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Accumulation of heavy metals in soil and tobacco after long-term mineral and organic-mineral fertilization

Съдържание на тежки метали в почва и тютюн след продължително минерално и органоминерално торене

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Abstract

The effect of long-term mineral and organic-mineral fertilization on accumulation of heavy metals in soil and plants was studied in stationary field experiment with continuous tobacco cropping system. Five treatments were selected in this study, including CK (control without fertilization), NP (nitrogen+phosphorus), NK (nitrogen+potassium), NPK (nitrogen+phosphorus+potassium) and NPK+FYM (nitrogen+phosphorus+potassium+manure). Total and available Pb, Cd, Ni, Mn, Zn and Cu content in soil, as well as concentration of the elements in tobacco leaves were determined. The application of mineral fertilizers did not significantly increase the total Pb, Cd, Ni, Zn and Cu content in the soil as compared to unfertilized control. The addition of manure (NPK+FYM treatment) decreased total Ni. There was significant increase of available Ni and Mn in treatment receiving NPK+FYM. A comparison of unfertilized treatment indicated that long-term mineral fertilization had no significant effect on studied metals in oriental tobacco leaves. A considerable decrease in Cd (2.2-2.8 times), Zn (1.7-3.3 times) and Cu (2.2-3.8 times) concentrations in the leaves compared with control without fertilization was detected due to long-term NPK+FYM fertilization. This study demonstrated that the risk of heavy metals' accumulation in tobacco leaves associated with long-term mineral and organic-mineral fertilization was low.

Keywords: heavy metals, long-term fertilization, soil, tobacco

Резюме

В условия на стационарен полски опит с непрекъснато монокултурно отглеждане на ориенталски тютюн е проучен ефектът от продължителното минерално и органо-минерално торене върху натрупването на тежки метали в почвата и растенията. В проучването са подбрани следните варианти: неторена контрола, NP (азот+фосфор), NK (азот+калий), NPK (азот+фосфор+калий) и NPK+FYM (азот+фосфор+калий+оборски тор). Определени са общите и подвижните форми на Pb, Cd, Ni, Mn, Zn и Cu в почвата, както и концентрацията на тези елементи в тютюневите листа. Прилагането на минерални торове не увеличава доказано общото съдържание на Pb, Cd, Ni, Zn и Cu в почвата в сравнение с неторената контрола. Органоминералното торене понижава общия Ni в почвата. Значимо е увеличението на подвижните форми на Ni и Mn при този вариант. Продължителното минерално торене е без доказан ефект върху съдържанието на Pb, Cd, Ni, Mn, Zn и Cu в листата на ориенталския тютюн. Системното органо-минерално торене понижава значително концентрацията на Cd (2,2-2,8 пъти), Zn (1,7-3,3 пъти) и Cu (2,2-3,8 пъти) в листата в сравнение с неторената контрола. Това проучване показва, че рискът от натрупване на тежки метали в тютюневите листа от системното минерално и органо-минерално торене е нисък.

Ключови думи: почва, продължително торене, тежки метали, тютюн

Подробно резюме

Торенето с минерални и органични торове е важно мероприятие за увеличаване продуктивността и качеството на тютюна. Многобройни са въпросите относно влиянието на продължителното системно торене върху свойствата на почвата и акумулирането на тежките метали в растенията.

В настоящото проучване се проследява влиянието на продължителното минерално и органо-минерално торене върху натрупването на някои тежки метали (Pb, Cd, Ni, Mn, Zn и Cu) в почвата и в листата на ориенталскя тютюн.

Изследванията са проведени върху стационарен полски опит със системно торене и непрекъсната монокултура тютюн. Заложен е върху Хумусно-карбонатна почва в Института по тютюна и тютюневите изделия в с. Марково през 1966 г.

В проучването са подбрани следните варианти: неторена контрола, NP (азот+фосфор), NK (азот+калий), NPK (азот+фосфор+калий) и NPK+FYM (азот+фосфор+калий+оборски тор). Почвени проби от слоя 0-25 cm са анализирани за: pH във вода - потенциометрично, общ хумус – по Тюрин (Totev et al., 1987). Определени са общите форми на Pb, Cd, Ni, Mn, Zn и Cu - след разлагане на почвата с царска вода (ISO 11466:1995) и подвижните форми на същите елементи – в извлек с 0,005 M DTPA+0,1 M TEA, pH 7,3 (ISO 14870:2001). За растителен анализ са използвани технически зрели листа от долен, среден и горен беритбен пояс. Съдържанието на тежките метали в тютюна е определено след сухо изгаряне на растителния материал и разтваряне на пепелта в 3 M HCl. За отчитане концентрацията на елементите в почвените и растителните проби е използван атомен абсорбционен спектрометър.

