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Use of induced phytoextraction for elimination of cadmium content in soil*

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ABSTRACT

We used induced phytoextraction to follow the effect of ethylene glycol tetraacetic acid (EGTA) on increase of cadmium transfer from contaminated soil ($5mg_{Cd}\cdot kg^{-1}$ and $10mg_{Cd}\cdot kg^{-1}$) to maize plants (*Zea mays* L.). In the pot experiments, uptake of cadmium into roots and green parts increased after application of the chelating agent. Addition of $6mmol_{EGTA}\cdot kg^{-1}$ affected 90% of Cd increase in roots in comparison with EGTA non-treated variant. In green parts, addition of 6 and $12mmol_{EGTA}\cdot kg^{-1}$ resulted in 100% increase at the same level of Cd contamination of soil substrata. The efficiency of cadmium uptake by maize doubled after addition of $6mmol_{EGTA}\cdot kg^{-1}$ into contaminated soil. Application of $12mmol_{EGTA}\cdot kg^{-1}$ in the soil, contaminated with $10mg_{Cd}\cdot kg^{-1}$, resulted to twofold enhancement of cadmium uptake by the maize. The results showed that induced phytoextraction can decrease cadmium content in the soil to the level convenient for growing of hygienic clean crops.

INTRODUCTION

The area of Slovakia belongs to the long term polluted areas. Transfer of contaminants from large industrial and power plant complexes with regional impact on the accumulation of pollutants, resulted in the amounts of heavy metals, in soils that are on the edge or exceed their safe limits. This particularly influences arable soils and their ability to produce safe foodstuffs, which is endangered by accumulation of biotoxic elements (Hegedűsová et al. 2000).

Our previous studies showed that cadmium (Cd) appeared to be the most dangerous accumulative toxic metal in the Slovak territory (Hegedűsová et al. 2000, 2003, 2006).

Cadmium does not belong to essential elements but it is highly toxic for animals and humans. The element belongs to accumulative poisons and its content in organism increases during the life (Toman et al. 2003). Target organs of Cd accumulation in animals are kidney, liver, and reproductive organs (Kirkham 2006). Accumulation of Cd in animals and humans comes from its high migration ability within the food chain (Hunter et al. 1987).

Uptake and accumulation by crop plants then used in producing human and animal food is the main entry pathway for potentially health-threatening of toxic metals. Most of the discussion has been focused on the risk of cadmium, because this toxic metal has by far been the most widely studied in plants and algae (Clemens 2006).

In soil-plant system, Cd belongs to the metals whose ions are most readily taken up by plant roots. It has a higher propensity for accumulation in tissues other than the roots. Still, there is usually more Cd in roots than in leaves, and even less in fruits and seeds (Wagner 1993). This is a very important phenomenon, which has been studied in phytoremediation techniques for reduction of Cd content in contaminated soils.

Decontamination of areas polluted with toxic metals is one of the very important research topics. Cleaning up of the areas by conventionally used physico-chemical methods is financially very expensive and often non-ecological (Hegedűsová et al. 2009).

Modern decontamination technologies, generally called phytoremediation, offer the possibilities of removing of heavy metals from soils and water (Chen and Cutright 2001; Huang and Cunningham 1996; Lasat 2002; Simon 2004; Simon et al. 2003). The most often used phytoremediation techniques are phytostabilitation and phytoextraction. Phytostabilisation is the process in which metal tolerant plants are used to reduce the mobility of a heavy metal

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(Raskin 1997). In phytoextraction the plants absorb contaminants from soil through the root system and store them mainly in the green biomass and partially in roots (Hernandez-Allice et al. 2008; Salt et al. 1995).

Effectivity of phytoextraction process can be enhanced by application of various chelating agents, which increase the bioavailability of the element (Blaylock et al. 1997; Chen and Cutright 2001; Kos et al. 2003; Luo et al. 2005). The process is known as an induced phytoextraction. According to previous research, Blaylock et al. (1997) recommend application of ethylene glycol tetraacetic acid (EGTA) to increase the efficiency of phytoextraction of Cd from polluted soils.

The phytoextraction efficiency can be also enhanced using the plant species with larger biomass production (McGrath et al. 2002; Simon et al. 2003; Van Engelen 2007).

Nowadays, the content of cadmium in arable soils of southern Slovakia is on the edge of limit values set for agricultural soils. Especially in this area it is important to reduce Cd content to the level below the limit values, therefore phytoremediation using one-year plants with high biomass production and at least of medium ability to accumulate heavy metals is the best choice in solving the problem within a relatively short time.

Maize (*Zea mays* L.), a common and important agricultural crop worldwide, has been used in several studies of metal pollution including phytoremediation of Cd (Huang et al. 2006; Komárek et al. 2007; Li et al. 2009; Perriguey et al. 2008). However, maize is considered as a moderate accumulator of heavy metals, its advantages come from the production of a high plant biomass and from relative tolerance to a high heavy metal contamination (Li et al. 2009). Therefore we selected maize as an experimental plant for our purposes.

The aim of this work was to examine the efficiency of selected chelating agent – EGTA in decontamination of cadmium polluted soil, using the induced phytoextraction technique and maize (*Zea mays* L., variety Quintal) as an experimental plant.

MATERIALS AND METHOD

Model pot experiments to follow the induced phytoextraction of cadmium by maize were established in growth chamber in seven variants of experimental conditions with nine replications of each (Table 1). A non-contaminated soil, characterized by a very low content of metals, was used as the growing medium in amount of 400g per pot. The chelating agent EGTA was used in two different concentration levels: 6 and 12mmol·kg^{-1}. Cadmium was applied as a water solution of $CdSO_4$ four weeks before seeding by spraying of soil substrata with two different Cd solutions to achieve concentration levels of 5 and 10mg·kg^{-1}. Upper soil layer in the pots was sprayed with the mechanical sprayer, filled up with 100ml of Cd water solutions. Water capacity of soil was adjusted to 75%.

Maize was sown directly to the pots in amount of 7 seeds per pot. Growing conditions in the growth chamber were following: air temperature 20°C, relative air humidity 60–70%, photoperiod set to 12h light/12h dark, maximum light intensity 50,000lx.

After four weeks of growth, chelating agent EGTA was added to the pots with Cd addition. The growth experiment was finished after five weeks of the plants growth.

Table 1. Cadmium content in roots and aerial parts of maize subjected to different additions of Cd and EGTA.

Variant	Experimental conditions – additions into soil		Content of Cd in dry mass (mg·kg^{-1})			
	Cd (mg·kg^{-1})	EGTA (mmol·kg^{-1})	roots		aerial parts	
			x_m	s_d	x_m	s_d
Control	0	0	0.28	0.103	0.24^{a}	0.094
5/0	5	0	8.11^{b}	0.907	5.55^{b}	0.789
5/6	5	6	15.42^{c}	3.325	11.22^{d}	1.886
5/12	5	12	13.70^{bc}	2.598	11.19^{c}	2.249
10/0	10	0	27.25^{d}	4.004	9.01^{bc}	1.997
10/6	10	6	60.04^{e}	9.685	18.69^{d}	3.533
10/12	10	12	61.40^{e}	8.445	17.61^{d}	2.927

x_m – arithmetic mean, $^{a, b, c, d, e}$ – analysis of variance with one variable, Tukey *b* test – statistically significant difference was between the mean values, which have in the same group different indexes, significant level $p<0.05$, s_d – standard deviation, n = 9.

Plant parts (roots, aerial parts) and soil substrata were submitted for analysis after previous sample pretreatment, as follows: soil and plant samples were dried at 80°C to constant weight, dry mass was calculated and both plant and soil samples were milled to powder. Plant material was wet digested at 140°C in the mixture of HNO_3 and H_2O_2 in a high-pressure vessels (type ZA-1, JZD Pokrok Zahnašovice, Czech Republic) for 120min. Soil extract was prepared in aqua regia according to a technical standard STN ISO 11466:2001 Soil quality. