TRACE ELEMENT CONCENTRATION IN ORIENTAL TOBACCO AS AFFECTED BY LONG-TERM NITROGEN FERTILIZATION

Radka BOZHINOVA*

**Agricultural Academy, Tobacco and Tobacco Products Institute, BG-4108 Markovo, Bulgaria*
Correspondence author: Radka Bozhinova, Tobacco and Tobacco Products Institute, Markovo; phone:+359 898 994 412, e-mail: rbojinova@yahoo.com

Abstract. This research was carried out to assess the effect of long-term fertilization with different N rates on the concentration of Fe, Mn, Zn, Cu, Pb, Ni and Cd in soil and in oriental tobacco plants. The long-term fertilizer experiment was initiated in 1966 in Markovo, Bulgaria on rendzina soil (Rendzic Leptosols). For the purposes of the study, three N rates (0, 50, and 100 kg $N \cdot ha^{-1}$) were selected. Each treatment had three replicates with a completely randomized block design. Results showed that long-term N fertilization caused a slight decrease in soil pH compared to the unfertilized control and thus did not substantially influence trace elements' availability. N-fertilizer rate had no significant effect on the available Fe, Mn, Zn, Cu, Pb and Cd content in the soil. Plant-available Ni content significantly increased in plots with long-term use of nitrogen fertilizer. Nitrogen fertilizer application greatly increased tobacco yield and N content in the leaves. With the increase of N fertilization rate the average concentration of the nitrogen in leaves increased from 1.35% to 2.79%. With the exception of the iron content in the upper leaves, increasing levels of N fertilization had no significant effect on Mn, Zn, Cu, Pb, Ni and Cd concentrations in oriental tobacco. Therefore, long-term application of different rates of nitrogen fertilizer had no obvious effect on the accumulation of available Fe, Mn, Zn, Cu, Pb, and Cd in soil and concentrations of trace elements in tobacco grown on alkaline soil.

Key words: nitrogen rate; trace elements; tobacco; soil.

INTRODUCTION

Nitrogen is essential for tobacco growth, development, yield and quality. The ability to control both the amount and time of nitrogen availability during growth and ripening, coupled with moisture supply, has a greater effect on the agronomic and smoking properties of tobacco than other nutrient or management factors [17]. The N fertilizer amount impacted on relative wrapper yield through its influence on plant growth, nutritional status and chemical composition [2].

Some studies have documented that nitrogen fertilization can influence the soil properties such as pH, soil organic matter content, the available concentrations of micronutrients. Soil pH declines while other basic soil properties (Eh, EC, and CEC) increase with increasing N application rates [1]. The SOC (soil organic carbon) stock at the 0-100 cm depth did not significantly differ in the soils subjected to different N application rates [15]. Extractable Cu was significantly greater without N application and declined with the application of N fertilizer [22]. N rate had no direct influence on available soil micronutrients after 14 cropping years [16].

Nitrogen fertilization can potentially increase the concentration of N in plants and can influence the tissue concentrations of other elements. The content of nitrogen in tobacco leaves positively correlated with levels of N applied [2, 14]. Application of N fertilizer reduced the concentrations of Ca and Zn, and increased the concentration of Mn in the grains of tropical maize [6]. N fertilization increased the concentrations of the micronutrients (Fe, Zn, and Cu) in wheat grain, but did not affect the concentration of Mn [21]. The same authors concluded that proper management of N fertilization could enhance micronutrient concentrations in grains and could thereby help to reduce micronutrient malnutrition of humans (so called biofortification). In no-till continuous maize stover N, Cu and Mn increased substantially with increasing N fertilization, whereas grain Cu and Mn did not, suggesting that higher N rates could alleviate deficiencies for silage, but not for grain nutrition [16]. Except for the concentration of Mn, none of the micronutrients in wheat grain were affected by N application rate [22]. The soil Cd concentration and the crop N nutrition status affect Cd accumulation in spring wheat grain produced in eastern Canada [11]. N application rates influenced soil properties and played a decisive role in the transfer of Cd from soil to wheat grain by increasing soil acidification, soil salinity, oxidation reactions, and exchange capacity [1]. Each 10 kg increase in N application gave an increased Cd concentration in winter wheat grain of approximately 0.001-0.003 $mg \cdot kg^{-1}$ [26]. The content of Cd in corn grain was positively correlated to nitrogen fertilizer input, but content of Pb was negatively related to nitrogen rate [19]. These results show that changes in element concentration in plants tissues vary because of nitrogen fertilizers, plant species, region-specific differences in soil and climate conditions and other factors.

