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ACCUMULATION AND AVAILABILITY OF TRACE ELEMENTS FROM SOIL INTO ORIENTAL TOBACCO GROWN IN MACEDONIA

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Abstract

Tobacco is one of the most important agricultural products in the Republic of Macedonia. Elemental composition of tobacco and soil from different agricultural areas was studied for monitoring purposes. The main purpose was to determine the intensity of accumulation of various elements in tobacco plants and to determine possible relationships between certain chemical and physical properties of soils (pH, clay, cation exchange capacity - CEC, organic matters - OM and total organic carbon - TOC). Total and DTPA (diethylenetriaminepentaacetic acid) extractable concentration of eighteen elements (Ag, Al, Ba, Ca, Cr, Cu, Li, Fe, K, Mg, Mn, Na, Ni, Pb, P, V, Sr, and Zn) was analyzed by atomic emission spectrometry with inductively coupled plasma (ICP-AES). Element content in tobacco leaves showed weak correlation with DTPA extractable elements, soil properties and total element content in soil. Strong correlations were observed only within soil properties and with Ni content of tobacco leaves and DTPA extractable Cd and P. Elemental distribution varied in different parts of tobacco plants. The results of multielement analyses generally showed the highest concentrations in leaves. Only Cu, Zn and P had higher concentration in tobacco seeds, Na in root and K in stem in comparison to other tobacco organs. Higher concentrations of metals were determined in tobacco leaves grown in soils with increased content of the corresponding metal. Despite intensive tobacco production, concentrations of most of the studied elements in soil and plants were at typical levels of low anthropogenic pressure areas.

Key words: soil properties, trace element, tobacco

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1. Introduction

Plants are collectors of all air, soil and water pollutants, and their chemical composition is widely used as indicator for contaminated areas. A large variety of plant species, such as wheat, grass, lichens, mosses, even tobacco are investigated for their reaction towards pollutants, both for impact and monitoring purposes. Even when grown on unpolluted soil, tobacco (*Nicotiana tabacum* L.) is characterized as a high heavy metal accumulator. On the other hand, oriental tobacco is one of the most important and strategic agricultural products in Republic of Macedonia viewed from economic, trade, fiscal, social and demographic aspects.

Tobacco is intermediate reservoir through which trace elements from soils, and partly from water and air, move to final consumers, humans. The ability of trace elements to cause harmful effects separates them from other known pollutants and from the aspect of human health it is very important to make continuous investigation and assessment of the level of these elements both in the tobacco raw and soil. The concentration of various elements in tobacco is influenced by different factors, either natural, or anthropogenic. A number of soil properties, genetic properties of the plant, climate, stalk position,

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application of soil amendments, application of organic and mineral fertilizers, inorganic pesticides as well as pollution of the surrounding environment affect the chemical composition and trace elements bioaccumulation by tobacco (Adamu et al., 1989; Golia et al., 2007).

One of the major factors influencing the element concentration in tobacco leaves is soil pH (Bell et al., 1992; Golia et al., 2009; Khan et al., 1992; Zaprijanova, 2010). High levels of nitrogen fertilizers increase the nicotine and nitrate levels and reduce level of soluble sugars of the leaf, parameters that have decisive role in evaluating tobacco quality (Rodgman and Perfetti, 2009).

Having in mind the significance of tobacco for Macedonian agriculture, the main objective of this study is to survey trace elements contents both in tobacco raw and in soils where this crop is cultivated. Focus has been put on using tobacco crop as an indicator for providing information on possible contamination, determining the intensity of accumulation of various elements in tobacco plants, exploring the relationship with ambient soil properties, total and mobile forms of trace elements in soil and chemical content of different parts of tobacco plant such as: root, stem, leaf, flower and seed. Statistical multivariate analysis were employed to obtain these correlations and to distinct and determine the origin of studied elements in tobacco. This will provide an insight into partial quality of the tobacco grown in various production areas in Macedonia as essential for achieving sustainable agriculture and basis for further monitoring that will prevent the negative impact of heavy metals on soil, tobacco and tobacco products.

2. Material and methods

2.1. Sampling sites

A two year survey (2010-2011) was conducted on 150 sampling points - family farms that grow oriental tobacco. Soil and plant samples were collected from the three growing regions in Macedonia - Pelagonia (PR), Vardar Valley (VV) and South eastern region (SER) (Fig. 1).

Pelagonia is known as the largest tobacco production area, with moderate continental climate and annual precipitation of 640 mm. Alluvial and delluvial soil cover the municipalities of Prilep, Mogila, Kruševo, Krivogaštani, Bitola, Novaci, Demir Hisar and Dolneni, all of them recognized as tobacco production regions.

Second largest is South-eastern region with municipalities of Bosilevo, Vasilevo, Konče, Novo Selo, Radoviš, Strumica. Soils in the vicinity of Radoviš are of type Smolnitza (vertisol) and are very suitable for tobacco production. Bio monitoring of air pollution in this area is related to copper mine and flotation plant, "Bučim" (Balabanova et al., 2010; Balabanova et al., 2011; Stafilov et al., 2010b). Studeničani, Veles, Čaška, Lozovo, Negotino, Kavadarci and Sveti Nikole are part of Vardar valley tobacco production region. Several investigations of soil, vegetables and fruit from this area reveal the pollution impact of the lead and zinc industry in the recent past (Bačeva et al., 2011; Bačeva et al., 2012; Stafilov et al., 2010a; Stafilov et al., 2010b)

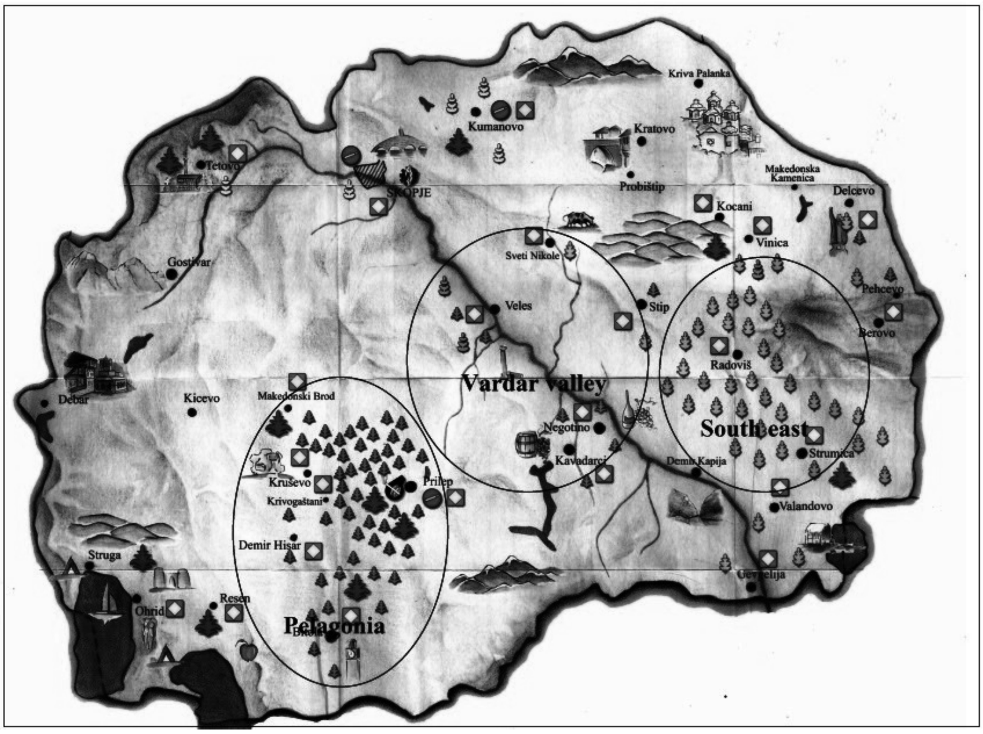


Fig. 1. Survey area of tobacco growing regions: Pelagonia (PR), Vardar Valley (VV) and South eastern region (SER) (Ministry of Agriculture, 1999)

2.2. Soil analyses

Soil composites were sampled at fixed depth (0-30 cm) in each field with two replicates (Hawks and Collins, 1987). Samples were taken, both: from arable - after tobacco harvesting, and grazing land in the nearest vicinity.

Soil properties were determined, such as: mechanical composition, pH, total nitrogen, organic matter, carbonate content, extractable phosphorus and potassium (Pelivanoska, 2012). Additionally total organic carbon (TOC) was determined by dry combustion (according to ISO standard 10694), electroconductivity was measured in a saturation extract, and the cation exchange capacity (CEC) by the methods described by Sumner and Miller (1996).

For determining total content of the elements, soil samples (0.2500 g) were placed in a Teflon digestion vessel and were digested on a hot plate (ISO 14869-1). Samples were analyzed for following elements: Al, Ba, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, P, Ni, Pb, Sr and Zn.

Plant available fractions of the elements were determined by extraction method using buffered solution of diethylentetraaminepentaacetic acid (DTPA) at pH 7.3 (ISO 14870).

2.3. Plant analyses

Tobacco samples (root, stems, leaves, blossoms and seeds) were selected from plants at the same sites where soils were sampled. Tobacco leaves from three harvesting zones with four replicates were collected. First harvest included lower leaves, second middle and third upper leaves – a total of 450 representative samples from 150 sampling points. Plant organs were washed carefully to remove any adhering soil particles and rinsed with redistilled water.

The plant material was dried to a constant weight at 75 ºC for 12 hours and homogenized. Plant samples (0.5000 g) were digested in Teflon vessels with HNO_3 and H_2O_2 using the Mars microwave system (CEM, USA) for elemental analysis. Determination of nicotine was performed according to ISO 2881:2010 - Determination of alkaloid content by Spectrometric method. Total nitrogen was estimated by modified Kjeldahl method and the reducing sugars content by Schmuk and Bertrand (Srbinoska, 2012).

2.4. Instrumentation

Determination of the elements was performed by ICP-AES (Varian, 715-ES). Plant material was analyzed by ICP-AES with ultrasonic nebulizer CETAC (ICP/U-5000 AT). Quality control was performed by replicates of filed duplicates, by standard addition method and using reference standard materials: JSAC 0401 (soil) and moss samples M-2 and M-3 (Steinnes et al., 1997).