Установено е, че прилагането на минерални торове не увеличава доказано общото съдържание на Pb, Cd, Ni, Zn и Cu в почвата в сравнение с неторената контрола. Органо-минералното торене понижава общия Ni в почвата. Значимо е увеличението на подвижните форми на Ni и Mn при този вариант.

Продължителното минерално торене е без доказан ефект върху съдържанието на Pb, Cd, Ni, Mn, Zn и Cu в листата на ориенталския тютюн. Продължителното органо-минерално торене понижава значително концентрацията на Cd (2,2-2,8 пъти), Zn (1,7-3,3 пъти) и Cu (2,2-3,8 пъти) в листата в сравнение с неторената контрола. Това проучване показва, че рискът от натрупване на тежки метали в тютюневите листа от продължителното минерално и органо-минерално торене е нисък.

Introduction

Fertilization is an important man-controlled factor for producing high quality tobacco. Mineral fertilizers and manure are added to soils to provide adequate N, P, and K for crop growth and to increase tobacco yield. There are numerous uncertainties about the impact of long-term fertilization on soil properties and accumulation of heavy metals in plants. According to Molina et al. (2009) the trace element concentration was very low in N, K, and NK fertilizers, but phosphorus fertilizers, especially triple superphosphate, carried significant As, Cd, Cu, Cr, Ni, V, and Zn concentrations. The addition of manure or P fertilizer increased total Zn, Fe, and Mn in soil, but had no significant effect on total Cu (Wei et al., 2006). The data of Raven et al. (1998) also showed that application of certain phosphate fertilizers inadvertently adds Cd and other potentially toxic elements to the soil. On the other hand, Richards et al. (1998) did not find Cd enrichment of either soil or crop after 29 years of P applications. Zhao et al. (2014) concluded that manure application led to accumulation of total Cd in the soil in corn seed production, which could become a risk for the safety of seed production in calcareous soils in Northwest China.

The bioavailability of metals to plants depends on total and DTPA-extractable metals' content in soils, pH, clay and hydrous oxide content, organic matter and redox conditions (Reichman, 2002; Golia et al., 2009).

This paper reports the results of the long-term mineral and organic-mineral fertilization on the total and available Pb, Cd, Ni, Mn, Zn and Cu content in soil. The accumulation of these metals in oriental tobacco leaves is reported as well.

Materials and methods

Three years data (2014-2016) were obtained from a trial with long-term fertilization. Stationary experiment with continuous tobacco cropping system was established in 1966 at Tobacco and Tobacco Products Institute – Markovo, Bulgaria (42°06' N and 24°70' E). The growing season for tobacco at this location is often characterized by warm and dry summers. The soil was classified as Rendzic Leptosols (World Reference Base for Soil Resources) (Teoharov, 2004). The experimental design was a randomized complete block replicated three times. 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*2.5 m). Tobacco seedlings were transplanted at a 0.5*0.12 m distance (166 000 plants*ha^{-1}). Five treatments were selected in this study, including CK (control without fertilization), NP (nitrogen + phosphorus), NK (nitrogen + potassium), NPK (nitrogen + phosphorus + potassium) and NPK+FYM (nitrogen + phosphorus + potassium + manure).

The fertilizer application rates of the treatments are shown in Table 1.

Table 1. Treatments and annual fertilizer application rates (kg*ha^{-1})

Таблица 1. Варианти и годишни норми на торене (kg*ha^{-1})

Fertilizer Тор	CK Неторена контрола	NP	N	NPK	NPK+FYM NPK+оборски тор
Nitrogen Азот (N)	-	50	50	50	50
Phosphorus Фосфор (P_2O_5)	-	75	-	75	75
Potassium Калий (K_2O)	-	-	75	75	75
Manure Оборски тор	-	-	-	-	20,000

Urea, triple superphosphate and potassium sulphate were used as sources of N, P and K. Manure came from cattle. Fertilizers were broadcast before transplanting and then incorporated in the topsoil layer.

