

PHYTOREMEDIATION POTENTIAL OF ENHANCED TOBACCO IN SOIL CONTAMINATED WITH HEAVY METALS

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Abstract: *A comparative research on the impact of organic meliorants on the uptake of heavy metals, micro and macroelements and phytoremediation potential of enhanced tobacco NBCu 10-8F3 has been carried out. The soil used in this experiment was sampled from the vicinity of the Non-Ferrous-Metal Works near Plovdiv, Bulgaria. The pot experiment was a randomized complete block design containing nine treatments and three replications (27 pots). The treatments consisted of a control (no organic meliorants) and compost and vermicompost meliorants (added at 5%, 10%, 15% and 30%, recalculated based on dry soil weight). Upon reaching commercial ripeness, the tobacco plants were gathered. Heavy metals, micro and macroelement contents in roots, stems and leaves of tobacco were analyzed by the method of the microwave mineralization. To determine the elements in the samples, inductively coupled emission spectrometry (Jobin Yvon Emission - JY 38 S, France) was used. The distribution of the heavy metals, micro and macroelements in the organs of the enhanced tobacco has a selective character and depended above all on the parts of the plants and the element that was examined. Pb, Zn, Cu, Fe, Mn, P and Mg distribution in tobacco decreases in the following order: roots > leaves > stems, and for Cd, K, and Ca - leaves > roots > stems. The high concentration of Cd in the leaves and the high translocation factor indicate the possibility of enhanced tobacco to be used in phytoextraction.*

Tested organic amendments significantly influenced the uptake of heavy metals, micro and macroelements by the roots, stems and leaves of tobacco. A correlation was found between the quantity of the mobile forms and the uptake of Pb, Zn and Cd by the enhanced tobacco. The compost and vermicompost treatments significantly reduced heavy metals concentration in leaves and increased uptake of K, Ca and Mg. The 30% compost and 30% vermicompost treatments led to the maximal reduction of heavy metals in enhanced tobacco NBCu 10-8F3. The addition of compost and vermicompost further reduces the ability to digest the heavy metals in the leaves, and phytoremediation potential of enhanced tobacco NBCu 10-8F3.

Keywords: *heavy metals, micro and macroelements, organic meliorants, enhanced tobacco NBCu 10-8F3, phytoremediation*

1. INTRODUCTION

Phytoremediation can be defined as the combined use of plants, soil amendments and agronomic practices to remove pollutants from the environment or to decrease their toxicity [1]. This technique has many advantages compared with other remediation procedures – low economic costs and the possibility of being applied to soils, causing a minimum environmental impact. As a technology based on the use of plants, the success of phytoremediation will mainly depend on the proper selection of plants. Many researchers

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examine the rapidly growing, high biomass plants, including agronomic crops for their ability to tolerate and accumulate metals in their shoots.

Tobacco is a crop that has an exceptional ability to accumulate more Cd in its leaves than any other crop [2-3] and is an efficient cadmium accumulator [4]. Guadagnini et al. [4] developed by means of conventional *in vitro*-breeding and selection techniques, 106 somaclonal variants of tobacco (*Nicotiana tabacum* ssp.) with increased metal tolerance, accumulation and extraction properties. The authors found that 17 of these somaclonal tobacco variants showed an improved heavy metal shoot uptake and phytoextracted 1.2–2.0 times more Cd and Zn than non-modified mother-plants. Herzig et al. [5] confirmed enhanced shoot metal removals up to a factor 1.8 for Cd, 3.2 for Zn and 2.0 for Pb in soil contaminated by industrial sewage sludge and up to a factor 12.4 for Cd, 13.7 for Zn and 13.5 for Pb in acid sandy soil contaminated by deposits from a zinc smelter for best tobacco variants NBCu 10-8F1 and NBCu 10-4F1 [6].

The addition of organic matter amendments, such as compost, fertilizers, and wastes, is a common practice for soil amelioration of contaminated soils [7]. The effect of organic matter amendments on heavy metal bioavailability depends on the nature of the organic matter, their microbial degradability, salt content and effects on soil pH and redox potential, as well as on the particular soil type and metals concerned [8].

The use of organic fertilizers influences the physicochemical properties of soils and improves the conditions for the development of crops. Organic fertilizers can neutralize or reduce the acidity of the soil and increase the content of certain microelements, such as Zn and Cu. The efficiency of organic fertilization is manifested in rapid growth, earlier maturation, improvement in the flammability of leaves, their mass and yield [9]. There is evidence that the use of combined inorganic and organic fertilizers can improve the growth and quality of tobacco [10].

