Cu, Zn, Mn AND Fe CONTENTS OF THE ORIENTAL TOBACOO MANUFACTURED IN REPUBLIC OF MACEDONIA

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ABSTRACT

Zn, Cu, Mn and Fe are essential components of proteins in plants, although are toxic in excess. The objectives of this study were to determine Cu, Zn, Fe and Mn concentrations in tobacco leaves produced in different growing areas in Macedonia. For that purpose oriental tobacco leaves were taken from 70 different locations from three primings of tobacco stalk, as well as corresponding soil samples. A correlation was made between some soil parameters (pH, clay, humus) and concentration of investigated metals in the tobacco leaves. The study showed a strong correlation between concentrations of the microelements in all three primings.

Key words: *metals, tobacco, soil, correlation*

INTRODUCTION

Territorial and natural conditions in Macedonia are well known to the world market as suitable soil for growing high-quality Oriental type tobacco [21]. Physiological and biochemical functions on Cu, Zn, Fe and Mn in the tobacco plants reveal to many cell proceses such as: photosynthesis, protein synthesis, regulation and consumption of sugars, carbohidrates, nitrogen, phosphorus exchange, etc. It is well known that micronutrients such as iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) are essential metals for plant growth and yield. Zn and Cu are essential components of thousands of proteins in plants, although are toxic in excess. Copper participates in numerous physiological processes and is an essential cofactor for many metalloproteins, however, problems arise when excess copper is present in cells. Excess copper inhibits plant growth and impairs important cellular processes [24]. Zn toxicity occurs in soils contaminated by mining and smelting activities, in agricultural soils treated with sewage sludge, and in urban and peri-urban soils enriched by anthropogenic inputs of Zn, especially in low-pH soils [7].

The need for Mn in mature plant leaves varied from 10 to 50 mg/kg dry mass, but its content in plant matter reached over 200 mg/kg (with soya 600, with cotton 700, and 1380 mg/kg dry mass with sweet potatoes) before the development of toxicity symptoms [14]. Golia et al. [11, 12] in his 3 year study on the heavy metal content in Burley and Virginia tobacco, cultivated in Central Greece showed that manganese content varied in very wide range 16.7 – 662.5 mg/kg with Burley and 4.1 – 897.5 mg/kg with Virginia. There is no data on manganese and iron toxicity display. But deficiency with these two elements leeds to formation of chlorotic spots, necrosis on young leaves and weakened turgor. Manganese deficiency in tobacco was demonstrated when leaf content was below 20-30 mg/kg, mostly when tobacco was grown on alkaline soils [16]. Manganese toxicity was usually observed when element content in tobacco leaves was over 1000 mg/kg [17, 18, 25,]. Mn concentration in tobacco leaves increased significantly with strong acid soil reaction and might reach 2400 mg/kg [4, 25]. The main source of these four elements are soils. The content of Cu, Zn, Mn and Fe in soil can vary widely - from deficit levels to toxic concentrations in acidic soils. Soil type, soil moisture, mineral and clay types and contents, diffusion and mass flow rates, weathering rates, soil organic matter, soil biota and plant uptake will also affect micronutrient distribution [5].

The tops of all plants are collectors for all air pollutants, and their chemical composition due the plant uptake form soils can be a good indicator for contaminated areas [19, 22]. As tobacco is in the group of agricultural products that play a significant role in the agricultural production of the Republic of Macedonia, we use it as indicator to provide an insight into possible contamination that may be occurring in the study area. The aim of this work was to analyze Cu, Zn, Fe and Mn content

in tobacco leaves from different locations in Macedonia, family farms that grow Oriental tobacco. Adequate soil samples were collected to survey content of these elements as well as basic soil properties such as humus, clay content, soil reaction, total nitrogen, phosphorus and potassium content.

