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CADMIUM UPTAKE BY DIFFERENT PARTS OF ORIENTAL TOBACCO GROWN IN THE REPUBLIC OF MACEDONIA

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ABSTRACT

Some plants have the ability to absorb and accumulate heavy metals that do not have important biological functions in their diet and development. Compared with other crops tobacco is well known accumulator of heavy metals even when grown on uncontaminated soil.The main aim of this investigation is to study cadmium uptake by oriental tobacco and its correlation between soil properties as well as the content of this element in different parts of tobacco. Samples were taken from 150 farms that grow oriental tobacco from three well known tobacco regions in Macedonia (Pelagonia, Southeastern Region and Vardar Valley). All analyzed samples are from the areas which are typically agricultural and with low anthropogenic pressure. It was found that the oriental tobacco has a high tendency for accumulation and transfer of Cd in top parts of the plant.

Keywords: cadmium, oriental tobacco, uptake, soil

ИЗВЛЕКУВАЊЕ НА КАДМИУМОТ ОД РАЗЛИЧНИ ДЕЛОВИ НА ОРИЕНТАЛЕН ТУТУН ОДГЛЕДУВАН ВО РЕПУБЛИКА МАКЕДОНИЈА

Одредени видови растенија имаат способност да апсорбираат и акумулираат тешки метали кои немаат значајни биолошки функции во нивната исхрана и развој. Во споредба со други земјоделски култури тутунот е познат акумулатор на тешки метали дури и кога се одгледува на незагадени почви. Главната цел на ова истражување е да се проучи извлекувањето на кадмиум кое го врши ориенталниот тутун, неговата корелација со почвените параметри, како и содржината на овој елемент во различните органи на тутунското растение. Примероци се земени од 150 локалитети од три познати реони во Македонија каде се одгледува ориентален тутун (Пелагонија, Југоисточниот и Вардарскиот тутуно-производен реон). Сите анализирани примероци се во граници кои се типични за земјоделски површини и ниски антропогени влијанија. Утврдено е дека ориенталениот тутун има голема тенденција за акумулација и пренос на Cd во антенските делови.

Клучни зборови: кадмиум, ориентален тутун, извлекување, почва

INTRODUCTION

Compared with other heavy metals, cadmium is characterized by considerable mobility in the system soil - plant and accumulates in the antenna parts of the plant to a much greater extent than other heavy metals (Symeonides & McRae, 1977). Cadmium is one of the most toxic and most harmful elements. Phytotoxicity of Cd is well known due to the high rate of transfer and the light accumulation in plants (Alloway & Ayres, 1994). It is well known that tobacco (*Nicotina tabacum*) easily accumulates Pb and Cd (Gondola & Kadar, 1995). According to some data (Golia et al., 2009) Cd content in tobacco variety Berlej is from 0.045 to 3.6 mg/kg, and variety Virginia has Cd content of 0.01–3.4 mg/kg. According to the results of Tso (1990), cadmium content of tobacco leafs exceeds 11.6 mg/kg. These values are much higher than the obtained values for most plant cultures. Usually its content is never above 0.05 mg/kg. Addition of nitrogen fertilizers, especially those containing chlorides, increases the availability of cadmium for plants (Alkorta et al., 2004). The absorption of macro and trace elements from plants and their accumulation in vegetative and reproductive organs has a very specific character both in terms of the types of plants, and in terms of the particular element.

The main aim in this investigation is to study cadmium uptake by oriental tobacco from the Republic of Macedonia and its correlation between soil properties as well as the content of this element in different organs of tobacco.

MATERIALS AND METHODS

Two years survey (2010 and 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. 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. Sample 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 (ISO 10390:2005), total nitrogen (ISO 11261:1995), organic matter (OM) by the wet oxidation method (Đamić, 1996), and calcium carbonate equivalent determined volumetrically (ISO 10693). Electroconductivity was measured in a saturation extract, the content of the extractable phosphorus and potassium according to the ammonium lactate method, while the cation exchange capacity (CEC) was measured by the method of Sumner and Miller (1996).