At the beginning of the experiment, the soil had a pH value of 8.5 and contained 3.01% humus and 15 mg P_2O_5*kg^{-1} soil (Vartanyan, 1979).

In March every year (2014, 2015 and 2016) soil samples were collected at a depth of 0–25 cm from all studied plots. The following soil characteristics were determined: pH in water, humus according to Tjurin (Totev et al., 1987), total Pb, Cd, Ni, Mn, Zn and Cu content by using Aqua Regia (HCl-HNO_3, 3:1) extraction method (ISO 11466:1995). A solution of 0.005 M DTPA+0.1 M TEA, pH 7.3 was used for extraction of the mobile forms of these elements from soils (ISO 14870:2001).

The concentrations of Pb, Cd, Ni, Mn, Zn and Cu in mature tobacco leaves from different stalk position (lower, middle and upper) 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 metals was done by means of dry ashing and dissolution in 3 M HCl. An atomic absorption spectrometer „SpektrAA 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 data from long-term experiment showed that by comparison with the control, soil $pH_{(H2O)}$ values were unaffected by the addition of inorganic fertilizers (Table 2). Organic-mineral treatment significantly decreased ($P<0.05$) the pH in the top layer. Similar results were obtained by Šimek et al. (1999) who concluded that in the absence of lime, the effects of the manure plus inorganic fertilizers were to reduce soil pH. According to Wei et al. (2006) the decrease in soil pH in the NP+manure treatments might have resulted from the release of organic acids and CO_2 into the soil during the decomposition of the manure.

In this study, different treatments significantly influenced the humus content compared to CK (Table 2). The level of humus was lower in the control plot than in soils under continuous fertilization. The combined organic and mineral fertilizer treatment resulted in highest humus increase. Šimek et al. (1999) concluded that the increases in soil C content were probably due to the combined effects of C addition in the manure and increased plant productivity as a result of combined manure and inorganic fertilizer additions.

The effect of the long-term fertilization on total soil contents of Pb, Cd, Ni, Mn, Zn and Cu is presented in Table 2.

Total Pb content in all plots was below the MAC (maximum allowed content) of 120 $mg*kg^{-1}$ (Ordinance № 3, 2008) for soils with $pH_{(H2O)}>7.4$. No significant differences were identified between control plot and treatments with continuous fertilization. Slightly higher Pb concentration was observed at the NPK+FYM treatment as compared to those receiving only mineral fertilizers. Results from this study differ from the findings of Atafar et al. (2010), who reported that total Cd and Pb concentrations increased in the cultivated soils due to fertilizer application.

Total Cd content in soil from all treatments did not exceed the maximum allowed content of 3 $mg*kg^{-1}$. The long-term fertilization had no recordable influence on the total amount of Cd in soil as compared to the control variant. Richards et al. (1998) have also found no significant treatment differences in total soil Cd and explained that Cd did not accumulate in the soil either because it was removed in crops or because it moved to below 50 cm depth.

The total Ni content of the experimental soils varied between 57.5-70.5 $mg*kg^{-1}$ and did not exceed the maximum allowed concentration of 150 $mg*kg^{-1}$. Significantly lower Ni concentration was observed at the organic-mineral treatment as compared to control plot.

Table 2. Soil reaction, humus and total Pb, Cd, Ni, Mn, Zn and Cu content in the soil (3-year average)

Таблица 2. Реакция на почвата, хумус и съдържание на общите форми на Pb, Cd, Ni, Mn, Zn и Cu в почвата (средно за 3 години)

Treatments Варианти	$pH_{(H2O)}$	Total humus Общ хумус (%)	Total forms Общо съдържание ($mg*kg^{-1}$)					
			Pb	Cd	Ni	Mn	Zn	Cu
CK Неторена контрола	8.17^{b*}	2.48^{d}	58.9^{a}	1.3^{a}	70.5^{a}	473^{b}	148.1^{ab}	74.1^{a}
NP	8.13^{b}	2.73^{bc}	57.6^{a}	1.2^{a}	68.6^{a}	546.5^{a}	157.6^{a}	69.6^{a}
NK	8.18^{b}	2.64^{cd}	57.3^{a}	1.1^{a}	68.5^{a}	565.8^{a}	147.4^{ab}	72.3^{a}
NPK	8.14^{b}	2.86^{b}	54.3^{a}	1.1^{a}	65.5^{ab}	470.7^{b}	139.6^{b}	71.3^{a}
NPK+FYM NPK+оборски тор	7.91^{a}	4.38^{a}	61.3^{a}	1.2^{a}	57.5^{b}	528.6^{ab}	152.1^{ab}	64.4^{a}
MAC** МДК			120	3	150	-	400	300

