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Pb AND Cd CONTENT IN SOIL AND TOBACCO AFTER 40 YEARS OF MINERAL AND COMBINED FERTILIZING

Radka Bozhinova, Penka Zaprjanova
Tobacco and Tobacco products Institute, Plovdiv
R. Bulgaria

INTRODUCTION

Intensification of the agriculture leads to accumulation of elements carrying risks for human health into the soil and plants. Therefore the development of ecologically safe mineral and organic fertilizing thresholds is particularly important in the case of systematic fertilization (Kurakov et al., 2006). The main negative effect of the heavy metals on the soil fertility is expressed as denaturation of metabolically important proteins, converting the phosphorus into insoluble forms and a competition in the uptake with the essential nutrition elements (Shkolnik, 1974; Yagodin and Kidin, 1996). Basic compounds used for producing P and K containing fertilizers are a major source for contaminating soils with ecologically hazardous microelements. The concentration of these elements could rise as well from the long-term use of organic fertilizers and plant protection compounds (Stoyanov et al., 1997). Phosphorous fertilizers that are used in tobacco growing contain heavy metals in relatively high concentrations, therefore making their use an important contributor for polluting agricultural soils (Golia et al., 2005). According to Kurakov et al. (2006) the concentrations of heavy metals in P and organic fertilizers are high, while N and K fertilizers are virtually clean from such contaminants.

Long-term fertilizing experiments could serve as reliable source of information on the ecological effects from the application of different fertilizing practices (Koteva and Stoyanov, 1993).

The aim of the present study was to investigate the effect of the long-term fertilizing with different amounts and ratios of mineral fertilizers and the application of combined (organic + mineral) fertilizing on the total and available Pb and Cd contents in the upper soil layer (0-25 cm). The accumulation of these metals in tobacco plants was followed as well.

MATERIAL AND METHODS

The studies were done at the long-term fertilizing experiment site that was established in 1966. In 2006 the effect of the 40-years systematic fertilizing on the total and available Pb and Cd contents in the soil and the accumulation of these elements in tobacco plants was studied. The long-term trial was established on rendzina soil with following characteristics: $pH_{(H2O)}$ - 8.5; humus - 3.01 %, clay - 47.3% (Vartanyan et al., 1968).

From the year of the site setup N, P and K (alone or in combination) were applied yearly in the following quantities: N_0, $N_{2.5}$, N_5, N_{10}; P_0, $P_{7.5}$, P_{10}, K_0 and $K_{7.5}$. Using full mineral nutrition the effect of the organic fertilizing is tested at 20 t/ha. The above fertilizers are applied once per vegetation (with the last spring cultivation) as follows: N as urea, P - as triple superphosphate and K - as potassium sulphate.

Soil samples were taken form the upper soil layer (0-25 cm) and the total content of the Pb and Cd was determined by HF, $HClO_4$ and

HNO_3 degradation (according to ISO 14869-1). The amount of the available forms was determined in DTPA extract with pH 7.3. The concentration of Pb and Cd in tobacco plants was determines by atomic absorption after dry ashing of the plant material and dissolving the ash in 20 % HCl.

Plant samples from oriental tobacco (variety Plovdiv 7) were collected at the following stages: beginning of the rapid growth (35 days after transplanting); maturity of the leaves from the first priming (56 days), second priming (77 days) and last priming (98 days).

Data was subjected to variation and correlation analysis using the SPSS statistical package.

RESULTS AND DISCUSSION

The results in the Table 1 demonstrate that the total content of Pb in the soil from the untreated control are above the average for the Bulgarian soils (39 mg/kg, referenced by Brashnarova - 1981), but below the MAC (maximum allowable content) of 80 mg/kg for soils with ðÍ(Í2Î)>6.5 according to Regulation ¹3 published in SG, No.39 from 16.04.2002 .

Table 1. Total and available content of Pb and Cd, mg/1000 g soil
Tabela 1 Sodr`ina na vkupni i podvi`ni formi na Pb i Cd, mg/kg po~va

Fertilization Ѓубрење	Total Pb Вкупно	Available Pb Достапно	Total Cd Вкупно	Available Cd Достапно
$N_0P_0K_0$ – Ø	60	10.2	1.8	0.34
N_5	68	10.3	2.1	0.30
N_{10}	68	9.8	2.0	0.35
$N_5P_{7.5}$	64	11.1	2.4	0.36
$N_5P_{7.5}K_{7.5}$	68	10.3	2.4	0.38
$N_5P_{7.5}K_{7.5}$+ manure	74	11.5	2.7	0.68
CV %	7.0	6.0	12.2	35.6
MAC	80.0		3.0	

The variation of the total Pb amount as related to the fertilization level is low (CV - 7.0%). The content of the Pb increases insignificantly as a result from the 40-year mineral fertilizing where for all the treatments the Pb content is below the MAC. Koteva and Stoyanov (1993) conclude that the total amount of Pb is a constant in all treatments and results from the clean (produced from clean compounds) macro fertilizers. This as well as the applied fertilizing with low levels could be the reason for lack of significant differences between the control and the treatments with mineral fertilizing. The increase of the total amount of Pb in the combined treatments (organic + mineral fertilizing) is more pronounced, but nevertheless remains below the MAC of 80 mg/ kg soil.

The quantity of the available lead is high in all treatments (Table 1). The variation of the available Pb content is low (CV - 6.0%). Continuous fertilizing does not result in increase of this element in the soil. Szalai et al. (2002) similarly found that the differences in available lead content between control and different treatments are insignificant. The data of Kurakov et al. (2006) shows that the quantity of available Pb depends on the applied treatment and increases by 32-73% as compared to the control. The same authors have found a significant effect of the season on the amount of the available Pb in the soil, that was related to the atmospheric pathway of the element entry into the soil. In our experiments we found a moderate positive correlation (r= +0.417) between the total and available Pb content in the soil. Mitsios et al. (2005) point to a statistically significant positive correlation between the total and available Pb content in the soil.

Data on the Pb content in plant organs is represented in Table 2. Pb content in the plants does not depend on the fertilization and during the entire vegetation period is the highest in the leaves. Tsotsolis et al. (2002) have found that the concentration of lead in the leaves of oriental tobacco is from 3.1 to 10.5 mg/kg, while the MAC for Pb in tobacco, conditionally introduced by Bojinova and Chuldjian (by Yancheva et al., 2007), is 30 mg/kg. Pb content in the leaves in our experiment varied between 5.5 and 14.2 mg/kg, remaining therefore below the MAC of 30 mg/kg. Pb content was the highest in the mature leaves from the first priming (56 days after transplanting), and the lowest in the mature leaves from the last priming (98 days). Similar observations on the effect of the priming on the Pb concentration in tobacco leaves were recorded by Golia et al. (2005).

Table 2. Lead content in the above-ground biomass of oriental tobacco as depending on fertilization, mg/kg

Tabela 2 Sodr`ina na Pb vo nadzemnata masa na orientalski tutun vo zavisnost od |ubreweto mg/kg

Fertilization level Дози на ѓубрење	35 day* den		56 day den			77 day den				98 day den				
	1	3	1	2	3	1	4	2	3	1	4	4a	2	5
$N_0P_0K_0$ – Ø	2.4	12.6	1.7	9.9	6.9	1.1	9.8	8.3	5.9	0.9	12.6	9.7	7.0	-
N_5	2.2	11.9	1.8	9.4	6.8	1.3	9.0	8.2	6.1	0.8	14.1	11.1	6.8	1.7
N_{10}	2.6	13.3	1.8	9.7	7.5	1.3	10.0	7.9	5.8	0.9	13.0	9.5	6.8	1.1
$N_5P_{7.5}$	2.8	10.1	2.0	10.0	8.6	1.5	10.6	9.0	6.4	1.1	13.3	10.0	7.3	1.3
$N_5P_{7.5}K_{7.5}$	3.2	13.0	2.2	10.4	8.0	1.1	9.3	8.1	6.7	0.7	12.5	10.3	6.5	1.7
$N_5P_{7.5}K_{7.5}$+ manure	2.3	10.0	1.8	8.7	6.8	1.3	9.5	7.7	5.5	1.0	14.2	12.0	6.2	1.4

*- days after transplanting (DAT); denovi posle rasaduvawe

1-stems; 2-mature leaves, 3-expanding leaves; 4-over mature leaves from first priming; 4a- over mature leaves from second priming; 5-raceme

1. -Stebla; 2- zreli listovi; 3- listovi vo porast; 4- prezreani listovi od dolen pojas; 4a - prezreani listovi od sreden pojas; 5-socvetija

As a result from the long-term fertilization the total Cd content in the soil slightly increases (Table1). According to Regulation N^{O} 3 published in SG, No.39 from 16.04.2002 MAC for the Cd in a soil with $pH_{(H2O)}$>=7.0 is 3 mg/kg. In our experiment the total amount of the Cd in soil from the control and other treatments remains below MAC. Some Cd increase in fertilized treatments was observed by other authors (Koteva and Stoyanov, 1993; Podkolizin et al., 2002), but it was so low that did not raise concerns about the environment and the quality of the plant production. The variation of the total Cd content as depending on the fertilization was not high (CV - 12,2%). The increase of the total amount of Cd with the application of mineral fertilizing is smaller than in the combined fertilization scheme. The amount of cadmium is higher in the treatments with phosphorus application.

A strong positive correlation was observed between the total and available Cd content in the soil (r= +0.757). The comparison with the unfertilized control shows that the changes in the Cd content as a result from the 40-years mineral fertilization are small and multilateral (Table 1). Therefore it is difficult to make any conclusions about the effect of mineral fertilization on the amount of available cadmium in the soil. Stoyanov et al. (1997) found small increases in the available Cd content due to continuous nitrogen fertilization. Szalai et al. (2002) did not find any relation between the fertilizing and the available Cd. The results published by Kurakov et al. (2006) and Lehoczky et al. (2004) similarly demonstrate that continuous fertilization does not affect the amount of available Cd in the 0-20 cm soil layer. The amount of available Cd in the combined treatment in our study is twice as high as that of the control. This duplication of the Cd quantity when $N_5P_{7.5}K_{7.5}$+ manure are applied leads to higher variation in the available Cd (CV - 35.6%).