For the determination of total content of cadmium in soil the samples were digested with mixture of 4 acids (HNO_3, HF, $HClO_4$ and HCl) according to ISO 14869-1.

Plant available fraction of Cd was determined by the extraction method using buffered solution of diethylentetraaminepentaacetic 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. 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. For the analysis of the total content of Cd, plant samples (0.5000 g) were digested in Teflon vessels with HNO_3 and H_2O_2 using the microwave digestion system (Mars, CEM, USA). 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 – ICP-AES (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).

RESULTS AND DISCUSSION

Descriptive statistics of analyzed soil properties are given in Table 1. Soil samples vary from silt loam (18.8 % clay) to silt clay loams (77.6 % clay). According to soil texture, average OM content is generally low to moderate (Table 1). Available phosphorus and potassium contents varied differently. Soils had an adequate CEC for agricultural production. All analyzed parameters pointed out the levels which are typical for agriculture except few sampled points from Pelagonia production region that had high content of available phosphorus.

Table 1. Basic soil properties, descriptive statistics of three tobacco production areas (Jordanoska et al., 2013)

	Pelagonia Region			South-eastern Region			Vardar Valley Region		
Soil properties	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, $cmol_c$/kg	10.6	2.6	6-19	9.7	2.3	6.5-16	11.8	1.9	8.2-15
EC, µS/cm	83	56	23-360	79	55	27-264	215	109	42-362

OM - organic matter, TN – total nitrogen, CEC - cation exchange capacity, EC – Electroconductivity, s_a - standard deviation, Min - minimum, Max - maximum

The total Cd content in soil samples was under the detection limits (<1 mg/kg) and therefore is not presented. Average values from different tobacco growing areas are given in Table 2. As it can be seen from data presented in Table 2, the mobile cadmium content varies from 0.02 mg/kg to 0.3 mg/kg. The content of Cd in tobacco leaves varies from 0.1-1.9 mg/kg, with an average value of 0.48 mg/kg. The highest content of Cd have samples from Vardar Valley.

Table 2. The content of Cd in tobacco leaves and the content of DTPA extractable Cd 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
Cd – leaves	0.005	0.35	0.17	0.09-0.89	0.45	0.25	0.18-1.27	0.8	0.5	0.3-1.9
Mobile Cd	0.005	0.02	0.01	0.01-0.07	0.06	0.04	0.05-0.2	0.2	0.1	0.1-0.3

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

Average content of Cd in tobacco leaves distributed by municipalities is given in Figure 1. As it can be seen, the samples from the municipality of Caška have the highest average values. All samples taken from this municipality have content in the range of 0.8-1.9 mg/kg Cd. This high content of Cd in the tobacco from this area is due to the polluted soil from the metallurgical activities of the former lead and zinc smelter located near the city of Veles (Stafilov et al., 2010). According to Tso (1990) tolerable content of Cd in tobacco is 3.0 mg/kg. No sampled tobacco leaves contains more than 2.0 mg/kg.

