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Accumulation of Metals on Tobacco Leaves (Primings) Grown in an Agricultural Area in Relation to Soil

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Introduction

Contamination of the soil-water-air-plant-animal-human system with metals has health, economic, and ecological importance (Oliver, 1997). The concentrations of metals in soils are associated with biological and geochemical cycles and are influenced by anthropogenic activities, such as agricultural practices, transport, industrial activities and waste disposal (Alloway, 1990; Kabata-Pendias and Pendias, 1992).

It is well-known that tobacco (*Nicotiana tabacum*) can easily accumulate certain metals and in particular cadmium in leaves (Clarke and Brennan, 1989; Tso, 1990; Gondola and Kadar, 1994). Cadmium is a non-essential, potentially toxic, largely pollutant ion, which is accumulated by tobacco plants and is transferred in tobacco smoke to humans (Bell et al., 1992; Sravrides, 2006). Phosphates fertilizers, which are used in tobacco cultivation, contain high concentrations of metals. The use of phosphate fertilizers in the fertilization of crops is one of the primary factors in the pollution of agricultural soils (He and Singh 1994a). Metal concentrations in tobacco have been found to be influenced by such factors as soil type and pH genotype, stalk position, soil and leaf residues resulting from the application of metal containing pesticides, and from soil amendments including fertilizers and municipal sludge (Adamu et al., 1989; Karaivazoglou et al., 2007).

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Although, a number of investigations have been carried out on metal concentration in tobacco leaves (Murty et al., 1986; Ruso et al., 2001; McNeill et al., 2006), there are no data concerning metal content in air-cured (Burley), flue-cured (Virginia) and sun-cured (Oriental-filling) tobacco priming produced in the Thessaly region of central Greece.

The objectives of the present study were: a) To survey metal concentrations in tobacco leaves produced in different growing areas of the Thessaly region and b) to determine the most important soil factors (chemical and physical), which influence the concentration of metals in Burley, Virginia and Oriental tobacco leaves.

Materials and Methods

A three-year survey (2002 to 2004) was conducted in the Thessaly region in order to determine the metal concentrations in soil (500 soil samples) as well as in leaves (290 leaves of Oriental, 130 of Virginia, and 80 of Burley tobacco) at the first, second and third priming. The sampling of soil as well as tobacco leaves was based on the extent of tobacco cultivation and the relative economic significance of the three types of tobacco (Burley, Virginia and Oriental) in the three prefectures of Thessaly (Karditsa, Larissa and Trikala). The Oriental, Virginia, and Burley tobacco types represents 60, 24, and 16% respectively of total Greek tobacco production.

Composite soil samples consisting of six subsamples were collected from the upper layer (0–30 cm) of each field with two replicates. The samples were air-dried and sieved through a 2-mm sieve. Soil properties such as clay content (%), organic matter (Walkley-Black method), pH (1:1) and electrical conductivity (1:1) were measured (Page et al., 1982). Plant-available fractions of metals were determined by using diethylene-triamine-pentacetic acid (DTPA) buffered at pH 7.3 (Lindsay and Norvel, 1978). The total concentration of metals was determined using the *Aqua Regia* (HCl-HNO_3, 3:1) extraction method (International Organization for Standarization, 1994) after digestion at 180°C for 2 h. All reagents were of analytical grade (Merck, Germany). The stock solutions of metals (1000 mg/mL) were prepared from ''titrisol'' Merck.

Cadmium and lead were measured by graphite furnace atomic-absorption spectophotometry (GFAAS). Zinc and Cu were determined by flame atomic-absoprtion spectrophotometry (FAAS). Deuterium background corrections were used in the analysis of Cd and Pb (Lajunen, 1992). The detection limits, based on three-times the standard deviation of the blank (n = 10), were found to be between 0.08 μg/L (Cd - GFAAS) and 0.3 mg/L (Cu - FAAS). A certified reference material [CRM, (No 141R, calcareous loam soil)] by the Community Bureau of Reference (BCR) was also analyzed for the verification of the accuracy of trace-element determination in soils. Recovery values were calculated as the ratio of the BCR results to those of the *Aqua Regia* digestion and ranged from 95 to 103%.

