

Figure 3. The distribution of the use of renewable energy sources by the municipality of Gyöngyös
Source: Own research and editing, 2017 n = 408

In case of the respondents, we considered that it is important to ask the younger generation of the issue. As the use of renewable energy sources are fundamentally a long-term investment, the positive impact on today's developments will be most appreciated by this generation. Wondering about what age it is considered necessary to educate themselves on environmental awareness. Figure 4 illustrates that the majority of young respondents (83.8%) consider environmental education as early as possible, even in a small age. Minor (10%) is the number of declarants that can solve the issue of environmental protection for involving the younger generation. The question was: *"What do you think, how important is the environmental educations?"*

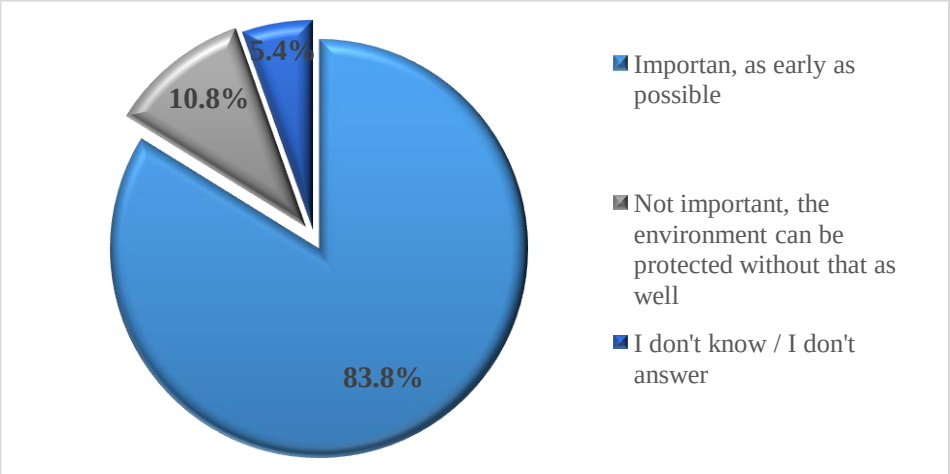

Figure 4. The students' answers about the importance of environmental education
Source: Own research and editing, 2017

Environmentally conscious thinking is indispensable to protect the environment and to maintain good living conditions for the next generation, but it is not enough. In addition to the way of thinking, it is necessary to have this view in our everyday lives. Figure 5 shows how young people live in everyday life, what they are doing and how they protect their environment. In case of Figure 5, it was an open question where the fillers could have given more answers. Generally speaking, the respondents wrote 2-3 answers in most cases, so the figure was summarized on this basis. On the figure, it can be found 9 different answers – 8 positive and 1 neutral ones.
The question was: *"What do you do in your home to protect the environment?"*

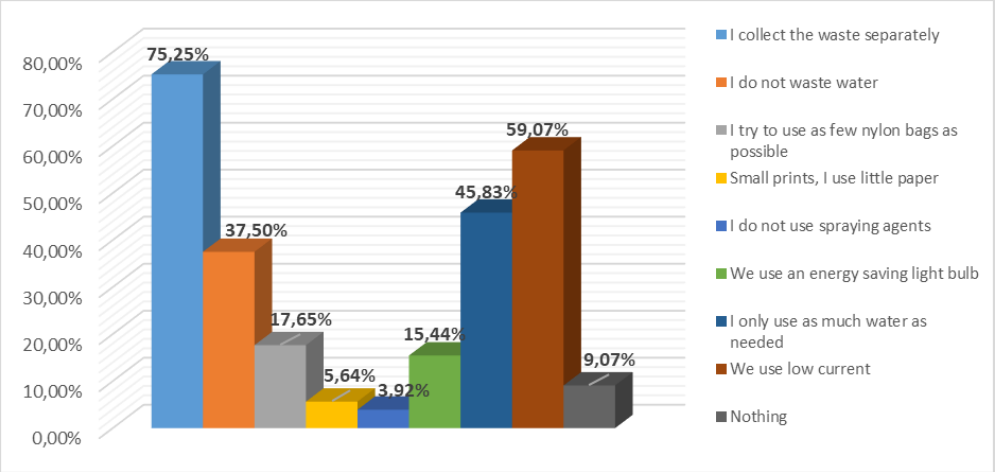

Figure 5. The distribution of answers about what the students do to protect the environment
Source: Own research and editing, 2017

From Figure 5 it can be seen that environmental awareness is the most important to those areas, which are related to utility services. These are selective waste collection, electricity supply and water consumption. Obviously, in these areas, environmental protection can be put into practice if the necessary conditions are met. In Hungary, in the majority of settlements – like in Gyöngyös as well – , the collection of selective waste is solved, so in this area the population has the opportunity to implement environmental protection in practice. This is also apparent to the responses, as more than three-quarters (75.25%) of young people are active in this area. Similarly high proportion (59.07%) was the ratio of the less electricity users. Conscious thinking is more obvious in this area as these consumers consume less electricity without the use of energy-saving light bulbs. The number of people in this category is much less than just 15.44%. The third outstanding category occurs to

water use. The 45.83% of the respondents try to minimize their water consumption. The reason why fewer (3.92%) spontaneous use of a healthy lifestyle may also be due to the fact that relatively few people in the settlement cultivate food (vegetables, fruits) for their own consumption. Apart from the practical environmental protection that we have put into our personal lives, we have also been curious about the environment within which the young people are employed today. Figure 6 shows the answers to the question: *"Rate on a 1 to 6 scale, how much do you care about the following topics?"*

On the scale 1 means: Not interested at all – 6 means: Very interested

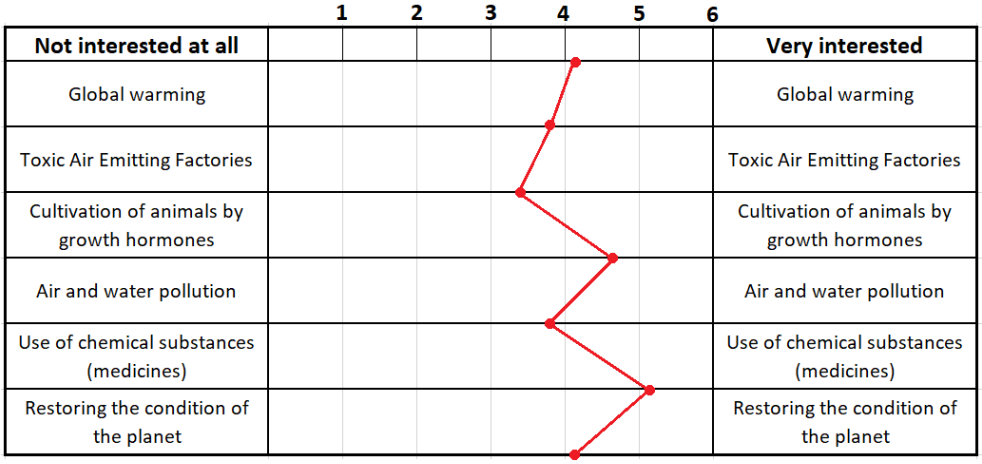

Figure 6. The average rating value of students' answers how much they care the mentioned topics
Source: Own research and editing, 2017

The rates were the following:

- Global warming (4.12)
- Toxic Air Emitting Factories (3.75)
- Cultivation of animals by growth hormones (3.43)
- Air and water pollution (4.56)
- Use of chemical substances [medicines] (3.75)
- Restoring the condition of the planet (5.17)

The greatest interest is the restoration of the planet's condition. This is followed by the issue of air and water pollution. This is what we have seen in the personal life of consciously less water use. Nearly similar concerns are given to global warming. At the same time, they are quite indifferent to the intake of toxic gases emitted by factories, the use of growth hormones used in animals and the use of medicines. The media's ongoing drug advertising campaigns can contribute to the latter's lack of interest as these products appear as part of everyday life. Regardless of the totality of the answers, unfortunately we can say that it is rather indifference to the subject. Despite the fact that the protection of the environment and the importance of it can be met more often by young people, both in print and in electronic media. It seems that this must be improved by involving other means and increasing the efficiency of addressing young people. Our results confirm our assumption that the residents of Gyöngyös and the Municipality of Gyöngyös, who have been interviewed by us, use the same amount of renewable energy sources. According to the survey, the main developments of the local government are focused on solar utilization, such as the use of solar collectors. The same can be observed for residential surveys, where solar energy has also proved to be the most widely used renewable energy source. The second most commonly used renewable energy source for biomass (14.8%) and third for geothermal energy (9.7%), while being moderately used, is biogas (7.7%) and biofuels (6.5%). The use of hydropower (3.9%) is negligible, and other sources of energy (2.6%) are used by the local government.

Conclusions

The question of energy consumption has been the most unsatisfactory situation in recent years (the vulnerable situation of European countries due to energy imports), not only at the national but also at the individual level (see Tóth et al, 2010). One of the most obvious ways of dealing with this situation lies in the use of alternative energies that take into account the natural potential of the given area and their potential for economic exploitation. The effective use of these opportunities and the appropriate institutional and legal background can provide a good basis for eliminating one-sided energy dependency. Our study shows that the Local Government of Gyöngyös lives and sets good examples for local residents regarding the use of alternative energy sources while contributing to reducing their own operating costs. The high percentage is associated with the decreasing state support, and therefore the municipality is forced to have more thoughtful and efficient management. It would be necessary to provide financial support for the population, including the local government, or to introduce local regulations that would encourage the use of alternative energies among residents. Within the population, the younger age group deserves special attention, who considers it necessary to preserve their environment. They are the ones who are willing to make efforts, therefore, for the place that means the cradle of future generations.

Acknowledgments

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CHARACTERISTICS OF WATER FROM FIRST AQUIFER BENEATH HYDROMORPHIC SOILS IN THE VOJVODINA PROVINCE

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Abstract
On the territory of the Vojvodina Province, the most common cause of soil salinization is the water in the first aquifer which is frequently saline and/or alkaline above the critical level. In this study, we analyzed water from the first aquifer sampled in locations classified as solonchaks in the soil map of Vojvodina (25 locations) and subsequently classified again into solonchak (5 locations), or reclassified into solonetz types of soil (9 locations) from the halomorphic order. The remaining eleven locations belonged to the soil types from the automorphic and hydromorphic order. Processes of desalinization and soil type change have occurred in these locations due to human activities (including the construction of drainage canals). But, based on salt or alkali levels above the critical and the quality of water in the first aquifer (especially high to very high sodium levels), it was concluded that there exists a serious risk of further continuous salinization and/or (especially) alkalization of the root zone of agricultural crops.

Keywords: critical (permissible) ground water level, salinization, alkalization, solonchaks, solonetz.

Introduction

One of the causes of salinization, i.e., accumulation of soluble salts in the topsoil due to the ascendent soil movement (ascendent movement prevails over descendent one in arid and semi-arid conditions), is the heavy mineralization of the shallow groundwater in the first aquifer. According to Miljković (1996) the immediate source of soluble salts is mineralized groundwater that rises by capillary movement and accumulates in the top layer of soil or on soil surface, thus causing the occurrence of salinization. The origin of salt is linked to the parent rocks that belong to aeolian, lacustrine and marine (paleogenic) sediments. Surface and ground waters seep through these rocks and dissolve the contained salts which mineralize the waters which become saline. The haloomorphic soil order includes soils whose profiles become waterlogged periodically or permanently due to surface or ground (most often) waters (Škorić et al. 1985). As the productivity of these soils is affected by ground water, it is important to examine ground water quality.

