ВЪЗМОЖНОСТИ НА НЯКОИ ЛЕЧЕБНИ И АРОМАТНИ РАСТЕНИЯ ЗА ФИТОРЕМЕДИАЦИЯ НА ЗАМЪРСЕНИ С ТЕЖКИ МЕТАЛИ ПОЧВИ
POTENTIAL OF SOME MEDICINAL AND AROMATIC PLANTS FOR PHYTOREMEDATION OF SOILS CONTAMINATED WITH HEAVY METALS

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Резюме

Проведено е сравнително изследване, което да ни позволи да определим количествата и депата на натрупване на Pb, Zn и Cd във вегетативните органи на някои лечебни и ароматни растения, в продуктите, получавани от тях, както и възможностите за използването им за фиторемедиация. Съществува ясно очертана видова способност в натрупването на тежки метали от изследваните култури. Основната част от тежките метали се инхибира от корените на *Valeriana officinalis L.* и *Melissa officinalis L.* и много малка част от тях се придвижва към надземните им части, докато при *Centranthus ruber L.*, *Nepeta cataria L.*, *Vinca minor L.* и *Datura stramonium L.* основната част се акумулира в надземните им части (стъбла и листа). Изследваните лечебни и ароматни растения може да се отглеждат на замърсени с тежки метали почви и имат потенциал за фиторемедиация.

Abstract

A comparative research has been carried out to allow us to (i) determine the quantities and the centers of accumulation of Pb, Zn and Cd in the vegetative organs of some medicinal and aromatic plants, as well as in their products, and (ii) evaluate the extent of tolerance and phytoextractability of selected plant species. A clearly distinguished species peculiarity existed in the accumulation of heavy metals in vegetative organs. *Valeriana officinalis L.* and *Melissa officinalis L.* accumulate heavy metals from the soil through the root system, and a great part of the heavy metals is inhibited by the roots and only a small quantity moves to the aboveground parts. In *Centranthus ruber L., Nepeta cataria L., Vinca minor L.* and *Datura stramonium L.* only a small part of the heavy metals is inhibited by the roots, and the great part of them is accumulated in the aboveground parts (stems and leaves). The obtained results show that the investigated medicinal and aromatic plants can be planted successfully on areas, contaminated by heavy metals and may have the potential for phytoremediation.

Ключови думи: фиторемедиация, тежки метали, лечебни и ароматни растения.
Key words: phytoremediation, heavy metals, medicinal and aromatic plants.

INTRODUCTION

Heavy metals are naturally present in the environment. They are harmful to humans, animals and tend to bioaccumulate in the food chain. Activities such as mining and smelting of metal ores, industrial emissions and applications of insecticides and fertilizers have all contributed to elevated levels of heavy metals in the environment (Alloway, 1994).

The clean-up of soils contaminated with heavy metals is one of the most difficult tasks for environmental engineering.Several technologies are available to remediate soils that are contaminated by heavy metals. Cleansing soil of heavy metals using traditional technologies such as excavation and chemical leaching of metals is expensive (Salt et al., 1998). Use of green plants to remove heavy metals from the contaminated soils, known as phytoremediation, is an emerging technique that offers the benefits being in situ, low cost and environmentally sustainable (Salt et al., 1998; Ma et al., 2001). One of the strategies of phytoremediation of metal-contaminated soil is phytoextraction, i.e. through uptake and accumulation of metals into plant shoots which can then be harvested and removed from the site. Another application of phytoremediation is phytostabil-ization where plants are used to minimize metal mobility in contaminated soils.

Since phytoremediation is a long-term technology, it is imperative to keep fields undergoing phytoremediation productive to achieve economically viable and socially acceptable decontamination. If the contaminated biomass may be further proceed for added value products (not only concentrated on deposits of hazardous wastes), then such fact represents an improvement of economical efficiency of phytoremediation technology.

