

**LEAD UPTAKE BY DIFFERENT ORGANS OF ORIENTAL TOBACCO GROWN IN THE REPUBLIC OF MACEDONIA**Biljana Jordanoska<sup>1</sup>, Valentina Pelivanoska<sup>1</sup>, Trajče Stafilov<sup>2</sup><sup>1</sup> *University "St. Kliment Ohridski" - Bitola, Scientific Tobacco Institute - Prilep, Republic of Macedonia  
e-mail: bi.jordanoska@gmail.com*<sup>2</sup> *University "Ss. Cyril and Methodius" - Skopje, Faculty of Science, Institute of Chemistry, Republic of Macedonia***ABSTRACT**

The study was conducted on 150 farms that grow oriental tobacco from three well known tobacco regions in Macedonia (Pelagonia, Southeastern Region and Vardar Valley). The total content of lead in soil samples was measured through decomposition by HF, HClO<sub>4</sub>, and HNO<sub>3</sub> acids. A solution of 0.005 mol/L diethylenetriaminepentaacetic acid (DTPA) and 0.1 mol/L triethanolamine at pH 7.3 was used for extraction of the element mobile form from soils. The digestion of plant samples was made with HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> using microwave digestion system. Atomic emission spectrometry with inductively coupled plasma (ICP-AES) was used for the determination of Pb content in the soil and plant samples. A correlation analysis was conducted between pH, organic matter content, total and mobile form of lead in the soil, and the concentration of this element in different organs of tobacco.

**Keywords:** lead, oriental tobacco, uptake, soil**ИЗВЛЕКУВАЊЕ НА ОЛОВОТО ОД РАЗЛИЧНИ ОРГАНИ НА ОРИЕНТАЛЕН ТУТУН ОДГЛЕДУВАН ВО РЕПУБЛИКА МАКЕДОНИЈА**

Студијата е спроведена на 150 локалитети од три познати реони во Македонија каде се одгледува ориенталентутун (Пелагонија, Југоисточниот Вардарскиот тутуно- произведен реон). Вкупната содржина на олово во примероците од почва е определена по нивното целосно разложување со HF, HClO<sub>4</sub>, и HNO<sub>3</sub>. За екстракција на подвижната форма на олово во испитуваните примероци почва е употребен раствор од 0.005 mol/L диетилентриаминпентаоцетна киселина и 0.1 005 mol/L триетаноламин со рН 7.3. Разложувањето на растителните примероци е извршена со HNO<sub>3</sub> и H<sub>2</sub>O<sub>2</sub> и примена на микробранов систем за разложување. Содржината на оловото во растителните и почвените примероци е определена со примена на атомската емисиона спектрометрија со индуктивно спрегната плазма (ICP-AES). Корелациони анализи се направени помеѓу рН на почвата, содржината на органска материја во почвата, содржината на вкупната и подвижна форма на олово во почвата и содржината на оловото во различните органи на ориенталскиот тутун.

**Клучни зборови:** олово, ориентален тутун, извлекување, почва

## INTRODUCTION

The toxic impact of Pb on the vital processes of almost all plants is well known (Rose et al. 2001; Ghaedi et al. 2007). It is confirmed that tobacco (*Nicotina tabacum*) can easily accumulate lead (Gondola and Kadar 1994). This crop is intermediate reservoir through which trace elements from soils, and partly from waters and air, move to final consumers, humans. Trace element content of the tobacco plants are variable and depend on many factors such as: soil properties, the climate, application of soil improvers and many more. Most of these properties, as well as environmental surroundings influence on the trace element uptake by tobacco plants (Adamu et al. 1989; Radojičić and Cvetković 2004; Golia et al. 2007). One of the parameter that has the

strongest impact on the intensity of metal accumulation is soil pH (Bell et al. 1992; Golia et al. 2007; Golia et al. 2009; Zaprijanova 2010). According to these authors trace element content in tobacco is influenced individually, or interactively and by several other parameters such as: organic matter content, clay content, etc.