Material and methods

Sampling of water from the first aquifer was carried out in 25 locations whose soils had been classified as solonchaks in the soil map of Vojvodina R 1:50000 (Nejgebauer et al. 1971).Based on field and laboratory research the soils in these locations were reclassified in accordance with the current classification (Škorić et al. 1985). This paper presents the analyses of ground water from the following locations classified to belong to the order of halomorphic soils:

- solonchak type

in the locations of Trešnjevac (profile 14), Bački Brestovac (profile 16), Mali Stapar (profile 17), Kljajićevo (profile 18) and Rančevo (profile 21)

- solonetz type

in the locations of Žabalj1 (profile 1), Žabalj2 (profile 2), Despotovo (profile 3), Novi Bečej (profile 7), Ogar (profile 10), Kula (profile 11), Ruski Krstur (profile 12), Stanišić (profile 19) and Gornji Breg

(profile 24). Ground water table was determined by digging a soil pit and probing from its bottom.Water quality was estimated on the basis of the following analyses: pH (potentiometrically), electrical conductivity - EC at 25°C (conductometrically), dry residue (evaporation at 105°C), ionic balance [HCO_3^- (titration with sulfuric acid in the presence of 1% solution of methyl orange), CO_3^{2-} (titration with sulfuric acid in the presence of 1% solution of phenolphthalein), SO_4^{2-} (gravimetrically, by precipitation with barium chloride), Cl^- (titration with a solution of silver nitrate in the presence of 5% potassium chromate), Ca^{2+} and Mg^{2+} contents (atomic absorption spectrophotometry), Na^+ and K^+ contents (atomic emission spectrophotometry)], sodium adsorption ratio to calcium and magnesium [SAR - sodium adsorption ratio (calculated)]. Based on the results of the analyses, water quality was determined and classified according to Stebler's irrigation coefficient, classification of US Salinity Laboratory, according to Nejgebauer and according to a modified FAO classification (Ayers and Westcot).

Results and discussion

Critical (permissible) ground water level is the depth at which ground water can have a detrimental effect on the soil. Based on the formula of Korda (quoted by Miljković, 2005) which takes into account the evaporation above the mean annual air temperature, critical ground water levels in the studied locations were found to be around 257-259 cm (Vasin, 2009). The results in Table 1 show that the first aquifer was above the critical level in all locations and that it represented a threat for the soils. The only exception were the locations of Žabalj2 (profile no. 2), and Ogar (profile no. 10) in which the ground water table was at a depth of 450 and 370 cm. The reaction of water from the first aquifer was alkaline and within a range considered as satisfactory, but for solonchack location near the limit of 8,5 pH unit (Ayers and Westcot 1985). The values of electrical conductivity (ECw) indicated a high salinity of ground water. All analyzed waters had high dry residue values, close, but somewhat lower values than the limit. That indicating their high mineralization. The chemical composition of groundwater (Table 2 and 3) was uniform and very low quality. Anion and cation sums were equal, corresponding to the rule. These sums for ground water beneath the soils from the hydromorphic order were slightly increased compared with the average for Bačka and Banat regions in the period 1959-1989 (Škorić, 1996). Among the cations in ground water, sodium ions were absolutely dominant. The negative effect of sodium is felt more by water-physical and chemical properties of soil than by agricultural plants. The other cations were present in the following order: magnesium, calcium and potassium. Bicarbonates were dominant anions in the analyzed ground waters. Water of such quality poses a risk of salinization and/or alkalization of soil in the root zone, especially when the ground water level stays above the critical level for an extended period. When ground water is above the critical level, salts may accumulate near soil surface on account of capillary movement, especially in the case of long spells without heavy rainfall. Ground waters beneath the soils of the halomorphic order (soil types solonchak and solonetz) tend to have poor quality score (on the vast majority of sites C4S4 class according to U.S. Salinity Laboratory, unsatisfactory and poor water according to Stebler, and unsuitable for irrigation according to Nejgebauer) (Table 4). Understandably, ground waters of such inferior quality are not used for irrigation, however, they pose a risk of salinization and alkalization of the upper part of the solum if they rise above the critical level. The data presented in Table 5 indicated that, according to the modified FAO classification (Ayers and Westcot, 1985), the risk of salinization of ground water is reduced if it is not already alkalized (if it has a low SAR value). Human action as a pedogenetic factor (construction of drainage canal systems, more dams and barrages on canals and rivers and drainage of waterlogged fields) caused desalinization and a change in soil type in the studied locations areas as compared with the situation encountered at the time when the soil map was made. However, because of a poor maintenance of the drainage systems, the ground water table has exceeded the critical level. In addition to the fact that the ground water quality is low (very saline and with a high sodium content) there is a serious risk of primary or even secondary salinization and alkalization of

soil in the root zone of agricultural crops (seeds or natural vegetation of meadows), which may be reflected in reduced crop production potential.

Table 1. Characteristics of ground water in the studied locations

Soil type	Profile no	Depth, cm	pH	ECw, S/m	Dry residue,mg/l
Solonchak	14	170	8,88	2,84	2.213
	16	123	8,35	3,60	2.833
	17	160	8,31	4,48	1.264
	18	165	7,90	3,12	1.563
	21	130	8,90	2,07	1.352
	average	150	8,47	3,22	1.845
Solonetz	1	200	7,57	2,16	503
	2	450	7,57	1,03	551
	3	130	8,49	4,60	2.886
	7	140	8,19	5,71	2.668
	10	370	8,15	1,32	883
	11	130	8,47	2,52	1.815
	12	115	8,30	6,54	1.905
	19	115	8,13	3,26	2.245
	24	150	8,30	4,51	3.038
	average	200	8,13	3,52	1.833
Acceptable values in irrigation water			**6,0-8,5**	**0-3,00**	**0-2.000**

Table 2. Cationic balance of ground water in the analyzed locations

Soil type	Profile no.	Cations (meq/l)					SAR
		Ca^{2+}	Mg^{2+}	K^{+}	Na^{+}	Sum	
Solonchak	14	0,19	0,28	0,02	33,08	33,57	68,2
	16	0,68	2,38	0,02	47,41	50,49	38,3
	17	0,17	0,24	0,02	26,82	27,25	59,2
	18	1,02	4,03	0,01	27,88	32,94	17,5
	21	0,11	0,24	0,01	29,66	30,02	70,9
	average	0,43	1,43	0,02	32,97	34,85	50,9
Solonetz	1	0,81	2,93	0,01	12,96	16,71	9,5
	2	3,94	1,85	0,07	4,24	10,10	2,5
	3	0,19	1,09	0,01	40,81	42,10	51,0
	7	1,29	3,60	0,01	32,08	36,98	20,5
	10	1,34	5,50	0,02	10,72	17,58	5,8
	11	0,26	0,85	0,28	27,54	28,93	37,0
	12	0,58	1,59	0,01	28,78	30,96	27,6
	19	0,31	0,63	0,01	43,31	44,26	63,2
	24	0,86	1,43	0,02	57,71	60,02	53,9
	average	1,06	2,16	0,05	28,68	31,96	30,1
Acceptable values in irrigation water		0-20	0-5	0-2	0-40	-	0-15

Table 3. Anionic balance of ground water in the analyzed locations

Soil type	Profile no.	Anions (meq/l)				
		CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}	Sum
Solonchak	14	0,75	30,65	2,55	2,77	36,72
	16	3,46	29,36	5,84	8,90	47,56
	17	2,94	17,02	1,17	1,13	22,26
	18	0,68	23,06	1,24	3,61	28,59
	21	3,64	16,09	1,50	1,23	22,46
	average	2,29	23,24	2,46	3,53	31,52
Solonetz	1	0,00	6,86	1,90	0,07	8,83
	2	0,00	10,74	1,06	0,67	12,47
	3	0,42	12,08	1,37	0,54	14,41
	7	0,00	19,26	13,68	6,14	39,08
	10	0,00	14,30	1,11	1,34	16,75
	11	0,00	24,03	3,72	5,22	32,97
	12	0,00	22,46	10,87	1,01	34,34
	19	3,84	29,51	8,37	3,53	45,25
	24	4,24	30,39	3,25	10,63	48,51
	average	0,94	18,85	5,04	3,24	28,07
Acceptable values in irrigation water		**0-0,1**	**0-10**	**0-30**	**0-20**	-

Table 4. Water quality assessment

Soil type	Profile no.	Water class (according to US Salinity Laboratory)	Stebler's classification		Water class according to Nejgebauer	
			Irrigation coeff.	Rated as	Irrigation coeff.	Rated as
solonchak	14	C4S4 - very high salinity, very high Na content	1,20	poor	IV b	unsuitable
	16	C4S4 - very high salinity, very high Na content	0,30	poor	IV d,e	unsuitable
	17	C4S4 - very high salinity, very high Na content	1,04	poor	IV b	unsuitable
	18	C4S4 - very high salinity, very high Na content	2,50	unsatisfactory	IV b	unsuitable
	21	C3S4 - salty, very high Na content	0,62	poor	IV d,e	unsuitable
	average	C4S4 - very high salinity, very high Na content	1,13	poor	IV b	unsuitable
solonetz	1	C3S2 - salty, medium Na content	1,41	unsatisfactory	IV b	unsuitable
	2	C3S1 - salty, small Na content	7,14	satisfactory	III b	additional examination
	3	C4S4 - very high salinity, very high Na content	0,95	poor	IV d,e	unsuitable
	7	C4S4 - very high salinity, very high Na content	3,21	unsatisfactory	IV b	unsuitable
	10	C3S2 - salty, medium Na content	0,98	poor	IV b	unsuitable
	11	C4S4 - very high salinity, very high Na content	1,39	unsatisfactory	IV b	unsuitable
	12	C4S4 - very high salinity, very high Na content	0,79	poor	IV b	unsuitable
	19	C4S4 - very high salinity, very high Na content	4,53	unsatisfactory	IV d,e	unsuitable
	24	C4S4 - very high salinity, very high Na content	7,14	satisfactory	III b	additional examination
	average	C4S4 - very high salinity, very high Na content	3,06	unsatisfactory	IV b	unsuitable

Table 5. Water quality assessment according to a modified FAO classification (*Ayers and Westcot*, 1985)

Soil type	Profile no.	Restricted use of water for irrigation according to:		
		Ecw dS/m	Dry residue, mg/l	SAR to Ecw ratio
Solonchak	14	moderate	possible	possible
	16	possible	possible	moderate
	17	possible	moderate	moderate
	18	possible	moderate	no
	21	moderate	moderate	possible
	average	possible	moderate	moderate
Solonetz	1	moderate	moderate	no
	2	moderate	moderate	moderate
	3	possible	possible	moderate
	7	possible	possible	no
	10	moderate	moderate	no
	11	moderate	moderate	possible
	12	possible	moderate	no
	19	possible	possible	moderate
	24	possible	possible	moderate
	average	possible	moderate	moderate

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RAPESEED (*BRASSICA NAPUS*, L.) – BIOLOGICAL REQUIREMENTS, GROWING CONDITIONS AND NEED FOR IRRIGATION

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Abstract
Rapeseed is one of the most important energy and food crops. The European agricultural producers are highly interested in it due to the obligations under the EU Directive for replacing the fossil fuels with biofuels as much as 20% by 2020, which has drawn the purchase prices considerably up. Rapeseed is widely used for production of cooking oil and rich in protein feed too. As regards to the environment, it contributes for restoration of degraded and contaminated lands owing to its capability for improving soil structure and leaving the area free of weeds. Rapeseed is one of the best pre-crops of winter wheat and contributes for some 20-30% increase of its yield. The paper contains an overview of different aspects of rapeseed growing: its biological requirements and the abiotic stressing factors in the Balkan geographic region; its sensibility to water and the impact of the water deficit on the yield and yield structural components; its yearly and monthly evapotranspiration and crop coefficients at different empirical evapotranspiration calculation methods; world data on its yields and the agricultural practices such as proper irrigation scheduling for its yield increase. The conclusions show that the soil and the climatic conditions on the Balkans are suitable for rapeseed growing and irrigation can contribute for obtaining sustainable yields from this crop.

Keywords: rapeseed, abiotic stressing factors, irrigation scheduling, yield and yield components.

Introduction

A number of research programs in Europe and in the world are devoted to rapeseed. It is known as a an energy crop rich in oil content. The interest in it grows in Bulgaria due to high purchasing prices and obligations of Bulgaria under the EU Directive on replacement of transport petroleum products by 2020 with biofuels up to 20%. Rapeseed is widely used for producing of household oil and high-protein fodder. Ecologically, it contributes to recultivation of eroded and contaminated terrains, improving the structure of the soil and leaving areas free of weeds. Rape is one of the best precursors to wheat. It contributes for increase of wheat yield by about 20-30%. The climatic conditions for growing winter oil rapeseed (*Brassica napus* L.) in Bulgaria are favorable but in some years they are risky. There is a potential risk of frost during the winter period and from drought during sowing, blooming and grain formation. World studies on the dynamics of rapeseed water consumption and its irrigation response are small in number, mostly in the countries with semi-arid and arid climate where drought is a constant or periodic phenomenon with a high probability. For the conditions of a moderate-continental climate, such as our country's climate, there is less research work, mostly for the US and Canadian conditions. In our country, such studies have not been made because the crop has been cultivated only in recent years and because of the presumption that its vegetation period is and the spring season, it is largely waterproofed by the autumn-winter spring rainfalls. However, our studies show that in 75% of the years the spring and summer rainfall totals are expected to be between 150 and 300 mm, while the rapeseed evapotranspiration, according to data in the literature, is 300 to 400 mm. Therefore, there are years in which the atmospheric precipitation provides only 50% of the water needed for the normal crop

development. In addition, the average annual rainfalls in the spring season are 190-200 mm with a 55-65% variation. The large variation shows the instability of the phenomenon and the risk of drought, resulting in yield instability. Under these conditions, it is logical to think of increasing the yield and stabilizing it by further introducing water by irrigation. For this purpose, we need knowledge about the evapotranspiration of the crop in the soil-climatic conditions of our countries, the sensitivity of its phenological stages to a water stress and the water use efficiency. The establishment of a proper irrigation scheduling is a step towards optimizing the farming practices to this crop, to controlling the management of the production process and is a basis for obtaining the desired economic results.

Rapeseed as a crop

Rape is an important oil crop with specific and favorable agronomical features: it is adaptable to different climatic conditions, has a good impact on wheat crops in field crop rotations and has high oil content. Rapeseed increases the crop yields in crop rotations by inhibiting the growth of weeds, diseases and pests, by reducing root disease, by improving soil structure (Hang et al., 2009). The yield of wheat, sawn after oil rapeseed is higher by 19 to 24-30% compared to the yields of wheat, sawn yield after wheat (Scott et al., 1999, Zentner et al., 1986, Larney, Lindwall, 1994, Brandt, Zentner (1995), cited by Johnston et al., 2002; Angus, 2002, cited by North, 2010). There are two types of winter rape - edible and industrial. The edible type contains less than 2% erucic acid and less than 30 μmol/g glycosinate. The average oil content of the seeds is 42% and the protein is 21% (DeClercq, Daun, 1999, cited by Johnston et al., 2002). The industrial rape contains 40% erucic acid and is used for the production of lubricants, rubber products, fibers, plastics, surfactants and others. (Gilliland, Hang, 2003).

Biological requirements and abiotic stressing factors

The crop has low resistance to extreme temperature impacts. The yields of winter rapeseed, however, are 1.3 times higher than those of the spring rapeseed (Bauder, 2006). Some authors have found that the seed yield when the crop is sawn in autumn is 3.0-4.0 Mg/ha, while the yield of a spring sawn crop is half of it (Evans and Luden (1987), Stefanova (1990), Slavankov (1991) cited by Ivanova, 2010). Rapeseed demonstrates the highest resistance to low temperatures during the rosette stage, with developed 6-8 leaves and when is well rooted. The temperature tolerance threshold of fully hardened plants is -15°C. It is essential that the autumn sowing date has to be chosen so as to enable the plants to grow up to the phenological stage required for successful wintering (Sovero, 1993). The temperatures during the spring vegetation have limiting effect on the yield, while the rainfalls have an increasing effect (Mailer and Cornish, 1987, Walton et al., 1999, Angadi et al., 2003, Faraji and Soltani, 2007, cited by Daneshvar et al., 2008). Rapeseed develops best at an air temperature of 21°C, and during its flowering and ripening, its requirements increase to 23°C (Ivanova, 2010). Plants are stressed at temperatures between 27°C and 30-32°C, which causes abortion of the flowers, the seed quality becomes poor, with low oil content (Sovero, 1993; Rahnema and Bakhshandeh, 2006). The thermal stress during flowering causes accelerated premature flowering, which limits the yield (Johnston et al., 2002). Negative effect was observed as a result of drought stress during flowering and seed maturing (Johnston et al., 2002). The rapeseed yield is mostly influenced by the temperature factor during flowering and by the rainfalls during seed ripening. Rapeseed has high requirements for soil and air humidity. In order to germinate and start their development, it is necessary to have about 20 mm available water in the upper soil layer (Tesfamariam, 2004; Masoud Sinaki et al., 2007). Rapeseed transpires a large amount of water. Its transpiration coefficient is in the range of 500-700 mm and 400-500 mm of precipitation is desirable to fall during the vegetation season (Ivanova, 2010). To obtain high yields, the air humidity during flowering should be high. Excessive rainfall and low temperatures during the reproduction stage are also unfavorable - they reduce the number of flowers, the number and size of the siliques and seeds in them. (Fabry, 1996, cited by Ivanova, 2010). The root system of winter oilseed rape extracts water from the deep soil layers - 115 to 165 cm. About 92-95% of the evapotranspiration is provided by the

water in the 0-120 cm layer. The most favorable soils for rapeseed production are the chernozems and the dark gray forest soils with clay-sandy mechanical composition. An important condition to be met by soils is not to form a soil crust. Sandy, over-wetted, marshy and acidic soils are inappropriate (Ivanova, 2010).

Rapeseed yields and factors for increasing the yields

According to a study by Istanbulloglu et al. (2010), the seed yields from different parts of the world (Australia, India, Iran in irrigated and rain-fed conditions are in the range of 1.0-5.3 Mg/ha (Taylor and Smith, 1992; Robertson et al., 2001; Rahnema and Bakhshandeh, 2006). According to Alberta Agriculture (1980) the rapeseed yields without irrigation are considered good when ranging from 1.0 to-2.6 Mg/ha, and with irrigation: when ranging from 3.2 to 4.0 Mg/ha. In order to be profitable, at an average multi-year price of $370/Mg, a yield of not less than 1.7 Mg/ha should be ensured. Yields without irrigation at NSW Central Murray (Australia) averaged 1.8 Mg/ha and farmers used to give up rapeseed growing. The best farmers there obtained 1.8-3.6 Mg/ha in conditions of irrigation, while the yields from the experimental fields were 3.8-5.2 Mg/ha (Wright et al., 1988; Taylor et al., 1991). The seed yield of irrigated winter canola in Nebraska was 2600 lb/A (=3 Mg/ha) (Aiken and Lamm, 2006). The average oil content of the seeds, according to the study of (Istanbulluoglu et al., 2010) varies between 31.0% and 46.4%. Henry & McDonald (1978) found that drought is a factor for reducing the seed oil content and increasing the protein content. A negative correlation between the oil content and the protein content has been demonstrated (Asare and Scarisbrick, 1995; Brennan et al., 2000; Danesh-Shahraki, 2008). Naderikharadji et al., 2008 established multiple regression where yield is a parameter and factors are different elements of yield:

Y = -21 + 0.32 (silique length) +0.045 (100-seeds weight) +0.13 (number of seeds in a silique)

From the equation we can see that the yield is mainly formed on the account of the length of the pods and the number of seeds in them. Irrigation and fertilization increase the seed yield. Canola reacts more strongly to nitrogen fertilization when irrigated. The dry matter and seed yield increase when irrigated and increased nitrogen fertilization. The oil content increases with irrigation and decreases with increasing the nitrogen fertilization (Smith et al., 1988). Under irrigation, Wright et al., 1988 obtained 1.2 Mg/ha oil yield and without irrigation: 0.8 Mg/ha. The maximum oil yield (about 1.6 Mg/ha) was obtained by irrigation combined with fertilization with 100 kg N/ha at sowing. Numerous studies have shown that the application of potassium in the form of fertilizer mitigates the negative impact of drought on growth (Fanaei et al., 2009). Irrigation has a greater impact on the number of seeds in a silique than on the other components of the yield (Masoud Sinaki et al., 2007). A feature of canola is that it uses soil moisture until the end of maturing (Bauder, 2006).Robertson et al. (2001) describe a model for the growth and development of winter rape as a module to the Australian Simulation Model of Agricultural Production (APSIM). Husson (1998) and Gabrielle et al. (1998), cited by Robertson et al. (2001) also developed simulation models from the family of CERES models. The Robertson model describes both dry and irrigation conditions. It simulates with sufficient accuracy the yields under different conditions, sowing dates, water supply, and nitrogen supply. Yields of 30 to 500 g/m^2 are simulated with 15% standard deviation.

Response to water. The effect of water deficit on the yield and the structural elements of the yield.

Drought is one of the most powerful abiotic stressors for rapeseed's growth and productivity. According to Fanaei et al. (2009), the relative yield reduction in abiotic stress conditions compared to optimum conditions ranged from 54 to 82%. The time of occurrence of drought stress is much more important than its intensity. Gan et al. (2004) found that plants undergoing drought stress in the early stages are easily recoverable while the drought stress during the formation of siliques causes substantial and irreversible reduction in the structural elements of the yield. The drought stress during flowering and seed maturing causes reduction in dry matter, seed yield and oil content (Nuttall et al., 1992; Mansour et al., 2005; Muhammad et al., 2007; Datesh-Shahraki et al., 2008;

Ahmadi and Bahrani, 2009). Colton and Sykes (1992) (cited by North, 2010) recommended that irrigation starts in spring without allowing drought stress. Bernadi (1996) recommends that full irrigation be applied. By applying drought stress during stem elongation and flowering, Richards, Thurling, 1978, cited by North, 2010) obtained seed yields 1.51 g/plant and 1.43 g/plant, respectively, while the yield at fully meeting the crop's water needs, was 3.30 g/plant. Tahir et al. (2007) obtained the highest yield when irrigating during the early vegetative stage, flowering and seed maturing. Masoud Sinaki et al. (2007) found that the reduction in yield was greatest at water stress during flowering, then during the siliques formation. Irrigation after the flowering stage by fully meeting the plant water needs, significantly increases the yield (North, 2010). Wright et al. (1988) recommended that rapeseed has to be irrigated, especially after flowering, in combination with high nitrogen fertilizers. The drought stress during flowering results in deviations in the leaf area index (LAI) and the photosynthetic potential (LAP) due to leaf wilting and dropping. This is accompanied by abortion and dropping of the flowers and the siliques (Gunasekara et al., 2003). According to observations by Gunasekara et al. (2006), the yield of biomass under moderate and strong water stress during the reproductive stage decreased by 17.9% and 32.1%, and the seed yield by 18.5% and 38.7%. Watering during the seed maturing has the greatest effect on the yield (Gilliland and Hang, 2001). The drought stress during late season does not affect the number of seeds but it affects the weight of seeds. It reduces the seed size, shortens the period of their maturing, and reduces the ability of plants to recover (Mendham, Salisbury, 1995). In Germany, Bilibio et al., 2009b found that the yield factor K_y, which is indicative of the sensitivity of the crop to water deficit, was low: <1. The most sensitive to water deficit is the seed yield. Water stress significantly influences the net productivity of the photosynthesis, the mouth cell conductivity and the cell concentration of CO_2 both during the vegetative and the flowering stage (Naderikharadji et al., 2008). According to Nasri et al. (2008), the irrigation scheduling at a 75% maximum allowable deficit provides for 43.1% seed oil content, while without irrigation or allowing 25% available water deficit provide for 40.2% seed oil content. With an increase of the drought stress from 75% to the maximum allowable deficit (without irrigation), the yield decreased from 3.3 to 1.8 Mg/ha, the amount of saturated fatty acids and glucosinolates in the seeds increased and the quality of the oil deteriorated. Brandt and McGregor (1997), cited by Johnston et al. (2002) have established an equation for the yield of spring canola as dependent on the meteorological factors:

Yield of *B. napus* L. = 4323 + 5.90 Precip. - 187.7 Temp.
R^2=0.76; SE = 221

where: Precip. - the precipitation total of the period from 21 June to 20 August (R^2=0.82), Temp. - the temperature total of the period 15 June to 15 August (R^2=0.58). The relationship showed that for each increase of the average daily temperature by 1°C, the yield was reduced by 188 kg/ha. For each mm increase of water in the soil, the yield increased by 5.9 kg/ha. The studies of Champolivier and Merrien (1996), cited by Moaveni et al. (2010) showed that the yield and the structural elements of the yield were significantly affected by the water shortages from the beginning of flowering to the end of seed maturing. The 1000-seeds weight was also affected by the smallest drought stress during the period of seed coloring. The same authors have found that the oil content decreased when the crop developed under water deficit conditions from flowering to maturity.

Evapotranspiration and crop factors

The requirements of rapeseed to water and the need for irrigation are well studied for different soil and climatic conditions (Choudhury et al., 1990, Francois, 1994). The potential rapeseed evapotranspiration during the spring period is around 300-400 mm. According to McKenzie (2009), rapeseed spends about 100-125 mm from germination to reaching the reproductive stage. In the initial stages it consumes 2-3 mm/d, during stem elongation: 3-5 mm/d. During flowering, which coincides with the summer period, from mid-June to late July or early August, spring rapeseed

consumes 7-8 mm/d. The 24-hour water consumption remains high throughout the flowering and seed formation period. It goes cuttingly down to the end of the seed stage when the maturing process is over. Spring canola grown under optimum conditions in South Alberta, Canada (well-watered, well-fertilized, on well-drained soils, by even stand and optimal density, weed-free) spends 400 to 480 mm of water for evapotranspiration. The average daily evapotranspiration ranges from 0.1 mm up to 7.0 mm during the flowering and seed-filling stage. Canola extracts 70% of the required water from the layer 0-50 cm. FAO 56 crop coefficients for canola in the southern hemisphere are 0.35, 0.36, 0.60, 0.93, 1.0, 0.95, 0.56 respectively in April, May, June, July, August, September and October (Allen et al., 1998). At plant height of maximum 0.60 cm, the coefficients K_c by stages are as follows: vegetative - 0.35, flowering - 1.00-1.15, maturation - 0.35. K_p to E_{pan} for the whole canola vegetation period is 0.75 (Nodson, 1978, cited by North, 2010), which corresponds to 0.94, converted to FAO PM (K_p=0.8K_c). The FAO Penman-Meyer coefficients for the months from April to November for two areas of Australia are: for Murray Valley - 0.3, 0.4, 0.6, 0.9, 1.0, 1.0, 0.9, 0.6 and for Griffith - 0.3, 0.4, 0.6, 0.75, 0.75, 0.7, 0.4 respectively (North, 2010).

Irrigation scheduling

Winter oil rapeseed is relatively new and unexplored in terms of the irrigation scheduling in the Balkan countries. Recent studies showed that in our soil-climatic conditions this crop is threatened by: 1) the negative effects of low winter and high summer temperatures, and 2) droughts in both autumn and spring seasons. In this case, irrigation must be applied to overcome these impacts and produce high yields. According to Krogman and Hobbs (1975), when increasing the soil moisture, both the leaves and the siliques increase their photosynthesizing area, resulting in an increased yield. Irrigation contributes for creation of a uniform crop stand, which is difficult to achieve in dry conditions. Because of the trends of climate warming and drought it is necessary to apply good irrigation management with high water use efficiency. Water availability is crucial for rapeseed the stages: germination, vegetative stage (before flowering: rosette formation, stem elongation and jointing), flowering and silique formation. Ideally, soil water should be maintained within 60-100% of the total available water, be readily available so as not to be a limiting factor for the formation of the yield. This is valid for the period of sowing too. The first irrigation application after sowing should be 15 mm for the 0-50 cm layer. The soil moisture during the vegetative stage should be monitored in the 0-100 cm layer. The irrigation application depths for the 0-100 cm layer should be 50 mm for sandy soils and up to 90 mm for sandy clayey and clayey soils. The last application should be given when the earliest siliques start to mature. Niazi and Fooladmand, 2006 concluded that the most favorable inter-application period for the experimental conditions was 10-12 days. In a field experiment of Sultana et al., 2009, the best results, i.e. the highest yield and the highest harvest index, were obtained under the largest number of applications. Irrigation contributes to formation of higher plants, more branching, much more and longer siliques, which automatically increases the yield of the biomass mass. According to Mondul et al (1988), cited by Sultana et al. (2009) the highest yield obtained was when giving an application during flowering and another one when silique forming (2.56 Mg/ha). In Tasmania, a 100 mm irrigation depth, given through two applications after flowering, increased the yield from 3.5 to 5.2 Mg/ha (Mendham and Salisbury 1995). Mendham and Salisbury (1995) cite authors who have obtained through 1, 2 and 3 applications of 50 mm respectively 275, 287 and 420 g/m^2. In Southern Brazil, it was concluded that establishing the soil moisture pressure of 20 kPa was a good indicator of starting irrigation. A factor of greater importance for increasing the rapeseed yield is the frequency of irrigation rather than the amount of water delivered (Bilibio et al., 2009a). According to Bauder (2006), maintaining a water supply of more than 75% of the available water content can cause to waterlogging.

Conclusions

The seed yield from different parts of the world under rain-fed conditions is in the range of 1.0-2.6 Mg/ha, and under irrigation: 3.2-4.0 Mg/ha. In experimental conditions the yield reaches 5.2 Mg/ha.

The average seed oil content ranges between 31.0% and 46.5% and the protein content is between 20% and 20.5%. Oil yield is 0.8-1.2 Mg/ha, and the yield of raw protein: up to 2.3 Mg/ha. Under good agricultural practices, the biomass can be 7.0 Mg/ha and the dry matter: 1.1 Mg/ha. The yields are mainly accumulated on account of the number and length of the siliques and the number of seeds in a silique. The water factor is crucial for releasing the productive potential of winter oil rapeseed as well as drought is one of the most powerful abiotic stressors for rape growth and productivity. Depending on the climatic and meteorological conditions, irrigation of rapeseed provides for an increase in the yields from several percent to several times: up to 560% in plant height, 116% in branching, 300% in silique number, 70% in silique length, 50% for seen number in a silique, 42% in 1000-seeds weight, 80% in oil yield. The leaf area, when meeting the crop needs in water, increases with 50%, the photosynthetic potential - up to 16%, the growth rate - up to 85% and the productivity of the photosynthesis - up to 50%. The most critical to water is the flowering stage, followed by the maturing stage (seed maturing) and the seed formation stage. Most affected by water deficiency during flowering are the seed and oil yields as well as the structural elements of the yield: silique number, seed number in a silique and the biometric features - plant height and number of branches per plant. The 1000-seeds weight is most affected by the water deficiency during seed maturing. Moderate and strong drought stress results in 14.5% and 32.0% reduction in the biomass yield and 18.5-38.0% reduction in seed yield. The seed number is mast affected. The drought stress has a stronger effect on seed yields than on the biomass yields. The drought stress after flowering reduces the oil content. The strong drought stress influences the negative relationship between oil and protein in the seeds in favor of fat. As the water stress increases, the harvest index decreases. With increasing of the water deficit, the amount of saturated fatty acids and glucosinolates in the seeds increases and the quality of the oil deteriorates. Waterlogging is harmful to rapeseed. It can occur if the water supply maintains the available water content over 75%. The rapeseed water requirements and its need in irrigation are well studied in the world. Under the conditions of the Mediterranean climate, evapotranspiration without irrigation is some 465 mm, and when meeting the crop water needs by irrigation, it is some 715 mm. The seasonal evapotranspiration during the spring period is around 300-400 mm. The maximum average daily evapotranspiration rate of 5 mm/d occurs during the flowering stage. In the same phenological stage, the spring rapeseed consumes 7-8 mm/d. In our countries there is no research on the water requirements of rapeseed. The FAO coefficients K_c have been established. At plant height of maximum 0.60 cm, FAO recommends the following coefficients per stages: vegetative - 0.35, flowering - 1.00-1.15, maturation - 0.35. A coefficient for the transition from evaporation from a class A evaporator to evapotranspiration of canola K_p=0.75 was established for the whole growing season. The elements of the irrigation scheduling for different climatic conditions have been estimated. The readily available water is within the 60-100% of the total available water. The first irrigations should be 15 mm for the 0-50 cm layer. If the water is readily available, watering can cause over-wetting, which is detrimental to the crop. If autumn is extremely dry, it is good to deliver an irrigation application to create a uniform crop. Some authors recommend that the first application be carried out before the leaf's water potential has fallen below 0.4 MPa. In spring vegetation, irrigation must be reintroduced from mid-April, when the crop is vegetative growing and continue during flowering. It is recommended that irrigation be carried out while the water potential of the soil is 20 kPa. Depending on the weather conditions, the water needs of the crop in moderate climates are met by 3-5 spring irrigation applications with an irrigation depth of 250-300 mm. The irrigation depths for the 0-100 cm layer are 50 mm for sandy and 90 mm for sandy clay and clayey soils. The last application should be given when the earliest siliques begin to mature. In case of water deficiency, it is advisable to irrigate during flowering, since irrigation can double the yield. The most favorable duration of the inter-application period is 10-12 days.

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PHYSICAL-CHEMICAL PROPERTIES OF WATER IN CRNA RIVER IN THE PELAGONIA REGION

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Abstract
The main purpose of this paper is to examine the physical-chemical properties of water in Crna River in the Pelagonia Region. Therefore, four locations along the Crna River in the Pelagonia Region were taken as target measuring points of the physical-chemical properties of water: measuring point 1 (near village Novaci), measuring point 2 (before the inflow of water to the fifth channel), measuring point 3 (Crna River after the inflow of water from the fifth channel) measuring point 4 (Skochivir). The water samples for analysis were taken in January and July in 2012, 2013 and 2014 and the following physico-chemical parameters were analyzed during our investigation: the water temperature (determined with a digital thermometer), turbidity (by turbidimeter), suspended solids, total organic carbon (TOC), biological oxygen demand (BOD), chemical oxygen demand (COD) and dissolved oxygen (determined with UV PASTEL-instrument). From the obtained results in our investigation it can be concluded that the highest average turbidity is noted in measuring point 1 (24,4 mg/L) in July. The highest average BOD is measured in point 3 (14,1 mg/L) in July, as a result of the water inflow from the fifth channel. The lowest average amount of TOC was noted in January in measuring point 1 (2,0 mg/L), while the content of dissolved oxygen in same measuring point show the highest value (11,53 mg/L).

Keywords: pollution, indicators for oxygen regime, turbidity, suspended solids.

Introduction

The rapid pace of development that is reflected in all segments of Tthe human life, increases the need for water. According to (Tanaskovic and Chukaliev 2013) the global warming leads to a worsening of problems with a lack of quality water for all purposes. The large deficit of clean water imposes a need for constant monitoring of surface and groundwater. This paper examines the water in Crna River in the Pelagonia region. The purpose of this research is to determine the state of the water in Crna River, which is the right tributary of the river Vardar. By determining the critical points to implement effective measures in the treatment of wastewater treatment before flowing into the rivers.

Material and methods

As a material for the work in the research was the water from the Crna River. The selection of the measuring points was at different locations along the Crna River in the Pelagonia Region.As target measuring points for taking sampling were the following: Measuring point 1 (village Novaci). This measuring point is 11 km away from the City of Bitola and according to the number of inhabitants 1 278 is the largest village. Measuring point 2 (before the water flows from the fifth channel in the Crna River). Measuring point 3 (Crna River after the water flows from the fifth channel in which flow the wastewater from the industrial sector, as well as part of the faecal waters from the city of Bitola and the village of Kravari) Measuring point 4 (Crna River in the village of Skocivir). The presence of rocks and stones in this part of the river contributes to the additional aeration of water. Samples for analysis were taken in January and July in duration for three years. In order to obtain a realistic

image of the state of the water in Crna River in the Pelagonia Region, analysis of the physical and chemical parameters were made: the water temperature was determined by a digital thermometer with an accuracy of ± 0,10C.
The measurement of turbidity was determined by a turbidimeter expressed with nephelometric units (NTUs), suspended solids, total organic carbon (TOC), biological oxygen demand (BOD), chemical oxygen demand (COD), and dissolved oxygen were determined With UV PASTEL - a tool for a direct reading of values.

Results and discussion

One of the physical indicators of water quality is temperature. With the rise in temperature, the chemical reactions catalyze which are of crucial importance in the aquatic ecosystems as they affect the biological activity of living organisms (Brutsaerd, 2005).