Information on the mineral composition of oriental tobacco as a function of N application rates is limited. The aim of the present study was to examine the effect of long-term fertilization with different N rates on the concentrations of trace elements (Fe, Mn, Zn, Cu, Pb, Ni and Cd) in soil and oriental tobacco plants.

MATERIALS AND METHODS

Experimental conditions. A long-term fertilization experiment with continuous tobacco cropping system was carried out at Tobacco and Tobacco Products Institute - Markovo, Bulgaria (42º06' N and 24º70' E).

Oriental tobacco plants *(Nicotiana tabacum* L., cv. Plovdiv 7) were grown in the stationary field. The experiment was established in 1966. Twenty-eight variants of fertilization with various rates and combinations of nitrogen, phosphorus and potassium, as well as organic-mineral fertilization are being tested. The nitrogen fertilization rates are 0, 25, 50, and 100 $kg \cdot ha^{-1}$, for phosphorus - 0, 50, 75, 100, and 225 $kg \cdot ha^{-1}$, and for potassium - 0, 75, and 450 $kg \cdot ha^{-1}$. The fertilizers are applied one-time before the last spring cultivation as follows: nitrogen - as urea, phosphorus - as triple superphosphate, and potassium - as potassium sulphate. The soil was classified as Rendzic Leptosols (World Reference Base for Soil Resources) [23]. The soil is representative for a significant part of the so-called tobacco-growing soils in the country. It is characterized with medium powerful humus horizon, heavy sandy-clay (the content of the clay and silt fraction (<0.02 mm) is 46%-49%) that gradually turns into carbonate base rock. The climate is transitional continental with early, mild spring, hot summer, late autumn and lasting sunlight.

For the purposes of the study, three N rates (0, 50, and 100 $kg\ N \cdot ha^{-1}$) were selected. Each treatment had three replicates in a completely randomized block design. The plot area was 6.25 m^2 (2.5 x 2.5 m). Tobacco seedlings were transplanted at a 0.5 x 0.12 m distance (166,000 $plants \cdot ha^{-1}$). Cultural practices were similar to those used by oriental tobacco producers. Commercial granular urea ($CO(NH_2)_2$), with a 46 percent nitrogen content, was applied at the rates 0 (0 kg N per hectare), 109 (50 kg N per hectare), and 218 (100 kg N per hectare) $kg \cdot ha^{-1}$ before transplanting. The fertilizer was uniformly broadcast over the soil surface of each plot before being incorporated.

Soil and plant analysis. Soil samples (0-25 cm depth) were collected in March 2014, 2015, and 2016. The following soil characteristics were determined: pH in water, humus according to Tjurin (2004) [25] and total N - by the Kjeldahl method (1999) [24]. A solution of 0.005M DTPA + 0.1M TEA, pH 7.3 was used for extraction of the mobile forms of Fe, Mn, Zn, Cu, Pb, Ni and Cd from soils [12].

Mature leaves (lower, middle and upper) were collected from all studied plots in every of the three experimental years. The samples were washed with tap water and rinsed with distilled water, after which they were dried at 75ºC for 12 h and ground. Total nitrogen in the plants was determined by the Kjeldahl method (1999) [24]. The preparation of plant samples for analysis of Fe, Mn, Zn, Cu, Pb, Cd and Ni was made by means of dry ashing and dissolution in 3 M HCl. An atomic absorption spectrometer „SpectrAA 220" (Varian, Australia) was used for determination of trace element concentration in the soil and plant samples.

Statistical analysis. Results were analyzed using the SPSS statistical package and differences were assessed with the Duncan's multiple range test at the 0.05 probability level.