Focus of this study has been given on exploring the relationship between soil properties, total and mobile forms of Pb in soil and its content in different parts of tobacco (root, stem, flower leaves and seed) from the oriental variety group of tobacco. This plant is used as indicator to provide information of the quality of the soil in which is grown and to determine the availability of the studied element for tobacco uptake.

## MATERIAL AND METHOD

Two years survey (2010-2011) was conducted in the well-known tobacco growing regions in Macedonia: Pelagonia region (PR), Southeastern region (SER) and Vardar Valley (VV) from 19 municipalities at 150 sampling sites (Figure 1). Soil composite samples from pedological profiles at fixed depth (0-30 cm) were taken from fields of each mentioned municipality. Samples were collected from 150 sites in each field with two replicates. Samples were taken from cultivated soil after tobacco harvesting, and from uncultivated soils in the nearest vicinity. Samples pretreatment was done in accordance to ISO 11464:2006. First the samples were air-dried and after that crushed and sieved through a 2-mm sieve.

The following soil properties were determined: mechanical composition (Đamić, 1996), pH (ISO10390:2005), total nitrogen (ISO11261:1995), organic matter (OM) with the wet oxidation method (Đamić, 1996), and calcium carbonate

equivalent volumetrically (ISO 10693). Electroconductivity was measured in a saturation extract, extractable phosphorus and potassium according to the ammonium lactate method, while the cation exchange capacity (CEC) was measured by the method described by Sumner and Miller (1996). Soil samples were digested with mixture of several acids (HNO<sub>3</sub>, HF, HClO<sub>4</sub> and HCl) for total digestion (ISO 14869-1). Plant available fraction of Pb was determined by extraction method using buffered solution of diethylenetetraaminepentaacetic acid (DTPA) at pH 7.3 (ISO 14870). Extracts were collected after filtration through Whatman No. 42 filter paper.

Tobacco samples (root, stems, leaves, blossoms and seeds) were selected from plants at the same sites where soils were sampled. Tobacco leaves from three primings were collected with the total of 450 representative samples from 150 sampling locations. Oriental tobacco

samples were washed carefully to remove any adhering soil particles and rinsed with redistilled water. The plant material was dried and homogenized to a constant weight after drying at 75 °C for 12 hours. Plant samples (0.5000 g) were digested in Teflon vessels with HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> using the Mars microwave digestion system (CEM, USA) (for elemental analysis. The plant samples were digested at 180°C. After cooling the digested samples were

quantitatively transferred into 25 mL calibrated flasks(Bačeva et al. 2012).

The investigated element was analyzed by the application of atomic emission spectrometry with inductively coupled plasma - AES-ICP (Varian, 715-ES). For the calibration a commercial standard mix solution (11355 - ICP Multi Element Standard IV, Merck) was used. The optimal instrumental conditions are given by Balabanova et al. (2010).

