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HEAVY METAL CONCENTRATIONS IN SOIL AND TOBACCO PLANTS FOLLOWING LONG-TERM PHOSPHORUS FERTILIZATION

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Abstract

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The effects of the different phosphorus rates (0; 75 and 225 kg ha^{-1}) on heavy metal concentrations in soil and tobacco plants have been studied in a stationary field trial. A long-term fertilizer experiment with continuous tobacco cropping system was established on rendzina soil (Rendzic Leptosols) in 1966. Although P fertilizers are considered a source of anthropogenic contamination of soil, the application of increasing P rates did not increase the total Cd, Pb and Cu content in the soil. Slightly higher Ni concentration was observed in plots receiving supplemental P fertilizer as compared to 0 kg P_2O_5 ha^{-1} treatment. The changes in the available Cd and Pb content as a result from the long-term P fertilization are insignificant. There was significant increase of available Ni and Cu in the plots treated with 225 kg P_2O_5 ha^{-1} compared to the 0 kg P_2O_5 ha^{-1} treatment. Increasing levels of P fertilization had no pronounced effect on Cd, Pb and Ni concentrations in leaves. Copper content in leaves decreased with the increase of P fertilizing levels. Therefore, the impact of phosphate fertilizer application on Cd, Pb, Ni and Cu content in soils and tobacco plants was proved to be limited. Under our experimental conditions, the risk of heavy metals' accumulation in soil and tobacco associated with long-term P fertilization was low.

Key words: cadmium, lead, nickel, copper, tobacco, phosphorus fertilization

Introduction

Maintaining an adequate N, P and K concentration in soils is necessary for improving yield and quality of tobacco. Tobacco farmers control the form, rate, time and methods of application of fertilizers. Concentrations of As, Cd and Pb in potassium and nitrogen fertilizers are low and are not regarded as significant contributors to their accumulation in soils (McBride and Spiers, 2001). The use of phosphate fertilizers is considered to be one of the primary factors in the pollution of agricultural soils (He and Singh, 1994). Phosphorus fertilizers, especially triple superphosphate, were found to have highest As, Cd, Cu, Cr, Ni, V, and Zn concentrations (Molina et al., 2009). Jiao et al. (2012) concluded that long-term use of phosphorus fertilizers could cause the As, Cd, and Pb contents of soils to rise if the products used contained high levels of these elements (for Cd, higher than 10 mg kg^{-1}). Cadmium impurities in superphosphate accumulated in the soil, mostly in the upper 10 cm, principally in a cation exchangeable form, and this increased the supply of available cadmium to plants (Williams and David, 1973). Although P fertilizers are considered a major anthropogenic source of soil pollution with Cd, Bogdanovic et al. (1999) have found that during the 30-year trial there was no significant increase of Cd in the plots treated with 50, 100 and 150 kg P_2O_5 ha^{-1} in comparison to the unfertilized plot. The data of Richards et al. (1998) also showed that there was no evidence of Cd enrichment of either soil or crop after 29 years of P applications. According to Martinovic et al. (1998) long-term fertilization with NPK fertilizers did not affect the content of total amounts of heavy metals (U, Pb, Cd, Ni and Cr) in the pseudogley soil.

Soil properties that affect the bioavailability of metals to plants include: total metal content in the soil; pH; clay and hydrous oxide content; organic matter and redox conditions (Reichman, 2002). Zaprjanova et al. (2010) reported that lead concentration in tobacco leaves and blossoms and cadmium concentration in the leaves increase linearly with the increase of the total element's content in the soil. Golia et al. (2009) have found significant positive correlations between DTPA extractable metals and Oriental tobacco leaves' heavy-metal

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content. The same authors concluded 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.

While soil factors have a large impact on the bioavailability of metals to plants, different species or varieties grown on the same soil can have different metal uptake (Miles and Parker, 1979).

This paper reports the results of the long-term phosphorus fertilization on the total and available Cd, Pb, Ni and Cu content in soil. The accumulation of the four metals in oriental tobacco plants is reported as well.

Materials and Methods

A long-term fertilizer experiment with continuous tobacco cropping system was established at Tobacco and Tobacco Products Institute - Markovo, Bulgaria on rendzina soil (Rendzic Leptosol) in 1966. The experimental design was a randomized complete block with three replicates. Oriental tobacco plants (*Nicotiana tabaccum* L. cv. Plovdiv 7) were grown in the stationary field. 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 ha^{-1}). Urea, triple superphosphate and potassium sulphate were used as sources of N, P and K. Soil samples from the following treatments: $N_{50}P_0K_{75}$, $N_{50}P_{75}K_{75}$ and $N_{50}P_{225}K_{75}$ were collected from the upper layer (0 - 25 cm) of each plot.

