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Relationships Between Soil Characteristics and Concentrations of Lead and Zinc in Soils and in Oriental Tobacco

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Abstract. Increasing efforts to curb tobacco use worldwide have been made in recent years. Harmful substances in tobacco raw material and cigarettes have been the primary focus of attention. Obtaining information regarding the relationship between soil characteristics and the absorption of heavy metals by tobacco plants could improve the estimations for metal uptake by the plants and the associated health risks with exposure to tobacco smoke. This study was conducted in the Eastern, Central, and parts of the Western Rhodope Mountains using Oriental tobacco plants. The determined soil characteristics were: pH, humus content, and texture. Inductively coupled plasma atomic emission spectroscopy was used to measure the concentration of Pb and Zn in mature tobacco leaves, and the total content and mobile forms of the same elements in the soil. The correlation/regression analyses showed statistically significant linear relationships between the total content and mobile forms of Pb and Zn in soils. The concentration of Zn in tobacco leaves was linearly proportional to the Zn content in soils. The power model adequately reflected the relationship between the Pb total content and mobile forms in soils. The relationships between clay content and the concentrations of Pb and Zn in tobacco leaves were statistically significant.

Key words: zinc, lead, heavy metals, oriental tobacco, soil.

Introduction

Bulgaria is a traditional producer of Oriental tobacco and despite problems in recent years, the tobacco sector continues to be of strategic importance to the country's economy. Oriental tobacco originated from tobacco plants imported through Persia directly from America. They have been planted in the poorest regions in arid climate conditions. *Nicotiana tabacum* L. is a mesophyte that has adapted to dry environments. Thus, two opposing adaptations were combined - those of mesophytic and xerophytic plants. The manifestation of one or the other depends on the water regime during growth and development (Donev et al., 1981).

There has been a lot of pressure in recent years to curb tobacco smoking worldwide. Much attention has been paid to the harmful substances in tobacco and cigarettes. Specialists have directed their

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research efforts to the alternative uses of tobacco. It has been ascertained that Oriental tobacco contains a high amount of rutin, of greater quantities than in thyme, mint, lemon balm, and chamomile. The content of chlorogenic acid, a powerful antioxidant, is also high. This makes tobacco especially useful in the pharmaceutical industry (Docheva et al., 2012; Popova et al., 2018). Tobacco leaves and stems can be utilized for the production of citric and malic acids, used in the food industry. Additionally, tobacco seeds can also be utilized as a biofuel source, and the stems are suitable both for feed and for the production of pellets (Mijailovic et al., 2014). Another possible application of tobacco could be in phytoremediation due to the ability of these plants to extract heavy metals from the soil (Stojanovic et al., 2012).

The use of tobacco in the pharmaceutical and food industries, as well as for the cigarette industry, requires a strict control over the content of potentially toxic elements in the tobacco products. In the cases where tobacco is grown in regions contaminated with heavy metals, it is crucial to verify the safety of the produce, and, if an increased risk to human health is identified, to offer possible alternative applications.

The content of heavy metals in such tobacco-growing areas is of interest, especially in the regions studied in this paper, due to the high demographic, economic and social importance of this culture (Yancheva et al., 2007).

The main objective of this study was to identify possible contamination with lead and zinc of soils from tobacco production areas, and of *Nicotiana tabacum* L., varietal group Basma, ecotype Krumovgrad grown on them. The statistical relationships between soil characteristics, total content and mobile forms of the heavy metals in soils, and their content in leaves of tobacco plants collected at maturity were investigated.

Material and Methods

The study area covers the eastern and middle parts of the Rhodope Mountains in Bulgaria, as well as parts of the Western Rhodopes. The terrain is mountainous and hilly. The eastern parts of the Rhodope Mountains have the lowest altitude, which increases gradually to the west. There is a shortage of arable land and most of the soils are eroded to varying degrees. According to the FAO soil classification, Fluvisols, Chromic Luvisols, Rendzic Leptosols, Planosols, and Cambisols soils (occurring at elevation of over 800-900 m) are widespread in the studied regions, and Vertisols are found in numerous small areas (Bozhinova & Zheleva, 2007; Yancheva & Stanislavova, 2006; Yancheva et al., 2007).