Soil reaction is one of the major factors influencing the metal concentration in tobacco leaves [1, 3, 12, 23, 25]. A significant negative correlation between pH of soil and heavy metal content in oriental tobacco was determined in Golia's studies [12]. Husnjak [10] stated the same, indicating that heavy metal content in tobacco is influenced individually, or interactively by several parameters such as soil reaction (pH), organic matter content (humus), mechanical content (percentage of clay), etc. [1, 25] found that humus content influences the heavy metal concentration. Based on the previous, another objective of this study was to determine the most important soil factors (chemical and physical), which influence the overall Cu, Zn, Mn and Fe concentration in the three primings of the Oriental tobacco grown in various parts of Macedonia.

MATERIALS AND METHODS

A survey was conducted in the well-known tobacco-growing regions in Macedonia (Prilep, Krivogaštani, Mogila, Novaci, Bitola, Demir Hisar, Kruševo, Dolneni, Veles, Caška, Studeničani, Skopje) and in some regions of Eastern Macedonia (Strumica, Vasilevo, Bosilevo, Novo Selo, Radoviš and Konče) from 70 samping sites. 210 leaves from oriental tobacco were collected from the lower, middle and upper primings. The lower primings included the sand and bottom leaves. The middle primings included the first, second and third middle leaves. In the upper primings were lower top and top leaves. The same localities were also used for sampling of 70 composite soil samples from pedological profiles at fixed depths of; 0 - 10 cm, 10 – 20 and 20-30 cm. Samples were taken during August - November, 2010 in each filed with two replicates. Samples pretreatment was done in accordance with ISO 11464:2006. First they were air-dried, and after that crushed and sieved through a 2-mm sieve. Physical properties were determined, such as; clay content (Korunović & S.V. Stojanović, 1989), pH (10390:2005), total nitrogen (11361:1995), humus (standard method developed by I.V. Tjurin, modified by Simakov), available phosphorus and potassium (Al-method, validated at the Scientific Tobacco Institute - Prilep, Macedonia, 2009). The metals contents was determined using the *Aqua Regia* (HCl-HNO_3, 3:1) extraction method (ISO 11047:1998) after digestion at 180ºC for 2 h. All reagents were of analytical grade (Merck, Germany). Appropriate blanks were included in all extractions.

Oriental tobacco leaves were washed to remove any adhering soil particles and rinsed with distilled water. After that, leaf samples were placed in paper bags, dried at 75 □C for 12 hours and ground using a mortar and pestle. Wet digestion of tobacco samples was performed using an oxi-acidic mixture of HNO_3/H_2O_2 (Handbook of reference methods for plant analysis, 1998) in a 100-mL beaker. This mixture was heated up to 130˚C for 1 hr and brought to a volume of 50 mL with deionized water. A blank was carried out in the same way.

The data was statistically analyzed using correlation analysis (Pearson correlation, two-tailed). Statistical analyses were performed using the SPSS 9.0 software. Correlation analysis was used to establish a relationship between physical and chemical characteristics of soil samples and the Cu, Zn, Mn and Fe content in the Oriental tobacco leaf samples from the three positions in the plant.

RESULTS AND DISCUSSION

It is known that soil is the fundamental base of agricultural production and its fertility is extremely important on the yield and quality of farmed crops. Soil fertility is described as the content of certain nutrients such as humus, total nitrogen, available phosphorus and potassium, carbonates, the reaction of the soil and some biogenic microelements. Descriptive statistics for basic soil properties are given in Table 1.

The analysis of soil composition shows a high variation of the major physical and chemical properties which define soil fertility (Table 1). The clay content varied from 19.50 to 77.6% and pH ranged from 6.00-8.50. According to classification, soils are from middle to hard loam, and from easy to middle clay. Humus content varied from 0.77 to 3.21 %. The literature data indicate that soils with lower humus content are a good environment for producing quality oriental aromatic tobacco [2, 8, 15, 19]. According to the results, 54% of the samples showed low humus content, 42% were with average content and 4% with very low and good content. The total nitrogen content was low and similar to that of the humus. The reaction of soil (pH) was neutral in 58% of the soils, 16% were weakly acid and 20% of the samples had poorly to moderately alkaline reaction. 80% of the soils were non-carbonate, 6% were poorly carbonate, 8% moderately and 6% strongly carbonate. 36% of the samples had low and extremely low concentration of phosphorus.