RESULTS

The soil reaction of the experimental plots varied from 8.05 to 8.17 (Table 1). The pH reduction in 50 $kg\ N \cdot ha^{-1}$ treatment was minimal as compared to unfertilized control. Soil pH declined slightly, but statistically significantly by long-term fertilization with 100 $kg\ N \cdot ha^{-1} \cdot y^{-1}$ (Table 1). Soil humus and total N content were increased with the increase in nitrogen fertilizer levels (Table 1).

With the exception of Ni, there was no significant effect of long-term N fertilization on DTPA-extractable Fe, Mn, Zn, Cu, Pb and Cd (Table 2). Zaprjanova & Hristozova (2018) [27] indicated that the humus-carbonate (Rendzic Leptosols) soil at the Tobacco and Tobacco Products Institute, Markovo was characterized as very highly supplied with mobile forms of copper and zinc, which did not correspond to the alkaline reaction and could probably be a result of industrial pollution (KCM Plovdiv). The same authors found that the content of mobile manganese and lead was high as well.

Long-term N fertilization greatly affected plant height, number of leaves per plant and dimensions of middle leaves (Table 3). The plant height and dimensions of the middle leaves changed significantly with increasing of nitrogen level from 0 to 50 $kg \cdot ha^{-1}$. No significant differences in studied plant characteristics were observed between 50 and 100 $kg\ N \cdot ha^{-1}$. Unfertilized treatment gave the lowest yield. Application of 50 and 100 $kg\ N \cdot ha^{-1}$ increased cured leaf yield, without any significant difference between them.

The concentration of nitrogen in the mature leaves ranged from 1.21% to 3.35% (Table 4). The nitrogen concentration in the leaves depends on their stalk position and the rate of N fertilizer. It rises from the lower to the upper leaves and the differences between them increased in response to increasing levels of N applied.

Tables 5 and 6 show the trace elements' concentrations in leaves from different stalk positions (lower, middle and upper) as dependent on long-term N fertilization, averaged over the period studied.

Iron concentration in mature leaves varied between 139.2 and 268.2 $mg \cdot kg^{-1}$. These values were within ranges observed by Golia et al. (2009) [7] for Oriental tobacco (140-520 $mg \cdot kg^{-1}$). The lower leaves accumulated more iron (231.7-268.2 $mg \cdot kg^{-1}$), compared with leaves from the middle (179.9-201.2 $mg \cdot kg^{-1}$) and upper stalk positions (139.2-184.9 $mg \cdot kg^{-1}$).

The concentration of Mn in leaves was from 46.6 to 66.8 $mg \cdot kg^{-1}$. These values are much lower than those established by Golia et al. (2009) [7] for Oriental tobacco (118-510 $mg \cdot kg^{-1}$), grown on acid soils. The mean concentrations of Mn had higher values at lower leaves than at the leaves from middle and upper stalk position. Manganese concentration in the leaves did not change significantly (P>0.05) with increasing nitrogen level.

Table 1. Soil pH, total humus and N content as dependent on long-term N fertilization (3-year average)

N rate (kg N·ha^{-1})	pH in H_2O	Total humus (%)	Total N (%)
0	8.17 ^{a}	2.48 ^{c}	0.143 ^{b}
50	8.12 ^{a}	2.59 ^{b}	0.157 ^{a}
100	8.05 ^{b}	2.70 ^{a}	0.166 ^{a}

Different letters within each column indicate that the means are significantly different (P<0.05) as determined by Duncan's Multiple Range Test (DMRT)

Table 2. Trace element concentrations in soil (mg·kg^{-1}), extracted by DTPA, as dependent on long-term N fertilization (3-year average)

N rate (kg N·ha^{-1})	DTPA-Fe	DTPA-Mn	DTPA-Zn	DTPA-Cu	DTPA-Pb	DTPA-Ni	DTPA-Cd
0	6.6 ^{a}	31.9 ^{a}	10.3 ^{a}	14.7 ^{a}	18.8 ^{a}	0.85 ^{b}	0.61 ^{a}
50	6.7 ^{a}	36.4 ^{a}	10.7 ^{a}	14.1 ^{a}	17.9 ^{a}	1.38 ^{a}	0.58 ^{a}
100	7.2 ^{a}	38.2 ^{a}	11.3 ^{a}	14.3 ^{a}	19.1 ^{a}	1.42 ^{a}	0.56 ^{a}