In three consecutive years (2016, 2017, 2018), Oriental tobacco samples from *Nicotiana tabacum* L., varietal group Basma, ecotype Krumovgrad were collected according to CORESTA Guide N° 13 (2012). As a modification to the protocol, aiming to improve the credibility of the data, 5 mature upper stalk leaves from 10 random plants were collected in the fields. For plant analysis, a mean sample was prepared to represent each sampling point. Subsequently, the data from the three years was averaged, as well.

Collocated soil samples (38 in total) were collected from the surface layer (0-30 cm) in accordance with ISO 10381, during the period 2016-2018, in the following municipalities: Kardzhali, Krumovgrad, Ivaylovgrad, Harmanli, Zlatograd, Ardino, and Kirkovo. Sample preparation was done according to ISO 11464.

The following soil and plant characteristics were determined:

1. pH in aqueous extract - potentiometric, ISO 10390;
2. Humus content - according to Tyurin – a titrimetric method, modified by Ponomareva and Plotnikova (Angelova et al., 2014);
3. Soil texture - pipette method; (Đamić et al., 1996);
4. Total lead and zinc content by *aqua regia* digestion (microwave mineralization), ISO 12914;
5. Concentration of mobile forms of lead and zinc - extraction with 0.005M DTPA + 0.1M TEA, pH 7.3, ISO 14870;

6. Content of Pb and Zn in Oriental tobacco leaves, ISO 14082.

Inductively coupled plasma atomic emission spectroscopy was used as an analytical technique for the determination of lead and zinc content in the soil and the plant samples.

It was determined that the measured values for the concentrations of Pb and Cd in the whole dataset followed a log-normal distribution, verified using a Sapiro-Wilk test ($p = 0.05$). Descriptive statistics and correlation/ regression analyses were performed in IBM SPSS Statistics for Windows, Vers. 19.0 (IBM Corp., 2010) to investigate the relationships between the soil characteristics, total and mobile forms of lead and zinc in soils, and the content of these elements in Oriental tobacco leaves.

Three standard reference materials were employed for accuracy and precision: *CRM045 Silt Clay Soil* was used in measurements of the total content of Pb and Zn in the soils, *NIM-GBW07412A Soil (Brown soil)* was used in measurements of their mobile forms, and *INGT-OBTL-5* (Samczyński et al., 2012) Polish reference material (Oriental Basma Tobacco leaves) was used for the plant analysis. The measured values were found to be in very good agreement with the certified data.

Results and Discussion

Soil

The results of the determined soil characteristics are shown in Table 1. In the studied areas, Oriental tobacco is grown on a wide variety of soils in terms of their texture and degree of erosion. Soils with clay and silt faction content between 10% and 50% (i.e. sandy and clayey-sandy soils) and humus content from 0.5% to 2.8% are considered suitable for Oriental tobacco production. Higher humus content (between 2.5% and 3.8%) could be used in the absence of better soil conditions (Tanov et al., 1978).

The content of the clay and silt fraction (<0.02 mm) varied between 6.3% and 82%. Predominant were the soils with a light texture, favorable for the cultivation of Oriental tobacco; and 27% of the soils were heavy.

Most soil samples were characterized by a slightly acidic to moderately acidic reaction (Fig. 1a). According to the used classification, soil reactions in the range of 5.1 to 6.0 are moderately acidic; between 6.1 and 6.9 – slightly acidic, and a pH of 7.0 is neutral. Very slightly alkaline soil reactions have values between 7.1 and 7.5, and pH levels of 7.6 to 8.0 are slightly alkaline. In some regions, the soils varied greatly in terms of their pH but all the samples had a soil reaction suitable for the normal growth and development of tobacco plants. Humus content of less than 1.0% is considered very low; values from 1.0 to 2.0% define low content; medium content is in range of 2.1 to 3.0%; and high content ranges from 3.1 to 5.0%. Values above 5% define very high humus content and a small proportion of soils, 3%, were characterized by an unsuitably high humus content (Fig. 1b).