In 2012 and 2013, the effect of long-term fertilization on the available Cd, Pb, Ni and Cu content in soil and the accumulation of these elements in tobacco plants were studied. A solution of 0.005M DTPA + 0.1M TEA, pH 7.3 was used for extraction of the mobile forms of these elements from soils (ISO 14870:2001).

In 2013, the following soil characteristics were determined: pH in water, humus according to Tjurin (Totev et al., 1987) and total Cd, Pb, Ni and Cu content by using Aqua Regia ($HCl\text{-}HNO_3$, 3:1) extraction method (ISO 11466:1995).

The concentrations of Cd, Pb, Ni and Cu in mature leaves from different stalk position (lower, middle and upper leaves), stems and blossoms were determined. All samples were rinsed once with tap water to remove any adhering soil particles and further rinsed with distilled water. Afterwards they were dried at 75°C for 12 h and ground. The preparation of plant samples for analysis of Cd, Pb, Ni and Cu was done by means of dry ashing and dissolution in 3 M HCl. An atomic absorption spectrometer „Spektra AA 220" (Varian, Australia) was used for determination of heavy metal content in soil and plant samples.

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 and Discussion

The long-term application of P fertilizers resulted in small changes of soil pH and total humus content, where the magnitude of these changes did not depend on phosphorus fertilizer rates (Table 1). The effect of the long-term P fertilization on total soil contents of Cd, Pb, Ni and Cu is presented in Table 1.

The concentrations of metals after long term-treatment were found to decrease as follows: Cu > Ni > Pb > Cd. Total Cd content in soil from all treatments did not exceed the MAC (maximum allowed content) of 3.0 mg kg^{-1} (Ordinance № 3, 2008) for soils with $pH_{(H2O)} > 7.4$. In our study, the content of the Cd did not increase as a result of long-term application of increasing phosphorus rates. Cadmium was found to accumulate in soils from fertilizer applications if the amount of Cd added with fertilizer is greater than the amount of Cd removal, whether in harvested crop removal or other loss pathways such as leaching, erosion, or bioturbation (Grant and Sheppard, 2008). According to Richards et al. (1998) one possible explanation for the lack of significant treatment differences in soil Cd content could be the fact that Cd did not accumulate in the soil either because it was removed by crops or because it moved below 50 cm depth.

Total Pb content in all plots was above the average for the Bulgarian soils (39 mg kg^{-1}, referenced by Brashnarova, 1981),

Table 1
Soil reaction, humus and total Cd, Pb, Ni and Cu content in the soil as dependent on P fertilizing rate

Treatments	$pH_{(H2O)}$	Total humus, %	Total Cd, mg kg^{-1}	Total Pb, mg kg^{-1}	Total Ni, mg kg^{-1}	Total Cu, mg kg^{-1}
$N_{50}P_0K_{75}$	8.20	2.62	1.3	53.3	54.9	63.2
$N_{50}P_{75}K_{75}$	8.17	2.68	1.2	48.0	58.9	65.4
$N_{50}P_{225}K_{75}$	8.23	2.70	1.3	51.0	56.3	61.2
MAC*			3.0	120	150	300

* MAC - Maximum allowed content

but below the maximum allowed content of 120 mg kg^{-1}. Increasing levels of P fertility had no effect on Pb content in the soil. The results of Koteva and Stoyanov (1993) similarly demonstrate that long-term fertilization does not change the total content of Pb in soil, as related to the use of clean (produced from clean compounds) macro fertilizers.

Total Ni content in soil from all treatments did not exceed the maximum allowed concentration of 150 mg kg^{-1} for our country. Slightly lower Ni concentration was observed at the 0 kg P_2O_5 ha^{-1} treatment as compared to those receiving supplemental P fertilizer.

The total amount of Cu in soil from all treatments remained below MAC. No clear accumulation trend was recorded in plots receiving long-term P fertilizer applications. Wei et al. (2006) have also found that addition of P fertilizer had no significant effect on total Cu in soil.

The concentration of DTPA extractable metals in soil was in the following order: Pb > Cu> Ni > Cd (Table 2).