The increasing application of organic fertilizers improves the soil's ability to supply nutrients, thus providing sufficient amounts of N, P, and K for the development of tobacco [11]. On the other hand, organic fertilizers can improve the resistance of the tobacco plant to diseases [12].

The main objective of this paper is to conduct a systematic study, which will help to determine the impact of organic soil amendments on the uptake of the heavy metals, micro and macroelements by enhanced tobacco NBCu 10-8F3, as well as the potential of the plant for phytoremediation of heavy metal contaminated soils.

2. MATERIAL AND METHODS

The soil used in this experiment was collected from the surface soil horizon (0-20 cm) from sites situated at a distance of 0.5 km from the vicinity of the area contaminated by the Non-Ferrous-Metal Works (MFMW) near Plovdiv, Bulgaria. The pot experimental was a randomized complete block design containing nine treatments and three replications (27 pots): 1 – addition of 5% of vermicompost to the soil, 2 - addition of 10% of vermicompost to the soil, 3 - addition of 15% of vermicompost to the soil, 4 - addition of 30% of vermicompost to the soil, 5 – addition of 5% of compost to the soil, 6 - addition of 10% of compost to the soil, 7 - addition of 15% of compost to the soil, 8 - addition of 30% of compost to the soil, 9- control variant.

Soils were passed through a 2-cm sieve. Amendments were added and thoroughly mixed by hand. The pots were filled with 3 kg soil. Three control pots were also set up without

amendment. Pots were watered and stored in a greenhouse, where they were left to settle a minimum of 6 weeks at room temperature before planting the enhanced tobacco.

Characteristics of soils and organic amendments are shown in Table 1. The soil used in this experiment was slightly alkaline, with moderate content of organic matter and essential nutrients (N, P and K). The pseudo-total content of Zn, Pb and Cd is extremely high (2544.8 mg/kg Zn, 2429.3 mg/kg Pb and 51.5 mg/kg Cd, respectively) and exceeds the maximum permissible concentrations (320 mg/kg Zn, 100 mg/kg Pb and 2.0 mg/kg Cd). Soil properties are a prerequisite for low to moderate metal mobility, as confirmed by the results of DTPA-extracted Pb, Cd and Zn (mean for Pb and Zn, and high for Cd).

Table 1: Characterization of the soil and the organic amendments used in the experiment

Parameter	Soil	Compost	Vermicompost
pH	7.6	6.9	7.5
Organic matter, %	3.99	72.10	38.58
N Kjeldal, %	0.24	2.22	1.57
Pseudo-total P, mg/kg	731	12654	10211
Pseudo-total K, mg/kg	4675	6082	10495
Pseudo-total Ca, mg/kg	10607.5	32158.7	31848
Pseudo-total Mg, mg/kg	9577.5	2086.5	7754.9
Pseudo-total Cu, mg/kg	124.8	43.23	53.33
Pseudo-total Fe, mg/kg	41650.9	3177.31	11813.7
Pseudo-total Mn, mg/kg	1244.5	360.48	423.3
Pseudo-total Pb, mg/kg	2429.3	12.0	32.3
Pseudo-total Zn, mg/kg	1430.7	170.8	270.3
Pseudo-total Cd, mg/kg	31.4	0.19	0.69
DTPA –extractable Pb, mg/kg	849.1		
DTPA –extractable Zn, mg/kg	236.8		
DTPA –extractable Cd, mg/kg	36.8		

The test plant was enhanced tobacco NBCu 10-8F3. The enhanced tobacco plants were grown in a climate chamber with regular watering and random rotation of the position of the pots. After 90 days, all plants were harvested.

The content of heavy metals, micro and macroelements was determined in the different parts of enhanced tobacco - roots, stems and leaves. The plant samples were treated by the method of microwave mineralization.

The pseudo-total content of metals in soils was determined in accordance with ISO 11466[13]. The available (mobile) heavy metals contents were extracted in accordance with ISO 14870 by a solution of DTPA [14].

To determine the heavy metal content in the plant and soil samples, inductively coupled emission spectrometer (Jobin Yvon Horiba "ULTIMA 2", France) was used.

3. RESULTS AND DISCUSSION

Table 2 presents the results obtained for the content of heavy metals, micro and macroelements in the vegetative organs of enhanced tobacco without amendments. The root system is a major route for the absorption of heavy metals into plants. Once they have entered the roots, they can be stored or moved to the stems. The results obtained show that a significant portion of Pb, Zn

and Cd is accumulated at the roots of the enhanced tobacco NBCu 10-8F3. These results are confirming the results obtained by [15], who found that Pb accumulated mainly in the roots of tobacco.