Three tobacco leaves from each priming, with two replicates, were selected from plants at the same sites where soils were sampled. First priming included lower leaves and third priming upper leaves. A total of 500 tobacco leaf samples were collected at three primings each. The 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. The extraction method described by He and Singh (1994a) was followed. The samples were then analyzed for metal concentrations as previously described. Appropriate blanks were included in all extractions.

The data were statistically analyzed using correlation analysis (Pearson correlation, two-tailed). Results from two replicates were averaged prior to statistical analyses. Correlation analysis was used to establish relationships between soil characteristics and metal concentration in soil samples and in Burley, Virginia, and Oriental tobacco leaf samples.

Results and Discussion

The soils in the region studied were silty loam (39%), sandy clay loam (33%) and clay loam (28%). In Karditsa, the soils were, according to soil taxonomy, alfisols with a mean soil pH value of 5.5. These soils have an ochric epipedon, an argillic horizon. In Trikala prefecture there were entisols with soil pH values between 6.5 and 7.5. In Larissa (Elassona) prefecture there were both alfisols as well as vertisols (pH > 7.5).

Table 1 Concentration of extractable metals in soils (DTPA method) cultivated with tobacco

	Zn (mg/kg dry soil)	Cu	Cd	Pb
Burley, n = 80				
Minimum value	0.2	0.3	0.001	0.1
Maximum value	2.0	2.2	0.8	2.1
Mean value	0.7	0.9	0.2	0.6
CV (%)	37.3	46.7	78.8	48.4
Virginia, n = 1 30				
Minimum value	0.1	0.2	0.0001	0.05
Maximum value	3.5	7.0	0.6	1.9
Mean value	0.6	1.0	0.14	0.3
CV (%)	61.8	60.2	87.3	56.3
Oriental, n = 290				
Minimum value	0.03	0.1	0.0001	0.04
Maximum value	5.5	4.2	0.5	2.2
Mean value	0.5	0.9	0.1	0.4
CV (%)	77.4	57.3	85.2	64.4

Soil electrical conductivity ranged between 104.0 and 3100 μS/cm, with a mean of 441.5 μS/cm. The mean values of organic matter and clay content (%) did not differ among soils where tobacco varieties were cultivated. The mean value of organic matter of soils was 1.4%, while the mean clay value was 23%.

The concentration of DTPA extractable metals in soils where Burley, Virginia and Oriental tobacco were cultivated is presented in Table 1.

Available Cd had the highest coefficient of variance. This is due to the different amounts of fertilizers that farmers in Thessaly use for tobacco cultivation. The mean values of Zn and Cu were similar among the three tobacco varieties. Soils where Burley tobacco was cultivated had higher mean Cd and Pb values. The levels of available concentration of metals were similar to those found in previous research at the same agricultural areas (Mitsios et al., 2005).

Table 2 shows the metal concentration extracted by *Aqua Regia* in soils cultivated with Burley, Virginia and Oriental tobacco.

Total Cu and Cd concentrations were similar in soils where the three tobacco varieties were grown. However, total concentrations of Zn and Mn appeared to be higher in soils cultivated with air-cured (Burley) tobacco.

The availability of metals was calculated as the ratio of available concentration to the total concentration for each metal ($C_{dtpa}/C_{aqua\ regia} \times 100$). That percentage (%) in soils where Burley tobacco was cultivated was: Zn: 1.8, Cu: 4.0, Cd: 25 and Pb: 10.9, in soils where Virginia tobacco was cultivated was: Zn: 1.9, Cu: 4.4, Cd: 23.3 and Pb: 5.9, and in soils where Oriental tobacco was cultivated was: Zn: 1.8,

Table 2 Concentration of extractable metals in soils (*Aqua Regia* method) cultivated with tobacco

	Zn (mg/kg dry soil)	Cu	Cd	Pb
Burley, *n* = 80				
Minimum value	16.6	8.1	0.06	2.5
Maximum value	69.9	40.5	1.3	9.4
Mean value	39.2	22.3	0.8	5.5
CV (%)	32.0	36.1	32.0	30.2
Virginia, *n* = 130				
Minimum value	11.9	9.0	0.03	1.9
Maximum value	72.8	52.9	1.1	8.4
Mean value	32.0	22.7	0.6	5.1
CV (%)	27.7	40.6	48.8	35.4
Oriental, *n* = 290				
Minimum value	7.0	5.6	0.06	2.0
Maximum value	131.3	60.9	0.9	7.9
Mean value	28.4	20.6	0.5	3.9
CV (%)	39.3	45.1	89.6	57.8

Cu: 4.4, Cd: 20 and Pb: 10.3. Therefore the element of primary concern regarding soil pollution, Cd, had the highest availability as related to DTPA extractability. This result agrees with other investigations (Adamu et al., 1989; Mitsios et al., 2005).