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

* Различните букви в една и съща колона показват, че средните са съществено различни ($P<0.05$)

** MAC Maximum allowed content (approved for Bulgaria)

** МДК Максимално допустими концентрации (установени за България)

Soil total Mn concentration significantly increased due to NP and NK treatments, but no significant difference was determined between control variant and combined fertilizer application (NPK+FYM).

The total Zn content in soil under different treatments varied from 139.6 to 157.6 $mg*kg^{-1}$ and was considerably lower than the accepted Bulgarian maximum allowed content of 400 $mg*kg^{-1}$. The plots receiving NP and NPK+FYM had a higher concentration of total Zn than the control treatment. Czarnecki and Düring (2015) reported that addition of fertilizer at all rates increased the soil total Zn level when compared to control plots.

The long-term fertilization had no significant influence on the total Cu in the soil. Results from this study agree with the findings of Wei et al. (2006), who reported that the addition of manure or P fertilizer had no significant effect on total Cu.

Mobile forms of Pb, Cd, Ni, Mn, Zn and Cu in soil from the examined treatments were further studied (data is presented in Table 3).

Table 3. Content of available (DTPA method) heavy metals as dependent on long-term fertilization (3-year average)

Таблица 3. Съдържание на подвижни форми (DTPA метод) на тежки метали в почвата в зависимост от продължителното торене (средно за 3 години)

Treatments Варианти	Pb ($mg*kg^{-1}$)	Cd ($mg*kg^{-1}$)	Ni ($mg*kg^{-1}$)	Mn ($mg*kg^{-1}$)	Zn ($mg*kg^{-1}$)	Cu ($mg*kg^{-1}$)
CK Неторена контрола	18.8[a*]	0.61[b]	0.85[c]	31.9[b]	10.3[a]	14.7[a]
NP	18.3[a]	0.56[b]	0.81[c]	35.3[b]	10.2[a]	16.8[a]
NK	20.7[a]	0.56[b]	1.48[b]	32.9[b]	10.7[a]	16.9[a]
NPK	21.4[a]	0.78[a]	1.12[bc]	39.9[b]	10.6[a]	13.7[a]
NPK+FYM NPK+оборски тор	18.4[a]	0.51[b]	2.01[a]	56.6[a]	14.7[a]	13.1[a]

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

* Различните букви в една и съща колона показват, че средните са съществено различни ($P<0.05$)

No significant differences were determined in available Pb content between control plot and fertilizer treatments. Szalai et al. (2002) similarly found that the differences in available lead content between control and different treatments are insignificant. Nonetheless, in this study the levels of available concentration of Pb (18.3-21.4 $mg*kg^{-1}$) were higher than values reported by other authors (Szalai et al., 2002; Lehoczky et al., 2004).

Mobile cadmium concentration in the studied treatments was in the range of 0.51-0.78 $mg*kg^{-1}$. The long-term fertilization with NP, NK and NPK+FYM had no significant influence on available Cd content as compared to the control treatment. Plots fertilized with NPK exhibited significant increases of available cadmium. Therefore it is difficult to make any conclusions about the effect of continuous fertilization on the amount of available cadmium in the soil. The results published by Kurakov et al. (2006) and Lehoczky et al. (2004) demonstrate that continuous fertilization does not affect the amount of available Cd in the 0-20 cm soil layer. Although the soil was acidified slightly by NPK+FYM treatment (0.26 pH unit reduction) compared to the CK variant, the extent of acidification did not have an effect on soil available Cd.