Phosphorus and potassium concentrations varied from 1.5 to 73.7, and 3.18- 60.93 mg/100g soil, respectively. Examined soils have medium total nitrogen that varies from 0.01 to 0.40 %. The general conclusion is that the variability of the soil properties is due to their geochemical origin represented by different types of land.

The Cu content in the examined leaves ranges from 1.3 to 15.8 mg/kg . Zn concentration varied from 2.2 to 34.1 mg/kg (Table 2). Mn and Fe, from 20 to 223 mg/kg and 22.5 to 438 mg/kg respectively.

The average concentrations of all four metals in tobacco and soil samples indicate that soil has higher concentrations of metal compared to tobacco. All elements had higer concentrations in the second priming of raw tobacco samples except Fe. Cu, Fe and Mn content in soil had a steady distribution in all three layers (0-10, 10-20 and 20-30 cm), while Zn concentration was higher in the II-nd and the III-rd layer (Table 3).

Table 1. Basic soil properties, descriptive statistics

Statistical index	Humus %	Total Nitrogen %	Soil reaction (pH)		Clay %	mg/100 g soil	
			H_2O	(pH) KCl		$P_2 0_5$	K_2O
Mean	1.64	0.08	6.97	5.82	39.41	17.96	22.96
Minimum	0.77	0.01	6.00	4.84	19.50	1.54	3.18
Maximum	3.21	0.40	8.50	7.23	77.60	73.77	60.93
CV, %	33.14	66.92	8.63	10.29	31.50	102.66	34.66

Table 2. Content of Cu, Zn, Mn and Fe in tobacco (n=70)

Heavy metals	Statistical index	1 st priming	2 nd priming	3 d priming
Cu, mg/kg	Mean, mg/kg	5.3	6.1	5.5
	Minimum, mg/kg	1.9	1.3	1.9
	Maximum, mg/kg	15.8	13.0	10.3
	CV, %	51.2	47.8	36.9
Zn, mg/kg	Mean, mg/kg	13.7	20.9	18.5
	Minimum, mg/kg	2.2	4.6	6.4
	Maximum, mg/kg	34.1	21.5	20.3
	CV, %	44.2	136.6	134.8
Mn, mg/kg	Mean, mg/kg	69.04	58.1	58.4
	Minimum, mg/kg	28.20	24.2	20.4
	Maximum, mg/kg	136.6	202.4	222.9
	CV, %	40.1	55.0	70.7
Fe, mg/kg	Mean, mg/kg	180.3	136.2	85.5
	Minimum, mg/kg	42.7	30.0	22.5
	Maximum, mg/kg	438.3	385.0	232.9
	CV, %	68.7	70.3	62.4

Table 3. Content of Fe, Mn, Zn and Cu in soil (n=70)

Heavy metals	Statistical index	(0-10) cm	(10-20) cm	(20-30) cm
Mn, mg/kg	Mean, mg/kg	1622.3	1428.1	1337.4
	Minimum, mg/kg	170,9	144.1	56.0
	Maximum, mg/kg	2999.4	3143.0	2886.6
	CV, %	53.0	58.7	65.9
Fe, mg/kg	Mean, mg/kg	14353.1	14547.2	14343.2
	Minimum, mg/kg	174.1	98.3	232.7
	Maximum, mg/kg	31674.9	30597.6	31830.9
	CV, %	48.3	49.7	48.2
Cu, mg/kg	Mean, mg/kg	17.6	17.7	17.8
	Minimum, mg/kg	5.1	6.1	6.3
	Maximum, mg/kg	45.0	44.2	41.5
	CV, %	50.4	50.8	48.7
Zn, mg/kg	Mean, mg/kg	43.2	46.0	49.6
	Minimum, mg/kg	15.5	15.6	17.5
	Maximum, mg/kg	81.7	117.6	127.7
	CV, %	40.1	49.0	44.6

Studies indicate that lower leaves seem to accumulate high amounts of metals, but in all cases the concentrations were lower than those reported by other investigators [19; 6, 4, 13 ,22 18].