Table 3 shows the concentration of metals in three tobacco types, averaged over the period studied. Correlation coefficients among soil parameters and the concentration of metals in Oriental tobacco are shown in Table 4. Correlation coefficients in Virginia and Burley tobacco were lower and in some cases not significant at either the 0.05 or 0.01 level (Pearson correlation, 2- tailed), so they are not presented.

In Burley, Virginia, and Oriental tobacco leaves the mean concentrations of Zn, Cu, Cd and Pb (as they are presented in Table 3) had the higher values at first priming and the lowest values at third priming. This finding is probably be explained by tendency for low metal mobilities through tobacco plants (Tso, 1990). Lower leaves seem to accumulate high amounts of metals, but in all cases the concentrations were lower than those reported by other investigators (Murty et al., 1986; Clarke and Brennan, 1989; Bell et al., 1992; Gondola and Kadar, 1994; Ruso et al., 2001; McNeill et al., 2006).

During the three years (2002–2004) of this research the concentrations of metals (Zn, Cu, Cd, Pb) were higher in Burley tobacco leaves. The levels of metals studied followed the order: Burley > Virginia > Oriental. Differences in metal concentration in the tobacco leaves examined seem to imply that different types of tobacco have different responses to metal accumulation. Also, it should be noted that Burley cultivation acquires higher amounts of both phosphate and nitrogen fertilizers than Virginia or Oriental tobacco. The use of fertilizers in crops is one of the primary factors in the pollution of agricultural soils as well as of tobacco plants (He and Singh, 1994a; Karaivazoglou et al., 2007).

Table 3 Concentration of metals at first, second and third priming of air-cured (Burley), flue-cured (Virginia) and sun-cured (Oriental-filling) tobacco leaves averaged over the years 2002–2004

	Zn			Cu			Cd			Pb		
	1st mg/kg dry matter	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Burley, *n* = 80												
Minimum value	6.8	8.9	12.4	4.6	3.1	1.1	0.1	0.1	0.04	0.2	0.1	0.04
Maximum value	161.6	133.6	125.5	54.5	37.3	27.6	3.7	2.9	3.6	9.1	3.3	1.3
Mean value	76.7	67.5	55.5	22.2	18.6	12.0	0.9	0.7	0.6	1.2	0.9	0.6
CV (%)	56.8	48.1	48.2	53.1	51.1	53.8	79.3	82.3	96.6	68.6	54.1	48.0
Virginia, *n* = 130												
Minimum value	9.2	7.4	4.8	1.9	1.1	1.7	0.02	0.01	0.01	0.04	0.1	0.01
Maximum value	135.6	118.8	99.7	42.8	33.2	26.0	3.0	2.4	1.3	10.2	8.0	4.8
Mean value	55.4	39.5	28.8	18.8	15.4	11.5	0.7	0.4	0.2	0.9	0.8	0.6
CV (%)	45.7	48.8	45.2	66.3	67.9	48.3	112.2	106.8	96.8	97.7	94.0	96.3
Oriental, *n* = 290												
Minimum value	2.1	10.0	8.2	0.3	0.6	0.2	0.01	0.01	0.01	0.02	0.01	0.02
Maximum value	114.4	109.4	95.6	37.8	30.8	25.3	2.5	2.6	1.9	14.4	7.2	7.7
Mean value	48.1	42.9	37.0	15.5	10.6	9.9	0.6	0.5	0.3	0.8	0.5	0.2
CV (%)	52.6	76.6	48.3	44.1	50.7	71.6	81.4	87.2	87.8	78.9	80.5	93.9

Table 4 Correlation among soil parameters and the concentration of metals in Oriental tobacco leaves (*n* = 290)