DTPA-Ni varied between 0.81 and 2.01 mg*kg^{-1}. The levels of available concentration of Ni were similar to those found by Golia et al. (2009) for the soils where Oriental tobacco is cultivated. No clear accumulation trend was recorded in plots receiving long-term mineral fertilizer applications. The highest amount was found in the NPK+FYM plot. The data of Uprety et al. (2009) also showed that the plant-available Ni concentration in arable layer was enhanced by long-term use of farmyard manure, combined with mineral fertilizer.

The available Mn content in soil was 31.9 to 56.6 mg*kg^{-1}. The plot receiving NPK+FYM had higher concentration of available Mn than the other treatments. According to Moharana et al. (2017) the lower pH may in the continuous FYM treatment have resulted in the release of previously non-available Mn from soil minerals. The same authors suggested that the decomposition of organic matter would have provided protons to the soil solution and also decreased soil Eh values and these changes could have resulted in the dissolution and reduction of Mn, thus increasing its availability.

The available Zn content in the soil varied from 10.2 to 14.7 mg*kg^{-1}. These values were higher than the mean DTPA extractable Zn content of 0.5 mg*kg^{-1} observed by Golia et al. (2009) for the soils where Oriental tobacco is cultivated. Applications of NP, NK and NPK fertilizers did not increase the available Zn content in the soils compared to non-fertilized treatment. More noticeable increase of DTPA-Zn was observed under plot receiving NPK+FYM fertilizer over unfertilized control. According to Wei et al. (2006) manure not only supplies large amounts of Zn to the soil, but also promotes biological and chemical reactions that result in the dissolution of otherwise non-available Zn.

There was no significant accumulation of DTPA-Cu in plots after long-term fertilization compared to the CK treatment. Moharana et al. (2017) reported similar findings. According to Wei et al. (2006), there are two possible explanations for this observation: either the fertilizers used contained very little Cu or the application of manure increased the amount of chelating agents in soil. In this case, Cu could become bound to organic matter and relatively unavailable to plants.

Tables 4, 5 and 6 show the heavy metals' concentrations in leaves from different stalk positions as dependent on long-term fertilization, averaged over the period studied.

The content of Pb in mature leaves was from 7.7 to 16 mg*kg^{-1} (Table 4). These values are similar or higher than those reported by Golia et al. (2007) for oriental tobacco (0.01-14.4 mg*kg^{-1}). The lead concentration in leaves did not significantly change among the different treatments ($P>0.05$).

The observed cadmium contents in the leaves (0.8-5.17 mg*kg^{-1}) (Table 4) were within the ranges reported by Lugon-Moulin et al. (2006) for Oriental tobacco samples (0-6.78 µg*g^{-1}). The Cd content in tobacco leaves from NPK+FYM plot was much lower than that from the other treatments.

Table 4. Heavy metals (Pb and Cd) concentrations of tobacco leaves (3-year average)

Таблица 4. Концентрация на Pb и Cd в листата на тютюна (средно за 3 години)

Treatments Варианти	Lower leaves Долни листа	Middle leaves Средни листа	Upper leaves Горни листа	Average Средно
		Pb concentration Концентрация на Pb (mg*kg^{-1} dry matter)		
CK Неторена контрола	7.7^{a*}	13^{a}	11.3^{a}	10.7^{a}
NP	9^{a}	14.3^{a}	11^{a}	11.5^{a}
NK	8.7^{a}	16^{a}	13^{a}	12.5^{a}
NPK	8^{a}	12^{a}	13^{a}	11^{a}
NPK+FYM NPK+оборски тор	10.3^{a}	13.3^{a}	11^{a}	11.5^{a}
		Cd concentration Концентрация на Cd (mg*kg^{-1} dry matter)		
CK Неторена контрола	5.17^{a}	3.03^{a}	1.7^{a}	3.3^{a}
NP	4.77^{ab}	3.07^{a}	1.23^{a}	3.03^{ab}
NK	3.47^{ab}	2.6^{a}	1.37^{a}	2.47^{ab}
NPK	3.1^{ab}	2.57^{a}	1.03^{a}	2.2^{ab}
NPK+FYM NPK+оборски тор	1.83^{b}	1.37^{a}	0.8^{a}	1.33^{b}

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

* Различните букви в една и съща колона показват, че средните са съществено различни (P<0.05)

Table 5. Heavy metals (Ni and Mn) concentrations of tobacco leaves (3-year average)

Таблица 5. Концентрация на Ni и Mn в листата на тютюна (средно за 3 години)

