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EFFECT OF COMPOST AND VERMICOMPOST AMENDMENTS ON HEAVY METALS UPTAKE BY TOBACCO

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Abstract

A comparative research regarding the impact of organic amendments on the uptake of heavy metals within Oriental tobacco Krumovgrad 90 has been carried out. Experiments have been implemented in controlled conditions. The soil used in this experiment was sampled from the vicinity of KCM – Plovdiv. The pot experiment was a randomized complete block design containing five treatments and three replications (15 pots). The treatments consisted of a control (no organic ameliorants) and compost, and vermicompost amendments (added at 2.5% and 5%, recalculated based on dry soil weight). Heavy metals were measured in roots, stems and leaves of tobacco. The photosynthetic and transpiration rates were measured during treatment using an LCA-4 portable photosynthesis system. Amendments favour the growth and development of tobacco and increased photosynthesis and transpiration intensity. Amendments dose not lead to effective immobilization of Cd, Zn and Cu phytoaccessible forms in soil. The effect of the amendments used for the reduction of the Cd and Zn content in tobacco is negligible. The use of vermicompost can result in a reduction of Pb content in tobacco leaves to 30-40%.

***Key words**: heavy metals, organic amendments, Oriental Tobacco, photosynthesis and transpiration rate.*

INTRODUCTION

Tobacco is a major agricultural crop for many countries, including Bulgaria. It is known that tobacco is a plant which, compared to other agricultural crops, accumulates higher amounts of heavy metals, even when grown on unpolluted soils (Tso, 1972).The content of metals in tobacco varies widely and depends on a number of factors such as soil type and pH, the use of metal-containing pesticides and fertilizers and others (Adamu et al., 1989; Bell et al., 1992; Khan et al., 1992). Some of the metals such as Fe, Mn, Zn and Cu are important micro elements for plant growth and yield. Other metals, such as Pb, Cd, Ni and Cr, are not relevant to plant development but can cause serious health and environmental problems. Cd content in tobacco ranges from 0.5 mg/kg to 3.5 mg/kg (Golia et al., 2007). Tso (1972) reports a Cd concentration in tobacco leaves reaching 11.6 mg/kg. These are much higher values than most cultures containing Cd (which is below 0.05 mg/kg). Pb content in tobacco leaves varies widely from 0 to 200 mg/kg depending largely on the soil characteristics, type and variety of tobacco, as well as its place of cultivation (Tso, 1972). Besides from the soil, Pb pollution can also be done by aerosol route.

Addition of organic matter amendments, such as compost, fertilizers and wastes, is a common practice for immobilization of heavy metals and soil amelioration of contaminated soils (Clemente et al., 2005). Organic amendments have the ability to improve the physical, chemical and biological properties of soil by: (i) raising the pH; (ii) increasing the content of organic matter; (iii) adding nutrients that are essential for plant growth; (iv) increasing the capacity for holding water; and (v) modifying the bioavailability of heavy metals (Walker et al., 2003; Walker et al., 2004; Angelova et al., 2013). Using organic fertilizers can improve biological activities and physicochemical properties of crop growth environment. Also, organic fertilizers can neutralize or decreases the soil acidity and supply some micronutrients such as zinc, and copper. Organic fertilizers could advance in weight of single tobacco leaf and yield. Fertilizer efficiency was required to induce rapid growth, improve leaf maturation and ripe, as well as leaf combustibility. It was believed that biological organic-inorganic mixed fertilizer could advance in growth, quality and output of tobacco (Hu, 2004). The

increasing application of organic fertilizers obviously improved soil capacity of supplying nutrients, thus enhancing the release of N, P, and K, providing enough K for tobacco (Cao et al., 2004). Noticeably, organic fertilizer could also improve resistance of tobacco plant in order to reduce diseases (Li, 2008).
The main objective of this article is to conduct a systematic comparative study to determine: (i) the influence of compost and vermicompost on the amount of Pb, Cu, Zn and Cd phytoaccessible forms, and (ii) to compare the effect of the selected amendments on the growth parameters, physiological status of Oriental tobacco and accumulation of Pb, Cd, Zn and Cu in Oriental tobacco when grown on highly contaminated soils.

