Uptake of heavy metals by enhanced tobacco grown in industrially polluted soils in Bulgaria

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Abstract

A field study was conducted to evaluate the efficacy of enhanced tobacco for phytoremediation of contaminated soils. The experiment was performed on an agricultural field highly contaminated (2544.8 mg/kg Zn, 2429.3 mg/kg Pb and 51.5 mg/kg Cd) by the Non-Ferrous-Metal Works near Plovdiv, Bulgaria. The concentration of heavy metals in different parts of three varieties of enhanced tobacco (NBCu108F3, NBCu104F3, BAGF3) was determined by ICP. A clearly distinguished species peculiarity existed in the accumulation of heavy metals in the organs of tobacco. Cd levels occur in the order, lower leaves > middle leaves > upper leaves > stalks > roots, Pb - middle leaves > lower leaves > upper leaves > stalks > roots, and Zn- upper leaves > middle leaves > lower leaves > stalks > roots. The translocation factor for all metals is greater than 1 (TF Pb -13.7-14.8, TF Zn -8.8-10.1, TF Cd -5.5-6.5). None of these varieties of enhanced tobacco was specified as a hyperaccumulator; nevertheless, all varieties show potential for phytoextraction of Pb, Zn, and Cd.

Keywords: heavy metals, enhanced tobacco, translocation factor, bioaccumulation factor, phytoextraction

Introduction

Remediation 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 (Salt et al., 1998). 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. An ideal plant for phytoextraction should possess several characteristics: tolerance to excessive heavy metals concentrations in soil, fast growth and high biomass and high efficiency in heavy metals uptake and translocation to aboveground parts, low water requirement, no invasiveness, resistance to pests and pathogens, phytomanagement with low inputs, and ease of harvest (Herzig et al. 2014). Phytoextraction efficiency can be significantly improved by combining selected and genetically improved cultivars of high biomass plants and relevant agronomical techniques (Nehnevajova et al. 2007, Herzig et al. 2014).

Tobacco is a crop that has an exceptional ability to accumulate more Cd in its leaves than any other crop (Mench and Martin, 1991, Mench et al.,1994) and is an efficient cadmium accumulator (Guadagnini, M., 2000). Guadagnini et al. (2000) 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. (2003) 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 NBCu108F1 and NBCu 104F1 (PHYTAC, 2005).

The main objective of this paper is to conduct a systematic study, which will allow us to determine the uptake of the heavy metals (Pb, Cd and Zn) by three varieties of enhanced tobacco (NBCu108F3, NBCu104F3, BAG F3), as well as the potential of the enhanced tobacco for phytoremediation of heavy metal contaminated soils.

Materials and methods

The test plant were three varieties of enhanced tobacco (NBCu108F3, NB104F3, BAGF3) grown at a distance of 0.5 km from the vicinity of the area contaminated by the Non-Ferrous-Metal Works (MFMW) near Plovdiv, Bulgaria. Field trials were set under the block method in four repetitions. Seeds of the plants were germinated in the greenhouse under controlled conditions. Three week old seedlings were transferred to pots, acclimatized outside before planting in the field. A planting distance of 15 cm in the row and 55 cm between the rows was followed. Technically senescenced leaves of various stem positions were taken for analysis. As the leaves of tobacco ripen sequentially, harvesting is done in stages /harvests/ by following the sequence of senescence of leaves. Harvesting of leaves is carried out for 3 harvests (1-lower leaves, 2-middle leaves, and 3- upper leaves, The collected technically senescenced leaves were strung on a single needle and dried in natural conditions, observing the technology of solar drying of oriental tobacco.

The concentration of heavy metals were determined in the different parts of three varieties of enhanced tobacco - roots, stalks and leaves (lower, middle and upper). The plant samples were treated by the method of microwave mineralization.

The pseudo-total concentration of metals in soils was determined in accordance with ISO 11466. The available (mobile) heavy metals concentration in soil were extracted in accordance with ISO 14870 by a solution of DTPA.

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

Results and discussion

Chemical characteristics of soils are shown in Tables 1 and 2. The soil used in this experiment was slightly alkalic, with moderate concentration of organic matter and essential nutrients (N, P and K).

Table 1. Characterization of the soil used in the experiment.

	pH	EC	Organic matter, %	N, %	P , mg/kg	K, mg/kg
Soil	7.6	0.3	3.99	0.22	731	4675

Table 2. Total and DTPA extractable Pb, Zn and Cd (mg/kg) in soil sample.

Parameter	Pb	Cd	Zn
Total concentration	2429.3	51.5	2544.8
DTPA extractable	868.0	31.7	279.8
DTPA/total, %	35.7	61.6	11.0
MPC (pH 6.0 -7.4)	100	2.0	320

The psedo-total concentration 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). The extremely high soil total metal concentration was a prerequisite for also high available concentration (for all investigated metals), as confirmed by the results of DTPA-extracted Pb, Cd and Zn (moderate for Pb and Zn, and high for Cd).