Treatments Варианти	Lower leaves Долни листа	Middle leaves Средни листа	Upper leaves Горни листа	Average Средно
		Ni concentration Концентрация на Ni (mg*kg^{-1} dry matter)		
CK Неторена контрола	2.6^{a}	1.4^{a}	1.6^{a}	1.87^{a}
NP	1.57^{a}	1.57^{a}	0.73^{a}	1.27^{a}
NK	1.2^{a}	1.47^{a}	0.43^{a}	1.03^{a}
NPK	2.4^{a}	1.23^{a}	0.77^{a}	1.47^{a}
NPK+FYM NPK+оборски тор	1.5^{a}	1.37^{a}	0.83^{a}	1.27^{a}
		Mn concentration Концентрация на Mn (mg*kg^{-1} dry matter)		
CK Неторена контрола	66.7^{a*}	53.3^{a}	46.6^{a}	55.5^{a}
NP	69.4^{a}	56.2^{a}	45.9^{a}	57.2^{a}
NK	59.1^{a}	52.7^{a}	45.6^{a}	52.5^{a}
NPK	55.3^{a}	52.9^{a}	46.6^{a}	51.6^{a}
NPK+FYM NPK+оборски тор	55.2^{a}	55.7^{a}	52.7^{a}	54.5^{a}

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

* Различните букви в една и съща колона показват, че средните са съществено различни (P<0.05)

Wang et al. (2014) reported that the higher soil organic C in the organic compost treatment caused more Cd adsorption in the soil, and consequently reduced the uptake of Cd by crops. The same authors concluded that the accumulations of Cd in crops were also reduced by its precipitation with phosphate in the soil. In a previous study, Bozhinova and Zapryanova (2007) have found that long-term mineral phosphorus fertilization increased 3-4 times mobile phosphorus in the soil compared to the unfertilized control. The accumulation of the mobile phosphorus was even higher (increased about 11 times compared to the control) when plot received NPK+FYM. Therefore, high levels of humus and available phosphorus in the plot receiving NPK+FYM could be important factors, which reduced Cd uptake by tobacco.

Ni concentration in mature leaves varied between 0.43 and 2.6 mg*kg^{-1} (Table 5). These values are lower than those found by Sebiawu et al. (2014) and similar to those reported by Stojanović et al. (2004). The long-term fertilization had no significant effect on nickel concentration in the leaves ($P>0.05$). According to Golia et al. (2009), significant positive correlations were observed between DTPA extractable metals and Oriental tobacco leaves' heavy-metal content and DTPA extraction supplies a reliable estimation of the plant availability of the metals studied in acid soils of central Greece. Results from this study showed non-significant relationship between leaf Ni concentrations and DTPA-Ni levels (correlation coefficients were -0.337, -0.333 and -0.192 for lower, middle and upper leaves, respectively). Consequently, it appeared that other factors must be influencing Ni uptake and its accumulation in the leaf.

The observed Mn values (45.6-69.4 mg*kg^{-1}) (Table 5) are similar to those reported by Apostolova (1985) for tobacco grown on alkaline soil and much lower than those established by Golia et al. (2009) for Oriental tobacco, grown on acid soils. Soil pH is the dominant factor controlling tobacco metal uptake and the bioavailability of metals increased with decreasing soil pH (Golia et al., 2009). Therefore, high soil pH values in the study lead to the decreased availability of Mn in tobacco plants and observed values in leaves were much lower than those determined by Golia et al. (2009). The amount of Mn in leaves was not significantly affected by the fertilization. Moharana et al. (2017) also concluded that the differences of wheat grain and straw Mn concentrations with different treatments were not significant. In spite of the higher amounts of available manganese in the soil in the NPK+FYM treatment, the content of this element in the lower and middle leaves is not higher than in the other treatments. The results of this study indicate that DTPA-Mn has not a significant impact on manganese concentration in tobacco leaves (correlation coefficients were -0.623, -0.030 and 0.047 for lower, middle and upper leaves, respectively. Evidently, Mn DTPA-extractable concentrations can not always be used as a predictor of Mn content in tobacco leaves. These results differ from the ones of Golia et al. (2009) who have found significant positive correlations between DTPA-Mn and concentration of Mn in Oriental tobacco leaves.