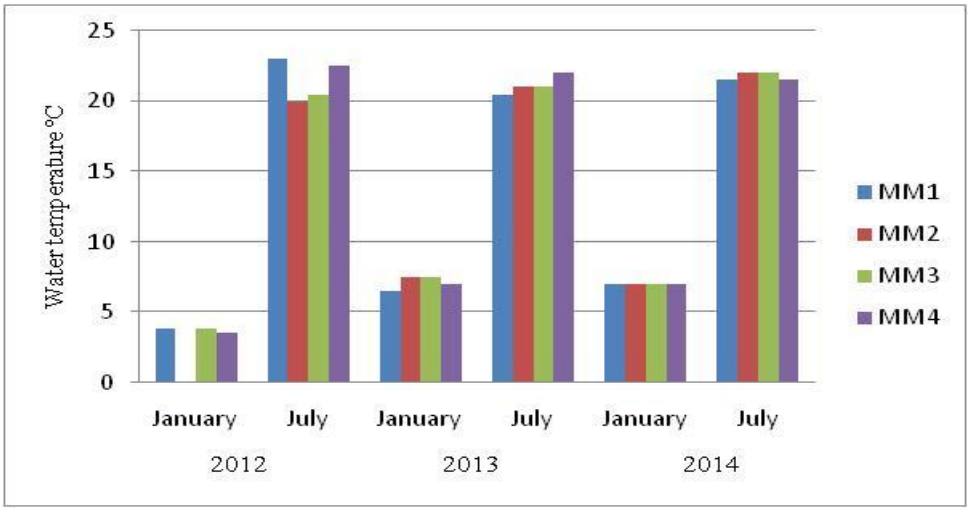

Chart 1. Water temperature in Crna River in January and July

From the results shown in Chart 1, it can be concluded that the higher values of the water temperature are typical for the summer period. The highest value is at the measuring point 1, 23C in July 2012. According to (Chukaliev et al. 2003), in the agricultural production, the temperature of the water for irrigation of crops plays a major role in their development. The turbidity of the water is one of the first visible indicators determining the water quality. Since the river ecosystem is concerned, the erosion processes have influence to the water turbidity, as well as the hydrometeorological conditions and the river bed. (Blazhevska, 2016).

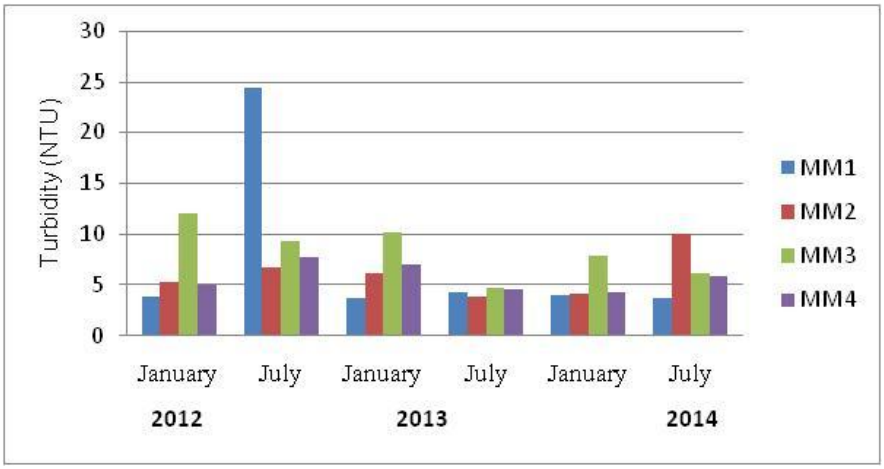

Chart 2. The turbidity of the water in Crna River in January and July 2012, 2013 and 2014

With the measurements of water turbidity in the three-year research period, it was found that Crna River has the highest value in the first year, at the measuring point 1 24,4mg / L in July, near the village of Novaci. This village, according to the number of inhabitants is the largest, therefore the quantity of waste water per resident is greater. In 2013, the highest value is at the measuring point 3, 10,2 mg / L, due to the wastewater from the fifth channel that is discharged into the Crna River. The high average value of turbidity found along the course of the Treska River in November was 34., mg / L according to (Stojanova, 2012) as a result of the heavy rains,inherent in that period of the year. In 2014, there are higher values at the measuring point 3, 7.9mg / L in January and at the measuring point 2, 10mg /L in July. The high value at the measuring point 2 is a result of the characteristics of the riverbed which contribute to the increase in the turbidity. From the obtained values for the turbidity of the water in Crna River according to the Decree on classification of waters in all measuring points the water is in IV and V class. Through the content of suspended solids can be determined the presence of organic and inorganic substances present in the water.

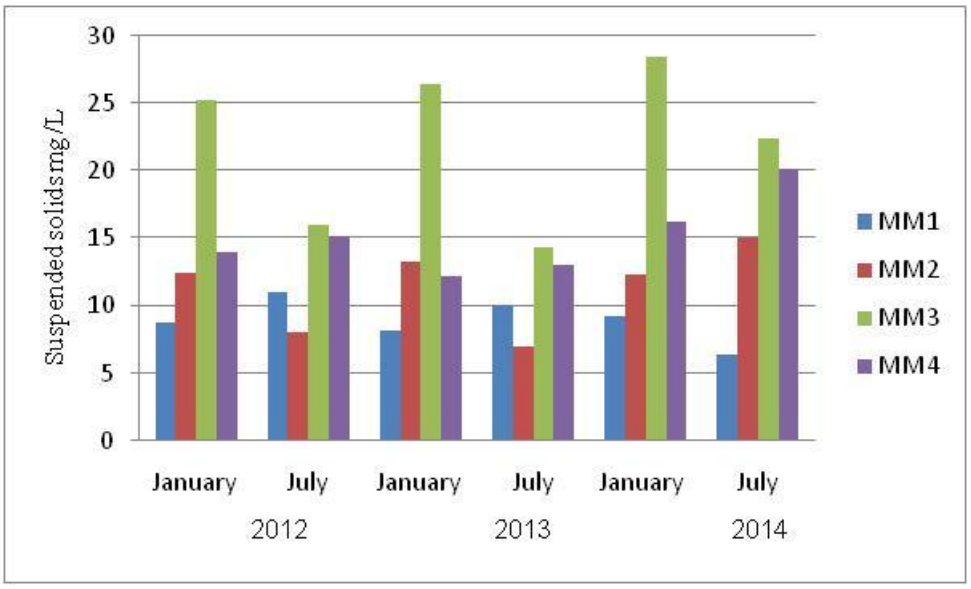

Chart 3. Suspended solids in the water of Crna River in January and July 2012, 2013 and 2014

According to the obtained results from the analysis of the quantity of suspended solids in the water of Crna River in the three years period, the highest values were determined at the measuring point 3, with epmphasised dynamics in January (25.2mg / L; 26.4mg / L; 28, 5mg / L) as a result of additional soil input in the water. As Crna River flows away from the populated areas, the value of the suspended solids in the measuring point 4, has a slight decline, as part of the organic and inorganic substances are sedimented, and part is carried through the stream of Crna River. From the values obtained for the presence of the suspended solids, according to the Water Classification Regulation, the measuring points 1, 2, 3 and 4 are in the II class.The dissolved oxygen in the water is one of the most important parameters for the survival of aerobic organisms.

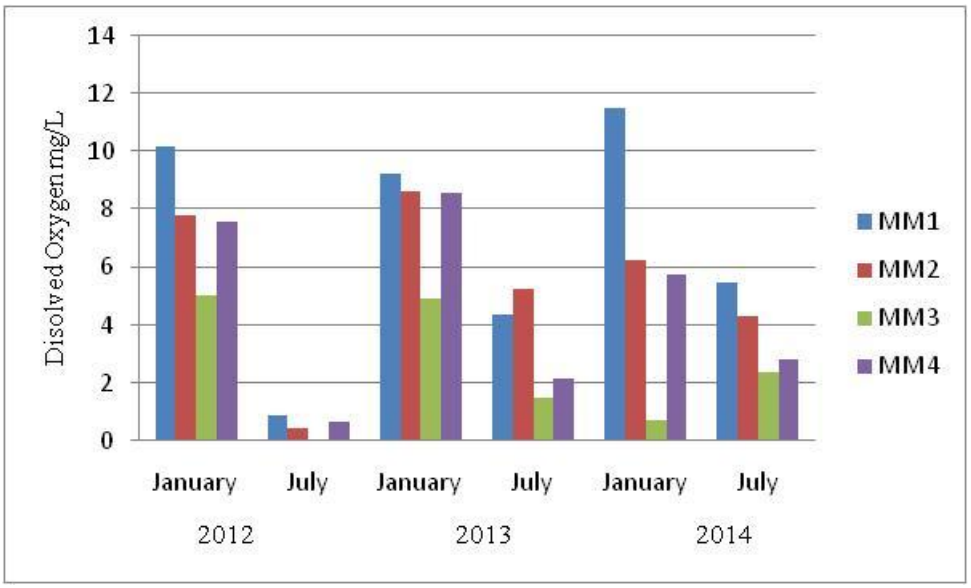

Chart 4. Dissolved oxygen in the water of Crna River in January and July 2012, 2013 and 2014

According to the obtained results of the performed measurements of oxygen in the water of Crna Reka, higher values in all measuring points are recorded at the measuring point 1, which collects the waters from the upper streams of the spring and then follows the measuring point 2. High values are recorded in January, while the lower ones in July, where, according to (Adebao, 2009), the reason is the low water level and the higher concentration of organic matter. This is confirmed in our research, where the lowest value is recorded at the measuring point 3 (0,0mg / L 2012; 1,51mg / L 2013; 2,4mg / L in 2 July 2014) after flowing of municipal and industrial wastewater in the river. According to (Malecka et al., 2006) the biological consumption of oxygen is correlated with the amount of organic matter in the aquatic ecosystem and the biological activity of microorganisms.

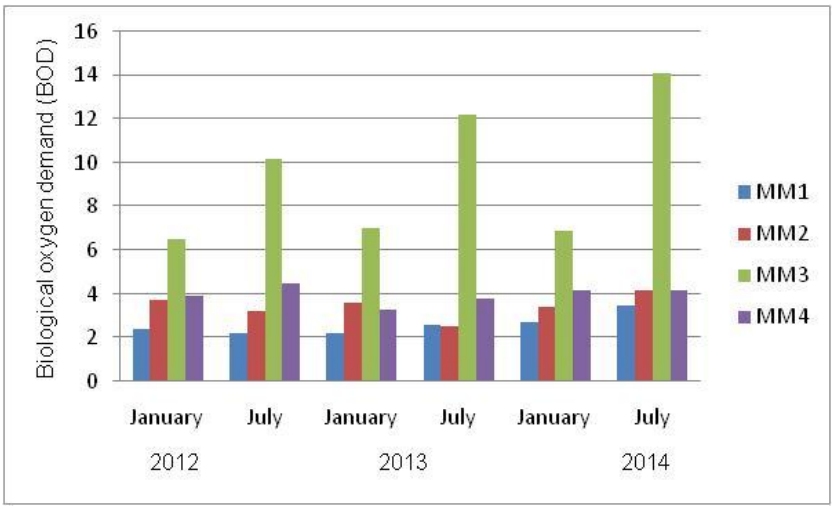

Chart 5. Biological oxygen demand in the water of the Crna River in January and July 2012, 2013 and 2014.

The highest BOD according to the obtained results from the measurements in all three years of examination, it was recorded at the measuring point 3 (10.2 mg / L 2012; 12.2 mg / L 2013; 14.1 mg / L 2014) in July due to the great anthropogenic influence in this part of the Crna River. At the same measuring point, the content of dissolved oxygen is the lowest. The lowest BOD values are recorded at the measuring point 1 (2.4mg / L2012; 2.2mg / L 2013; 2.7mg / L 2014) in January, where the content of dissolved Oxygen is the highest (10.2 mg / L 2012; 9.23 mg / L2013; 11, 53 mg / L2014) in

January. According to the Water Classification Regulation, the measuring points 1, 2, 4 are in the II class, and the measuring 3 of IV class.
The chemical oxygen demand is an indicator of water quality.