Different letters within each column indicate that the means are significantly different (P<0.05) as determined by Duncan's Multiple Range Test (DMRT)

Table 3. Plant height, number of leaves per plant, dimensions of the middle leaves (14-th leaf from the bottom) and yield of the oriental tobacco mature plant variety Plovdiv 7 (3-year average)

N rate (kg N·ha^{-1})	Plant height (cm)	Number of leaves per plant	Dimensions of the 14-th leaf (cm)		Yield (kg·ha^{-1})
			Length	Width	
0	77.9 ^{b}	27.0 ^{b}	20.2 ^{b}	9.5 ^{b}	73.2 ^{b}
50	94.7 ^{a}	28.2 ^{ab}	24.6 ^{a}	11.6 ^{a}	104.8 ^{a}
100	105.4 ^{a}	29.0 ^{a}	26.3 ^{a}	12.3 ^{a}	115.3 ^{a}

Different letters within each column indicate that the means are significantly different (P<0.05) as determined by Duncan's Multiple Range Test (DMRT)

Table 4. N concentration (%) of tobacco leaves (3-year average)

N rate (kg N·ha^{-1})	Lower leaves	Middle leaves	Upper leaves	Average
0	1.21 ^{c}	1.39 ^{c}	1.44 ^{c}	1.35 ^{c}
50	1.74 ^{b}	2.43 ^{b}	2.92 ^{b}	2.36 ^{b}
100	2.21 ^{a}	2.79 ^{a}	3.35 ^{a}	2.79 ^{a}

Different letters within each column indicate that the means are significantly different (P<0.05) as determined by Duncan's Multiple Range Test (DMRT)

Table 5. Trace elements (Fe, Mn, Zn and Cu) concentrations of tobacco leaves (3-year average)

N rate (kg N·ha^{-1})	Lower leaves	Middle leaves	Upper leaves	Average
	Fe concentration (mg·kg^{-1} dry matter)			
0	268.2 ^{a}	201.2 ^{a}	184.9 ^{a}	218.1 ^{a}
50	231.7 ^{a}	179.9 ^{a}	161.0 ^{ab}	190.9 ^{b}
100	238.0 ^{a}	201.1 ^{a}	139.2 ^{b}	192.8 ^{b}
	Mn concentration (mg·kg^{-1} dry matter)			
0	66.7 ^{a}	53.3 ^{a}	46.6 ^{a}	55.5 ^{a}
50	62.2 ^{a}	54.7 ^{a}	49.2 ^{a}	55.4 ^{a}
100	66.8 ^{a}	57.3 ^{a}	47.5 ^{a}	57.2 ^{a}
	Zn concentration (mg·kg^{-1} dry matter)			
0	122.5 ^{a}	87.7 ^{a}	55.3 ^{a}	88.5 ^{a}
50	89.6 ^{a}	70.1 ^{a}	52.2 ^{a}	70.6 ^{a}
100	78.5 ^{a}	73.3 ^{a}	58.3 ^{a}	70.0 ^{a}
	Cu concentration (mg·kg^{-1} dry matter)			
0	18.2 ^{a}	19.0 ^{a}	15.7 ^{a}	17.6 ^{a}
50	20.3 ^{a}	21.2 ^{a}	16.8 ^{a}	19.4 ^{a}
100	20.5 ^{a}	19.4 ^{a}	16.0 ^{a}	18.6 ^{a}

Different letters within each column indicate that the means are significantly different (P<0.05) as determined by Duncan's Multiple Range Test (DMRT)

Table 6. Trace element (Pb, Ni and Cd) concentrations of tobacco leaves (3-year average)