The content of Cd is higher in the tobacco leaves than in the root system and stems, which is in line with the results of other authors [16-17]. The greater accumulation of Cd in tobacco leaves is probably due to Cd absorption from the soil through the root system of the plant and their movement through the conductive system. It has been found that cadmium can be accumulated in tobacco leaves in an amount 10 times higher than that in the soil [18]. This is consistent with what was found by [19], who found that tobacco had an extraordinary ability to digest Cd as compared to other plants when grown on Cd contaminated soils.

Table 2: Content of heavy metals, micro and macroelements (mg/kg) in enhanced tobacco NBCu 10-8F3 (without amendments)

	Pb	Zn	Cd	Cu	Fe	Mn	K	Ca	Mg	P
Roots	152.0	405.9	50.5	72.2	629.7	28.4	12365	12341	2598.3	1132,3
Stems	24.4	86.9	20.1	6.0	12.6	1.6	10003	3994	366.1	419.6
Leaves	75.5	119.6	125.4	20.4	211.7	53.0	18569	14541	2280.0	778.8

The results for the influence of organic meliorants on the accumulation and distribution of heavy metals, micro and macroelements in the enhanced tobacco NBCu 10-8F3 are shown in Figure 1. The addition of compost and vermicompost results in a decrease in Pb, Zn and Cd content in the roots, stems and leaves of tobacco, as this decrease is more pronounced when 30% of compost and 30% vermicompost is imported. The results obtained may be explained by the amount of heavy metal mobile forms. The addition of compost and vermicompost to the soil leads to the decrease of mobile forms, resulting in lower accumulation of these elements in the leaves of the plants.

The distribution of heavy metals, micro and macroelements in the organs of enhanced tobacco has selective character specific for individual elements. The main part of heavy metals (except Cd), microelements and macroelements (P and Mg) are accumulated in roots. The distribution of heavy metals, micro and macroelements in the organs of the enhanced tobacco with the addition of compost and vermicompost follows the same relation observed in the control sample (Fig. 1).

The Pb content in tobacco leaves reached to 75.5 mg/kg in the control, and from 13.1 to 67.1 mg/kg in variants with incorporation of compost and from 17.4 to 42.0 mg/kg in variants with incorporation of vermicompost. According to Tso [20], the content of Pb in tobacco leaves varies widely - from 0 to 200 mg/kg, and depends largely on the soil characteristics, the type and variety of tobacco, as well as the place of cultivation [21]. The incorporation of compost and vermicompost leads to a decrease of the Pb content in tobacco leaves (Fig.1).

The Cd content in tobacco leaves reached to 125.4 mg/kg in the control, and from 40.7 to 94.1 mg/kg in variants with incorporation of compost and from 54.4 to 99.6 mg/kg in variants with incorporation of vermicompost. According to Golia et al. [22] the content of Cd in tobacco ranges from 0.5 to 3.5 mg/kg, while Tso [20] reports values reaching up to 11.6 mg/kg. According to Mench et al. [23] and Sappin-Didier et al. [24], Cd content in tobacco leaves varies from 40 to 120 mg/kg depending on the soil characteristics. The results obtained are consistent with the values reported in other literature sources [25-26] and higher than the concentrations considered

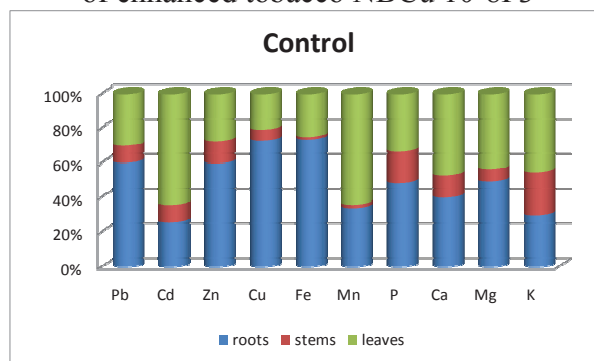
critical in plants (5-30 mg/kg) [27]. Incorporation of compost and vermicompost leads to a reduction of Cd in tobacco leaves.