The optimal instrumental conditions are given by Balabanova et al. (2010).

2.5. Statistical analyses

Statistical analyses were performed using the IBM SPSS v 19. The correlation between some soil characteristics and element content in plants is revealed by Pearson two tailed correlation coefficients. Factor analyses were used to establish basic structure of the variables in tobacco leaves.

3. Results and discussion

3.1. Soil properties

Main soil properties from three production regions are presented in Table 1. Soils showed a wide range of physical and chemical properties based on their geochemical origin and long cultivation period. Diversity in these properties has direct influence on the tobacco quality. In comparison with uncultivated control soil, treated soils show some statistical differences in their properties, although not very significant.

Average OM content of cultivated soil samples is low to moderate with 71 % showing low OM content, 24% average content and 2% very low and good content. According to Filipovski (1990) soils with lower OM content are a good environment for producing a good quality oriental aromatic tobacco.

The total nitrogen content in soils ranged from 0.01 to 0.40 %. The average values of soil pH were 6.5, 6.7 and 7.7 for PR, SER and VV respectively. Studied soils were mostly: silt loam and silt clay loams, non-carbonate and with high variations of available phosphorus and potassium content (Table 1). Clay content varied from 18.80 to 77.6 %. The low obtained values for CEC <10 $cmol_c$/kg can be explained with low levels of organic matter and clay.

Median values of obtained metals and metalloids (Table 2) are comparable to those obtained from the study on agricultural soils of Europe (Salminen et al., 2005; Soriano-Disla et al., 2012). Only Ni content measured in Vardar valley samples exceeds the optimum limit of 35 mg/kg in the new Dutch list (VROM, 2000). High content of nickel in most samples from this region is found and by Stafilov et al. (2010a), mostly explained by the dependence of the parent rock material, although anthropogenic influence cannot be overlooked.

Barium concentration in all three production regions was above the optimum value of 200 mg/kg (VROM, 2000) indicating anthropogenic influence, as most commonly used fertilizers for tobacco cultivation in Macedonia are P and K fertilizers. The Cr mean values are not high enough to indicate contamination, although high levels were detected in Pelagoni (Dolneni), Vardar Valley (Čaška) and South eastern region (Radoviš). The presence of high clay content and human activities can increase the normal content of Cr in soil (Micó et al., 2006).

Table 1. Basic soil properties, descriptive statistics of three tobacco production areas
(OM: organic matter;TN: total nitrogen; CEC: cation exchange capacity; EC: electrical conductivity; av. P -available P, av. K - available K, SD-standard deviation, CV-coefficient of variation)

	OM (%)	*TN (%)*	*pH (H_2O)*	*Clay (%)*	*av. P*	*av. K*	*CEC [$Cmol_c/kg$]*	*EC [µS/cm]*
					(mg/100 g)			
VARDAR VALLEY PRODUCTION REGION								
Mean	1.6	0.07	7.7	45.9	24.5	31.7	11.8	215
Median	1.6	0.07	7.8	44.9	17.0	26.0	11.4	269
SD	0.3	0.02	0.8	7.2	25.3	14.3	1.9	109
Minimum	1.0	0.05	6.5	31.9	1.8	22.3	8.2	42
Maximum	2.1	0.10	8.6	56.2	64.2	62.8	14.9	362
CV	22	21	10	16	103	45	16	51
SOUTH EASTERN PRODUCTION REGION								
Mean	1.3	0.06	6.7	36.4	12.5	21.8	9.7	79
Median	1.3	0.06	6.7	34.4	6.2	21.2	9.2	58
SD	0.3	0.02	0.5	11.9	23.5	4.6	2.3	55
Minimum	0.7	0.01	5.5	19.4	1.5	12.8	6.5	27
Maximum	2.1	0.11	8.0	63.7	154.3	32.2	15.6	264
CV	27	29	8	33	189	21	24	70
PELAGONIA REGION								
Mean	1.5	0.08	6.5	37.7	19.7	20.4	10.6	83
Median	1.5	0.07	6.4	35.8	9.1	19.8	10.1	70
SD	0.5	0.04	0.6	11.1	32.2	7.4	2.6	56
Minimum	0.8	0.02	5.0	18.8	0.5	3.2	6.0	23
Maximum	3.2	0.40	8.3	77.6	198	63.6	19.2	360
CV	31	52	10	29	164	36	24	68