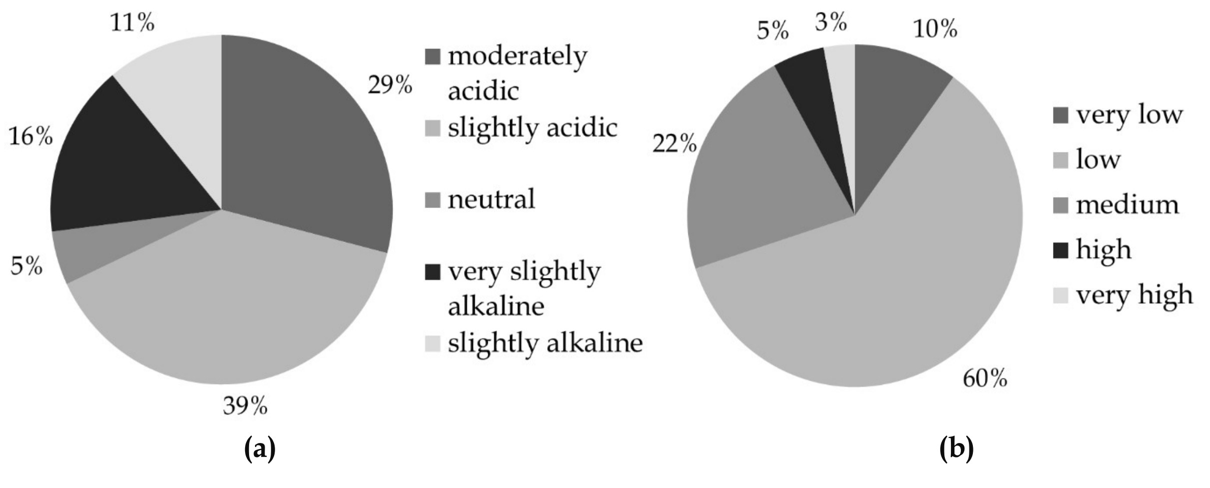

Fig. 1. Soil reaction (a) and humus content (b).

Table 1. Descriptive statistics of the soil characteristics determined in the studied regions.

Statistical Parameters	Soil texture fraction content, %					pH	Humus content %
	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	39.93	13.53	23.03	36.58	6.50	1.85
Standard deviation	17.79	20.09	9.69	16.20	23.48	0.72	1.02
Range	56.68	77.73	33.73	56.66	73.40	2.72	5.27
Minimum	3.35	4.67	3.27	3.68	8.54	5.15	0.86
Maximum	60.03	82.40	37.00	60.34	81.94	7.87	6.13
CV, %	75.73	50.31	62.40	70.34	64.19	11.08	55.14

It was ascertained that 26% of the soil samples were characterized by the optimal for Oriental tobacco-growing sandy and clayey-sandy texture and humus content between 0.8% and 2%. They were collected in the vicinity of the villages: Gluhar, Gradinka, Dryanova Glava, Opalchentsi, Panichkovo, Kondovo, Oreshino, Lyaskovo, Draganovo, and Konush.

Table 2 shows the summarized results for the total lead and zinc content in the soils. The soil status was determined in accordance with Ordinance #3 (2008), which indicates the permissible content of harmful substances in soils and specifies the maximum permissible concentrations (MPCs) and intervention concentrations (ICs) for several heavy metals and metalloids. 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. The concentrations defined in Ordinance #3 (2008) are different depending on the soil reaction, location and land use (i.e. industrial/production sites, permanent grasslands, pastures, in settlements, arable land).

No instances of exceedance of the ICs were determined. Exceedance of the maximum permissible concentration for Pb, set at 60 mg/kg, 100 mg/kg or 120 mg/kg depending on the soil reaction, was observed in samples collected near Visegrad, Kirkovo, and Panichkovo. The MPC for Zn (200 mg/kg, 320 mg/kg or 400 mg/kg based on the soil reaction) was exceeded in one of the soil samples collected in a small tobacco-growing field near the Kardzhali municipal landfill site, however, the IC (900 mg/kg) was not exceeded. Contamination with both Pb and Zn was ascertained in soil samples collected near the Studen Kladenets Reservoir – in the village of Ostrovitsa, located opposite to the non-operational Kardzhali lead-zinc plant, beyond the reservoir (Jeleva et al., 2012).