Medicinal plants appear to be a good choice for phytoremediation since these species are mainly grown for secondary products (essential oils) thus the contamination of the food chain with heavy metals is eliminated. Researches on certain medicinal and aromatic plants (mint, lavander, thyme, st. john's wort, pot marigold, hollyhock, garden sorrel, black nightshade sp. etc) showed that they can be more resistant to some heavy metals and other pollutants than other crops. Investigations has shown that heavy metals accumulated by aromatic and medicinal plants do not appear in the essential oil (Scora and Chang, 1997; Zheljazkov and Nielsen, 1996; Angelova et al., 2005) and that some of these species are able to grow in metal contaminated sites without significant yield reduction.

The aim of this experiment was to carry out a comparative research, which to allow us to (i) determine the quantities and the centers of accumulation of Pb, Zn and Cd in the vegetative organs of some medicinal and aromatic plants (*Valeriana officinalis L., Centranthus ruber L., Nepeta cataria L., Melissa Officinalis L., Vinca minor L. and Datura stramonium L.*)., as well as in their products, and (ii) evaluate the extent of tolerance and phytoextractability of the selected plant species.

MATERIALS AND METHODS

1.Soils

Soils used in this experiment were sampled from the vicinity of the area contaminated by the Non-Ferrous-Metal Works (NFMW) near Plovdiv, Bulgaria. Soils were collected from the surface (0-20 cm depth) of fields located at 0.1 km from the NFMW. The test soils were alluvial meadow soils with 37.6% clay, 2.2% humus, 3.85% $CaCO_3$ and pH 7.4. The soil samples were air dried, homogenized in an agitate mortar and sieved. A fraction with particle size < 1 mm was taken for analysis.

2.Plants

In the study were included medicinal and aromatic plants (*Valeriana officinalis L., Centranthus ruber L., Nepeta cataria L., Melissa Officinalis L., Vinca minor L. and Datura stramonium L.*). The experimental plots were situated at 0.1 km from the source of pollution - the Non-Ferrous Metal Works near Plovdiv. The field tests were set after the block method in four replications. The plants were grown in accordance with the standard technology. 5 plants from each replications were used in the analysis.

On reaching flowering stage, the plants were gathered and the contents of Pb, Zn and Cd in their different parts - roots, stems and leaves - were determined. Under laboratory conditions we extracted essential oil from the roots of *Valeriana officinalis L.* and *Centranthus ruber L.* and above-ground parts of *Nepeta cataria L., Melissa Officinalis L., Vinca minor L.* and Datura stramonium L. by means of steam distillation. The contents of heavy metals (Pb, Zn and Cd) in the plant material (roots, stems and leaves) and in the essential oils were determined by the method of the dry mineralization.

3. Heavy metal analysis

3.1. Soils.

3.1.1. Total content of heavy metals

Total content of heavy metals in soils was determined after the soil sample was decomposed on a sand bath heater for 3 h with 21 ml of concentrated HCl + 7 ml of concentrated HNO_3. After cooling the sample, the residue was transferred into a 50-ml flask, and water was added to the mark.

3.1.2. Available forms of heavy metals

For extraction of the available for plants heavy metals we used the following extractant 0.005 M diethylentriaminepentaacetic acid (DTPA) +1.0 M NH_4HCO_3, pH 7.6 (Soltanpour and Schwab, 1977).

Soil samples were shaken for 15 minutes at 20°C. After shaking, the soil-solution system was centrifuged and filtered. The ratio soil to liquid was 1:2 by weight to volume.

3.2. Plants

A 1 g sample was weighed into a quartz crucible and put into a furnace (400°C) until ashing occurred. After cooling to a room temperature 1 ml HNO_3 (1:1) was added, evaporated in a sand bath and put again into the furnace (400°C). The procedures were repeated until the ash was white. It was finally dissolved in 2 ml 20% HCl (v/v), transferred in a graduated 10 ml flask and brought to volume with doubly distilled water.

3.3. Oil

A 10-g sample was weighed into a quartz crucible and put into a furnace (400°C) for 2 hours. After cooling to a room temperature, the residue was dissolved in 2 ml 20 % HCl (v/v), transferred in a graduated 10 ml flask and brought to volume with doubly distilled water.

3.4. Heavy metal determination

To determine the heavy metal content in the samples, inductively coupled emission spectrometer (Jobin Yvon Emission - JY 38 S, France) was used (20). The working wave lengths were as follows: Zn - 213.9 nm; Pb - 220.4 nm; Cd - 214.4 nm.

RESULTS

1. Soils

The results given in table 1 showed that with increasing the depth of the horizon a well exhibited tendency for reducing the content of heavy metals in the soil was observed.

In the soil samples taken from the region situated at 0.1 km from the NFMW were established values for Pb exceeding the maximum permissible concentration (80 mg/kg) - 913.5 mg/kg in the layer 0-20 cm and 724.8 mg/kg in the layer 20-40 cm. Analogous were the results for Cd and Zn. In the region of the NFMW were established 1903.8 mg/kg Zn and 26.2 mg/kg Cd, whose values exceeded considerably the maximum permissible concentrations. With increasing the depth of the soil horizon the contents decreased more intensively (up to 1467.9 mg/kg Zn and 20.0 mg/kg Cd). In Table 1 there are also specified the obtained results from the mobile forms of lead, zinc and cadmium, presented in the soil test. The obtained results indicate that DTPA extracts up to 43.1% of the total quantity Cd, less Pb (26.9%), and Zn (15.8%).

2. Medicinal and aromatic plants

As can be seen in Table 2 considerable differences were found in the distribution of the metals in the separate parts of the plants.

Metal concentrations in plants vary with plant species (Alloway et al, 1994). Plant uptake of heavy metals from soil occurs either passively with the mass flow of water into the roots, or through active transport crosses the plasma membrane of root epidermal cells. Under normal growing conditions, plants can poten-tially accumulate certain metal ions an order of magnitude greater than the surrounding medium (Kim et al., 2003). The combination of elevated soil pH and moderate organic matter in the study site may have played a role in the limited plant availability of heavy metals in the soil, resulting in low plant uptake of these metals (Jung and Thornton, 1996; Rosselli et al., 2003).

Pb concentrations in the plants ranged from 4.7 mg/kg in the roots of *Datura stramonium L.* to 48.5 mg/kg, with the maximum being in the roots of *Valeriana officinalis L* (Table 2). In addition, the roots of *Melissa officinalis L.* and *Nepeta cataria L.* also contained significant amounts of Pb (38.2 and 28.8 mg/kg). Cadmium concentrations in the plants varied from 0.36 mg/kg in the roots of *Datura stramonium L.* to 1.67 mg/kg, with the maximum being in the roots of *Vinca minor L.* In addition to *Vinca minor L.,* the roots of *Melissa officinalis L., Valeriana officinalis L.* and *Nepeta cataria L.* also contained significant amounts of Cd (0.82–1.4 mg/kg). The Zn contents in the plants ranged from 15.9 mg/kg in the roots of *Datura stramonium L.* to 98.8 mg/kg (Table 4). Like Pb the maximum values were again found in the roots of *Valeriana officinalis L.* Also the roots of *Melissa officinalis L.* and *Nepeta cataria L.* contained significant amounts of Zn (71.8–91.3 mg/kg). The obtained results could be explained with the anatomic and biologic peculiarities of the plants. The greater part of the heavy metals, that had entered the soil, are fixed and accumulated in the roots of *Valeriana officinalis L, Vinca minor L.*, *Melissa officinalis L.* and *Nepeta cataria L.*, as these plants formed a shallow root system. The lower values, established in datura, were in correlation with the deeply penetrating tap root systems.

The obtained results showed that the movement and the accumulation of the heavy metals in the vegetative organs of the studied plants differed considerably. In *Melissa officinalis L.* and *Valeriana officinalis L.* the root Pb concentrations were much greater than those of the shoot Pb contents, indicating low mobility of Pb from the roots to the shoots and immobilization of heavy metals in roots. Similar to Pb, Cd and Zn concentrations were greater in the roots of *Valeriana officinalis L.* and *Melissa officinalis L.* than the shoots. However, it is observed a contrary tendency in the *Nepeta cataria L., Datura stramonium L, Centranthus ruber L.* and *Vinca minor L.* Probably via the conductive system the heavy metals had been moved to