MATERIALS AND METHODS

The soils were collected from the surface soil horizon (0-20 cm) of sites located at 0.1 km from the source of pollution (KCM - Plovdiv, Non-Ferrous Metal Plant). The studied soils are slightly sandy loam and are characterized by alkaline reaction (7.7), average content of carbonate (7.3%) and humus (2.2%).
Soil properties are a precondition for low to medium mobility of the metals, as confirmed by the results for DTPA-extracted Pb, Cd, Zn and Cu (low mobility for Pb, Zn and Cu, and medium for Cd).
The total content of Pb, Cd, Zn and Cu is very high and significantly exceeds the permissible concentrations (Table 1).

Table 1. Total content and DTPA - extractable (mg/kg) mobile forms of Pb, Cd, Zn and Cu in soil

Element	DTPA-extractable	Total content	DTPA - extractable/ total content, %	MPC*
Pb	401.3	10293	3.8	80
Cd	65.5	174.7	37.5	2,5
Zn	260.1	11795	2.2	340
Cu	45.3	629.6	7.2	280

*MPC - maximum permissible concentrations

Organic soil amendments were tested in the study. A major consideration when choosing them was the requirement not to pollute the soil further and to favour soil fertility. Compost and vermicompost obtained from the processing of natural fertilizer and other organic waste from the California worm were selected. Characteristics of soils and organic amendments are shown in Table 2.

Table 2. Characterization of the soil and the organic amendments used in the experiment

Parameter	Soil	Compost	Vermicompost
pH	7.7	7.5	7.8
EC, dS/m	0.3	0.2	2.2
Organic matter, %	2.2	53.8	34.9
N Kjeldal, %	0.23	1.81	1.96
Pseudo total P, mg/kg	2961	1005	1009
Pseudo total K, mg/kg	5882	2452	5684
Pseudo total Ca, mg/kg	31029	420	893
Pseudo total Mg, mg/kg	11495	168	410
Pseudo total Pb, mg/kg	10293	10.5	26.0
Pseudo total Zn, mg/kg	11795	93.2	191.8
Pseudo total Cd, mg/kg	174.7	0.47	0.43
Pseudo total Cu, mg/kg	629.6	30.3	53.0

After the soils sieving through a 2 cm^2 mesh sieve, organic soil amendments (compost and vermicompost) were added at 2.5% and 5% (recalculated based on dry soil weight) and gently hand-mixed with the soil. The total amount of mix used in all variants was 9 kg. All treatments were performed in three replications. Additionally, three test pots were prepared for the control samples (no. amendments).
Oriental tobacco plants (Krumovgrad 90) were used as test plants. When the plants had developed three pairs of leaves, they were transferred to the pots, where they were left to grow in a climate chamber for 67 days, with regular watering and random rotation of pots position. All plants were grown successfully, with some of the plants reaching blooming phase (control sample and variants with vermicompost - 2.5 and 5%). Tobacco plants were harvested and the content of Pb, Cu, Zn and Cd in different parts - roots, stems and leaves was determined. Since Pb, Zn and Cd were accumulated less in the leaves of the lower belt compared to the leaves of the middle and upper belt (Lugon-Moulin et al., 2004), only the leaves from the lower belt were analyzed. The plant samples were dried at 60°C.
The photosynthetic and transpiration rates were measured during treatment using an LCA-4 (ADC, England) portable photosynthesis system.
The content of heavy metals in Oriental tobacco leaves was determined by the method of dry mineralization. The pseudo total content of metals in the soil was determined in

accordance with ISO 11466. The mobile forms were extracted by a solution of 0.005 M DTPA and 0.1 M TEA (pH 7.3) (ISO 14870). The quantitative measurements were carried out with inductively coupled plasma emission spectrometry (ICP) (Jobin Yvon Emission - JY 38 S, France).

RESULTS AND DISCUSSIONS

The Table 3 shows the amounts of mobile forms of DTPA-extracted Pb, Cd, Zn and Cu in the control sample from anthropogenically contaminated soil used in the experiment and their changes in 6 weeks after the addition of organic soil amendments.

Table 3. Impact of soil organic amendments on the amount of DTPA-extractable mobile forms of Pb, Cd, Zn and Cu

Element	Control	Compost		Vermicompost	
		2.5%	5.0%	2.5%	5.0%
Pb	401.3	452.0	453.6	446.5	432.5
Cd	65.5	64.2	64.6	62.3	66.9
Zn	260.1	262.4	255.8	298.5	278.6
Cu	45.5	41.3	43.7	45.2	47.4

The results presented in the table shows that the influence of soil amendments on the mobile forms of Pb, Cd, Zn and Cu is diversely and relatively poorly expressed, with up to 15% deviations from the control sample. The addition of compost and vermicompost resulted in an increase of mobile Pb by 13%. Adding compost practically does not affect the amount of mobile Zn, and the biofertilizer leads to an increase in the amount of mobile Pb by 13%. The quantities of Cd and Cu remained practically unchanged.

After harvesting, the mass and height of the plants were measured (data are not shown). The results shown that the amount of fresh (dry) biomass as well as the height of the plants are influenced by the type and amount of the amendments used. Adding vermicompost leads to their more intense growth, and this effect is more visible in 5.0% vermicompost. In practice, the addition of 2.5% and 5% compost does not have a significant effect on plant growth.

Greater biomass and differences in tobacco development (the earlier flowering of variants with the addition of 2.5% and 5% vermicompost) may be due to the improvement of soil physical characteristics favouring better root development and greater absorption of macro and microelements compared to the control sample. The photosynthetic response of the plants to the effect of heavy metals was thoroughly analyzed by determining the parameters of *leaf gas exchange*, including photosynthesis rate, transpiration intensity and stomata conductivity. The results are presented in Table 4.

Table 4. Influence of organic soil amendments on the rate of photosynthesis (μmol (CO_2). m^{-2}. s^{-1}), the transpiration intensity (mmol (H_2O). m^{-2}. s^{-1}) and stomata activity (mol. m^{-2}. s^{-1})

Treatment	Parameters		
	Photosynthesis rate, μmol(CO_2).m^{-2}.s^{-1}	Transpiration, Mmol (H_2O). m^{-2}.s^{-1}	Stomata conductivity mol.m^{-2}.s^{-1}
Control	5-6	2.7-2.9	0.07-0.08
2.5% Compost	3-4	3.0-3.5	0.07-0.09
5% Compost	6-7	3.6-4.0	0.10-0.12
2.5% Vermi compost	8-10	4.0-5.2	0.16-0.18
5% Vermi compost	10-14	4.0-5.3	0.17-0.19

The results shows that changes in the physiological status of the plants are observed depending on the type and amount of organic additive used. The increase in the photosynthesis rate in plants after the addition of vermicompost compared to the control sample is visible, with the experiment of 5% vermicompost reaching 50-60%. Reverse but less obvious is the tendency for tobacco after the addition of 2.5% compost, where the photosynthesis rate reduction reaches 30%.

On the other hand, the addition of compost and vermicompost results in an increase in transpiration intensity and in the stomata conductivity compared to the control sample, with this increase being more pronounced with 5% compost and 5% vermicompost.

The influence of organic amendments on the accumulation and distribution of Pb, Zn, Cu and Cd in the plants tested are presented in Table 5. The change in heavy metal content is also presented in comparison with the control sample (%).