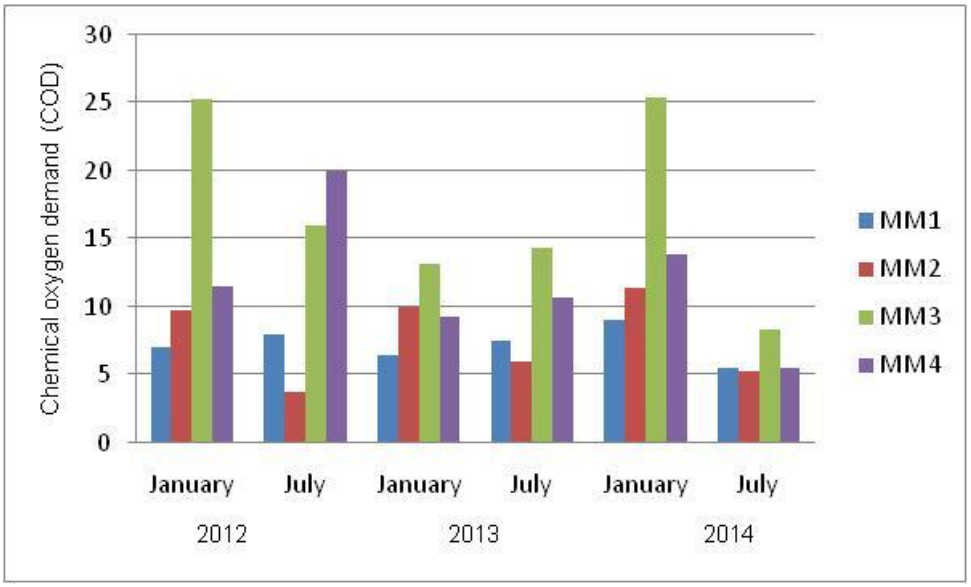

Chart 6. Chemical oxygen demand in the water of Crna River in January and July 2012, 2013 and 2014

The highest chemical oxygen demand in the water of Crna river is registered at the measuring point 3 25.4mg / L in January 2014 as a result of the high content of organic matter present in this measuring point. Higher values have been recorded by (Ziberovski, 2000) on the river Lepenec before entering into the river Vardar, where the value of COD is 32.4mg / L, and such water is not intended for irrigation. There is a low COD content at the measurement point of 2 6,0 mg / L in July 2013 and the measurement point 1 6,5 mg /L in January. These are measuring points where the content of BOD is lower and the content of dissolved oxygen is higher. According to the Decree on the classification of waters the measuring points 1 and 2 are in the III class and the measuring points 3 and 4 are in the IV class. According to (Agbaba et al., 2005), the total organic carbon present in the water is an emitter for the creation of new organic compounds.

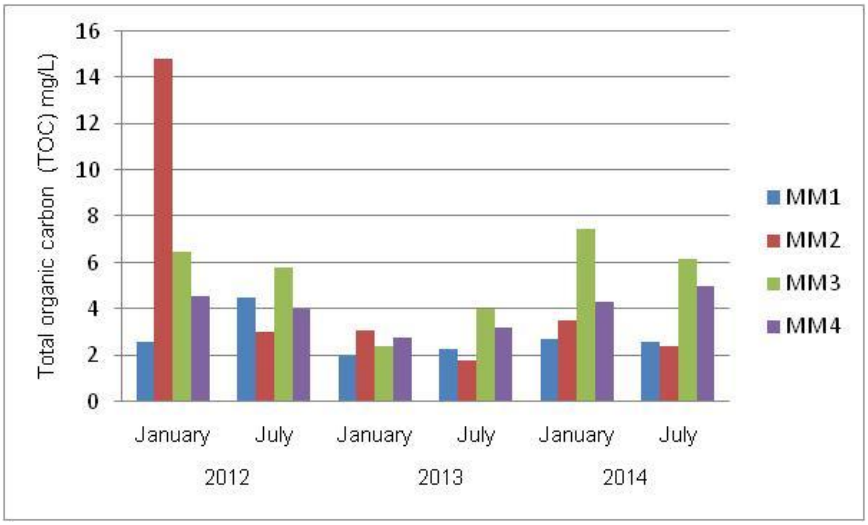

Chart 7. Content of total organic carbon in the river Crna River in January and July 2012, 2013 and 2014

From the obtained values of the TOC in the water of Crna River, the highest value was determined at the measuring point 2 14.8 mg / L in January 2012. In the next two years in January and July at the

measuring point 3, there is a continuous increase in the value of TOC as a result of the constant burden on organic matter from the waste industrial and fecal waters. The lowest value was recorded at the measuring point 2 1.8mg / L in July 2013 and the measuring point 1 2.0mg / L in January 2013. At these measuring points the content of BOD and COD is the lowest. According to the Water Classification Regulation, the measuring points 1 and 4 are in the II class, and the measuring points 2 and 3 in the III class.

Conclusions

In addition to the presence of nutrients in the water, as a limiting factor that determines the development of microorganisms is also the temperature. The established values of the suspended solids coincide with the values for the water turbidity. In the measuring point 3, the anthropogenic influence can be felt the most, because there is a continuous release of waste industrial and fecal waters. All of this suggests that the cleaning of the river beds should become an obligation of the competent institutions, to undertake measures for wastewater treatment before they flow into the canals and rivers.

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EFFECTS OF DIFFERENT GRAFTING METHODS AND TIMES ON GRAFTING SUCCESS AND PLANT DEVELOPMENT IN SARI ALIÇ HAWTHORN GENOTYPE (*Crataegus azarolus* L.)

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Abstract

The study was carried out to determine the different grafting methods and times on the grafting success and plant development in 'Sarı Alıç' hawthorn genotype (*Crataegus azarolus* L.) on *Crataegus monogyna* L. rootstock in Hatay province, eastern Mediterranean region of Turkey. T and chip budding and whip grafting methods were conducted on February 15, March 01, March 15, April 01, April 15, May 01 and May 15 dates in 2016. The grafting take and bud sprout percentages, graft shoot development and shoot diameter were also investigated. The results of the study showed that graft success and plant development were affected by grafting times and methods. The mean highest bud sprout percentage (71.91%) was found in whip grafting whereas mean graft shoot development (55.18 cm) and diameter (5.57 mm) were detected in T budding. The bud sprout ratio was the highest on April 01 and April 15 (84.44% and 77.77%, respectively). The results showed that whip grafting method applied within March and April months was very successful for growing of 'Sarı Alıç' hawthorn genotype in Hatay, Turkey ecological conditions.

Keywords: Hawthorn, budding, grafting, plant development.

Introduction

Crataegus species form genus known as hawthorns that belongs to subfamily Maloideae of the Rosacea (Evans and Campbell 2002). The genus is closely related to the genera *Pyracantha* M. Roem, *Mespilu*s L. and *Hesperomeles* Lindl. (Hummer and Janick 2009). Several hawthorn species are grown for their eatable fruit in Asia, America, and the Mediterranean countries. In Europe, the fruit, leaves, and flowers of the hawthorn are traditionally employed in the treatment of heart problems due to their antispasmodic, cardiotonic, hypotensive, and antiatherosclerotic effects, as well as being ingredients in wine, conserve and sweets (Ljubuncic et al. 2005, Caliskan 2015).

Turkey have different climatic conditions for the hawthorn species in the diverse regions. The regions have at least one characteristic species and other secondary or common species with domestic genotypes. *C. monogyna* Jacq is widespread in Turkey. Hawthorn species are mainly distributed in temperate zone areas of Turkey. Some hawthorn species are also growing in low altitudes such as 300 and 500 m areas of subtropical ecology such as Hatay in Turkey (Caliskan et al. 2016). Caliskan et al (2012) reported some genotypes of the *C. azarolus* are grown for large and delicious fruits in Belen, Hatay province, eastern Mediterranean region of Turkey. The promising genotypes are found in the region because of its fruit size (fruit weight >10 g), as well as high total soluble solids, total phenolic, antioxidant activity and antioxidant capacity contents. The genotypes of this species are commonly grown in Belen County and the fresh fruits have been sold with the higher prices in local markets.

A most important limiting factor for the hawthorn growing is propagation. Vegetative propagation of hawthorn species is not a usefully method because of their rooting percentage is very lower. At the present time, rootstock production is curried out with seeds, and then grafting and budding methods are used for new plants. However, there is no detail studies for the grafting methods and times on the grafting success and plant development in hawthorn. This study was designed to determine optimal grafting methods and times for hawthorn grown in Hatay, Turkey ecological conditions.

Material and methods

The study was conducted at Fidanlı located Samandağ, Hatay province (Turkey). The scions of 'Sarı Alıç' hawthorn genotype was taken from main plants which were selected Belen, Hatay in February and stored at 4°C in a refrigerator. In the study, *Crataegus monogyna* L. seedling produced with seed propagation was used as rootstock.

T and chip budding and whip grafting methods were conducted on February 15, March 01, March 15, April 01, April 15, May 01 and May 15 dates in 2016. The budding and grafting methods on rootstock were applied from soil surface at a height 15-20 cm. Cultural applications such as removal of suckers below graft point, weeding, irrigation and fertilization were fulfilled at regular intervals. A mini data logger (Testo 174T Temp/RH, Germany) was used for the daily mean temperature and relative humidity measurements (eight hours intervals).

The grafting take and bud sprout percentage (%), graft shoot length (cm) and diameter (mm) were investigated. The bud sprout percentage was observed within 30 days following grafting. The shoot length and diameter were measured at the end of vegetation period (December 15). The shoot length was measured by meter from the graft point and the shoot diameter was evaluated by digital caliper at a height 5 cm above the graft point.

Data were analyzed using SAS software and procedures (SAS 2005). Variance analysis was formed with Tukey's Honestly Significant Difference (HSD) method at $p<0.05$. The two-factorial arrangement on a completely randomized design was constructed with three replications. Each replication included 15 plants. The data expressed as percentage were transformed using the to the arc-sin√x transformation.

Results and discussion

Climatic data included daily mean temperature (°C) and relative humidity (%) were recorded during February 10 to December 31, 2016 year (Figure 1). In the experimental area, daily mean temperature ranged between 2.8 and 38.5 (°C) and daily mean relative humidity varied between 22.8 and 99.9%. Generally, mean temperature and relative humidity varied throughout the year except for June, July and August. Hartmann et al. (2011) indicated that temperature and relative humidity values are very critical pending the 30 days following grafting methods. The period is important for formation of callus cells and wound closure. In addition, Yilmaz (1992) showed that the temperature at the grafting times should be 26 and 28°C for optimum development of callus cells. The maximum mean temperature (38.5°C) was recorded in June whereas the mean temperatures were ranged between 10 and 20°C in February and March, ranged between 12 and 25°C in April and ranged between 15 and 29°C in May at the grafting application times. The relative humidity values changed from 30 to 70% at the same times.

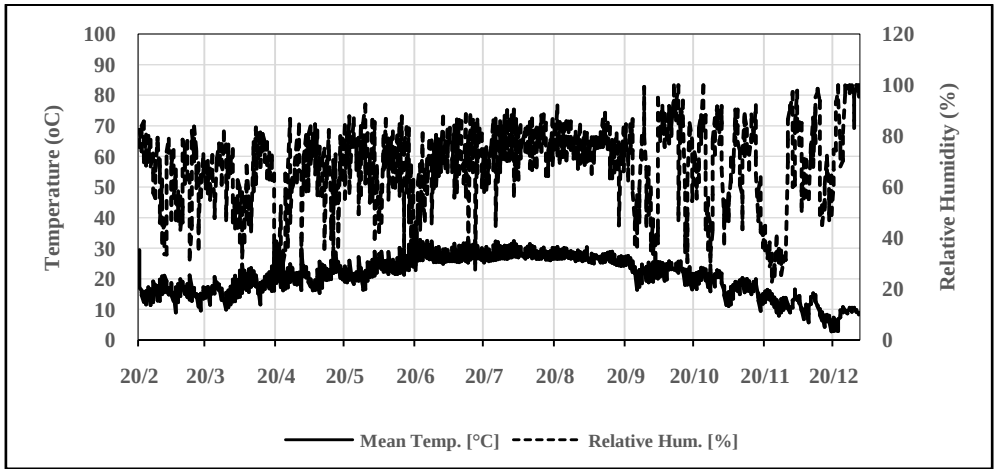

Figure 1. Variation of mean temperature (°C) and relative humidity (%) during the days after grafting in 2016.