N rate (kg N·ha^{-1})	Lower leaves	Middle leaves	Upper leaves	Average
	Pb concentration (mg·kg^{-1} dry matter)			
0	7.7 ^{a}	13.0 ^{a}	11.3 ^{a}	10.7 ^{a}
50	8.7 ^{a}	12.0 ^{a}	11.0 ^{a}	10.6 ^{a}
100	8.3 ^{a}	14.7 ^{a}	11.0 ^{a}	11.3 ^{a}
	Ni concentration (mg·kg^{-1} dry matter)			
0	2.60 ^{a}	1.40 ^{a}	1.60 ^{a}	1.87 ^{a}
50	1.43 ^{a}	1.13 ^{a}	0.53 ^{a}	1.03 ^{a}
100	1.63 ^{a}	1.40 ^{a}	0.73 ^{a}	1.25 ^{a}
	Cd concentration (mg·kg^{-1} dry matter)			
0	5.17 ^{a}	3.03 ^{a}	1.70 ^{a}	3.30 ^{a}
50	3.57 ^{a}	1.77 ^{a}	1.00 ^{a}	2.11 ^{a}
100	3.50 ^{a}	2.63 ^{a}	0.93 ^{a}	2.36 ^{a}

Different letters within each column indicate that the means are significantly different (P<0.05) as determined by Duncan's Multiple Range Test (DMRT)

The zinc concentration in tobacco leaves (52.2-122.5 $mg \cdot kg^{-1}$) in our experiment was higher than the values (3.2-50 $mg \cdot kg^{-1}$) observed by Golia et al. (2009) [7] who reported significant negative correlations between Zn concentrations in Oriental tobacco leaves and soil pH. N rates had no significant influence on Zn concentration in leaves.

Concentration of Cu in mature leaves ranged from 15.7 to 21.2 $mg \cdot kg^{-1}$. Observed concentrations were similar to mean values for leaves at first and second priming reported by Golia et al. (2009) [7].

The lead concentrations in the leaves varied between 7.7 and 14.7 $mg \cdot kg^{-1}$, similar to the values reported by Zaprjanova and Hristozova (2018) [27], but higher than the ranges (0.02-8.56 $\mu g \cdot g^{-1}$) observed by Lazarević et al. (2012) [10].

Ni concentrations in mature leaves (0.53-2.60 $mg \cdot kg^{-1}$) were much lower than those found by Golia et al. (2009) [7] for oriental tobacco (20-88 $mg \cdot kg^{-1}$). The concentration of Ni in leaves was not affected by the N fertilization.

The concentration of Cd in mature leaves was from 0.93 to 5.17 $mg \cdot kg^{-1}$. The lower leaves had higher Cd concentration than the middle and upper leaves. King (1988) [9] has found similar discrepancies in the Cd concentration in the leaves as depending on the leaf stalk position. The observed cadmium concentrations in the leaves were within the ranges reported by Lugon-Moulin et al. (2004) [13] for Oriental tobacco samples from Bulgaria.

DISCUSSION

The established minimal changes in soil pH in treatments with application of N fertilizer (Table 1) may be due to the high buffering capacity in rendzina soil with its high carbonate content. The soil pH was lowest in the treatment with 100 kg $N \cdot ha^{-1}$ followed by 50 kg $N \cdot ha^{-1}$, and control, respectively. Miao et al. (2018) [15] reported that the soil pH for the entire 100 cm profile was reduced under the highest N treatment. Miner et al. (2018) [16] found that there was no effect of N rate on soil pH, indicating that moderate to heavy N fertilization did not impact soil acidity on high-pH, calcareous soil with high buffering capacity.

The fertilizer treatments show significant increase in soil humus and total N content when compared to the control plot (Table 1). According to Bundy (2003) [4] long-term N use increased soil organic C and N content and N availability, likely due to higher residue amounts and N concentrations. The higher amount of total soil N in 100% N treatment may be due to less uptake of nitrogen by plant due to the imbalance nutrients application in this treatment [5].

The concentration of mobile Fe, Mn and Zn increased with the increase in nitrogen fertilizer levels, but differences were very small (Table 2). Shi et al. (2010) [21] similarly found that the differences in DTPA extractable Fe, Mn, Cu and Zn between control and different N treatments are insignificant. Available soil micronutrients were not impacted by long-term nitrogen fertilization, despite widely differing rates of nutrient harvest and return [16]. On the other hand, the data of Schwab et al. (1990) [20] showed that the plant-available Fe concentration was significantly increased and concluded that the solubility and extractability of Fe would increase as pH decreased due to long-term fertilization with ammonium. The data from long-term experiment showed that in N treatments the pH decreased by 0.6% to 1.5% compared to the unfertilized control plot. The small variations in trace element concentrations in soil can be explained by the slightly decreased soil reaction after the application of different amounts of N fertilizer in the 50-year period.