Table 2. Total content of selected elements in soil samples

Element, unit	***Pelagnia region***			***Vardar valley region***			***South eastern region***		
	Mean	***SD***	***Range***	***Mean***	***SD***	***Range***	***Mean***	***SD***	***Range***
Pb, mg/kg	14	4.8	10-30	14	4.5	10 -22	15	5.5	10-29
Ni, mg/kg	22	13.6	7-124	51	29	5-117	25	20	5-117
Cr, mg/kg	48	31	15-292	63	58	12-176	48	29	13-180
Cu, mg/kg	17	9	5-49	16	4.3	9-26	20	11	6-53
Zn, mg/kg	59	84	16-593	62	24	35-101	93	228	23-1534
Li, mg/kg	14	7.6	3.4-53	15	4.1	6-20	13	6.5	12145
P, %	475	320	140-2098	299	151	73 -531	447	251	134-1309
K, %	1	0.3	0.4-2	0.7	0.2	0.4-0.9	0.9	0.3	0.4-2
Ca, %	1.1	0.6	0.2-3	1	0.8	0.2-2.6	0.8	0.7	0.2 -4
Mg, %	1	0.2	0 .4-2	0.5	0.1	0.2-0.7	0.6	0.2	0.2-1.1
Ag, mg/kg	0.5	0.2	0.2-1.1	0.4	0.2	0.2-0.7	0.5	0.3	0.2-1
Al, %	5	0.9	2.8-6.8	3.7	0.6	2.5-4.4	4.1	0.9	2-6
Ba, mg/kg	358	121	137-764	235	59	133-332	251	61	149-471
Fe, %	2	0.8	0.9-5	1.8	0.5	0.9-2.7	2.4	0.7	1-4
Mn, mg/kg	551	171	218-1268	542	161	366-878	480	139	239-814
Na, mg/kg	1	0.3	0.1-2	0.5	0.2	0.3-0.9	0.7	0.2	0.2-1.1
Sr, mg/kg	151	89	26-537	64	29	23-109	72	29	26-147
V, mg/kg	66	32	27-217	43	14	20-67	61	20	24-112
Zn, mg/kg	59	84	16-593	62	24	35-101	93	228	23-1534

Anthropogenic influence in these areas is evident, due to obtained low levels of clay. Anomalies for Zn and Cu were measured in the municipalities of Konče and Kruševo. Influence from the open coal deposit in the Pelagonia area was detected by higher concentrations of Fe, above 5 %, measured at Bitola and Mogila sampling areas. These values are higher than the Fe content in European topsoil average of 2.2 % (Salminen et al., 2005), and 2.4 % in Vardar Valley agricultural soils (Stafilov et al., 2010a).

Although there is no single extraction that would represent the true availability of elements in all plant species and predictions of plant uptake mostly employ the regression models based on concentration of elements in the soil, their characteristics and concentrations within plants, the most widely used extraction reagent for trace elements estimation is DTPA solution (Lindsay and Norvell, 1978). The median concentration of DTPA extractable elements are given in Table 3. The mean values of most of the extracted elements differ within the production regions and highest concentrations were extracted from Vardar valley samples. Concentrations of extractable Ag, Cr, Li and V were below detection limits. Availability of studied elements calculated as a ratio of available concentration (DTPA extraction for trace elements and ammonium lactate extraction for P and K) and total concentration of each element in soil, given in percentage (%) are given in Fig. 2.

Aluminum, although analyzed had negligible percentage of availability. As we can see, the percentage sequence follows the following order: Cu>Mn>Pb>Ni>Zn>Sr>Ba>Fe>Na>Al. According to Adamu et al. (1989) and Golia et al. (2007) the highest availability of Cd was detected in soils where oriental tobacco was cultivated. In our study, total Cd concentration in soil was below detectable limits, but small significant concentration of DTPA extractable Cd was detected in all investigated areas.

3.2. Plant analyses

The descriptive statistics of the content of analyzed elements in the tobacco leaves is given in Table 4. In comparison with other literature sources, concentration of all studied elements is within the range for oriental tobacco leaves (Golia et al., 2009; Gondola and Kadar, 1994; Metsi et al., 2002; Pelivanoska et al., 2011; Tso, 1990). Only Fe, Cr, Ni, Sr and Ba had higher mean concentration than values reported by Tso 1990, but they are all below the contamination limit for plants (Kabata-Pendias, 2011). Medium nicotine content, high content of soluble sugars and low to medium nitrogenous and acid constituents are some of the parameters that define quality of the oriental tobacco.

Table 3. Concentrations of DTPA extractable elements in soil from different tobacco growing areas given in mg/kg (LOD is limits of detection, *SD*-standard deviation)

	LOD (mg/kg)	***PR***			***SER***			***VV***		
		Mean	***SD***	***Range***	***Mean***	***SD***	***Range***	***Mean***	***SD***	***Range***
Al	0.01	1.35	1.26	0.11-5.67	2.7	3.1	0.1-13.4	0.6	0.5	0.2-1.6
Ba	0.03	0.66	0.20	0.20-1.26	1.6	1.0	0.2-4.2	2.4	1.4	1.1-5.3
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
Cu	0.02	1.23	0.63	0.50-3.71	2.9	1.6	0.5- 7.4	2.7	0.5	2.0-3.7
Fe	0.006	20	9	3-39	32.7	20.1	3.7-96	20.4	13	6-44
Mn	0.0015	27	10	5-48	57	35	10-172	70.5	41	28-124
Na	2.5	3.5	2.7	1.6 -15	10	5	2-19	9	3.6	4.7-16
Ni	0.25	0.64	0.30	0.19-1.59	1.2	0.7	0.1-2.6	4.7	3.6	1.3-11
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
Sr	0.025	1.88	0.98	0.70-5.40	3.3	1.6	1-8	4.6	2.5	1.7-10
Zn	0.003	1.26	1.70	0.15-8.62	1.8	1.9	0.4-9	4.1	3.7	1-11