The influence of T and chip budding and whip grafting on the grafting take and bud sprout percentages, graft shoot length and diameter values were shown in Table 1. The data showed that grafting success and plant development in 'Sarı Alıç' hawthorn genotype were statistically significant ($p<0.05$) depend on grafting method and time. Grafting success was not obtained from T budding method on February 15. The result may be due to the application date was not enough for activity of plant sap in phloem cells. However, the highest bud sprout ratio (86.67%), graft shoot length (94.33 cm) and shoot diameter (8.40 mm) were found in T budding application on April 15. Grafting take and bud sprout ratios were showed differences in chip budding. The highest grafting ratio was detected for chip budding on March 01 and March 15 whereas the highest bud sprout ratio for chip budding was observed on April 01. Bud sprout ratio for chip budding was the lowest on May 01. The highest graft shoot length and shoot diameter values were measured for chip budding on April 01 while it was lowest on February 15 (50.67 cm and 3.15 mm, respectively) and May 15 (41.33 cm and 3.75 mm, respectively). Grafting take ratio for whip grafting was ranged between 26.66 (May15) and 100.00% (March01 and April 15) and bud sprout ratio was ranged between 23.33 (May 01) and 93.66% (April 15). The longest graft shoot length was detected for whip grafting on February 15 (72.08 cm) whereas it was the lowest on May 15 (26.44 cm). Graft shoot diameter values for whip grafting based on various grafting times did not found statistically important.

Table 1. The influence of T, chip and whip grafting based on grafting times on grafting success and plant development in 'Sarı Alıç' hawthorn genotype

Grafting Method	Grafting Time	Grafting take ratio (%)	Bud sprout ratio (%)	Graft Shoot length (cm)	Graft Shoot diameter (mm)
T budding	15 February	0.00 b	0.00 c	0.00 d	0.00 d
	01 March	80.00 a	60.00 ab	50.33 c	6.66 ab
	15 March	86.67 a	26.67 bc	46.78 c	4.83 b
	01 April	100.00 a	86.68 a	61.50 bc	2.74 c
	15 April	93.33 a	86.67 a	94.33 a	8.40 a
	01 May	100.00 a	53.33 ab	70.43 b	8.36 a
	15 May	86.70 a	66.67 ab	62.89 bc	8.00 a
Chip budding	15 February	91.67 ab	46.67 abc	50.67 c	3.15 e
	01 March	100.00 a	60.00 ab	32.44 e	4.04 cd
	15 March	100.00 a	40.00 bc	56.04 b	4.21 bc
	01 April	93.33 ab	80.00 a	63.00 a	5.64 a
	15 April	86.67 ab	53.33 abc	54.00 bc	4.50 b
	01 May	80.00 ab	20.00 c	53.44 bc	3.97 cd
	15 May	73.33 b	53.33 abc	41.33 d	3.75 d
Whip Grafting	15 February	46.68 b	43.22 b	72.08 a	5.46
	01 March	100.00 a	80.00 a	56.22 b	5.47
	15 March	80.00 a	80.00 a	49.22 c	5.17
	01 April	86.67 a	86.67 a	53.33 bc	5.04
	15 April	100.00 a	93.33 a	53.67 bc	4.72
	01 May	86.66 a	66.67 a	37.11 d	4.64
	15 May	26.66 b	23.33 b	26.44 e	4.61
HSD_T		29.81	40.35	20.28	1.85
HSD_{Chip}		25.99	36.51	4.75	0.41
HSD_{Whip}		32.19	41.71	5.24	ns

Different letters within columns indicate significant differences by Tukey's at $p<0.05$; ns: not significant

The mean grafting take and bud sprout ratios, graft shoot length and diameter values were presented in Table 2. The mean grafting take ratio was the highest for chip budding (89.29%). The lowest grafting take ratio was observed in whip grafting (75.24%). However, the mean bud sprout ratio was the highest for whip grafting (71.91%). Actually, some researchers indicated that whip

grafting is one of the successfully methods than others due to the callus formation and graft union are better in whip grafting (Miller and Crocker 1994, Bellini 2002).

The highest mean grafting take ratios were found between March 01 and May 01 (from 88.80 to 93.50%). The lowest grafting take and bud sprout ratios for all grafting methods were detected in February 15 (46.11 and 40.00%, respectively). The mean bud sprout ratio was the highest in April 01 (84.44%) and April 15 (77.77%). The climatic data showed that daily mean temperatures between 15 and 25°C at the grafting application time (April) were very successfully for hawthorns. Grafting take and bud sprout ratios were adversely affected by low temperatures in February and March. Similar to our results, low temperatures at the March were showed negative results on grafting success in persimmon (Zenginbal 2015) and kiwi (Öztürk et al. 2012). In addition, April is the stage of speed shoot development on hawthorns in Hatay, Turkey ecological conditions. This time can be perfect time for grafting because of growth hormones are concentrated in the buds and these may be induce differentiation of vascular tissues in the graft union point (Hartmann et al. 2011).

Grafting methods and times had significant effect ($p<0.05$) on the mean graft shoot length and diameter values (Table 2). The highest mean graft shoot length were found in T budding (55.18 cm), followed by chip budding (50.13 cm) and whip grafting (4.73 cm). The-mean graft shoot length was the highest for April 15 and April 01 (67.33 and 59.28 cm, respectively) whereas the lowest mean graft shoot length was found in February 15 (40.92 cm) and May 15 (43.56 cm). T budding had the highest mean graft shoot diameter with 5.57 mm. The lowest mean graft shoot diameter was measured in chip budding (4.18 mm). The mean graft shoot diameter was effected by grafting time. The highest shoot diameter was obtained in April 15, May01 and May 15 (5.88, 5.65 and 5.45, respectively).

Table 2. The effects of various grafting methods and times on mean grafting take, bud sprout ratio, graft shoot length and shoot diameter in 'Sarı Alıç' hawthorn genotype

Variable	Grafting take ratio (%)	Bud sprout ratio (%)	Graft Shoot length (cm)	Graft Shoot diameter (mm)
Grafting method				
T budding	78.10 b	54.29 b	55.18 a	5.57 a
Chip budding	89.29 a	50.48 b	50.13 b	4.18 c
Whip Grafting	75.24 c	71.91 a	49.73 b	5.02 b
Grafting time				
15 February	46.11 c	40.00 c	40.92 e	2.87 d
01 March	93.33 a	66.67 ab	46.33 de	5.39 ab
15 March	88.89 a	48.89 bc	50.68 cd	4.74 bc
01 April	93.35 a	84.44 a	59.28 b	4.47 c
15 April	93.50 a	77.77 a	67.33 a	5.88 a
01 May	88.80 a	46.67 bc	53.66 bc	5.65 a
15 May	62.22 bc	47.78 bc	43.56 e	5.45 a
HSD_{method}	7.91	10.64	3.33	0.36
HSD_{time}	15.40	20.71	6.49	0.69

Different letters within columns indicate significant differences by Tukey's at $p<0.05$.

Conclusions

This study provided the first data indicating the grafting methods and times in hawthorn culture. The results showed that considerable variation in bud sprout percentage, graft shoot length and diameter values based on grafting methods and times. According the results, we can say that whip grafting can be used successfully from March 01 to April 15 for hawthorn nursery production in Hatay, Turkey ecological conditions. Chip budding may be preferred if there are not enough graft shoots on February 15, March 01 and March 15. T budding can be suggested for high budding success and plant development on April 01 and April 15.

Acknowledgments
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CHARACTERIZATION OF CAPRIFIG (*Ficus carica* var. *caprificus*) ACCESSIONS SELECTED FROM VARIOUS LOCATIONS IN THE EASTERN MEDITERRANEAN REGION OF TURKEY

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Abstract
The study was conducted to investigate the some morphological and pollinizer characteristics on caprifig accessions selected from five locations (Adana, Hatay, Kahramanmaraş, Mersin, and Osmaniye) in eastern Mediterranean region and six standard cultivars ('Ak İlek', 'Armut İlek', 'Elma İlek', 'Hamza', 'Küçük Konkur', and 'Taşlık') from Aydin location in the Aegean Region of Turkey. A total of 27 characteristics included profichi crops of caprifigs were observed in Adana (12 accessions), Hatay (37 accessions), Kahramanmaraş (17 accessions), Mersin (22 accessions), and Osmaniye (9 accessions) locations and six standard cultivars in 2014 and 2015 years. The average fruit weight per profichi crop of the cultivars (30.33 g) in Aydın location and caprifigs (27.41 g) in Hatay location were the highest compared with the caprifigs selected from Adana (23.14 g), Mersin (19.84), Kahramanmaraş (17.57 g), and Osmaniye (16.10 g) locations. The caprifigs in Mersin location had the darkness fruit skin color. The number of gall flowers was highest in cultivars (634.07), whereas the number of male flowers was found to be highest caprifigs (142.41) in Adana location. The pollen viability of caprifigs ranged between 65.76% (Mersin) and 90.51 (Cultivars). The highest of pollen number per profichi fruit were found caprifigs in Adana (824.188). The cluster analysis showed that Hatay caprifigs and standard cultivars and also Kahramanmaraş and Osmaniye caprifigs were partly similar to each other according to morphological and pollinizer characteristics.

Keywords: Caprifig, morphology, location, pollinizer characteristics.

Introduction

Fig is a functionally gynodioecious genus that includes the hermaphrodite (caprifig) and the female (edible fig) flowers (Beck and Lord 1988). The fig has an interesting and special pollination biology. Fig cultivars are grouped into four types based on their pollination requirement and cropping stages (Flaishman et al. 2008). The caprifig crops are not occur edible fruit. The caprifigs are important as a pollen source both for fruit set in the edible figs and fig breeding. The second and third types contain the two groups of edible figs, Smyrna and San Pedro, require caprification, or the pollination of edible figs with pollen carried from caprifig fruits by *Blastophaga psenes* wasps (Galil and Neeman 1977), to set their main crops of fruit. The fourth type, the common figs, are called 'parthenocarpic' fig because they can occur one or two crops per season with or without caprification. Caprification is a common application in fig-growing areas and has a significant effect on the fruit set and quality parameters of edible figs (Condit, 1947, Ferrara et al. 2016). On the other hand, all of caprifigs are not useful in caprification because of important parameters in their special fruit characteristics. The critical characteristics of the caprifig for profichi crops include fruit size, fruit number per shoot, the time of *Blastophaga* wasps exit from caprifig fruits, ripening period, coinciding with the female flowers in edible figs, amount of gall and male flowers, amount of pollen production, pollen viability and germination ratios, inclusion of the mammoni and mamme crops, and free from disease and pests (Ilgin et al. 2007, Caliskan et al. 2016a). Anatolia is one of the center of origin for figs from where they were transplant to other areas (Condit 1947). The native figs in the country serve as a

rich genetic resource for fig breeding. In addition, Turkey is the world's leading fig-producing and numerous cultivated and wild species of fig, including caprifigs, with considerable diversities of fruit color, form and maturing times are found (Caliskan et al. 2016b). 'Sarılop' and 'Bursa Siyahı' figs are major cultivated cultivars. The cultivars and most local cultivars in Turkey, desire caprification for fruit set. Thus, it has become essential to establish a germplasm evaluation program for caprifigs. The study was conducted to characterize some of the plant, fruit and pollinizer qualities of selected caprifig accessions from different locations, in the eastern Mediterranean region of Turkey.