Table 2. Descriptive statistics of the total content of Pb and Zn in soil (mg/kg).

Statistical Parameters	Pb	Zn
Mean	43.48	82.85
Standard deviation	31.36	72.55
Range	110.00	435.00
Minimum	6.60	17.90
Maximum	116.60	452.90
CV, %	72.12	87.58

Background and precautionary concentrations of heavy metals and metalloids are also established in the referenced Ordinance #3 (2008). By definition, the exceedance of the precautionary concentrations would not lead to inhibition of the soil functions, nor to endangerment to the environment and human health. The values vary according to the soil texture. The percentage of collected samples characterized by very low concentrations of Pb was 37% (lower than the background values) and 74% for Zn. Concentrations of Pb lower than the precautionary values were determined in 63% of the samples, and for Zn – in 92% of the samples. It should be noted that soils (and soil-forming rocks) on the Balkan Peninsula are characterized by the relatively high mineral content (Milev et al., 2007) and this is reflected in the concentration classification system in Ordinance #3 (2008).

Table 3 contains the summarized results for the DTPA-extractable forms of Pb and Zn. According to the classification for concentrations of mobile forms of elements in soils (Horneck et al., 2011; Jones Jr., 2001), 53% of the soil samples had medium Zn concentration, 18% were characterized by low Zn concentration, and 3% had a very low concentration. High concentrations were determined in 8% of the samples, and a very high concentration – in 18%. The highest concentrations of mobile Zn forms were determined in samples from the villages of Ostrovitsa (in the vicinity of the Kardzhali Pb-Zn plant), Vishegrad (near the municipal landfill), Gluhar, Zheltusha, Lyaskovo.

A classification system for the concentrations of mobile forms of Pb was not found but the results could be compared to data from neighboring countries. According to Golia et al. (2008), the concentration of mobile Pb ranges between 2.5 and 4.8 mg/kg in agricultural land, and increases to 7.9 mg/kg in industrial areas. Similar concentrations of mobile Pb were determined in the industrial area of Heraklion, Crete, Greece, by Papafilippaki et al. (2008). According to Jordanoska et al. (2018), the DTPA-extractable Pb content in several Oriental tobacco growing areas of North Macedonia ranged between 0.5 and 4.4 mg/kg.

Table 3. Descriptive statistics of DTPA-extractable forms of trace elements in soils (mg/kg).

Statistical Parameters	Pb	Zn
Mean	2.83	5.18
Standard deviation	3.03	9.87
Range	17.07	50.10
Minimum	0.31	0.39
Maximum	17.37	50.50
CV, %	107.09	190.72

In the present study, the Pb content ranged between 0.31 and 17.37 mg/kg. The highest values were determined in samples from Ostrovica (located opposite to the Pb-Zn smelter in Kardzhali) and from Vishegrad (near the Kardzhali municipal landfill). With the exception of these samples, the determined values for DTPA-extractable concentrations of Pb were similar to those reported in the previously referenced sources (Golia et al., 2008; Jordanoska et al., 2018; Papafilippaki et al., 2008).

Oriental tobacco leaves

Summarized results for the determined Pb and Zn concentrations in tobacco leaves collected at maturity are shown in Table 4.

Plants grown in unpolluted areas have a Pb content in the range of 0.1 to 10 mg/kg, and concentrations in the range of 30 to 300 mg/kg are considered critical (Kabata-Pendias & Pendias, 1992). Bozhinova (2016) ascertained that phosphorus fertilizers do not have a pronounced effect on the concentration of Pb in tobacco leaves, nor in soils (both mobile forms and total content). In this study, high values (maximum concentrations) were ascertained in small crop fields located 10 m away from the Kardzhali municipal landfill, and in the village of Ostrovitsa. However, no visible symptoms of toxicity of the plants were observed.