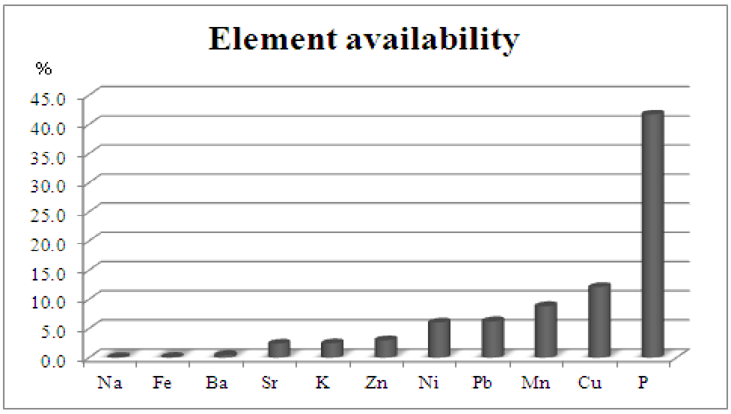


Fig. 2. Availability of studied elements calculated as a ratio of available concentration (DTPA extraction for trace elements and ammonium lactate extraction for P and K) and total concentration of each element in soil, given in percentage (%)

Levels of soluble sugars, nicotine level, total nitrogen and Shmuk number are within the high quality range (Rodgman and Perfetti, 2009). For determination of chemical associations between the element contents in tobacco leaves multivariate factor analyses was used. The matrix of rotated factor loadings is given in Table 5. Six components were extracted explaining 74.95 % of the total variance.

According to visual inspection of spatial distribution of the elements in tobacco leaves and corresponding soil, as well as the results of factor analyses, two geogenic, two mixed (geogenic-anthropogenic) and two anthropogenic associations were defined.

Factor 1 represents 27.30 % of the total variance and is presented by Fe, V, Al and Pb. This is the strongest factor and is combination of naturally distributed and anthropogenic components because of the presence of Pb in this group. Fe, V and Al are associated to natural distribution, but Pb in the ecosystems usually is introduced through anthropogenic activities.

Table 4. Descriptive statistics of chemical composition of tobacco leaves (n=450). The values for nicotine, TN and SS are given in %, for the rest of the elements in mg/kg (X_g is geometrical mean, Md-median, *SD*-standard deviation, *A*-skewness, *E*-kurtosis, P_{10} 10 percentile, P_{90} 90 percentile, TN- total nitrogen, SS- soluble sugars, SN- Shmuk number)

	X_g	*Md*	*SD*	*A*	*E*	*Minimum*	*Maximum*	P_{10}	P_{90}	*CV*
Al	1472	1305	822	0.93	0.70	158	4527	540	2658	56
Ba	45	44	16	0.57	0.44	13	103	26	65	36
Ca	14920	14603	3398	0.53	0.87	7707	28065	10751	19090	23
Cd	0.41	0.3	0.3	2.40	8.59	0.1	1.9	0.2	0.72	64
Cr	2.7	1.8	4	5.46	38.16	0.3	33	0.8	5.42	132
Cu	12	11	4	1.76	6.61	4	35	7.0	16	37
Fe	871	732	484	1.00	0.80	147	2679	340	1633	56
K	7610	7653	2225	0.17	-0.17	2575	13292	4654	10371	29
Li	13.9	11	10	1.47	2.05	2	49	5	26	71
Mg	1305	1311	231	0.02	0.06	744	1883	1010	1577	18
Mn	68	57	62	9.12	98.67	27	749	35	101	91
Na	64	59	20	1.84	4.74	38	150	44	85	31
Ni	2.5	2.1	2	3.79	21.02	0.6	16	1.1	4.2	72
P	834	807	239	1.44	4.36	439	1991	565	1130	29
Pb	1.0	0.9	1	1.86	4.31	0.5	4.0	0.4	1.9	65
Sr	48	48	16	0.28	0.58	12	109	26	68	34
V	2.6	2.1	2	1.66	3.89	0.5	11.1	1.0	5.1	67
Zn	22	21	7	1.20	2.35	8	52	14	32	34
Nicotine	1.7	1.7	0.5	0.56	0.37	0.7	3.1	1.1	2.3	29
TN	2.4	2.4	0.4	0.06	-0.08	1.4	3.6	2.0	3.0	16
SS	14	13.9	4	0.31	-0.04	5.8	25	9.6	19.6	26
SN	1.9	1.88	1	0.78	1.10	0.5	4.6	1.1	2.9	36

Table 5. Rotate component matrix (n=150) for 18 selected elements in tobacco leaves Com - Communality (%), Var- Variance (%)