Table 4. Correlation among soil parameters and concentration of studied metals in Oriental tobacco leaves T is for tobacco leaves (n = 70)

	Cu Soil	Mn soil	Fe soil	Zn soil	Cu T	Mn T	Fe T	Zn T
pH	-0.223	-0.411*	0.017	-0.525**	0.113	-0.658**	-0.239	-0.163
Humus	0.353	0.124	0.017	0.564**	0.214	0.236	0.171	0.392*
Clay	-0.147	-0.073	0.086	-0.200	0.261	-0.530**	-0.292	0.261
Cu Soil	1.000	0.516**	0.576**	0.807**	0.329	0.342	0.482*	0.042
Mn soil		1.000	0.585**	0.467*	0.058	0.426	-0.077	0.017
Fe soil			1.000	0.300	-0.120	0.032	0.160	-0.142
Zn soil				1.000	0.317	0.369	0.455*	0.155
Cu T					1.000	-0.067	0.192	0.177
Cd T						0.606	-0.319	0.115
Mn T						1.000	0.232	0.105
Fe T							1.000	-0.140
Zn T								1.000

1st - lower primings 2nd – middle primings 3rd – upper primings

* Correlation is significant at 0.05 level (2-tailed). ** Correlation is significant at 0.01 level (2-tailed)

Correlation coefficients between total metal concentrations in tobacco and soil parameters (Table 4) showed a strong relationship among the concentrations of Mn in soil, and Mn in tobacco leaves with pH of the soil. No significant correlation was noticed between pH and other metal contents in our investigations, which does not conclude with those of some authors, who reported strong correlation [1; 12, 25].

Zn in the plants matterial has a significant correlation at 0.01 level, with humus content of the soil. Clay had influence (correlation is significant at 0.01 level) on the concentration of Mn content of the Oriental tobacco.

According to the results, it can be stated that most of the soils are ideal for producing high quality Oriental tobacco. Concentrations of Cu , Zn, Mn and Fe in investigated tobacco plants and soils are below permissible limit values in conventional and ecological agriculture. Comparing the results of the investigation, it can be concluded that Fe, Zn, Cu and Mn content in soil has a negligible or no influence upon the same metals cncentration in tobacco.

REFERENCES

1. Adamu C. A , C.L. Mulchi and P.F. Bell. (1998). Relationships between soil pH, clay, organic matter and CEC (cation exchange capacity) and heavy metal concentration in soils and tobacco. *Tob. Sci*., 33: 96-100.

2. Alloway, B.J. (1999). Heavy metals in soils. John Wiley and Sons, Inc. New York, ISBN 0470215984 EC.Integrated Pollution Prevention and Control (IPPC) Best available techniques reference document on the production of iron and steel. Publ: EC European IPPC Bureau 370 pp.1999.

3. Anderson PR, Christensen TH (1988). Distribution Coefficients of Cd, Co, Ni, and Zn in Soils. J. Soil Sci., 39, 15-22.

4. Bell, P.F, C.L. Mulchi and R.Z. Chaney.(1992). Microelement content in Maryland air cured tobacco. *Commun. Soil Sci. Plant anal*., 23(13-14): 1617-1628.

5. Broadley MR, Willey NJ, Wilkins JC, Baker AJM, Mead A, White PJ. 2001. Phylogenetic variation in heavy metal accumulation in angiosperms. *New Phytologist* **152**: 9–27.

6. Clarke B. B and E. Brennan. (1989). Differential cadmium accumulation and phytotoxicity in sixteen tobacco cultivars. *JAPCA J Air Waste Ma* 39:1319–1322

7. Chaney, R.L. and J.A. Ryan. 1993. Heavy metals and toxic organic pollutants in MSW-composts: Research results on phytoavailability, bioavailability, fate, etc. p.451-506. In Keener (ed) Science and Engineering of Composting: Design, Environmental, Microbiological and Utilization Aspects. Renaissance Publ., Worthington, OH.