Table 3 presents the results obtained for the concentration of heavy metals in the vegetative organs of three varieties of enhanced tobacco NBCu108F3, NBCu104F3 and BAGF3. 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 obtained results show that a significant portion of Pb, Zn and Cd is accumulated at the roots of the enhanced tobacco NBCu108F3, NBCu104F3 and BAGF3. The lead concentration in tobacco roots varies from 69.8 mg/kg in NBCu108F3 to 132.2 mg/kg in BAGF3, Cd from 17.7 mg/kg in NBCu108F3 to 28.1 mg/kg in BAGF3, Zn – from 97.6 mg/kg in NBCu108F3 to in 185.1 mg/kg in BAGF3. These results are contrary to the results obtained by del Piano

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(2008), who found that Pb accumulated mainly in the roots of tobacco.

The concentration of heavy metals was higher in tobacco stalks than in the root system, with the exception for the BAGF3 cultivar for Pb and Zn. The lead concentration in tobacco stalks varies from 75.6 mg/kg in NBCu104F3 to 100.2 mg/kg in BAGF3, Cd from 28.2 mg/kg in NBCu104F3 to 33.4 mg/kg in NBCu108F3, Zn stalks 164.4 mg/kg in NBCu104F3 to 184.3 mg/kg in BAGF3 (Table 3).

Table 3. Concentration of Pb, Cd and Zn (mg/kg) in vegetative organs of enhanced tobacco (NBCu108F3, NB104F3, BAGF3).

	Roots	Stalks	Leaves			
			Lower	Middle	Upper	Average
Pb						
NBCu108F3	69.8	96.9	927.8	1062.6	888.4	959.6
NB104F3	74.2	75.6	1062.5	1403.1	838.0	1101.2
BAGF3	132.3	100.2	1074.5	1044.3	930.2	1016.3
Cd						
NBCu108F3	19.8	33.4	155.6	120.9	66.4	114.3
NB104F3	17.7	28.2	155.8	129.0	61.1	115.3
BAGF3	28.1	29.2	132.9	135.2	75.8	114.6
Zn						
NBCu108F3	97.6	176.3	860.1	936.7	1147.9	981.6
NB104F3	124.9	164.4	922.2	1173.0	1134.5	1076.6
BAGF3	185.1	184.4	949.2	1000.7	1065.1	1005.0

The concentration of heavy metals were higher in the tobacco leaves than in the root system and stalks, which is in line with the results of other authors (Wagner and Yergan, 1986; Mench et al., 1989; Keller et al., 2003; Fässler et al., 2010). The average Pb concentration in tobacco leaves varied from 959.6 mg/kg in NBCu108F3 to 1101.2 mg/kg in NB104F3 (Table 3). The concentration of lead was the highest in the middle leaves with the exception for the BAGF3 cultivar. Visible symptoms, caused by high levels of Pb, which occur in tobacco (dark green leaves, leafroll of old leaves, dark brown and short roots), were not observed in our experiments. Obviously, Pb can be incorporated in plant tissues in enhanced tobacco in a way so that they do not pose an issue for plant physiological processes. According to Tso (1991), the concentration 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 and total concentration of Pb in soil (Lugon-Moulin, 2004).

The average Cd concentration in tobacco leaves varies from 114.3 mg/kg in NBCu108F3 to 115,3 mg/kg in NB104F3. Visible symptoms caused by the increased concentration of Cd in plants, such as growth arrest, damage to the root system, chlorosis on leaves, reddish to dark brown colour on their edges, were not observed. Obviously, Cd can be incorporated in plant tissues in enhanced tobacco in a way so that they do not pose an issue for plant physiological processes. According to Golia et al. (2007) the concentration of Cd in tobacco ranges from 0.5 to 3.5 mg/kg, while Tso (1991) reports values reaching up to 11.6 mg/kg. According to Mench et al. (1994) and Sappin-Didier et al. (1997), Cd concentration in tobacco leaves varies from 40 to 120 mg/kg depending on the soil characteristics and Cd soil total concentration, plant cultivar and environmental conditions (which affect temperature, moisture, etc.). The results obtained were consistent with the values reported in other literature sources (Adamu et al., 1989, Bell et al.,1992) and higher than the concentrations considered critical for plants to have developmental issues (which were not visually observed) (5-30 mg/kg) (Kabata Pendias and Pendias, 2001). 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 (Anonymous, 1995). This is consistent with what was found by Yeargan et al.(1992), who found that tobacco had an extraordinary ability to accumulate Cd as compared to other plants when

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grown on Cd contaminated soils.

The concentration of cadmium is the highest in the lower leaves with the exception for the BAGF3 cultivar. Our results confirm the results of Wagner (1994), according to whom the highest values of Cd are found in the leaves from the lower zone of tobacco and significantly lower in the leaves of top zone, assuming gradual accumulation over time.