Material and methods

This study was carried out to determine the caprifig accessions collected from Adana, Hatay, Mersin, Osmaniye and Kahramanmaraş provinces located in the eastern Mediterranean region of Turkey, during 2014 and 2015. A total of 22 characteristics included profichi crops of caprifigs were observed in Adana (12 accessions), Hatay (37 accessions), Kahramanmaraş (17 accessions), Mersin (22 accessions), and Osmaniye (9 accessions) locations and six standard cultivars from Aydın province ('Ak İlek', 'Armut İlek', 'Elma İlek', 'Hamza', 'Küçük Konkur' and 'Taşlık'). The characterization of plant material was carried out using the IPGRI (International Plant Genetic Resources Institute) and CIHEAM (International Centre for Advanced Mediterranean Agronomic Studies) descriptors for *F. carica* L. (IPGRI and CIHEAM 2003). A total of 27 morphological characteristics were investigated. The pomological analysis used this study have been described previously by Caliskan and Polat (2012). Fruit weight (FW, g) was investigated with a scale sensitive to 0.01 g (Precisa XB 2200 C, Precisa, UK). Digital calipers (0–150 mm; BTS Tools, Malaysia) were used to determine fruit lengths (FL, mm) and widths (FW, mm), fruit neck lengths (NL, mm) and ostiole widths (OW, mm). Fruit skin colors were evaluated by a colorimeter (Chroma Meter CR-300, Minolta Co., Osaka, Japan). Color parameters were expressed as L*, a*, b*, *C* and *h°* (Francis 1980). Fruit skin color was measured at two opposite locations per fruit. Fruit yields per mamme, profichi and mammoni crops were classified based on fruit numbers per shoot according to the standard fig descriptor. Mature profichi fruits from which *Blastophaga* wasps had not exited and in which anthers had not dehisced and leaves were collected for these examinations. The morphological analyses were conducted on 30 profichi fruits and 30 leaves for each accession. Three replicates, each consisting of 10 fruits or leaves, were performed. Also, 20 profichi fruits were evaluated for estimate pollinizer traits. Pollen viability and germination tests were investigated in ten fruits per accession. The mature profichi fruits were taken to room temperature with dry environment and then split open longitudinally and spread out to dry on aluminum foil on a laboratory bench. After 1 or 2 days, fruits had dried and all anthers had dehisced. The pollens were placed in glass jars (25 ml), then stored at 4 °C in a refrigerator (Storey 1975). Pollen viability was observed using 2,3,5-triphenyl tetrazolium chloride (TTC) staining. Pollen was evenly dusted onto the surface of the TTC solution and held at room temperature for 2 h under daylight. Pollen grains were investigated using a light microscope (x40; Nikon Eclipse E200, Japan). Pollen with bold red color was calculated as viable and colorless as nonviable. In vitro pollen germination was examined using Petri dish method (Eti 1991). Pollens were sown on culture with a clean brush, and incubated under dark conditions at 25 °C for 24 h. The culture medium included 1% agar, 3% sucrose, 100 ppm H_3BO_3, 300 ppm $Ca(NO3)_2$, 200 ppm $MgSO_4$ and 100 ppm KNO_3 at pH 5.0 (Caliskan et al., 2016b). Pollen germination was scored in four random fields of three Petri dishes for each caprifig. A pollen grain was evaluated germinated when the pollen tube length was equal to or greater than the pollen grain width. The numbers of anthers from three replications of 20 flowers per caprifig accession were counted, and after pollen shed, the number of pollen grains was observed using a hemocytometer (Eti, 1990). Pollen production per flower was calculated by multiplying the number of anthers per flower by number of pollen grains per anther. Pollen production per fruit was then investigated by multiplying the number of flowers per fruit by the number of pollen grains per flower. The numbers of male flowers per fruit, numbers of anthers per flower and numbers of gall flowers per profichi fruit that contain *Blastophaga* were also examined for each accession. These investigations were performed using five fruits per accession. The fruits

were stored at -20 °C until investigations. Each profichi fruit was divided into five or six pieces and the numbers of gall flowers that included *Blastophaga* wasps were counted. The numbers of anthers were also evaluated using five male flowers per fruit. Data were analyzed using SAS software (SAS, 2005). Analysis of variance tables were established with Duncan multiple range test at $p<0.05$. To evaluate dissimilarity among locations, cluster analysis was carried out using UPGMA (Unweighted Pair-group Method with Arithmetic Mean).

Results and discussion

Morphological and pollinizer characteristics of caprifig accessions were shown in Table 1. The effects of the locations on the caprifig characteristics except for anther number per flower, shoot length and profichi number per shoot were statistically significant ($p<0.05$). The mean fruit weight, fruit diameter and fruit length values were highest in the standard cultivars of Aydın location (30.33 g, 45.21 mm and 56.12 mm, respectively). The longest fruit neck length was found caprifigs in Osmaniye (13.47 mm) whereas the neck length was the lowest caprifigs in Mersin (9.58 mm). The lowest ostiole width was observed in Mersin location (0.93 mm). Khadivi-Khub and Anjam (2014) estimated that fruit weight varied from 11.52 to 38.12 g, fruit width varied from 18.60 to 34.70 mm, fruit length varied from 25.30 to 55.60 mm and ostiole width varied from 5.80 to 15.00 mm in Iranian caprifigs.

Table 1. Morphological and pollinizer characterization of caprifig accessions selected from different locations in the eastern Mediterranean region of Turkey.

Variable	Adana	Hatay	Kmaras	Mersin	Osmaniye	Aydın
FW	23.14 bc	27.41 ab	17.57 cd	19.84 cd	16.08 d	30.33 a
FD	40.70 a	44.32 a	38.95 ab	41.55 a	32.98 b	45.41 a
FL	52.55 ab	54.95 a	41.06 c	40.48 c	45.61 bc	56.12 a
FNL	13.05 ab	12.20 ab	11.66 ab	9.58 b	13.47 a	12.34 ab
OW	1.56 cd	1.90 bc	2.92 a	0.93 d	1.75 cd	2.47 ab
L	57.43 a	49.96b	48.53 b	47.07 b	51.62 ab	52.14 ab
a	-15.45 b	-15.32 b	-13.59 b	-6.80 a	-16.46 b	-16.46 b
b	39.97 a	40.38 a	24.97 c	33.35 b	40.96 a	42.84 a
C	43.72 b	47.65 ab	41.32 bc	36.19 c	47.11 ab	51.21 a
h°	106.69 ab	91.91 bc	110.79 a	80.61 c	94.17 bc	82.76 c
GFN	484.85 bc	521.48 ab	376.31 c	423.88 bc	521.68 ab	634.07 a
MFN	142.41 a	134.03 ab	98.28 b	128.34 ab	116.08 ab	131.01 ab
AN^{ns}	4.42	4.49	4.32	4.21	4.30	4.45
PV	87.20 ab	86.72 ab	84.92 ab	65.76 c	73.06 bc	90.51 a
GP	56.54 a	38.17 bc	44.74 ab	25.22 c	46.43 ab	42.34 ab
PNA	1343 a	1225 a	1417 a	703 b	1068 ab	1414 a
PNFL	5789 a	5236 a	5855 a	3030 b	4723 ab	5827 a
PNFR	824.188 a	708.809 a	543.852 ab	390.296 b	599.557 ab	722.090 a
SL^{ns}	17.73	12.99	12.32	14.82	13.75	13.76
SD	8.24 bc	8.63 b	6.91 c	7.89 bc	8.14 bc	10.08 a
LN	5.53 bc	5.10 bc	6.12 b	5.30 bc	4.73 c	9.88 a
LL	19.11 ab	19.23 ab	12.79 c	19.16 ab	17.82 b	21.23 a
LW	16.20 b	15.59 b	11.03 c	16.57 ab	14.90 b	18.48 a
LA	313.38 b	306.55 b	137.85 c	335.00 ab	287.22 b	396.58 a
PN^{ns}	3.78	3.89	4.01	3.71	3.51	4.82
MN	0.15 b	0.07 b	0.00 b	0.10 b	0.11 b	2.58 a
MAN	0.16 bc	0.22 bc	0.04 c	0.36 b	0.14 bc	1.42 a

FW; fruit weight (g), FD; fruit diameter (mm), FL; fruit length (mm), FNL; fruit neck length (mm), OW; ostiole width (mm), GFN; gall flower number, MFN; male flower number, AN; anther number, PV; pollen viability (%), GP; germination percentage (%), PNA; pollen number per anther; PNFL; pollen number per flower, PNFR; pollen number per fruit, SL; shoot length (cm), SD; shoot diameter (mm), LN; leaf number per shoot; LL; leaf length (cm), LW; leaf width (cm), LA; leaf area (cm^2), PN; profichi number per shoot, MN; mammoni number per shoot, MAN; mamme number per shoot.
^{ns}; non-significant.

The lightness (L) caprifig fruit skin colors were measured in Adana location (57.43). The highest a* value was detected in Mersin location (-6.80). The lowest b* values was found in Kahramanmaraş location (24.97). Caliskan and Polat (2012) indicated that lowest L*, C and h° values are correlated with darkest fig skin colors. The darkness fruit skin colors were measured caprifigs in Mersin location with the lowest chroma (*C*) and hue (*h°*) values (36.19, and 80.61, respectively). However, most of the caprifigs had the green skin colors. Some researchers showed that the fruit skin colors of caprifigs are mainly green (Condit 1955, Khadivi-Khub and Anjam 2014). The number of the gale flowers per profichi varied from 376.31 (Kahramanmaraş) and 634.07 (Aydın). The number of male flowers per profichi was highest in Adana location (142.41) while it was lowest in Kahramanmaraş location (98.28). Khadivi-Khub and Anjam (2014) displayed that the number of *Blastophaga* in profichi fruits ranged between 4.0 and 267.0 in Iranian caprifigs. Previous reports showed that when the pollen viability was over 50% (Gaaliche et al. 2013) and pollen germination was over 30% (Dokuzoguz 1953), the pollen can be considered functional. Pollen viability ranged between 65.76 (Mersin) and 90.51% (Aydın) and pollen germination ranged between 25.22 (Mersin) and 56.54% (Adana). The pollen numbers per anther, per flower and per fruit were the highest Aydın, Adana and Hatay locations (1414, 5827, 722.090; 1343, 5789, 824.188; and 1225, 5236, 708.809, respectively).
Shoot diameter was ranged from 6.91 (Kahramanmaraş) to 10.08 mm (Aydın). Leaf number per shoot, leaf length, leaf width, leaf area values of caprifigs were the highest in Aydın location (9.88, 21.23 cm, 18.48 cm and 396.58 cm^2). The highest mammoni number per shoot was found in Aydın location (2.58) whereas mammoni number per shoot was not observed in Osmaniye location. Mamme number per shoot was ranged between 1.42 (Aydın) and 0.04 (Kahramanmaraş).
The UPGMA dendrogram based on morphological and pollinizer variables measured 96 caprifig accessions selected from six locations were presented in Figure 1. The six locations can be divided into two main groups. The subgroup A included the locations of Adana, Hatay and Aydın. The caprifig accessions in Hatay and Aydın locations were showed very similar to each other depend on morphological characteristics such as fruit size, fruit neck length, pollen numbers. The caprifigs selected from Kahramanmaraş, Osmaniye and Mersin locations were classified in the group B. Kahramanmaraş and Osmaniye with similar fruit size, fruit skin color, pollen viability and germination and shoot length values were in the same subgroup.

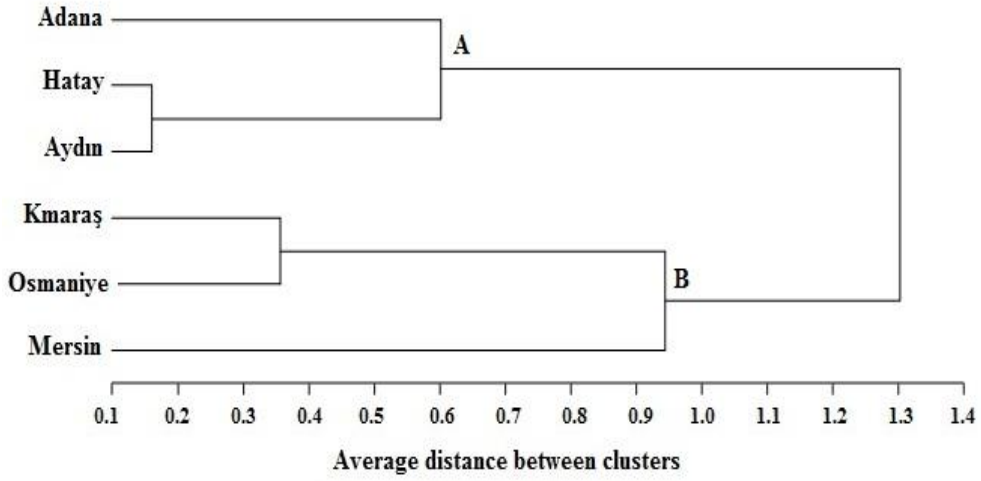

Figure 1. UPGMA dendrogram by using morphological parameters in the caprifig locations

Conclusions

Caprification is very critical for the edible fig culture. Unfortunately, there has been a few study into the genetic resources possible for caprifig germplasm. Further, the morphological and pollinizer parameters of caprifig accessions have not been characterized in detail. The study showed great variation in morphological parameters of caprifig accessions from different locations, eastern Mediterranean region of Turkey. According to these results, the caprifig accessions of Adana, Hatay,

Kahramanmaraş, Mersin and Osmaniye locations found in the eastern Mediterranean region of Turkey can be commonly used for caprification.

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