The zinc content in leaves from CK, NP, NK and NPK treatments ranged from 48.5 to 122.5 mg*kg^{-1} (Table 6). These values are higher than the average values reported by Golia et al. (2007), which for the oriental tobacco leaves at first, second and third priming was 48.1, 42.9 and 37 mg*kg^{-1} respectively.

Table 6. Heavy metals (Zn and Cu) concentrations of tobacco leaves (3-year average)

Таблица 6. Концентрация на Zn и Cu в листата на тютюна (средно за 3 години)

Treatments Варианти	Lower leaves Долни листа	Middle leaves Средни листа	Upper leaves Горни листа	Average Средно
	Zn concentration Концентрация на Zn ($mg*kg^{-1}$ dry matter)			
CK Неторена контрола	122.5^{a}	87.7^{a}	55.3^{a}	88.5^{a}
NP	84.7^{ab}	68^{a}	56^{a}	69.5^{ab}
NK	94.9^{ab}	74.5^{a}	52.7^{a}	74.1^{a}
NPK	77^{ab}	71.4^{a}	48.5^{a}	65.6^{ab}
NPK+FYM NPK+оборски тор	37.3^{b}	37.8^{b}	33.4^{a}	36.2^{b}
	Cu concentration Концентрация на Cu ($mg*kg^{-1}$ dry matter)			
CK Неторена контрола	18.2^{a}	19^{a}	15.7^{a}	17.6^{a}
NP	18.5^{a}	19.9^{a}	14.2^{a}	17.5^{a}
NK	17^{a}	20.1^{a}	16.5^{a}	17.9^{a}
NPK	12.3^{ab}	13.7^{ab}	12.5^{ab}	12.8^{ab}
NPK+FYM NPK+оборски тор	6.4^{b}	5^{b}	7.3^{b}	6.2^{b}

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

* Различните букви в една и съща колона показват, че средните са съществено различни (P<0.05)

High levels of zinc in leaves in the present experiment can probably be explained by the high content of available Zn in the soil. The zinc concentration in the tobacco leaves was the lowest in NPK+FYM treatment (33.4-37.8 mg*kg^{-1}), although the mobile zinc content in the soil was the highest in this treatment. This can be explained by the Zn uptake by plants depending not only on the levels of soil available zinc. Phosphorus fertilization causes a reduction of Zn concentrations in plants, which is possibly due to P-Zn interactions in soil (Lambert et al., 2007). Therefore, high content of available P_2O_5 in the soil in NPK+FYM treatment could be important factor, which reduced Zn uptake by tobacco.

The content of Cu in leaves was from 5 to 20.1 mg*kg^{-1} (Table 6). These values were similar or higher than the average concentrations for the oriental tobacco (9.9-15.5 mg*kg^{-1}) reported by Golia et al. (2007). The treatment receiving NPK+FYM had the lowest concentrations of Cu in the leaves. One possible explanation for this contradiction could be found in the high content of available phosphorus in these plots. According to Touchton et al. (1980) Cu concentrations in the wheat tissue generally decreased as applied and residual soil P levels increased. The long-term mineral fertilization had no significant influence on the Cu concentration in tobacco leaves.

Conclusions

The application of mineral fertilizers did not significantly increase the total Pb, Cd, Ni, Zn and Cu content in the soil as compared to unfertilized control. The addition of manure (NPK+FYM treatment) decreased total Ni, but had no significant effect on total Pb, Cd, Mn, Zn and Cu content in soil. The total concentrations of the studied metals did not exceed the maximum allowed in Bulgaria content. Available Pb, Zn and Cu in the soil were not significantly influenced by long-term fertilization. There was significant increase of available Ni and Mn in treatments receiving NPK+FYM.

A comparison of unfertilized treatment indicated that long-term mineral fertilization had no significant effect on studied metals (Pb, Cd, Ni, Mn, Zn and Cu) in oriental tobacco leaves. A remarkable decrease in Cd (2.2-2.8 times), Zn (1.7-3.3 times) and Cu (2.2-3.8 times) concentrations in the leaves compared with control without fertilization was detected due to long-term NPK+FYM fertilization.

This study demonstrated that the risk of heavy metals' accumulation in tobacco leaves associated with long-term mineral and organic-mineral fertilization is low.

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