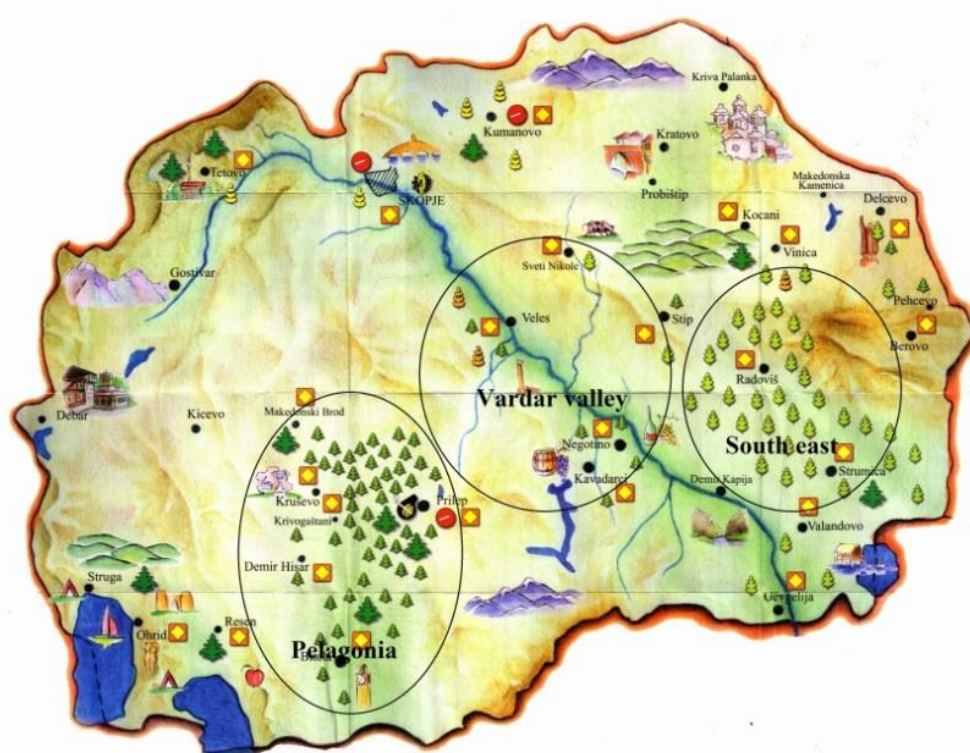


Figure 1. The location of the investigated tobacco growing regions in Macedonia (Pelagonia, South-eastern Region and Vardar Valley) provided by the Ministry of Agriculture, Forestry and Water Economy, Economic Chamber of Macedonia and Scientific Tobacco Institute – Prilep, 1999

## RESULTS AND DISCUSSION

Tobacco quality is highly influenced on the soil physical and chemical properties. Descriptive statistics of analyzed soil properties are given in Table 1. According to texture, the analyzed soilsamples vary from silt loam (18.8 % clay) to silt clay loams (77.6 % clay). Clayed soils were found only in the municipality of Krivogaštani (Pelagonia region). Average values of soil pH were 6.5 and 6.7 for PR

and SER, respectively. Average OM content is generally low to moderate, according to soil texture (Table 1). Available phosphorus and potassium concentrations varied differently. Extreme concentrations of available phosphorus (60-198 mg/100 g) were recorded in some sampling spots from municipalities of Novaci, Demir Hisar and Prilep (PR), Studeničani, Veles and Caška (VV). In the

same sampling spots the anomalies for available phosphorus were detected (Prilep and Veles); concentrations of available potassium of 60.93 mg/100 g and 63.64 mg/100 g were recorded, respectively. None of the examined soil samples displayed a low capacity for nutrient storage ( $CEC < 10 \text{ cmol}_c/\text{kg}$ ).

All soils had an adequate CEC for agricultural production. According to EC most of the samples are non-saline, except for some samples from Vardar Valley production region from municipality of Veles ( $EC > 360 \mu\text{S}/\text{cm}$ ).

**Table 1. Basic soil properties, descriptive statistics of three tobacco production areas**

Soil properties	Pelagonia Region			South-eastern Region			Vardar Valley Region		
	Mean	$s_a$	Min-Max	Mean	$s_a$	Min-Max	Mean	$s_a$	Min-Max
OM, %	1.5	0.5	0.8-3.2	1.3	0.3	0.7-2.1	1.6	0.3	1-2.1
TN*, %	0.08	0.04	0.02-0.4	0.06	0.02	0.01-0.11	0.07	0.02	0.05-0.1
pH	6.5	0.6	5-8.3	6.7	0.5	5.5-8	7.7	0.8	6.5-8.6
Clay, %	37.7	11.1	18.8-78	36.4	11.9	19.4-64	45.9	7.2	32-56
Available P, mg/100 g	19.7	32.2	0.5-198	12.5	23.5	1.5-154	24.5	25.3	1.8-64
Available K, mg/100 g	20.4	7.4	3.2-64	21.8	4.6	13-32	31.7	14.3	22-63
CEC, $\text{cmol}_c/\text{kg}$	10.6	2.6	6-19	9.7	2.3	6.5-16	11.8	1.9	8.2-15
EC, $\mu\text{S}/\text{cm}$	83	56	23-360	79	55	27-264	215	109	42-362