8. Francisco S. F, N.M. B.Nelson do Amaral Sobrinho.(2010). Background levels of some trace elements in weathered soils from the Brazilian Northern region. Sci. *agric. (Piracicaba Braz.)* vol.67 no.1

9. Filiposki, K. (1990). Ефекти од наѓубрувањето и наводнувањето врз приносот и квалитетот на тутунот од типот Прилеп, Tobacco. 1-6 стр. 53-442.

10. Husnjak S, I. Turšić, S. Žalac, M. Boić, D. Vrhovec and V. Kozumplik. (2009). Heavy metal content in soil and in tobacco leaf in Croatia. *Tutun/Tobacco*, 58: 13-17.

11. Golia E. E, I. K. Mitsios and C. D. Tsadilas. (2001). Concentration of heavy metals in burley, virginija and oriental tobacco leaves in the thessaly region of central Greece. CORESTA, Agro-Phyto meeting, Cope Town, Sout Africa.

12. Golia E. E, A. Dimirkou and K. I. Mitsios. (2007). Accumulation of Metals on Tobacco Leaves (Primeings) Grown in an Agricultural Area in Relation to Soil. *Bull Environ Comtam Toxicol*., 79:158-162

13. Gondola I and I. Kadar (1993). Relationship of heavy metal concentrations in flue-cured tobacco leaf to certain environmental factors in Hungary. CORESTA Meeting, Agro-Phyto Group, Budapest.

14. Jones, J., Jr. Wolf, H. Mills. (1991). Plant Analysis Handbook, *Micro – Macro Publishing*, Ins.

15. Kabata-Pendias, A. and H. Pendias. (1992). Trace Elements in Soils and Plants, 2nd ed. CRC Press, Boca Raton. 365 p.

16. Miner GS, Tucker MR. (1990). Plant analysis as an aid in fertilizing tobacco. In: Westerman RL, editor. Soil testing and plant analysis. 3rd ed. Madison (WI): Soil Science Society of America, Inc [SSSA]. p 645-57. (SSSA book series; 3)

17. Metsi T. N. Tsotsolis, N. Barbayani, S. Miele, E. Bargiacchi, O. Kosmidou and O. Olivieri.: Heavy metal levels in soil, irrigation waters and five tobacco types. CORESTA, Congress, New Orleans.2002.

18. McNeill A, R . Beri, S. Islam, M. N. Alkhatib and R. West**.** (2006**).** Levels of toxins in oral tobacco products in the *UK*. Tob Control, 15:64–71.

19. Murty K S N, J.C. Tjell and N. C. Gopalachari**.:** Lead and cadmium content of Indian flue-cured tobacco. *Plant Soil*, 95:281–284. 1986

20. Pelivanoska, V. (2005). Влијание на хранливиот и поливниот режим врз хемискиот состав на ориенталските сорти *П-65 и НС-72. Tobacco*. 5-6, pp. 108-115. 2005

21. Pelivanoska V, B. Jordanoska, T. Mitkova and M. Markoski. (2011). Heavy metal content in soil and oriental tobacco manufactured in Republic of Macedonia. *International Conference 100 years Bulgarian Soil Science,* p.754-758 Sofia, Bulgaria.

22. Ruso J, J. Japata, M. Hernadez, M.A. Ojeda, M. Benlloch, E. Prats-Perez, M. Tena, R. Lopez and J. Jorrin. (2001): *Toxic metals accumulation and total soluble phenolics in sunflower and tobacco plants*. Minerva Biotechnol., 13:93–95.

23. Sanders JR, McGrath S, Adams TMcM. (1986). Zinc, Copper and Nickel Concentrations in Ryegrass Grown on Sewage Sludge-Contaminated Soils of Different pH. *J. Sci. Food and Agriculture*., 37, 961-968.

24. Yruela I (2005). Copper in plants. Braz J Plant Physiol 17: 145-146.

25. Zaprjanova P, K. Ivanov, V. Angelova and L. Dospatliev. (2010). Relation between soil characteristics and heavy metal content in Virginia tobacco Soil Solution for a Changing World., 19 th World Congress of Soil Science: 205-208.