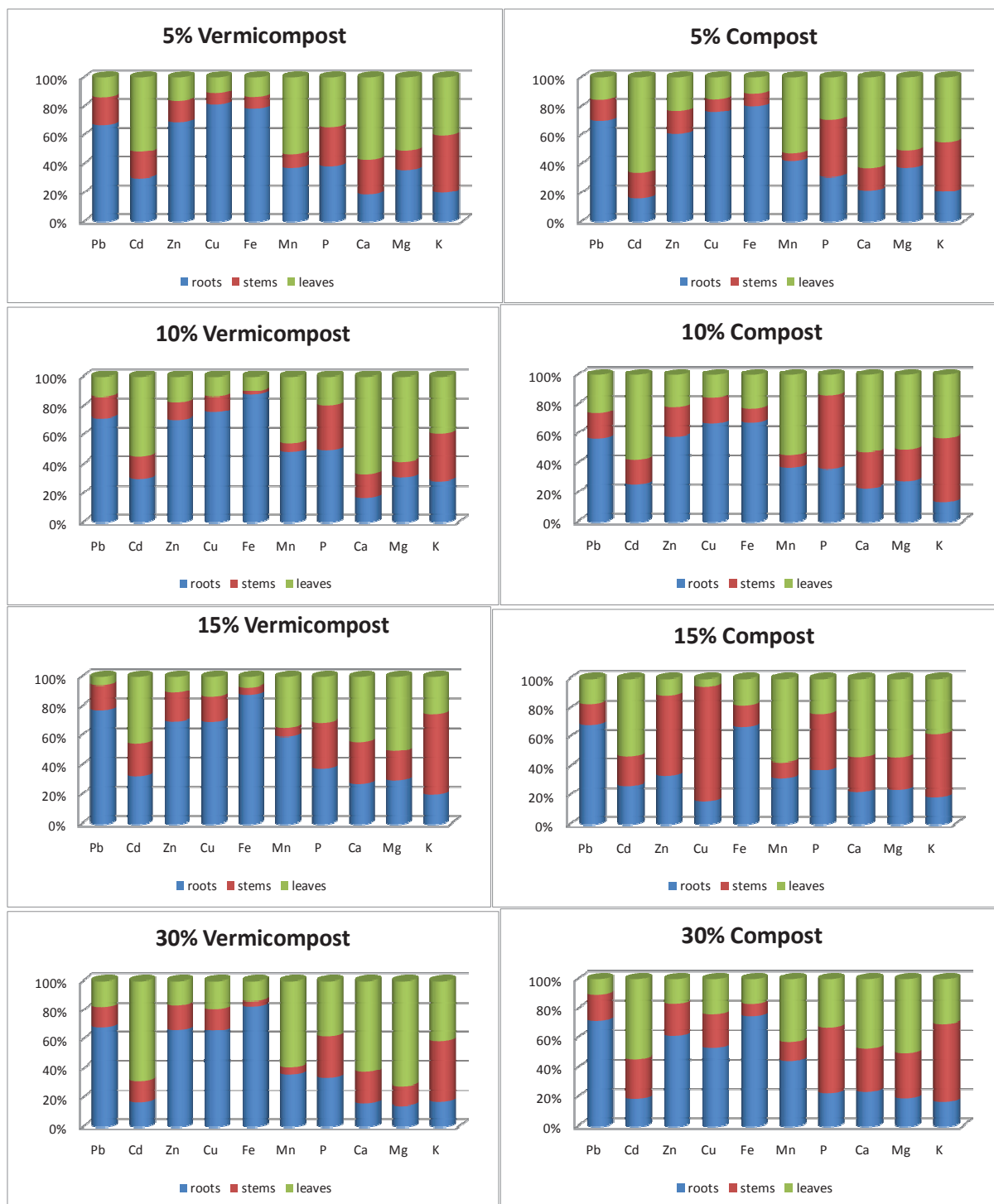
The Zn content in tobacco leaves reached to 183.6 mg/kg in the control, and from 105.0 to 197.8 mg/kg in variants with incorporation of compost and from 77.4 to 138.9 mg/kg in variants with incorporation of vermicompost. According to Jones et al.[28] and Campbell [29] the optimum amount of Zn in the tobacco is in the range from 20 to 60 (80) mg/kg, and excess were observed in values above 80 to 100 mg/kg.

According to Campbell [29] the optimum values of Cu for tobacco are 5-10 mg/kg, whereas Yancheva [30] found that level of Cu in tobacco is in the range 11.9-13.3 mg/kg. The values identified by us for Cu in the leaves from untreated tobacco plants reached to 20.4 mg/kg. The incorporation of compost decreases the content of Zn and Cu in tobacco leaves (Fig. 1).