Element	*F1*	*F2*	*F3*	*F4*	*F5*	*F6*	*Com*
Fe	**0.96**	0.03	0.05	-0.02	0.15	0.02	94.91
V	**0.92**	0.13	0.03	-0.06	0.11	0.04	88.94
Al	**0.92**	0.19	0.16	0.05	0.08	0.06	92.84
Pb	**0.65**	0.00	0.17	0.22	0.04	-0.08	50.34
Sr	-0.05	**0.91**	-0.04	0.08	-0.04	0.15	85.87
Mg	0.13	**0.77**	0.30	-0.11	0.15	-0.19	76.93
Na	0.22	**0.64**	0.13	0.34	-0.07	0.01	60.03
Ba	0.16	**0.64**	0.16	0.09	0.02	0.43	66.07
Zn	0.24	0.11	**0.80**	0.26	0.09	0.01	78.56
Cd	0.01	0.03	**0.77**	0.11	0.13	0.33	72.26
Cu	0.16	0.25	**0.75**	-0.10	0.08	0.04	67.09
P	0.00	-0.02	-0.14	**0.86**	-0.01	-0.02	76.03
K	0.16	0.04	0.26	**0.74**	0.01	0.22	69.60
Ca	0.01	0.37	0.19	**0.66**	0.01	0.02	60.75
Cr	0.18	-0.03	-0.01	-0.02	**0.93**	-0.09	90.76
Ni	0.13	0.06	0.31	0.04	**0.88**	0.09	90.75
Mn	0.12	0.08	0.02	-0.02	0.05	**0.81**	67.65
Li	-0.20	0.04	0.27	0.19	-0.09	**0.66**	59.65
Var	27.34	14.90	9.95	9.40	7.43	5.94	74.95

The natural concentration of Pb in plants grown in unpolluted regions is in the range of 0.1-10 mg/kg (Kabata-Pendias, 2011).

Soluble soil vanadium appears to be easily taken up by roots and some plant species show a great ability to accumulate this metal (Kabata-Pendias, 2011). Vanadium average concentration in tobacco leaves is 2.6 mg/kg and as it can be seen in Table 7, leaves of studied tobacco plants have the highest availability to absorb this element, rather than other investigated plant organs. The same distribution was also observed in Al, Fe and Pb content in leaves compared to other above ground mass of oriental tobacco plants (Table 7, Fig. 3a-b). Second factor or 14.90 % of the total variance is explained by Sr, Mg, Na and Ba.

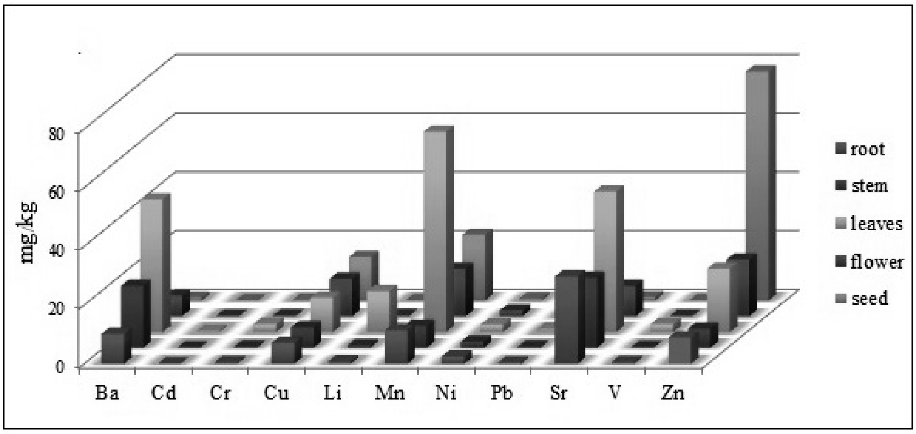
The spatial distributions of the elements content in this group are strictly connected to the nature of the pedogenic materials and to the evolution processes of the different types of soil. Compared to other production regions, the highest levels of this association are measured in both, tobacco leaves and soils from Pelagonia area (Tables 2 and 6). None of the measured concentration of all elements presenting factor 2 exceeded the deficient or contaminate level for plants.

Table 6. Descriptive statistics for total concentrations of chemical elements in tobacco leaves from three tobacco growing regions given in mg/kg

