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Cadmium Content in Soils and Oriental Tobacco Leaves: a Study in Tobacco-Growing Regions of Southeast Bulgaria

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Abstract. There is an extensive body of research on the content of harmful substances in tobacco raw materials and cigarette smoke. Data on the statistical relationships between the heavy metal content in soils and in tobacco plants are of interest. For this study, 38 soil samples and 38 samples from mature Oriental tobacco leaves (*Nicotiana tabacum* L., varietal group Basma, ecotype Krumovgrad) from the Eastern, Central, and parts of the Western Rhodope Mountains in Bulgaria were collected. Inductively coupled plasma atomic emission spectroscopy was used to measure the mobile (DTPA-extractable) forms and total content of the element Cd in soil, as well as the concentration of Cd in tobacco leaves. The basic soil characteristics: pH and texture were determined. Regression analysis was employed to study the statistical relationships between all measured variables. The total content of Cd in the soils ranged from 0.15 mg/kg to 3.30 mg/kg; and the mobile forms – from 0.02 mg/kg to 1.48 mg/kg. The Cd concentration determined in Oriental tobacco leaves varied between 0.05 mg/kg and 15.95 mg/kg. No visible symptoms of phytotoxicity were observed. Therefore, Oriental tobacco plants exhibited accumulating properties.

INTRODUCTION

Over the last decades, concerted efforts to decrease tobacco consumption worldwide have been made. Harmful substances in cigarette smoke have been studied thoroughly and alternative uses of tobacco raw materials have been a recent focus of attention. It has been ascertained that Oriental tobacco contains a rather high amount of rutin and chlorogenic acid, which implies possible applications in the pharmaceutical industry [1, 2]. The content of citric and malic acids in tobacco leaves and stems could be utilized in the food industry. Stems have been shown to be suitable both for feed and for the production of pellets, whereas seeds are a possible biofuel source [3]. It has been demonstrated that tobacco plants could be used for the purposes of phytoremediation, to extract heavy metals from the soil [4].

Determination of the content of potentially toxic elements in raw plant materials is a prerequisite for their utilization in the pharmaceutical and food industries. Verification of the safety of the produce is especially important in cases where tobacco has been grown on soil contaminated with heavy metals.

Monitoring Cd content in soils from agriculturally important areas is of great importance, as this non-essential element is a high priority toxicant which bioaccumulates in consumable plant tissues and could pose a health risk. According to the International Agency for Research on Cancer (IARC), Cd is classified as a Group 1 carcinogen. It enters into the human organism mainly through food consumption, and yet it has been shown that the content of Cd in smokers' blood is 4 times higher than in non-smokers [5]. Due to the lack of specificity of certain transport proteins, with the uptake of Zn by the tobacco plants, which is essential, Cd is also taken up and accumulated, and most of it is translocated to the leaves [5,6].

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Even though production has dwindled over the last decades, the tobacco sector continues to be of strategic importance to Bulgaria's economy [7]. The focus of the study were areas of the country, where Oriental tobacco has been grown traditionally and heavy metal contamination of the air and soils had been registered in the past due to industrial activities [8].

The aim of this study was to identify possible contamination with Cd of soils and Oriental tobacco plants grown on them (*Nicotiana tabacum* L., varietal group Basma, ecotype Krumovgrad), as well as to determine the basic soil parameters known to affect Cd take-up by plants and to ascertain the soil-plant relationships.

STUDY AREA. MATERIALS AND METHODS

The study area included the Eastern, Central, and parts of the Western Rhodope Mountains in Bulgaria. The specific terrain, deforestation, and monoculture practices in this area led to soil erosion. Oriental tobacco production has high social importance and is strategic to the economy in these regions of Bulgaria, where soil fertility and possible contamination of the crops are topical issues. In the past, this part of the country was affected by industrial contamination with metals related to the Kardzhali lead-zinc plant (non-operational since 2011) and open tailings. They were the main source of atmospheric emissions of Pb and Cd aerosols, fine particulate matter, and sulphur dioxide, as reported by the regulatory bodies [8]. *Nicotiana tabacum* L., varietal group Basma, ecotype Krumovgrad was chosen to be studied as a typical Oriental tobacco plant traditionally grown in Sothern Bulgaria.

In 2016, 2017, and 2018, surface soil samples (0-30 cm) and Oriental tobacco leaves at technical maturity were collected at 38 sites in the municipalities of Kardzhali (Krumovgrad, Zlatograd, Ardino, Kirkovo) and Haskovo (Ivailovgrad, Harmanli). Oriental tobacco samples were collected according to CORESTA Guide No. 13 [9]. The leaves were dried and homogenized, and a mean sample representing each sampling point was prepared. Cd content was determined in accordance with ISO 14082 [10] using inductively coupled plasma atomic emission spectroscopy.