Таблица 1. Съдържание на Pb, Zn и Cd (mg/kg) в почвите
Table 1. Content of Pb, Zn and Cd (mg/kg) in soils

Дълбочина Depth, cm	Pb x±sd		Zn x±sd		Cd x±sd	
	Общо Total	DTPA	Общо Total	DTPA	Общо Total	DTPA
0-20	913.5±6.0	245.6±2.5	1903.8±4.7	338.9±1.8	26.9±0.2	11.6±0.1
20-40	724.8±5.1	211.9±2.0	1467.9±3.2	298.7±1.4	20.0±0.2	8.2±0.1
ПДК/МРС	**80**		**340**		**2.5**	

x - средна стойност (mg/kg) от измерването на 5 успоредни проби; sd – средно стандартно отклонение;
ПДК – пределно допустими концентрации
x - average value (mg/kg) from 5 repetitions; sd - mean standard deviation
MPC - maximum permissible concentration (approved for Bulgaria)

Таблица 2. Съдържание на Pb, Zn и Cd (mg/kg) в изследваните растения и масла
Table 2. Content of Pb, Zn and Cd (mg/kg) in plants and oil

Растение Plant	Корени/Root x±sd	Стъбла/Stems x±sd	Листа/Leaves x±sd	Масло/Oil x±sd
Pb (ПДК масло/MPC oil – 0.1 mg/kg)				
Melissa officinalis L.	38.2±1.2	14.2±0.4	25.4±1.0	nd
Nepeta cataria L.	28.8±1.0	64.7±2.0	137.2±5.0	0.05±0.002
Valeriana officinalis L	48.5±1.5	8.0±0.4	16.98±1.0	0.05±0.002
Centanthus ruber L.	10.7±0.4	99.1±3.4	343.6±6.1	nd
Datura stramonium L.	4.7±0.5	19.7±1.0	120.2±3.8	0.05±0.002
Vinca minor L.	11.4±1.0	52.8±2.0	200.9±3.5	0.05±0.002
Cd (ПДК масло/MPC oil - 0.05 mg/kg)				
Melissa officinalis L.	0.99±0.03	0.21±0.01	0.44±0.03	nd
Nepeta cataria L.	1.36±0.1	2.70±0.2	5.69±0.2	0.01±0.001
Valeriana officinalis L	0.82±0.03	0.07±0.01	0.38±0.01	0.02±0.001
Centanthus ruber L.	0.38±0.01	3.45±0.2	7.31±0.3	nd
Datura stramonium L.	0.36±0.01	1.45±0.1	4.76±0.1	0.02±0.001
Vinca minor L.	1.66±0.1	4.15±0.3	4.98±0.1	0.02±0.001
Zn (ПДК масло/MPC oil - 10 mg/kg)				
Melissa officinalis L.	71.8±0.8	30.3±0.2	39.7±0.3	1.5±0.1
Nepeta cataria L.	91.3±0.9	151.5±1.0	321.4±3.5	2.4±0.1
Valeriana officinalis L	98.8±1.2	21.5±0.1	26.4±0.3	2.1±0.1
Centanthus ruber L.	31.2±0.3	170.3±1.5	202.2±3.4	0.9±0.1
Datura stramonium L.	15.9±0.3	49.4±0.3	234.5±3.3	2.1±0.1
Vinca minor L.	55.0±0.5	145.5±1.8	143.7±1.5	1.9±0.1

x - средна стойност (mg/kg) от измерването на 5 проби; sd – стандартно отклонение
x - average value (mg/kg) from 5 repetitions; sd - mean standard deviation

Таблица 3. Акумулиране и придвижване на Pb, Cu, Zn and Cd (mg/kg) в изследваните растения
Table 3. Accumulation and translocation of Pb, Cd and Zn in selected plants

Растение Scientific name	Фактор на биоконцентрация Bioconcentration factor (BCR)*			Фактор на транслокация Translocation factor (TF)*		
	Pb	Cd	Zn	Pb	Cd	Zn
Melissa officinalis L.	0.042	0.037	0.038	**1.036**	0.658	0.974
Nepeta cataria L.	0.032	0.051	0.048	**7.01**	**6.17**	**5.18**
Valeriana officinalis L	0.053	0.030	0.047	0.515	0.539	0.539
Centanthus ruber L.	0.012	0.014	0.016	**41.37**	**28.54**	**11.95**
Datura stramonium L.	0.005	0.013	0.008	**29.77**	**17.25**	**17.86**
Vinca minor L.	0.012	0.062	0.029	**22.25**	**5.5**	**5.26**