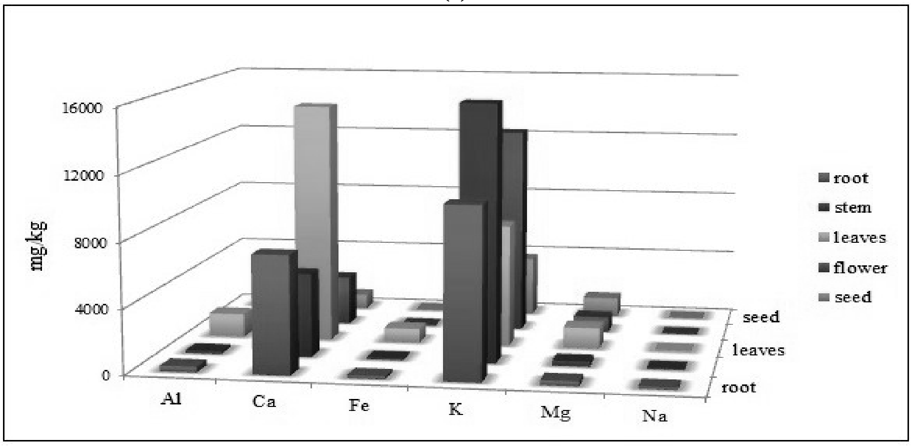
	LOD	***PR***			***SER***			***VV***		
	(mg/kg)	X_g	s_a	***Min-Max***	X_g	s_a	***Min-Max***	X_g	s_a	***Min-Max***
Al	0.01	1426	849	158-3686	1675	872	420-4527	1371	467	688-2194
Ba	0.03	48	16	16-103	40	14	13-81	40	16	18-79
Ca	0.025	14287	2836	7707-19914	14981	3549	8904-24029	19296	3802	12969-28065
Cd	0.005	0.35	0.17	0.09-0.89	0.45	0.25	0.18-1.27	0.8	0.5	0.3-1.9
Cr	0.05	2.1	1.9	0.3-16	4.2	5.8	0.5-33	2.9	2.2	0.9-6.9
Cu	0.02	11	4	4-35	13	4	6-29	12	4	7-19
Fe	0.006	820	492	147-2367	1053	491	296-2679	762	292	346-1423
K	5	7306	2430	2575-13292	7785	1537	407-10809	9234	1845	6102-12490
Li	0.05	13	10	2-49	13	8	2-42	21	11	9-40
Mg	0.025	1332	224	749-1883	1251	233	744-1684	1308	273	1018-1859
Mn	0.0015	64	27	28-172	78	109	27-749	69	22	39-117
Na	2.5	66	22	38-150	60	14	38-117	59	8	47-79
Ni	0.25	2	1	1-6	3	2	1-15	4	3	1-11
P	0.5	795	213	439-1529	860	275	497-1991	1018	203	813-1542
Pb	0.5	0.9	0.6	0.5-4	1.1	0.6	0.5-2.7	1.6	1.0	0.5-3.8
Sr	0.025	53	15	16-109	38	14	12-74	42	15	22-64
V	0.05	3	2	0.2-9	3	2	1-11	2	1	1-3
Zn	0.003	20	6	8-48	23	7	13-38	29	10	18-52

Table 7. Concentration of studied elements in different parts of oriental tobacco given in mg/kg, BAF is Biological Accumulation Factor defined as the ratio between total content of the elements in all parts of studied plant and corresponding soil, * -not calculated because soil Cd content is above detection limits

		Root		***Stem***		***Flowers***		***Seed***	
	Mean	*Min-Max*	*Mean*	*Min-Max*	*Mean*	*Min-Max*	*Mean*	*Min-Max*	*BAF*
Al	338	200-549	170	90-297	57	43-69	15	10-23	0.05
Ba	10	5-14	21.1	6.3-49	7	6-7	0.87	0.47-1.22	0.27
Ca	7266	5681-10318	5194	3973-6495	3071	2194-4052	926	830-989	0.32
Cd	0.07	0.03-0.12	0.09	0.02-0.22	0.09	0.03-0.15	0.06	0.03-0.08	*
Cr	0.48	0.28-0.70	0.4	0.2-0.6	0.27	0.18-0.46	0.19	0.17-0.22	0.08
Cu	7.0	3.8-9.9	7.0	4.3-9.5	13	8-18	15	13-18	2.96
Fe	176	103-255	105	46-199	76	67-81	73	64-80	0.06
K	10508	9829-11158	15855	11246-24674	12893	12368-14056	3739	3497-3928	5.06
Li	0.8	0.3-1.1	0.9	0.1-2.3	0.4	0.1-0.7	0.07	0.04-0.11	1.14
Mg	293	181-357	320	190-542	951	826-1012	1207	1182-1243	0.82
Mn	11	8-17	7.5	4.0-13.2	16	14-18	22	20-24	0.24
Na	148	128-169	75.3	67-83	37	33-42	39	38-42	0.05
Ni	2.5	0.9-5.4	2.1	0.4-5.2	1.9	0.3-5.7	0.6	0.5-0.7	0.38
P	425	369-489	587	440-680	1659	1326-1852	2694	2587-2954	13.7
Pb	0.8	0.4-0.8	0.6	0.5-0.6	0.6	0.5-0.6	0.5	0.6-0.5	0.17
Sr	30	19-36	23.9	13-43	10	10-11	1.1	0.9-1.4	0.93
V	0.6	0.4-1.1	0.2	0.1-0.5	0.1	0.1-0.2	0.05	0.05-0.06	0.06
Zn	8.9	6.0-11	6.5	4.6-10	19	15-22	78	63-99	2.07



(a)



(b)

Fig. 3. (a) Distribution of Ba, Cd, Cr, Cu, Li, Mn, Ni, Pb, Sr, V and Zn in different organs of the tobacco plant (given in mg/kg), (b) Distribution of Al, Ca, Fe, K, Mg and Na in different organs of the tobacco plant (given in mg/kg)

According to distributions in different parts of tobacco plant (Table 7, Fig. 3a-b), seeds, leaves and flowers seem to accumulate similar amounts of Mg. Sodium had higher concentration in root compared to other plant organs.