\*TN – total nitrogen,  $s_a$ -standard deviation, Min-minimum, Max-maximum

The total lead content in soils varies from 10 mg/kg to 30 mg/kg (Table 2). According to production region distributions, samples had similar Pb concentration (Table 2). Although Veles region is known for its lead and zinc industrial activity in the nearest past (Stafilov et al. 2010), concentration of Pb in soils from all production regions is not exceeding the contamination limit of 85

mg/kg for optimal value and 530 mg/kg for intervention value (The new Dutch list, (<http://www.contaminatedland.co.uk/std-guid/dutch-1.htm#KEYWORD-ONE>)).

As it can be seen from data presented in Table 2, the mobile lead content varies from 0.5 mg/kg to 4.4 mg/kg. The highest values were recorded in soils with the highest total Pb content, respectively.

**Table 2. Total and DTPA extractable Pb in soil from different tobacco growing areas (given in mg/kg)**

LOD*	Pelagonia Region			South-eastern Region			Vardar Valley			
	mg/kg	$X_g$	$s_a$	Min-Max	$X_g$	$s_a$	Min-Max	$X_g$	$s_a$	Min-Max
Total Pb	10	14	4.8	10-30	15	5.5	10-29	14	4.5	10-22
Mobile Pb	0.5	0.5	0.17	0.5-1.0	0.9	0.5	0.5-2.2	2.2	1.1	0.9-4.4

\*LOD- limits of detection,  $X_g$ -geometrical mean,  $s_a$ -standard deviation, Min-minimum, Max-maximum

Distribution of Pb content in different tobacco organs are given in Figure 2. Highest accumulation with average value of 1.5 mg/kg was recorded in leaves from the first harvesting zone (first priming). According to Tso (1990) and Kabata-Pendias (2011) lead concentration in tobacco leaves and plant material may reach up to 200 mg/kg. According to regulative in Macedonia, maximum permitted concentrations for Pb in tobacco leaves are 10 mg/kg. Availability of 6.2 % of the studied element was calculated as a ratio ( $C_{DTPA}/C_{total} \times 100$ ) of available concentration (DTPA extraction) and total concentration of Pb in soil.

Using determined concentrations in different parts (root, stem, flower, leaves and seed) of tobacco plant and total element content of examined soils the Biological Accumulation Factor (BAF) was calculated. This factor is defined as the ratio between total content of the elements in all parts of studied plant and corresponding soil. Calculated average values for Pelagonia Region and South-eastern Region are 0.16 meaning tobacco is capable to accumulate small amounts of this element. Higher average values of BAF of 0.21 were obtained from samples of Vardar Valley region.