**BCR=съдържание на метала в корените/съдържание на метала в почвата*
TF=съдържание на метала в надземната маса/съдържание на метала в корените
**BCR =metal concentration ratio of plant roots to soil and TF=metal concentration ratio of plant shoots to roots*

the above-ground parts of these plants and were being predominantly accumulated there. Pb concentrations in the plants ranged from 16.98 mg/kg in the leaves of Valeriana officinalis L. to 343.6 mg/kg, with the maximum being in the leaves of *Centanthus ruber L.* (Table 2). In addition, the leaves of *Datura stramonium L., Nepeta cataria L.* and *Vinca minor L.* also contained signif-icant amounts of Pb (120.2 mg/kg, 137.2 mg/kg and 200.9 mg/kg). None of the plant species accumulated Pb above 1000 mg/kg in the shoots, the criteria for a hyperaccumulator (Baker and Brooks, 1989).

Cadmium concentrations in the plants varied from 0.38 to 7.3 mg kg. Like Pb the maximum values were again found in the leaves of *Centranthus ruber L..* In addition to *Centranthus ruber L,* the leaves of *Datura stramonium L., Vinca minor L.* and *Nepeta cataria L.* also contained significant amounts of Cd (4.76 – 5.76 mg/kg). None of the plant species accumulated Cd above 100 mg/kg in the shoots, the criteria for a hyperaccumulator. The Zn contents in the plants ranged from 26.4 to 321.4 mg/kg (Table 2). The maximum values were found in the leaves of *Nepeta cataria L.* and no plant species accumulated Zn above

10000 mg/kg. Also the leaves of *Centranthus ruber L.* and *Datura stramonium L.* contained significant amounts of Zn (202.2-234.5 mg/kg).

Accumulation in the leaves was likely due to the uptake of heavy metals (from the contaminated soil) by roots and the movement of the heavy metals through the conductive system, as well as due to airborne heavy metals that fall onto the leaves as a result of their dispersion into the air by aerosols. Their stronger accumulation in *Centranthus ruber L.* was probably due to the fact that the leaves of centransus were with wax coating in contrast with *Valeriana officinalis L.*, which distinguishes by smooth leaves, which contributed to the fixing of the aerosol pollutants and for their accumulation there.

In general, all three heavy metals occurred at elevated levels in aboveground parts of tested plants. Normal and phytotoxic concentrations of Pb, Zn and Cd were reported by Kabata-Pendias and Pendias (1992), which were 0.5-10 and 30-300 mg kg for Pb, 3-30 mg kg for Cd, and 10-150 and >100 mg/kg for Zn. Almost all tested plant species showed heavy metal concentra-tion higher than the normal or phytotoxic levels. These results may indicate that these plants were tolerant of these metals.

The content of heavy metals in essential oils of selected aromatic and medicinal plants was also determined. The obtained results showed that the main part of the heavy metals contained in the roots of *Valeriana officinalis L.* and *Centranthus ruber L.* and above-ground parts of Nepeta cataria L., *Melissa Officinalis L., Vinca minor L.* and *Datura stramonium L.* was not transferred in the oil during the processing, due to which their content in the oil was considerably lower. The heavy metals content in the essential oil were very low and near the detection limits of the techniques used (ICP). The Pb contents in the essential oil reached to 0.05 mg/kg, Cd to 0.02 mg/kg and Zn to 2.4 mg/kg. The obtained results show that the quantities of Pb, Zn and Cd in essential oil are lower than the accepted Maximum Permissible Values and they meet the requirements for ecologically clean product. The obtained results matched well with those from Zheljazkov and Nielsen (1996), who found that the contents of heavy metals in the essential oils were very low and were not affected by the level of soil pollution with heavy metals.