The determined values for Zn were lower than the critical concentrations for plants (100-400 mg/kg) and consistent with literature data (Kabata-Pendias & Pendias, 1992). According to Stamatov et al. (2015), the concentration of trace elements in oriental tobacco leaves depends on the variety of the plant.

Table 4. Descriptive statistics of Pb and Zn concentrations (mg/kg) in Oriental tobacco leaves. Summarized results for samples collected in 2016, 2017, and 2018.

Statistical Parameters	Pb	Zn
Mean	11.46	89.98
Standard deviation	46.79	66.14
Range	272.83	315.80
Minimum	0.27	22.90
Maximum	273.10	338.70
CV, %	408.29	73.51

Statistical relationships between the data for soils and Oriental tobacco leaves

Table 5 contains the Pearson correlation coefficients for the determined Pb and Zn concentrations in Oriental tobacco leaves and in soils. The significant correlation between the total content of Pb and Zn in the soil ($R = 0.475$) is explained by the geological features of the region and the presence of polymetallic ores. A significant correlation between the mobile forms of the elements in the soil was observed as well ($R = 0.697$). Additionally, the total content of the elements and their mobile forms correlated significantly. The concentrations of Pb and Zn determined in Oriental tobacco leaves correlated significantly as well ($R = 0.508$). The relationships between the mobile forms of the elements and their concentrations in tobacco leaves are of interest. Significant and strong correlations were expected due to the accumulating properties of the studied crop and the availability of the mobile metal forms to plants. The Zn concentration determined in the tobacco leaves and the mobile forms of both Pb and Zn in the soils showed stronger correlations than those of the Pb concentration in tobacco and the mobile forms of Pb and Zn in the soils, however, all were significant.

Regression analyses were performed to investigate possible linear and non-linear statistical relationships between the data. Table 6 contains the regression equations for the whole set of determined soil characteristics, the total content and mobile forms of Pb and Zn in the soils, and their content in Oriental tobacco leaves.

A strong and significant linear relationship between the DTPA-extractable forms of Zn and the Zn concentration in leaves was observed. A similar relationship was ascertained in a study conducted in Greece (Golia et al., 2009). For Pb, the association between the mobile forms and the concentration in the leaves was described by the quadratic regression model. Despite the known effects of soil pH on the mobility and uptake of metals in plants (Kabata-Pendias & Pendias, 1992), no statistically significant relationships were ascertained between the soil reaction and the mobile forms of Pb and Zn in this study. The DTPA-extractable forms of the elements had significant relations to the humus content, described by the power regression model for both. The strong and significant relationships with the total content for the two metals were best described by non-linear regression equations. The power regression model suited the relationship between the mobile forms and the total content of Pb, whereas the cubic model described the dependencies between the total content of Zn and the Zn mobile forms.

The relationships between the soil characteristics and the determined concentrations for the two metals both in the leaves and in the soils were rather similar to those determined in Virginia tobacco grown in Bulgaria (Zaprjanova et al., 2010). The lack of a statistical relationship between the humus content and the Pb concentration in tobacco leaves was an exception. Additionally, the same authors found a linear relationship between the Pb concentration in the leaves and the total content of the element in the soil, whereas, in this study, the relationship was described by the logarithmic regression model.

In a similar study conducted in North Macedonia, it was shown that the composition of the tobacco leaves rarely correlates significantly with the soil characteristics, with the exception of the clay fraction (Jordanoska et al., 2018). The results from this study revealed weak yet significant statistical relationships between the Pb content in the tobacco leaves and both the clay and clay and silt fractions of the soil, both described by the logarithmic regression model.

Table 5. Correlation (Pearson correlation coefficients) between the determined Pb and Zn concentrations in Oriental tobacco leaves and in soils (total *C* – total concentration, mf – mobile forms). Legend: ** Statistical significance at $p \leq 0.01$; * Statistical significance at $p \leq 0.05$.