The studied plant characteristics and cured leaf yield were enhanced when the N application was increased from 0 to 50 kg $N \cdot ha^{-1}$, but they were not increased significantly by increasing the rate from 50 to 100 kg $N \cdot ha^{-1}$ (Table 3). It is well known that plant N uptake largely depends on the soil moisture content. Except in extreme heat and drought conditions, avoiding irrigation is recommended to produce quality Oriental tobacco. In our experiment the plots were irrigated only when the available soil moisture was limiting tobacco growth. Under long-term trial conditions, water deficit could be factor, which reduces nitrogen uptake by tobacco and therefore, continuous fertilization of Oriental tobacco with 100 kg $N \cdot ha^{-1}$ is not an effective practice.

The rates of nitrogen fertilizer have a strong effect on the N concentration in the mature leaves (Table 4). The N concentration in the leaves (lower, middle and upper) was significantly lower in the N0 treatment than in the N50 and N100 treatments. Borges et al. (2012) [2] also have found a strong and positive linear relationship between tobacco leaf total N concentration and N fertilizer supply.

The concentrations of Fe had the higher values at lower leaves and the lowest values at upper leaves (Table 5). This is probably because of the low mobility of iron in plants - only a small part of it is in soluble form, while most of it is related to stable organic structures. The iron concentration in lower and middle leaves was not significantly influenced by N addition. The control treatment had the significantly higher Fe concentrations in the upper leaves as compared to the 100 kg $N \cdot ha^{-1}$ treatment, suggesting a dilution of Fe concentrations by leaf dry matter increase. Our findings oppose the results from Miner et al. (2018) [16], who reported that grain Fe increased in response to N fertilization, suggesting that Fe nutrition is improved with N fertilization.

In the present study, the concentrations of Mn in tobacco leaves were much smaller than the values reported by Golia et al. (2009) [7] for Oriental tobacco, grown on acid soils in Greece. The same authors found that soil pH is the dominant factor controlling tobacco metal uptake and the bioavailability of metals increased with decreasing soil pH. Therefore, the availability of Mn in tobacco plants tended to decrease when oriental tobacco was grown on alkaline soils conditions. No significant differences were noted on Mn concentration in tobacco leaves among treatments (Table 5). Our results differ from those of Miner et al. (2018) [16] who have found a clear synergistic effect of N on stover Mn probably due to the integration of increased root interception and uptake as well as higher transpiration rates.

Higher levels of zinc in tobacco leaves (Table 5) as compared to the values found by Golia et al. (2009) [7] can probably be explained by the high concentration of available Zn in the soil in our experiment. The available Zn concentration in the soils studied varied from 10.3 to 11.3 $mg \cdot kg^{-1}$. These values were much higher than the mean DTPA extractable Zn content of 0.50 $mg \cdot kg^{-1}$ observed by Golia et al. (2009) [7] for the soils in Greece, where Oriental tobacco is cultivated. In the current study, N fertilization did not change significantly the zinc concentration in tobacco. Our results differ from findings of Bruns and Ebelhar (2006) [3] who have found that Zn concentrations in ear leaves of maize increased significantly as N fertility rates increased.

The concentration of Cu in leaves was not affected by the application of different nitrogen rates (Table 5), and thus no relationship appeared between these elements. These results differ from the ones of Bruns and Ebelhar (2006) [3] who have found that the Cu concentrations in maize leaves were positively affected by N fertilization.

The lead concentration was not significantly influenced by N addition (P>0.05) (Table 6). Rui et al. (2009) [19] found that correlation between N fertilizer input and content of Pb in corn grain was negative. They assumed the influence of N fertilization on plants Pb content was likely dependent on nitrogen fertilizer used. According to Rodríguez-Ortíz et al. (2006) [18], increasing additions of ammonium nitrate to soil (50, 100, and 150 mg $N \cdot kg^{-1}$ soil) significantly increased aboveground Pb accumulation in tobacco during a 50-day experimental period, whereas increasing additions of urea to soil (50 and 100 mg $N \cdot kg^{-1}$ soil) did not show these effects at the same significance levels.