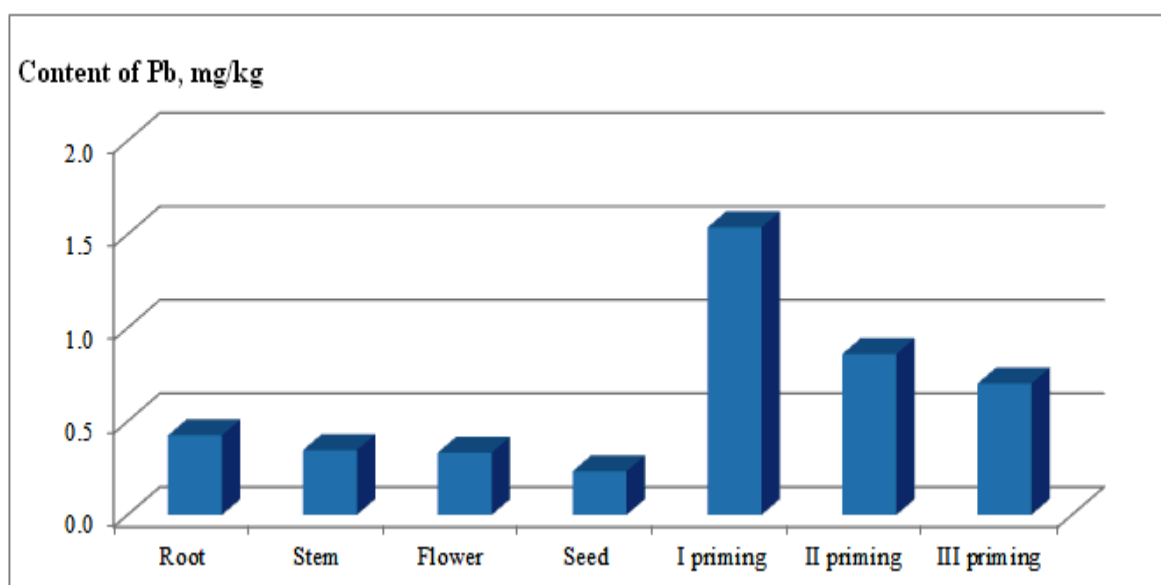


Figure 2. Pb content in different tobacco organs

The correlation dependencies among important parameters of soil and total quantities of Pb in soil and tobacco leaves as well DTPA extract were analyzed using correlation analysis (Pearson correlation, two-tailed). The obtained results are given in Table 3. As it can be seen no significant correlation was observed among Pb content of tobacco leaves and soil parameters. Gondola and Kadar (1994), Golia et al. (2009) and Zaprijanova et al. (2010) observed correlations among soil properties and

element concentration in tobacco harvesting zones, finding significant negative correlation between pH and organic matter of soil and Pb content in oriental tobacco. All these authors found a statistically important relation between the mobile forms of Pb extracted from the soil and their concentration in tobacco leaves. This is in accordance with the results of our study where significant correlation was observed between most of the soil properties and mobile form of Pb.

**Table 3. Correlation coefficients of Pb content in tobacco leaves and soil properties**

	Pb soil	Pb DTPA	Pb I priming	Pb II priming	Pb III priming
Pb DTPA	0.26	1			
Pb I priming	-0.09	-0.21	1		
Pb II priming	-0.12	-0.10	0.15	1	
Pb III priming	0.07	-0.10	0.08	0.11	1
OM	0.20	<b>0.43</b>	-0.12	-0.10	0.03
TN	0.07	<b>0.43</b>	0.06	-0.03	0.03
pH	0.02	<b>0.45</b>	-0.16	-0.08	-0.16
Clay	0.22	<b>0.42</b>	-0.10	-0.10	0.11
P <sub>2</sub> O <sub>5</sub>	0.00	-0.01	0.09	0.00	0.00
K <sub>2</sub> O	0.00	0.21	-0.08	-0.08	0.00
CEC	0.25	<b>0.50</b>	-0.13	-0.12	0.09
EC	0.09	<b>0.62</b>	-0.17	-0.07	0.06

Bold numbers present significant correlation at 0.01 levels

## CONCLUSIONS

According to soil properties, generally all analyzed samples are a good base for production of the high-quality oriental tobacco. Moreover, some adjustments are needed that can largely be achieved through appropriate fertilization rate recommendations. The soil concentration of lead in all analyzed samples pointed out levels which are typical of agricultural and low anthropogenic pressure areas. Contents of lead in all plant samples are under limits

considered critical. According to results of correlation analysis there are no statistically significant dependences determined between the soil parameters and the lead concentration in the plant organs of oriental tobacco. Regression dependencies of some significance are observed between the mobile lead in the soil and most of the soil parameters except available forms of phosphorus and potassium.

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