	Zn 1st	Zn 2nd	Zn 3rd	Cu 1st	Cu 2nd	Cu 3rd	Cd 1st	Cd 2nd	Cd 3rd	Pb 1st	Pb 2nd	Pb 3rd
pH	-0.827**	-0.855**	-0.648**	-0.815*	-0.748*	-0.741*	-0.746**	-0.671**	-0.640**	-0.762*	-0.582*	-0.581*
EC	-0.623**	-0.503**	-0.512**	-0.655**	-0.518**	-0.415**	-0.447*	-0.444*	-0.398*	-0.547*	-0.547*	-0.417*
OM %	0.655**	0.635**	0.747**	0.525**	0.517*	0.411*	0.222**	0.236*	0.247*	0.355*	0.342*	0.232*
Clay %	0.425**	0.452**	0.347	0.423**	0.445**	0.458*	0.447*	0.435**	0.447*	0.351*	0.323*	0.311*
Zn Aqua	0.585*	0.559*	0.532*	0.333*	0.339*	0.337*	0.314*	0.348*	0.316*	-0.041	0.143	-0.015
Zn DTPA	0.699**	0.681**	0.666**	0.440**	0.525*	0.598**	0.491*	0.487*	0.348*	0.011	-0.048	-0.006
Zn 1st		0.845**	0.890**	0.645*	0.522*	0.586*	0.258**	0.238*	0.270*	0.190	0.193	0.213*
Zn 2nd			0.865**	0.435*	0.335*	0.439*	0.361*	0.319*	0.320*	0.151	0.054	0.086
Zn 3rd				-0.040	0.277**	0.277*	-0.012	-0.012	0.255**	0.423**	0.445**	0418**
Cu Aqua				0.323*	0.326*	0.333*	0.237*	0.027	0.041	-0.189	-0.177	-0.144
Cu DTPA				0.876**	0.701**	0.739**	0.416**	0.284**	0.106**	0.083	0.058	0.061
Cu 1st					0.745**	0.745*	0.355**	0.455**	0.325*	0.212*	0.219	0.259*
Cu 2nd						0.756*	0.323*	0.412**	0.323*	0.123	0.214*	0.215*
Cu 3rd							0.147	0.123	0.255*	0.147	-0.012	0.122
Cd Aqua							0.368*	0.260*	0.394*	0.195	0.136	-0.207
Cd DTPA							0.786**	0.749**	0.728**	0.220*	0.343*	0.214*
Cd 1st								0.655**	0.625**	0.523*	0.258*	0.213*
Cd 2nd									0.633**	0.250*	0.123	0.145
Cd 3rd										0.256	0.223	0.256*
Pb Aqua										0.0236*	0.328*	0.323*
Pb DTPA										0.420*	0.484*	0.466
Pb 1st											0.512**	0.522**
Pb 2nd												0.555**

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

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

Correlation coefficients among metal concentrations in tobacco and soil parameters (Table 4), show that there is a strong relationship among the concentrations of each tobacco priming (first, second and third) for every metal studied. Strong correlation was also recorded among the available concentrations of metals and their respective concentrations in all tobacco primings. This suggests that the concentrations of metals in the soil measured by the DTPA method can be used as a predictor of metal concentration in tobacco leaves. As there is also a relationship among available (DTPA method) and total (*Aqua Regia* method) concentration, the total concentration may possibly be calculated from the available concentration. This may be advantageous, as the *Aqua Regia* extraction method is very laborious and more expensive than the DTPA method.

Soil pH appeared to be the most important of the factors measured which influenced the concentration of Cd, Pb, Zn and Cu at three primings of air-cured (Burley), flue-cured (Virginia) and sun-cured (Oriental-filling) tobacco produced in the Thessaly region of central Greece. This generally agrees with other investigations in other agricultural soils (Adamu et al., 1989; Gondola and Kadar, 1994; Karaivazoglou et al., 2007).

In conclusion, the metal concentrations found in air-cured (Burley), flue-cured (Virginia) and sun-cured (Oriental-filling) tobacco priming produced in the Thessaly region are lower than those reported by other investigators. Therefore, tobacco plants grown in the area studied can be used for cigarettes without health risks depending on metal accumulation. Respective soils from the areas of central Greece studied were also less polluted, as most of the metals were present at concentrations lower than the critical limits (Alloway, 1990; Kabata-Pendias and Pendias, 1992).

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