The surface soil samples were collected in accordance with ISO 10381 [11], and sample preparation was carried out in accordance with ISO 11464 [12]. Examined were the following soil parameters: pH in aqueous extract (ISO 10390 [13]), soil texture (according to Wigner), total Cd content by aqua regia digestion (ISO 12914 [14], microwave mineralization), concentration of mobile forms of Cd (extraction with 0.005M DTPA + 0.1M TEA, pH 7.3 – ISO 14870 [15]).

Three SRMs were employed for quality control: CRM045 (Silt Clay Soil) in the analysis of the total content of Cd in the soils, NIMGBW07412A Soil (Brown soil) in the analysis of mobile forms of Cd in the soil samples, and INGT-OBTL-5 (Oriental Basma Tobacco leaves) – for plant analysis. The measured values were found to be in very good agreement with the certified data.

RESULTS

It was determined that in the studied area Oriental tobacco is grown on a wide variety of soils (Table I). The most suitable soils for Oriental tobacco production are reported to have clay and silt fraction (<0.02 mm) content between 10% and 50% (sandy and clayey-sandy soils) and humus content from 0.5% to 2.8% [16]. Of the studied soil samples, 73 % were characterized by a favourably light texture and the content of the clay and silt fraction varied between 6.3% and 82% (Table I).

TABLE I. Soil texture determined in the studied regions: soil fractions.

Statistical index	Coarse sand (2 – 0.2 mm)	Sand (0.2 – 0.02 mm)	Silt (0.02-0.002 mm)	Clay <0.002 mm	Silt + clay <0.02 mm
Mean	23.49	39.94	13.54	23.03	36.58
Standard deviation	13.79	20.1	9.70	16.20	23.48
Range	56.68	77.73	33.73	56.66	73.40
Minimum	3.35	4.67	3.27	3.68	8.54
Maximum	60.03	82.40	37.00	60.34	81.94

The soil reaction ranged from slightly alkaline (pH between 6.1 and 6.9) to moderately acidic (pH from 5.1 to 6.0) for the majority of the samples (Fig. 1). The soils varied greatly in terms of their reaction but it was found that based solely on this parameter, all studied soils were suitable for the normal growth of Oriental tobacco plants.

The Cd content determined in the soil and plant samples are shown in Table II. An assessment of the soil status could be made using Bulgarian legislation (Ordinance No. 3 of 2008 [17]). The soil reaction, land use and location were taken into account in establishing different background, precautionary, maximal permissible (MPC) and interventional (IC) levels. MPC is defined as the content of a harmful substance in the soil (in mg/kg), which, if exceeded, under certain conditions could inhibit soil functions and pose danger to the environment and human health. IC is the content of a harmful substance in the soil (in mg/kg), which, if exceeded, leads to inhibition of the soil functions and endangerment of the environment and human health.

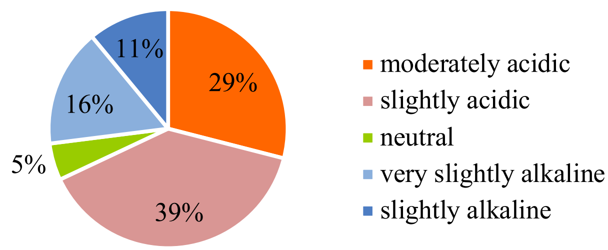

FIGURE 1. Soil reaction

No instances of exceedance of the IC (12 mg/kg) and MPs (agricultural land: 1.5 mg/kg for pH <6.0, 2 mg/kg for pH >6.0 to 7.4, and 3 mg/kg for pH >7.4) were observed. The highest content was determined in soil samples collected in small tobacco-growing fields near the Kardzhali municipal landfill and near the Studen Kladenets Reservoir – in the village of Ostrovitsa, located opposite to the currently non-operational Kardzhali lead-zinc plant, beyond the reservoir. It was observed that according to Ordinance No. 3 of 2008 [17], the majority of the soil samples were characterized by very low Cd content: in 76 % it was lower than the set precautionary level, and of this 76%, 45% had a Cd content below the background level. Therefore, as regards the Cd content in the studied soils, they could be considered clean even though in the past this region was an "ecological hot spot" [18].

TABLE II. Cd content (mg/kg): total content and mobile (DTPA-extractable) forms in soil, and Cd content in mature tobacco leaves (averaged data for 3 consecutive years).