The average Zn concentration in tobacco leaves varies from 981.6 mg/kg in NBCu108F3 to 1076.6 mg/kg in NB104F3. According to Jones et al. (1991) and Campbell (2000) 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. Symptoms of zinc toxicity which manifest themselves as chlorosis and necrosis at the edges of the leaves, inter-veinal chlorosis in young leaves, plant growth arrest as a whole, damage to the roots, were not observed as well. The concentration of zinc is higher in the upper leaves, in comparison with the leaves of the lower and middle leaves with the exception for NB104F3.

The distribution of heavy metals in the organs of enhanced tobacco has selective character specific for individual elements. Cd levels occur in the order, lower leaves > middle leaves > upper leaves > stalks > roots, Pb - middle leaves > lower leaves > upper leaves > stalks > roots, and Zn- upper leaves > middle leaves > lower leaves > stalks > roots (Table 3).

For comparing phytoextraction efficiencies of enhanced tobacco, translocation and bioconcentration factors were calculated. The translocation Factor (TF=Cshoots/Croots) provides information on the ability of plants to uptake heavy metals through the roots and to move them to the above-ground mass (leaves). The translocation factor for all metals is greater than 1. The results we obtained show that, with respect to Pb, the translocation factor for plants varies from 7.7 to 14.8, for Cd from 4.1 to 6.2 and for Zn from 5.4 to 10.1 (Table 4).

Table 4. Translocation (TF) and Bioconcentration factors (BCF roots, BCF shoots) in enhanced tobacco (NBCu108F3, NB104F3, BAGF3).

	TF			BCF		
	NBCu108F3	NB104F3	BAGF3	NBCu108F3	NB104F3	BAGF3
Pb	13.7	14.8	7.7	1.1	1.3	1.2
Zn	10.1	8.6	5.4	3.5	3.8	3.6
Cd	5.8	6.5	4.1	3.6	3.6	3.6

Translocation factor (TF=Cshoots/Croots),
Bioconcentration factor (BCFshoots=Cshoots/Cavailable soil concentration)

When compared with the BAGF3, the NBCu108F3 and NB104F3 showed an improved translocation from roots to shoots that was 2 times greater for lead and zinc and 1.5 times for cadmium, the bioconcentration factor (BCFshoot =Cshoots/Cavailable soil concentration) values reached to 1.1-1.3 for Pb, 3.5 - 3.8 for Zn and 3.6 for Cd. Higher values for Pb, Cd and Zn are probably a consequence of the greater ability of these elements to accumulate in the above-ground mass than in the roots, which is consistent with the results of Fässler et al. (2010) for Cd. The high concentration of Cd, Zn and Pb in the leaves and the high translocation factor indicate the possibility of enhanced tobacco to be used in phytoextraction of Pb, Cd and Zn from highly contaminated soil. Higher root to shoot translocation of investigated metals indicated that enhanced tobacco have vital characteristics to be used for phytoextraction of these metals. None of these varieties of enhanced tobacco was specified as a hyperaccumulator; nevertheless, all varieties show potential for phytoextraction of Pb, Zn, and Cd. Further studies are required to determine the utilization of residual products of the enhanced tobacco when growing on industrially polluted soils.

Conclusions

Based on the results obtained regarding 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. A clearly distinguished species peculiarity existed in the accumulation of heavy metals in the organs of enhanced tobacco. Generally, Cd levels occur in the order, lower leaves > middle leaves > upper leaves > stalks > roots,

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Pb - middle leaves > lower leaves > upper leaves > stalks > roots, and Zn - upper leaves > middle leaves > lower leaves > stalks > roots.

2. The high concentration of Cd, Pb and Zn in the leaves and the high translocation factor (TF Pb - 13.7-14.8, TF Zn - 8.8-10.1, TF Cd -5.5-6.5) indicate the possibility of enhanced tobacco to be used in phytoextraction of these metals.
3. The two varieties of enhanced tobacco NBCu108F3 and NB104F3 showed an improved translocation from roots to shoots than BAGF3, that was 1.8 times greater for lead and zinc and 1.5 times for cadmium.
4. The concentration of Cd in upper, middle, and lower leaves reached to 62.1-75.8 mg/kg, 120.9-135.2 mg/kg, 132.0-155.8 mg/kg; Pb - to 838.0-930.2 mg/kg, 1044.3-1403.1 mg/kg, 927.8-1074.5 mg/kg and Zn - to 1065.1-1147.9 mg/kg, 936.7-1173.0 mg/kg, 860.1-949.2 mg/kg, respectively. None of these varieties of enhanced tobacco was specified as a hyperaccumulator; nevertheless, all varieties show potential for phytoextraction of Pb, Zn, and Cd. Further studies are required to determine the utilization of residual products of enhanced tobacco when growing on industrially polluted soils.

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