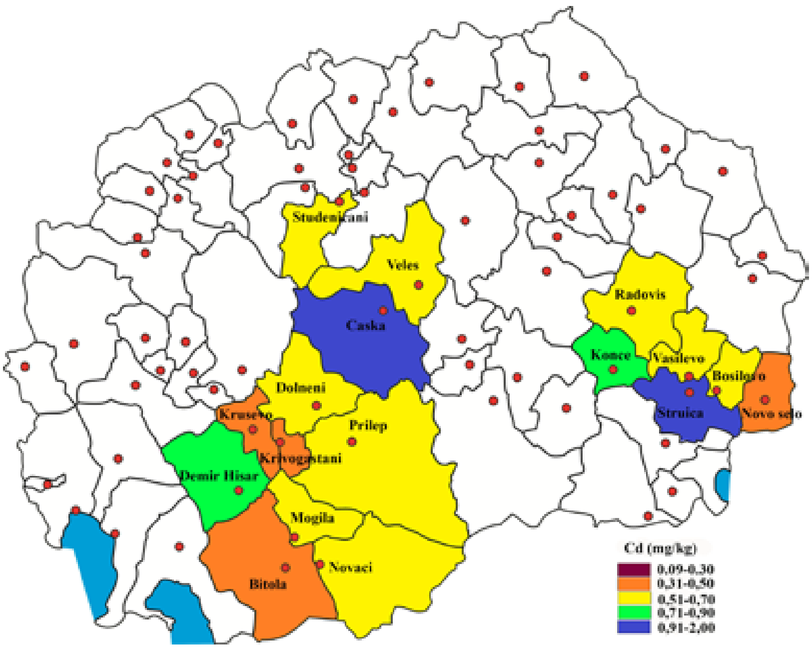

Figure 1. Ranges of content of Cd in tobacco leaves in samples from different studied municipalities in the Republic of Macedonia

The average content of Cd in different tobacco parts are given in Figure 2. The highest accumulation was recorded in leaves from the first harvesting zone (first priming). The content of Cd in the leaves is almost five times higher than the content in other vegetative organs. Compared to the other organs, stem and flower have almost equal distribution of 0.08 mg/kg. Although cadmium is a non-essential element for plants, it can easily be accumulated through the root system and by foliar feeding. Cadmium is easily transported from the roots to the aerial organs. Its transfer through the whole plant may be limited because it can easily be kept in places where there are active components found in cells (Kabata-Pendias, 2011).

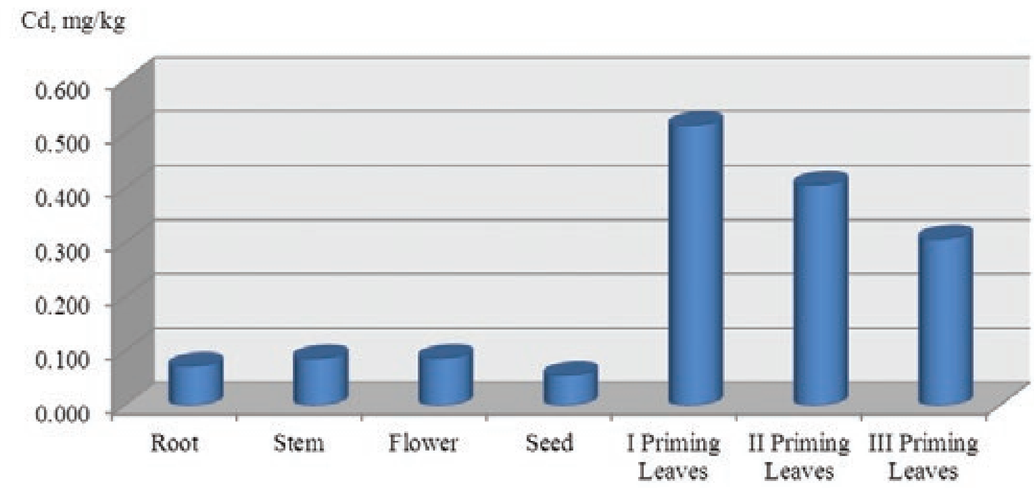

Figure 2. The average Cd content in different tobacco parts

Using determined contents in different parts (root, stem, flower, leaves and seed) of tobacco plant and total element content of corresponding 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 in corresponding soil. The BAF for Cd is calculated taking the value which corresponds to the half of the limit of detection obtaining BAF of 2.58. Compared to other trace elements only Cu (BAF=3.2) has higher factor than Cd. This means that oriental tobacco accumulates high content of this observed element.

To assess the bioaccumulation ability of certain elements in the parts of the tobacco plant, despite biological accumulation factor, another parameter - Biological Transfer Factor (BTF) is also used. This factor represents the ratio of the content of the element in the aerial tissues of the plant and the root. This factor indicates the efficiency of transfer of the elements to the antenna parts of the plant. According to the values of this factor we found that Cd has the biggest BTF compared to all analyzed micro and macro elements. This means that the biggest transfer in the aerial organs of oriental tobacco has Cd. Also, this means that the oriental tobacco has a high tendency for accumulation of Cd in the antenna parts (BTF = 20.2), similar to that of Li (BTF = 20.0).