The available Cd content in the soils varied from 0.45 to 0.49 mg kg^{-1}. These values were higher than the mean DTPA-extractable Cd content of 0.10 mg kg^{-1} observed by Golia et al. (2009) for the soils where Oriental tobacco is cultivated. The changes in the available Cd content resulting from the long-term P fertilization are insignificant. The results published by Szalai et al. (2002) similarly demonstrate that different rates of NPK had no significant effect on the available Cd content in the 0-20 cm soil layer due to the use of P fertilizers with low cadmium content.

The available lead content in soil was 13.5 to 14.2 mg kg^{-1}. These values are higher than those reported by other authors (Szalai et al., 2002; Lehoczky et al., 2004). Increasing levels of P fertilization had no significant effect on available Pb content in the soil. Lehoczky et al. (2004) have also found that the different rates of NPK fertilizer treatments applied for 28 years have not resulted in significant differences in available Pb content in the experimental soils.

The levels of available concentration of Ni in our study were lower than mean values reported by Golia et al. (2009) for acid soils of central Greece where tobacco is traditionally cultivated. DTPA-Ni concentration was significantly increased with an increase in phosphorus fertilizer levels from 0 to 75 kg P_2O_5 ha^{-1}.

DTPA-Cu varied between 11.7 and 12.4 mg kg^{-1} and showed small, but significant changes between the 0 and 225 kg P_2O_5 ha^{-1} treatments. Szalai et al. (2002) have found that nutrient supply had no significant influence on the available Cu content of the soil. The levels of available concentration of Cu in the study were higher than those found by Golia et al. (2009).

Table 3 shows the heavy metals' concentrations in the stems, leaves and blossoms as dependent on P fertilizing rate, averaged over the period studied.

Tobacco can accumulate relatively high concentrations of Cd in its leaves. The variation in Cd concentration depends on various factors, such as agricultural practices, soil characteristics, climatic conditions, and plant varieties (Lugon-Moulin et al., 2004). The observed cadmium contents in the leaves were about or slightly higher from the ones determined by Tsotsolis et al. (2002) for oriental tobacco (0.5-3.0 mg kg^{-1}) and within the ranges reported by Lugon-Moulin et al. (2006) for Oriental tobacco samples from Bulgaria. The concentration of Cd in lower and middle leaves was not significantly affected by the P fertilization. Significant increase was observed in Cd concentration of upper leaves between 0 and 225 kg P_2O_5 ha^{-1} treatments. When comparing the Cd concentrations in leaves from different stalk positions (lower, middle and upper leaves) the higher concentration of the element in the lower leaves becomes apparent, together with its lowest concentration in the upper leaves. King (1988) similarly found that the cadmium concentration was at maximum in the lower leaves and decreased with leaf position up the stalk. Increasing levels of P fertilization had no pronounced effect on Cd content in stems. The blossoms' concentrations of Cd tended to increase as phosphorus fertilization increased from 0 to 225 kg P_2O_5 ha^{-1}.

The accumulation of Pb in tobacco organs occurred in the following order: leaves > blossoms > stems. The content of Pb in mature leaves was from 9.1 to 12.1 mg kg^{-1}. These values are similar to those reported by Tsotsolis et al. (2002) for oriental tobacco (3.1-10.5 mg kg^{-1}). Pb content was the highest in the leaves from the first priming (lower leaves). Similar obser-

Table 2
Content of available (DTPA method) heavy metals as dependent on P fertilizing rate (2-year average)

Treatments	Cd, mg kg^{-1}	Pb, mg kg^{-1}	Ni, mg kg^{-1}	Cu, mg kg^{-1}
$N_{50}P_0K_{75}$	0.47a	13.5a	0.61b	11.73b
$N_{50}P_{75}K_{75}$	0.45a	14.2a	0.85a	12.10ab
$N_{50}P_{225}K_{75}$	0.49a	14.0a	0.87a	12.43a

* - Different letters within each column indicate that the means are significantly different ($P<0.05$)

Table 3

Heavy metals concentrations of tobacco plants as dependent on P fertilizing rate (2-year average)