The amount of Cd in plants was not affected by the fertilization (Table 3). In spite of the higher amounts of available cadmium in the soil in the combined treatment, the content of this element in the plants is not higher than in the other treatments. Gondola and Kadar (1993) similarly found that the higher amount of available Cd in the soil does not lead to increase in this element in tobacco leaves. The recorded Cd content in tobacco organs when $N_5P_{7.5}K_{7.5}$+ manure was applied often is lower than that in the other treatments. This is explained by Stoyanov et al. (1997) with the biological dissolving of the elements when higher yields are obtained. In our experiment the amount of the element was lower in the stems and racemes. Leaves that have finished their development (over-mature and mature leaves) have higher Cd content than the expanding leaves.

Tso (1989) has found similar discrepancies in the Cd concentration in the leaves as depending on the leaf position on the stem. When comparing the Cd concentrations in mature leaves from first (56 day after transplanting), second (77 day) and last (98 day) priming the higher concentration of the element in the lower leaves becomes apparent, together with its lowest concentration in the mature leaves of the last priming. The observed cadmium contents in mature leaves from first and second priming were about or slightly higher from the ones determined by Tsotsolis et al. (2002) for leaves of oriental tobacco (0.5-3.0 mg/kg), but well below the conditionally introduced by Bojinova and Chuldjian (by Yancheva et al., 2007) MAC of 5 mg/kg.

Table 3. Cadmium content in the above-ground biomass of oriental tobacco as depending on fertilization, mg/kg

Tabela 3 Sodr`ina na kadmium vo nadzemnata masa na orientalski tutun vo zavisnost od |ubreweto mg/kg

Fertilization level Дози на ѓубрење	35 day* den		56 day den			77 day den				98 day den				
	1	3	1	2	3	1	4	2	3	1	4	4a	2	5
$N_0P_0K_0$ – Ø	2.0	3.6	1.8	3.8	2.1	1.3	3.9	2.7	2.0	1.1	4.0	2.3	1.5	-
N_5	1.5	3.9	1.6	3.9	2.4	1.1	3.9	3.3	2.5	0.6	3.8	3.4	2.1	0.7
N_{10}	1.3	3.1	1.4	4.2	2.5	0.9	3.9	3.2	2.3	0.7	3.6	3.0	2.0	1.1
$N_5P_{7.5}$	1.8	3.7	1.6	4.3	2.4	1.2	4.3	3.1	2.5	0.4	4.3	3.2	2.3	1.2
$N_5P_{7.5}K_{7.5}$	1.8	3.6	1.5	3.5	2.3	0.9	3.5	3.0	2.0	0.4	3.5	2.5	2.0	0.8
$N_5P_{7.5}K_{7.5}$+ manure	1.5	2.5	1.2	3.0	1.9	0.9	2.7	1.8	1.2	0.5	2.4	1.6	1.6	0.7

*- DAT;

1-stems; 2-mature leaves, 3-expanding leaves; 4-over mature leaves from first priming; 4a- over mature leaves from second priming; 5-raceme

1. -Stebla; 2- zreli listovi; 3- listovi vo porast; 4- prezreani listovi od dolen pojas; 4a - prezreani listovi od sreden pojas; 5-socvetija

CONCLUSIONS

1. The amount of total Pb and Cd increases less in the treatments with mineral fertilizing as compared to the combined ($N_5P_{7.5}K_{7.5}$+manure) as a result from 40-years long-term experiment. In all treatments the observed amounts were below the MAC.

2. The content of available Pb is high in all treatments, but the continuous fertilization does not lead to its increase in the soil. The amount of available Cd changes insignificantly as a result from 40-years fertilizing with mineral fertilizers, while in the combined ($N_5P_{7.5}K_{7.5}$+manure) fertilization the amount of available Cd is twice as high as that in the control.

3. The effect of the fertilization on the Cd and Pb content in the tobacco organs is insignificant. The concentration of Cd and Pb is the highest in the leaves, while in the stems and racemes it is lower.

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SODR@INA NA Pb I Cd VO PO^VATA I TUTUNOT PO 40 GODI[NO MINERALNO I KOMBINIRANO \UBREWE

R. Bo`inova, P. Zaprjanova

Institut za tutun i tutunski preraboteki - Plovdiv, Bugarija

REZIME

Vo ova istra`uvawe e prou~uvano vlijanieto na |ubreweto vrz sodr`inata na te{ki metali (Pb i Cd) vo po~vata i tutunskite rastenija. Pri toa e utvrdeno deka ~etirieset godi{noto |ubrewe predizvikalo slabo zgolemuvawe na vkupnata sodr‘ina i na podvi‘nite formi na Pb i Cd. Malku e zgolemena sodr`inata na Pb i Cd vo tehni~ki zrelite listovi od dolniot pojas.

Author's address:
R. Bozinova, P. Zaprajanova
Tobacco and Tobacco Products Institute
Plovdiv, Bulgaria