The correlation dependencies among the important parameters of soil and total quantities of Cd in tobacco leaves as well in the DTPA extract were processed using correlation analysis (Pearson correlation, two-tailed). The obtained results are given in Table 3 and 4. According to the data of the regression and correlation analysis, significant correlation was observed only among soil clay and Cd content in the leaves from the third priming (Table 3). Soil pH and Cd content of all vegetative organs of oriental tobacco have no significant correlations. This is not in accordance to literature data that study mainly the relation of pH and element content of the leaves (Gondola & Kadar, 1993; Adamu, 1989). There are no significant correlations between the organic matter and Cd in all parts of the tobacco plant, as well as its available fraction. Significant correlations were determined with some moving elements from the soil and Cd in leaves from the first and second harvest belt and are given in Table 3.

Flower and root, and leaves from second and third priming have significant correlation at level 0.01 of significance (Table 4). Cadmium content in flower has linear dependence with the amount of Cd in the root. From the determined coefficients we can conclude that around 80% of the Cd in the flower depends of its content in the root.

Table 3. Significant correlation coefficients of Cd content in tobacco leaves and soil properties

Parameter	Leaves			Root	Stem	Flower	Seed
	I priming	II priming	III priming				
Cd-DTPA	**0.309**	**0.265**	**0.462**	0.088	-0.050	0.084	0.051
Clay	**0.311**	0.081	0.190	1.000	0.000	0.081	**0.274**
P_2O_5	**-0.317**	-0.108	-0.118	0.035	-0.158	0.037	0.097
K_2O	**0.267**	0.015	0.054	-0.211	0.125	-0.207	-0.103

Bold numbers present significant correlation at 0.01 levels

Some weak correlations are also found between the total content of Al, Na, Ni and Sr in the soil and the content of Cd in the second harvest belt. Negative significant correlation coefficients are determined and between Cd content in the leaves of the first priming and total content of Ba, Ca, Na and Sr of the soil samples.

Table 4. Significant correlation coefficients of Cd content in tobacco parts and micro and macro elements in the soil samples

Parameter	Leaves			Root	Stem	Flower	Seed
	I priming	II priming	III priming				
Cd II priming	**0,469**	**0,561**	1,000	0,190	-0,022	0,185	-0,042
Cd stem	-0,162	0,081	0,190	1,000	0,000	**0,991**	0,034
Al	**-0,419**	-0,184	-0,275	-0,009	0,035	-0,012	-0,026
Ba	**-0,287**	-0,055	-0,129	-0,063	-0,046	-0,060	0,112
Ca	**-0,282**	0,002	-0,222	0,110	-0,112	0,117	0,040
Na	**-0,349**	**-0,271**	**-0,274**	0,085	0,137	0,080	-0,073
Ni	0,148	**0,314**	**0,258**	0,019	-0,153	0,027	0,200
P	**-0,317**	-0,108	-0,118	0,035	-0,158	0,037	0,097
Sr	**-0,326**	-0,172	**-0,232**	0,118	-0,003	0,127	0,067

Bold numbers present significant correlation at 0.01 levels

CONCLUSIONS

According to the determined soil properties, all analyzed soil samples are in good condition for the production of the high-quality oriental tobacco. Cadmium content of soil samples war under the detection limits (<1 mg/kg), so all analyzed samples pointed out levels which are typical of agricultural and low anthropogenic pressure areas. All plant samples had Cd content under limits considered critical. According to distribution of this element in tobacco vegetative organs, the highest accumulation was recorded in leaves from the first harvesting zone (first priming). According to the value for BAF it can be concluded that oriental tobacco plant accumulates high content of P, K, Cu and Cd. From all analyzed samples and calculated BTF it can be concluded that oriental tobacco has a high tendency for transfer and accumulation of Cd in antenna parts. According to data of the regression and correlation analysis, significant correlation was observed only among the content of soil clay and the content of Cd in the leaves from the third priming. Regression dependencies of some significance are observed between the mobile cadmium in the soil and most of the soil parameters except with the available forms of phosphorus and potassium.

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