Factor 3 (Zn - Cd - Cu) associates elements affected by human activity. Concentrations of the elements from this factor are below permissible limit values in conventional and ecological agriculture and are comparable with the results from the same investigated areas (Jordanoska et al., 2012; Pelivanoska 2007). As we can see in Table 6, Zn and Cd concentration in tobacco leaves from Vardar valley are higher than those in the other production regions. Veles region is known for its lead and zinc industrial activity in the recent past. Elevated levels of Cu concentration in the SER can be correlated with the presence of a copper mine and flotation plant. This was noted by some anomalies measured in soil samples, but none of the above-mentioned elements exceeded the contamination level in leaves. Cd content of different tobacco organs seems to be equally distributed among stem – flower, and root – seed (Table 7, Fig. 3a). Relatively high concentration of Zn was measured in the tobacco seeds in comparison with all other plant organs.

Fourth factor (P-K-Ca) represents association correlated to natural and anthropogenic activity origin. Three major nutrients Ca, P, K are known to play a significant role in the normal growth and development of tobacco. The content of these elements in tobacco plants varies strongly depending on its representation of the nutritional environment. According to tobacco growing areas distribution, K and P higher contents are measured in VV, and Ca highest content in SER (Table 6). K has greater concentration in tobacco stem, and P in tobacco seed in comparison with other plant organs.

Fifth factor consisted Cr and Ni that are associated with air pollution. According to Tso (1990), Ni content of tobacco varies from 0.2-1.6 mg/kg and Cr content is found in traces. Results from our study point out to a significantly higher content for both elements. Higher values for Cr were observed in SER, and higher Ni content was observed in VV. Elevated concentrations of these elements were also found and in the corresponding soil samples. Obtained values are comparable with Cr and Ni content of lichens and mosses analyzed in this area (Balabanova et al., 2012; Barandovski et al., 2008; Barandovski et al., 2012).

Finally, the sixth factor is associated with Mn and Li and they are naturally distributed. Levels of both elements are below contamination limit. Most of the studied elements have the highest concentration in tobacco leaves (Fig. 3b). It is noticeable that Mn content of leaves is significantly higher compared to other tobacco plant organs.

Bivariate correlation was used to establish the relationships between soil physical and chemical properties and metal concentrations in plant and soil samples. Correlations between pH, EC, TOC and most of the total elements in soil were generally not significant ($P<0.05$). A strong positive correlation, however, was observed between soil parameters: organic matter content with CEC (r = 0.809) and CEC with clay content (r = 0.874). Only clay showed moderately significant positive correlation with total Cu content in soil (r = 0.414), and negative correlation with total Na (r = -0.563).

Soil parameters generally do not correlate significantly with chemical composition of tobacco leaves. Statistically significant correlation ($P<0.01$) were established only among clay and Al, Cd, Fe, Mg and Zn contents of tobacco leaves. Soil pH was negatively correlated with Ba and positively with Ca and K, and CEC with Mg and Zn content tobacco leaves. Significantly moderate correlations ($P<0.01$), were observed between leaf Fe content and total Cu (r = 0.380), Fe (r = 0.486), Mg (r = 0.324), Na (r = 0.430) and V (r = 0.383) in soil.

Similar correlations were observed with Al concentration in the tobacco leaves and total Cu, Fe and Na in the soil. Leaf V content show moderate correlation with total soil Cu and Na and strong correlation with total soil Fe.

Element content in tobacco leaves showed weak correlations with DTPA extractable elements. Strong correlations were observed only between Ni content of tobacco leaves and DTPA extractable Cd (r = 0.500) and P (r = 0.650).

Total nitrogen content of the tobacco leaves shows a significant positive correlation with nicotine (r = 0.625), but a negative relation with soluble sugars (r = -0.707). This confirms that the amount of nicotine depends on nitrogen fertilizers, as nitrogen is constituted in the nicotine molecule. With incrising nitrogen fertilizer, the amount of soluble sugar was reduced.

Tobacco is sensitive crop to nitrogen fertilizers that influence its yield, leaf area and physiological activity that lasts long (Hawks and Collins, 1987). Studies have shown that tobacco during its vegetation period is constantly accumulating nitrogen and potassium, and their concentration in the tobacco leaf is constantly increasing. In contrast, tobacco accumulates phosphorus during the whole growing period. Using all determined concentrations in different parts of tobacco plant (root, stem, flower, leaves and seed) and the total element content of investigated soils, we calculated the biological accumulation factor (BAF). This factor is defined as the ratio between total content of the elements in all parts of studied plant and corresponding soil and obtained values are given in Table 7. The obtained values show high factors for P, K and Cu, which indicates that tobacco, is capable of accumulating high amounts of these elements. According to this, impact of uncontrolled fertilizer rate cannot be neglected, as the most common fertilizer used for tobacco growing in Macedonia is complex nitrogen, phosphorus and potassium fertilizer, NPK 8-16-24.

4. Conclusions

The goals of our study were to provide information on possible pollution, spatial trends of trace elements in tobacco raw and determine the quality of agricultural soils used for tobacco cultivation in the Republic of Macedonia.

Despite intensive tobacco production, content of all determined elements pointed out levels, which are typical of agricultural and low anthropogenic pressure areas. Element content in tobacco leaves showed weak correlation with soil properties and total element content in soil. Elemental distribution varied in different parts of tobacco plants, but generally highest concentrations are observed in tobacco leaves.

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