Golia et al. (2009) [7] reported that the concentrations of DTPA-Ni in the acid soils in Greece, where Oriental tobacco was grown, were between 0.88 and 4.2 $mg \cdot kg^{-1}$. Although these values are close to the concentration of mobile Ni in our plots (0.85-1.42 $mg \cdot kg^{-1}$), the differences in the nickel concentration of tobacco from our experiment and from Greece are very high. The comparison of data for leaf Ni concentrations in Oriental tobacco, grown on acid and alkaline soils confirm the conclusion by Golia et al. (2009) [7] that agronomic practices that reduce soil acidification may be helpful in minimizing the bioavailability of heavy metals in soil, thus resulting in reduced plant uptake and accumulation in plant tissues. In spite of the higher amounts of available nickel in the soil in the N50 and N100 treatments, the concentration of Ni in the leaves was not higher than in plots without fertilization (Table 6).

The long-term N fertilization had no significant effect on cadmium concentration in the leaves (P>0.05) (Table 6). These results differ from the findings of Li et al. (2011) [11], who found that wheat grain Cd concentration increased significantly with increasing N application rates at 11 of the 12 site-years. The Cd concentration in tobacco leaves from unfertilized plot was higher than that in the other treatments. According to Jarrell and Beverly (1981) [8], fertilization usually increases the yields and this can lead to a dilution of micronutrients if dry matter increases with yield faster than micronutrient uptake or accumulation.

Acknowledgments. The study has been carried out with the support of the Agricultural academy, Bulgaria, Project POZM-188.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

REFERENCES

[1] Ata-Ul-Karim, S.T., Cang, L., Wang, Y., Zhou, D., (2020): Interactions between nitrogen application and soil properties and their impacts on the transfer of cadmium from soil to wheat (*Triticum aestivum* L.) grain. Geoderma, 357: 113923.

[2] Borges, A., Morejón, R., Izquierdo, A., Monzón, L., Ortega, E., Rodés, R., (2012): Nitrogen fertilization for optimizing the quality and yield of shade grown Cuban cigar tobacco: Required nitrogen amounts, application schedules, adequate leaf nitrogen levels, and early season diagnostic tests. Beiträge zur Tabakforschung International, 25: 336-349.

[3] Bruns, H., Ebelhar, M., (2006): Nutrient uptake of maize affected by nitrogen and potassium fertility in a humid subtropical environment. Communications in Soil Science and Plant Analysis, 37: 275-293.

[4] Bundy, L., (2003): Long-term nitrogen fertilization effects on corn yields and soil properties. Proceeding of the Conference „Thirty-Third North Central Extension-Industry Soil Fertility". Published by Potash & Phosphate Institute, 19: 145-150.

[5] Chaudhury, J., Mandal, U.K., Sharma, K.L., Ghosh, H., Mandal, B., (2005): Assessing soil quality under long-term rice-based cropping system. Communications in Soil Science and Plant Analysis, 36: 1141-1161.

[6] Feil, B., Moser, S.B., Jampatong, S., Stamp, P., (2005): Mineral composition of the grains of tropical maize varieties as affected by pre-anthesis drought and rate of nitrogen fertilization. Crop Science, 45: 516-523.

[7] Golia, E.E., Dimirkou, A., Mitsios, I.K., (2009): Heavy-metal concentration in tobacco leaves in relation to their available soil fractions. Communications in Soil Science and Plant Analysis, 40: 106-120.

[8] Jarrell, W., Beverly, R., (1981): The dilution effect in plant nutrition studies. Advances in Agronomy, 34: 197-224.

[9] King, L.D., (1988): Effect of selected soil properties on cadmium content of tobacco. Journal of Environmental Quality, 17: 251-255.

[10] Lazarević, K., Nikolić, D., Stošić, L., Milutinović, S., Videnović, J., Bogdanović, D., (2012): Determination of lead and arsenic in tobacco and cigarettes: an important issue of public health. Central European journal of public health, 20: 62-66.

[11] Li, X., Ziadi, N., Bélanger, G., Cai, Z., Xu, H., (2011): Cadmium accumulation in wheat grain as affected by mineral N fertilizer and soil characteristics. Canadian Journal of Soil Science, 91: 521-531.

[12] Lindsay, W., Norvell, W., (1978): Development of DTPA soil test for zinc, iron, manganese and copper. Soil Science Society of America Journal, 42: 421-428.

[13] Lugon-Moulin, N., Zhang, M., Gadani, F., Rossi, L., Koller, D., Krauss, M., Wagner, G.J., (2004): Critical review of the science and options for reducing cadmium in tobacco (*Nicotiana tabacum* L.) and other plants. Advances in Agronomy, 83: 111-180.

[14] Marchetti, R., Castelli, F., Contillo, R., (2006): Nitrogen requirements for flue-cured tobacco. Agronomy journal, 98: 666-674.

[15] Miao, C., Chen, Z., Zhou, J., Han, J., Shi, Q., (2018): Effects of long-term cultivation practices and nitrogen fertilization rates on carbon stock in a calcareous soil on the Chinese Loess Plateau. Journal of Arid Land, 10: 129-139.

[16] Miner, G.L., Delgado, J.A., Ippolito, J.A., Barbarick, K.A., Stewart, C.E., Manter, D.K., Del Grosso, S.J., Halvorson, A.D., Floyd, B.A., D'Adamo, R.E., (2018): Influence of long-term nitrogen fertilization on crop and soil micronutrients in a no-till maize cropping system. Field Crops Research, 228: 170-182.

[17] Peedin, G.F., (1999): Production practices. Flue-cured tobacco. pp. 104-142. In: Davis, D.L., Nielsen, M.T. (eds.): Tobacco: production, chemistry, and technology. Blackwell Publishing Ltd., Oxford, UK.

[18] Rodríguez-Ortíz, J.C., Valdez-Cepeda, R.D., Lara-Mireles, J.L., Rodríguez-Fuentes, H., Vázquez-Alvarado, R.E., Magallanes-Quintanar, R., García-Hernández, J.L., (2006): Soil nitrogen fertilization effects on phytoextraction of cadmium and lead by tobacco (*Nicotiana tabacum* L.). Bioremediation Journal, 10: 105-114.

[19] Rui, Y.K., Zhang, F.S., Shen, J.B., (2009): Effects of nitrogen fertilization on heavy metal content of corn grains. Phyton-International Journal of Experimental Botany, 78: 101-104.

[20] Schwab, A.P., Owensby, C.E., Kulyingyong, S., (1990): Changes in soil chemical properties due to 40 years of fertilization. Soil Science, 149: 35-43.

[21] Shi, R., Zhang, Y., Chen, X., Sun, Q., Zhang, F., Römheld, V., Zou, C., (2010): Influence of long-term nitrogen fertilization on micronutrient density in grain of winter wheat (*Triticum aestivum* L.). Journal of Cereal Science, 51: 165-170.

[22] Shiwakoti, S., Zheljazkov, V.D., Gollany, H.T., Kleber, M., Xing, B., (2019): Micronutrients decline under long-term tillage and nitrogen fertilization. Scientific Reports, 9: 12020.

[23] Teoharov, M., (2004): Correlation of soils indicated map and classification of Bulgaria with world reference base (WRBSR, 2002). Soil Science, Agrochemistry and Ecology, 4: 3-13 [in Bulgarian].

[24] Tomov, T., Rachovsky, G., Kostadinova, S., Manolov, I., (1999): Manual for Agrochemistry. Agricultural University Plovdiv, Academic Press, 110 p. [in Bulgarian].

[25] Totev, T., Gribachev, P., Nechev, H., Artinova, N., (1987): Manual for Soil science. Zemizdat, Sofia, 112 p. [in Bulgarian].

[26] Wångstrand, H., Eriksson, J., Öborn, I., (2007): Cadmium concentration in winter wheat as affected by nitrogen fertilization. European Journal of Agronomy, 26: 209-214.

[27] Zaprjanova, P., Hristozova, G., (2018): Microelement content of oriental tobacco varieties grown under the same agro-ecological conditions. Agricultural sciences, 10: 41-47.

Received: October 21, 2020
Accepted: May 20, 2021
Published Online: May 22, 2021
Analele Universităţii din Oradea, Fascicula Biologie
http://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
University of Oradea Publishing House