A major route for the entry of heavy metals in plants is the root system. Once they have entered the roots, they can be stored or moved to the stems. As can be seen from the data presented in Figure 1, the major portion of Pb, Zn and Cu is retained by the tobacco roots, and a smaller portion of them moves to the stems and leaves. In cadmium case, however, the main portion is accumulated in the leaves. These trends are observed both in the control sample and in the cultivated plants after the addition of organic amendments.
The Cd content in tobacco leaves is higher than the root system and the stems, which is also consistent with the results of other authors (Mench et al., 1989; Keller et al., 2003; Angelova et al., 2006). Their higher accumulation in tobacco leaves is probably due to the absorption of heavy metals from the soil through the root system of the plant and their movement through the conductive system. This is consistent with the results of Yeargan et al. (1992) and Angelova et al. (2006), who found that tobacco has an extraordinary ability to digest Cd in comparison with other plants when grown on highly Cd-contaminated soils. Our results are significantly higher than the data on Cd content in leaf tobacco (Lugon-Moulin et al., 2004) reported in literature. According to Mench et al. (1994) and Sappin-Didier and Gomez (1994), the Cd content in tobacco ranges from 40 to 120 mg/kg depending on the soil characteristics.
Cd content in Oriental tobacco leaves reaches 261.8 mg/kg in the control sample and is significantly higher than the concentrations considered being critical for plant growth - 5 to 10 mg/kg (Kabata Pendias, 2001). The visible symptoms caused by the increased Cd content in plants such as growth inhibition, root system damage, leaf chlorosis, reddish-brown colour at the edges were not observed.
The Pb content in tobacco leaves reaches 92.3 mg/kg in the control sample and is lower than the concentrations considered to be critical for plant growth - 30 to 300 mg/kg (Kabata Pendias, 2001). The visible symptoms caused by the increased Pb content occurring in dark green leaves, deformation of old leaves, dark brown and short roots in tobacco were not observed.

The Zn content in tobacco leaf reaches 304.6 mg/kg in the control sample and is below the critical concentrations for plants - 100-400 mg/kg (Kabata Pendias, 2001). Symptoms of zinc toxicity, such as chlorosis and leaf-edge necrosis, interveinal chlorosis on young leaves, inhibition of plant growth as a whole, root damage, were not observed as well.
Cu content in tobacco leaves reaches 22.4 mg/kg in the control sample and is lower than the concentrations considered to be critical for plant growth - 30 mg/kg (Kabata Pendias, 2001).
The distribution of heavy metals in Oriental tobacco organs has a selective character which decreases in order: Pb - roots> stems> leaves, Cd - leaves> stems> roots, Zn and Cu - roots> leaves> stems (Figure 1).

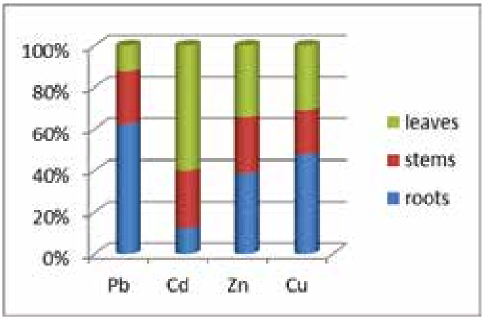

Figure 1. Distribution of Pb, Cd, Zn and Cu (%) in the Oriental tobacco organs

Our results show that the absorption of Pb, Zn and Cd from tobacco is not one-sided and depends on soil amendments and treatment (type and norm).
The addition of 2.5% and 5% compost results in a decrease in Pb content in tobacco stems and leaves; this decrease being more pronounced with 5% compost. Adding vermicompost also leads to a decrease in Pb content in stems and leaves compared to the control sample, this decrease being more pronounced with a 2.5% additive. The influence of the organic amendments used on the accumulation of Pb in tobacco roots is more complex and substantially dependent on their quantity. While in the addition of 2.5% compost and vermicompost, the reduction in Pb content is relatively high (16.2% and 10.5%), in 5.0% amendments the reduction is

significantly lower and is 10.0% and 7.7%, respectively. The probable cause for this is the much stronger root system in the second case.
The trends in the change of Cd and Zn content in tobacco organs are quite complicated and controversial. The content of both elements in the root system increases in all variants of the experiments. A similar but less pronounced trend is observed for cadmium in stems and leaves. The exception is only the case of using a 5.0% vermicompost additive in which the Cd content in the stems decreases by 7.9% and it's in contradiction with the results of Chlopecka and Adriano (1997) who found that amendments the soil are low, probably because the high Cd content in the leaves remains close to that of the control sample. The reason is probably the amount of biomass. The result obtained reveals the number of added enhancers is not sufficient to immobilize the Cd amount.
The increase in Zn content in leaves compared to the control sample is higher, as in the experiment with 5.0% vermicompost and it reaches 50%. Reverse but less pronounced is the trend in stems where the reduction in Zn content is from 4.4 to 24.4%.
The addition of 2.5% and 5.0% compost results in a decrease in the copper content in tobacco stems and leaves, this decrease being more pronounced with 5.0% compost.
The increase in copper content in leaves compared to the control sample is higher, as in the experiment with 5.0% vermicompost it reaches 57%. Reverse but less pronounced is the trend in stems where the reduction in copper content is 10% to 31%.

Table 5. Influence of organic amendments on the accumulation of Pb, Zn, Cu and Cd (mg/kg) in the Oriental tobacco

Element	**Plant part**	**Control**	**Compost 2.5%**	**Change %**	**Compost 5.0%**	**Change %**	**Vermicompost 2.5%**	**Change %**	**Vermicompost 5.0%**	**Change %**
Pb	Roots	475.8	398.5	- 16.2	430.2	- 10.0	425.9	- 10.5	439.2	- 7.7
	Stems	195.3	149.4	- 23.5	120.2	- 38.5	125.9	- 35.5	144.9	-25.8
	Leaves	92.3	87.1	- 5.6	65.6	- 28.9	52.1	- 43.6	59.5	-35.5
Cd	Roots	59.2	75.1	+26.9	85.0	+43.6	132.4	+123.6	63.5	+ 7.3
	Stems	121.3	137.8	+13.6	142.4	+17.4	125.8	+ 3.7	111.7	- 7.9
	Leaves	261.8	303.1	+15.8	266.5	+ 1.8	285.9	+ 9.2	260.1	- 0.6
Zn	Roots	340.7	348.8	+ 2.4	468.8	+37.6	569.5	+67.2	431.9	+26.8
	Stems	240.4	201.9	- 16.0	187.1	- 24.4	229.8	- 4.4	197.9	- 17.7
	Leaves	304.6	408.3	+34.0	327.7	+ 7.6	316.8	+ 4.0	460.0	+51.0
Cu	Roots	34.5	32.3	- 6.4	47.3	+ 37.1	53.8	+ 55.9	37.7	+ 9.3
	Stems	15.5	12.3	- 20.6	10.7	- 31.0	12.8	- 17.4	14.0	- 9.7
	Leaves	22.4	22.8	+ 1.8	17.0	- 24.1	25.9	+ 15.6	35.1	+ 56.7

CONCLUSIONS

On the basis of the results obtained, the following conclusions can be drawn:

1. Tobacco is a crop tolerant to heavy metals and it develops normally when grown on soils contaminated with heavy metals. The distribution of heavy metals in Oriental tobacco organs has a selective character which decreases in order: Pb - roots> stems> leaves, Cd - leaves> stems> roots, Zn and Cu - roots> leaves> stems.
2. Organic ameliorants influences the amount of fresh (dry) biomass as well as the height of the plants. The addition of 5% vermicompost leads to tobacco plants more intense growth. The addition of 2.5% and 5% compost in practice does not have a significant effect on plant growth.
3. Organic ameliorants influences tobacco physiological status. The addition of compost and vermicompost leads to increased photosynthesis rate, transpiration intensity and in the stomata conductivity.
4. The use of organic amendments does not lead to effective immobilization of Cu, Zn and Cd phytoaccessible forms in heavily polluted soil. This contradicts the results of other authors who worked with slightly or moderately contaminated soils. Explaining

this contradiction requires the accumulation and analysis of more experimental results.

5. The effect of the compost and vermicompost used for the reduction of the Cd and Zn in tobacco is negligible. The use of vermicompost can result in a reduction in Pb content in tobacco leaves to 30-40%.

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