Statistical index	Cd: mobile forms (soil)	Cd: total content (soil)	Cd: concentration (tobacco)
Mean	0.17	0.58	2.17
St. dev.	0.3	0.55	2.80
Variance	0.1	0.31	7.8
Range	1.46	3.15	15.50
Minimum	0.02	0.15	0.05
Maximum	1.48	3.30	15.95

A classification system for the concentrations of mobile forms of Cd was not found but the results could be compared to data from neighbouring countries in which Oriental tobacco is grown. According to Golia et al. [19], in Almyros region, Central Greece, the concentration of mobile Cd ranges between 0.8 and 2.25 mg/kg in agricultural land and increases to 2.6 mg/kg in industrial areas. Papafilippaki et al. [20] surveyed the industrial area of Heraklion, Crete, Greece and reported concentrations of mobile Cd between 0.053 and 0.13 mg/kg. Jordanoska et al. [21] found that the mobile forms of Cd ranged between 0.01 and 0.3 mg/kg in different Oriental tobacco growing areas in the Republic of North Macedonia. As with the total content of Cd, the highest values among the determined mobile forms of Cd were found in samples collected in small tobacco-growing fields near the Kardzhali municipal landfill and near the Studen Kladenets Reservoir.

According to Kabata-Pendias [22], plants grown in unpolluted areas have a Cd content of 0.005 to 0.252 mg/kg. In this study, the maximum value was 15.95 mg/kg and the sample was collected tobacco-growing fields near the

Kardzhali municipal landfill. No visible symptoms of toxicity of the plants were observed at any sampling site.

To determine possible linear and non-linear statistical relationships between the variables, regression analysis was performed. Table III shows the regression equations for the determined soil characteristics, the total content and mobile forms of Cd, and the content of the same element in Oriental tobacco leaves. A significant correlation between the mobile forms of the element and the total content in the soil was observed. Strong and significant relationships between the mobile forms of Cd in soils and tobacco leaves suggest that that the easily determinable mobile forms (as compared to the total content) could be used as a predictor of the concentration of Cd in the tobacco plants.

TABLE III. Regression/correlation analysis. Legend: R^2 – coefficient of determination, ** Statistical significance at p≤0.01; * Statistical significance at p≤0.05.

Variable x	Variable C – concentrations of mobile forms of Cd in soil	R^2	*p*
Clay fraction	$C = 0.044\ e^{0.031\ x}$	0. 244**	0.002
Clay + silt	$C = 0.053\ e^{0.015\ x}$	0.244*	0.035
pH	no significant relationships	-	-
Cd total	C = - 0.106 + 0.480 x	0.908**	0.000
Variable x	**Variable C – averaged concentrations of Cd in tobacco leaves**	R^2	*p*
clay fraction	$C = 0.236\ x^{0.584}$	0.183**	0.007
silt + clay	no significant relationships	-	-
pH	$C = 68.9\ e^{-0.618\ x}$	0.155*	0.015
Cd total	C = - 0.389 + 4.444 x	0.775**	0.000
Cd mobile	C = 0.678 + 8.763 x	0.764**	0.000
Variable x	**Variable C – concentrations of mobile forms of Cd in soil**	R^2	*p*
Clay fraction	$C = 0.044\ e^{0.031\ x}$	0. 244**	0.002
Clay + silt	$C = 0.053\ e^{0.015\ x}$	0.244*	0.035

The performed correlation/regression analyses revealed significant positive linear relationships between the total content and mobile forms of Cd in soils (p<0.001). The concentration of Cd in the tobacco leaves was linearly proportional to the Cd content in soils: both for the total content and the mobile forms (p<0.001). The exponential model described the relationship between the soil pH and the Cd concentration in tobacco leaves; as well as the relation between the mobile forms of Cd with the clay (<0.002 mm) and the silt + clay (<0.02 mm) fractions of the soil. The power model adequately reflected the relationship between the clay content and the Cd concentration in the tobacco leaves. The relationships between the soil characteristics and the determined concentrations both in the leaves and in the soils were similar to those determined in Virginia tobacco grown in Bulgaria [23].

Prohibition to plant *Nicotiana tabacum* L., varietal group Basma, ecotype Krumovgrad in close proximity to known point sources of heavy metal contamination should be considered.

CONCLUSION

The content of Cd was determined in collocated soil samples and tobacco leaves collected at maturity (*Nicotiana tabacum* L., varietal group Basma, ecotype Krumovgrad). Based solely on the content of Cd in the soil samples, no instances of exceedance of the intervention concentrations and maximal permissible content were observed. According to the Bulgarian legislation, the majority of the soil samples can be considered clean, since the content of Cd was determined to be lower than both the precautionary and background concentrations. The sets of collocated tobacco and soil samples collected in crop fields located at a distance of 10 m to 600 the municipal landfill in Vishegrad and the village of Ostrovitsa were characterized by high concentrations of Cd. At high Cd concentration in the leaves such as 15.95 mg/kg, no visible symptoms of phytotoxicity of the plants were observed, confirming that Oriental tobacco plants possess accumulating properties.

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