3. Accumulation and translocation of metals in plants

In this study, none of the plant species showed metal concentrations > 1000 mg/kg for Pb, 10000 mg/kg and Zn and >100 mg/kg for Cd in the shoots (Table 2), i.e. none of them are hyperaccumulators (Baker and Brooks, 1989). However, the ability of these plants to tolerate and accumulate heavy metals may be useful for phytoremediation. Both bioconcentration factors (BCF) and translocation factors (TF) can be used to estimate a plant's potential for phytoremediation purpose.

A plant's ability to accumulate metals from soils can be estimated using the BCF, which is defined as the ratio of metal concentration in the roots to that in soil. A plant's ability to translocate metals from the roots to the shoots is measured using the TF, which is defined as the ratio of metal concentration in the shoots to the roots. The process of phytoextraction generally requires the translocation of heavy metals to the easily harvestable plant parts, i.e. shoots. By comparing BCF and TF, we can compare the ability of different plants in taking up metals from soils and translocating them to the shoots. Tolerant plants tend to restrict soil—root and root-shoot transfers, and therefore have much less accumulation in their biomass, while hyperaccumulators actively take up and translocate metals into their aboveground biomass. Plants exhibit-ing TF and particularly BCF values less than one are unsuitable for phytoextraction (Yoon, 2006). Only those species that show values of BCFs and TFs greater than one, have the potential to be used for the phytoextraction (Yoon et al., 2006). In this present work, many species have TF greater than one. None of the plants were suitable for the natural phytoextraction because no hyperaccumulator was identified. Although no heavy metal hyperaccumulators were found, heavy metal-tolerant species with high TF can be used for induced phytoextraction of contam-inated sites. Examples of such plants in our study included *Centranthus ruber L., Nepeta cataria L., Vinca minor L. and Datura stramonium L.* The plants most effective in the translocation of metals are *Valeriana officinalis L.* (TF=11.9), *Centranthus ruber L.* (TF=11.9) and *Datura stramonium L.* (TF=17.9) for Cd*, Datura stramonium L. (TF= 17.3) and Centanthus ruber L.* (TF=28.5) for Zn, Vinca minor L. (22.25), *Datura stramonium L.* (TF=29.8) and *Centanthus ruber L.* (TF=41.4) for Pb. (Table 3).

Heavy metal-tolerant species with low TF can be used for phytostabilization of contam-inated sites. Examples of such plants in our study included *Melissa officinalis L.* and *Valeriana officinalis L.* (Table 3). Phytostabilization can be used to minimize migration of contaminants in soils (Salt et al.,1998). This process uses the ability of plant roots to change environmental conditions via root exudates. Plants can immobilize heavy metals through absorption and accumulation by roots, adsorption onto roots, or precipitation within rhizosphere. This process reduces metal mobility and leaching into ground water, and also reduces metal bioavailability for entry into the food chain. One advantage of this strategy over phytoextraction is that the disposal of the metal-laden plant material is not required (Salt et al., 1998).

CONCLUSION

A clearly distinguished species peculiarity existed in the accumulation of heavy metals in vegetative organs of the studied plants. *Valeriana officinalis L.* and *Melissa officinalis L.* accumulates heavy metals from the soil through the root system, and a great part of the heavy metals is inhibited by the roots and only a small quantity moves to the surface sections. However, in *Centranthus ruber L., Nepeta cataria L., Vinca minor L.* and *Datura stramonium L.* only a small part of the heavy metals is inhibited by the roots, and the great part of them is accumulated in the aboveground parts (stems and leaves).The obtained results show that investigated medicinal and aromatic plants can be planted successfully on areas, polluted by heavy metals. The studed crops represent a good compromise, where accumulation of toxic metals is comprensated by the possibility of processing of contaminated biomass. Favorable is also the fact that heavy metals do not influence the development of the studied crops, as well as on the quality and quantity of the oils obtained from them. The possibility of further industrial processing will make *Valeriana officinalis L., Centranthus ruber L., Nepeta cataria L., Melissa Officinalis L., Vinca minor L. and Datura stramonium L.* economically interesting crops for farmers of phytoremediation technology.

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