The Fe content in tobacco leaves reached to 211.7 mg/kg in the control, and from 70.2 to 221.1 mg/kg in variants with incorporation of compost and from 69.9 to 108.4 mg/kg in variants with incorporation of vermicompost. In scientific literature the optimal Fe content in tobacco leaves is 50 to 300 mg/kg [28], whereas 40-50 mg/kg is considered low concentration depending on the stage of plant development [30].

Figure 1: Effect of organic amendments on distribution of heavy metals in vegetative organs of enhanced tobacco NBCu 10-8F3





The incorporation of compost leads to decrease of the Fe content in the tobacco leaves.

The Mn content in tobacco leaves reached to 53.0 mg/kg in the control, and from 37.6 to 66.5 mg/kg in variants with incorporation of compost and from 29.6 to 46.5 mg/kg in variants with incorporation of vermicompost. The incorporation of 15% and 30% compost results in increased Mn content in leaves (Figure 1).

The content of P in the leaves of tobacco plants reached to 778.8 mg/kg in the control, and from 793.1 to 1953.7 mg/kg in variants with incorporation of compost and from 652.6 to 1689.6 mg/kg

in variants with incorporation of vermicompost. Phosphorus content in tobacco leaves, depending on the stage of development, ranges from 0.1 to 1% [28]. The incorporation of compost and vermicompost results in increased P content in leaves.

The content of K in the leaves of tobacco plants reached to 1.85% in the control, and from 2.5 to 6.2% mg/kg in variants with incorporation of compost and from 2.4 to 3.6 mg/kg in variants with incorporation of vermicompost. The incorporation of compost and vermicompost results in increased K content in leaves.

The Ca content in tobacco leaves reached to 1.5% in the control, and from 0.9 to 1.8% in variants with incorporation of compost and from 1.3 to 2.0% in variants with incorporation of vermicompost. The incorporation of 30% compost in soil results in a decrease of the Ca content in tobacco leaves (up to 0.9% in the leaves).

The minimum Mg content in tobacco leaves, where symptoms of insufficiency do not appear, is about 0.25% [20]. According to McCants and Woltz [31] the content of Mg in technically senescence leaves of oriental tobacco is changed in a narrow range - from 0.33 to 0.69% and depends mainly on the location of the leaves to the stem. The organic additives lead to increase in the Mg content in tobacco leaves (Fig. 1).

The Translocation Factor ($TF = \frac{[Metal] \text{ shoots}}{[Metal] \text{ roots}}$) provides information on the ability of plants to digest heavy metals through the roots and to move them to the above-ground mass (leaves). The results we obtained show that, with respect to Pb, the translocation factor for plants without the importation of additives reaches up to 0.50, for Cd up to 2.48 and for Zn up to 0.45. Higher values for Cd are probably a consequence of the greater ability of this element to accumulate in the above-ground mass than in the roots, which is consistent with the results of Wagner and Yergan [16] and Fässler et al. [17]. The results of the study show that enhanced tobacco accumulates significant amount of Cd in the leaves and has a relatively higher potential for phytoextraction. The high concentration of Cd in the leaves and the high translocation factor indicate the possibility of enhanced tobacco to be used in phytoextraction. However, the addition of compost and vermicompost reduces the ability to digest the heavy metals in the leaves, and phytoremediation potential of enhanced tobacco NBCu 10-8F3.

CONCLUSIONS

Based on the results obtained regarding the impact of organic soil amendments on the uptake of the heavy metals by enhanced tobacco, as well as the potential of the plant for phytoremediation of heavy metal contaminated soils, the following conclusions can be made:

1. The distribution of the heavy metals, micro and macroelements in the organs of the enhanced tobacco NBCu 10-8F3 has a selective character and depended above all on the parts of the plants and the element that was examined. Pb, Zn, Cu, Fe, Mn, P and Mg distribution in tobacco decreases in the following order: roots > leaves > stems, and for Cd, K, and Ca - leaves > roots > stems.
2. The incorporation of compost and vermicompost leads to decrease in the content of heavy metals in the tobacco leaves and increase uptake of K, Ca and Mg.
3. The high concentration of Cd in the leaves and the high translocation factor indicate the possibility of enhanced tobacco to be used in phytoextraction. However, the addition of compost and vermicompost further reduces the ability to digest the heavy metals in the leaves, and phytoremediation potential of enhanced tobacco NBCu 10-8F3.

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