Statistical parameters	Pb tobacco	Zn tobacco	Pb total *C*, soil	Zn total *C*, soil	Pb mf, soil	Zn mf, soil
Pb tobacco	1					
Zn tobacco	0.508**	1				
Pb total *C*, soil	0.490**	0.532**	1			
Zn total *C*, soil	0.227	0.755**	0.475**	1		
Pb mf, soil	0.409*	0.742**	0.658**	0.511**	1	
Zn mf, soil	0.369*	0.863**	0.567**	0.925**	0.697**	1

Table 6. Regression equations and significance level the determined soil characteristics, Pb and Zn concentrations in Oriental tobacco leaves and in soils. Legend: ** Statistical significance at $p \leq 0.01$; * Statistical significance at $p \leq 0.05$.

Variable X	Variable C – concentrations of the mobile forms of Pb in soil	R^2	*p*
Clay fraction	$C = 18.222 + 7.505 . \ln X$	0.187**	0.007
Clay + silt	no significant relationships	-	-
pH	no significant relationships	-	-
Humus	$C = 1.431 . X^{0.224}$	0.203**	0.004
Pb total	$C = 22.928 . X^{0.586}$	0.506**	0.000
Variable X	**Variable C – averaged concentrations of Pb in tobacco leaves**	R^2	*p*
Clay fraction	$C = 21.185 + 5.113. \ln X$	0.194**	0.006
silt + clay	$C = 33.510 + 6.503. \ln X$	0.144*	0.019
pH	no significant relationships	-	-
Humus	no significant relationships	-	-
Pb total	$C = 38.836 + 12.874 . \ln X$	0.329**	0.000
Pb mobile	$C = 1.992 + 0.237. X - 0.001 . X^2$	0.709**	0.000
Variable X	**Variable C – concentration of the mobile forms of Zn in soil**	R^2	*p*
Clay fraction	no significant relationships	-	-
clay + silt	no significant relationships	-	-
pH	no significant relationships	-	-
Humus	$C = 1.052 . X^{1.515}$	0.378**	0.000
Zn total	$C = 4.021 – 0.141 . X + 0.002 . X^2 - 2.91.10^{-6} . X^3$	0.917**	0.000
Variable X	**Variable C – averaged concentrations of Zn in tobacco leaves**	R^2	*p*
Clay fraction	$C = 32.684. X^{0.292}$	0.173**	0.009
clay + silt	no significant relationships	-	-
pH	$C = 737.205 . 0.704^{X}$	0.190**	0.006
Humus	$C = 155.339 - 154.826 . X + 79.717 . X^2 - 9.386 . X^3$	0.255*	0.017
Zn total	$C = 32.931 + 0.689 . X$	0.571**	0.000
Zn mobile	$C = 60.062 + 5.778 . X$	0.744**	0.000

A significant statistical relationship was determined between the Zn concentration in tobacco leaves and the clay fraction of the soil described adequately by the power regression model. The compound regression model described the relationship between the reaction of the soil and the Zn concentration in tobacco leaves.

Conclusions

The content of lead and zinc was determined both in soils and in tobacco leaves collected at maturity. In terms of the total content of the metals in soil, no instances of exceedance of the intervention concentrations were observed. The majority of the soil samples can be considered clean, as the determined content of the elements was lower than the precautionary and background concentrations according to the Bulgarian legislation.

The maximum permissible concentration for lead was exceeded in samples collected in the area of Vishegrad, Kirkovo, and Panichkovo. Soil contamination with zinc was ascertained near the village of Ostrovitsa, in the vicinity of the non-operational Kardzhali Pb-Zn plant. Remediation of the affected soils is recommended.

The tobacco samples collected in crop fields located at a distance of 10 m to 600 m from the municipal landfill in Vishegrad and in the village of Ostrovitsa were characterized by high concentrations of both lead and zinc. At the same sites, the concentrations of the heavy metals in the soils were also high (both total content and mobile forms). No symptoms of toxicity of the plants were observed. It was confirmed that Oriental tobacco exhibits accumulating properties, therefore planting in close proximity to known point sources of heavy metal contamination should be prohibited. The observed strong and significant relationships between the DTPA-extractable forms of Pb and Zn in soils with their concentrations in the tobacco leaves show that the mobile forms could be used as a predictor of the metal concentration in the tobacco plants.

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