Treatments	Stems	Lower leaves	Middle leaves	Upper leaves	Blossoms
Cd concentration (mg kg^{-1} dry matter)					
$N_{50}P_0K_{75}$	1.1b	3.8a	2.9a	1.4b	1.2b
$N_{50}P_{75}K_{75}$	1.7a	3.7a	3.5a	1.8ab	1.4b
$N_{50}P_{225}K_{75}$	1.3ab	4.0a	3.2a	2.3a	2.2a
Pb concentration (mg kg^{-1} dry matter)					
$N_{50}P_0K_{75}$	1.0a	10.8a	10.4b	9.5a	1.6a
$N_{50}P_{75}K_{75}$	1.1a	12.1a	11.2a	9.1a	1.4a
$N_{50}P_{225}K_{75}$	1.2a	11.2a	9.9b	9.4a	1.4a
Ni concentration (mg kg^{-1} dry matter)					
$N_{50}P_0K_{75}$	0.1a	1.7b	1.4b	0.3a	0.1a
$N_{50}P_{75}K_{75}$	0.1a	1.6b	1.6ab	0.5a	0.1a
$N_{50}P_{225}K_{75}$	0.1a	2.3a	1.8a	0.5a	0.1a
Cu concentration (mg kg^{-1} dry matter)					
$N_{50}P_0K_{75}$	11.6a	28.1a	25.0a	16.6a	23.2a
$N_{50}P_{75}K_{75}$	12.2a	14.8b	16.7b	15.0ab	22.6a
$N_{50}P_{225}K_{75}$	12.0a	12.4b	13.8c	12.6b	22.2a

* - Different letters within each column indicate that the means are significantly different ($P<0.05$)

vations on the effect of the priming on the Pb concentration in tobacco leaves were recorded by Golia et al. (2007). The lead concentration in stems and blossoms, lower and upper leaves was not significantly influenced by P addition ($P > 0.05$).

Nickel concentration in mature leaves varied between 0.3 and 2.3 mg kg^{-1}. These values are lower than those reported by Golia et al. (2009). The same authors concluded that soil pH is the dominant factor controlling tobacco metal uptake and the bioavailability of metals increased with decreasing soil pH. Therefore, high soil pH values under our experimental conditions lead to the decreased availability of nickel in tobacco plants and observed values in leaves were much lower than those established by Golia et al. (2009). The concentration of Ni in stems, blossoms and upper leaves was not significantly affected by the application of different phosphorus rates. For lower leaves, 225 kg P_2O_5 ha^{-1} treatment resulted in highest Ni concentrations. There was no significant difference among plants supplied with 0 and 75 kg P_2O_5 ha^{-1}. Similar trend was observed for nickel content in the middle leaves.

The content of Cu in mature leaves was from 12.4 to 28.1 mg kg^{-1}. Observed values were similar or higher than the average concentrations reported by Golia et al. (2007), which for the oriental tobacco were 15.5, 10.6 and 9.9 mg kg^{-1} respectively for leaves at first, second and third priming. Copper concentration of the leaves decreased with increase of P fertilizing level. Copper concentrations were significantly ($p < 0.05$) greater in lower, middle and upper leaves that received no supplemental P fertilizer as compared to 225 kg P_2O_5 ha^{-1} treatment. According to Golia et al. (2009), to predict leaf Cu concentrations in Oriental tobacco, grown on acid soils of central Greece, the following equation can be used: $Y = 26.266x + 5.7437$, where x is DTPA-extractable copper in the soil. Based on this regression model, the predicted values in our study for Cu content in Oriental tobacco leaves (313.8; 323.6 and 332.2 mg kg^{-1} for 0; 75 and 225 kg P_2O_5 ha^{-1} treatment, respectively) are much higher than measured values, shown in Table 3. The comparison of our data with predicted values shows that under our experimental conditions, this linear regression equation based on DTPA-extracted copper in the soil was inaccurate to predict tobacco Cu concentrations. Therefore, for tobacco plants grown on alkaline soil with very high available Cu, copper concentration in leaves seems to depend on pH, organic matter content and other soil factors.

Conclusions

Although P fertilizers are considered a source of anthropogenic contamination of soil, the application of increasing P rates did not increase the total Cd, Pb and Cu content in the soil. Slightly higher Ni concentration was observed in plots receiving supplemental P fertilizer as compared to 0 kg P_2O_5 ha^{-1} treatment. The total concentrations of the studied metals did not exceed the maximum allowed content for our country. The changes in the available Cd and Pb content as a result from the long-term P fertilization were found to be insignificant. There was significant increase of available Ni and Cu in the plots treated with 225 kg P_2O_5 ha^{-1} as compared to the 0 kg P_2O_5 ha^{-1} treatment. Long-term fertilization had no significant effect on Pb, Ni and Cu concentrations in stems and blossoms. Increasing levels of P fertilization had no pronounced effect on Cd, Pb and Ni concentrations in leaves. Copper content of the leaves decreased with the increase of P fertilizing. Therefore, the impact of phosphate fertilizer application on Cd, Pb, Ni and Cu content in soils and tobacco plants is limited. Under our experimental conditions, the risk of heavy metals' accumulation in soils and tobacco associated with long-term P fertilization was low.

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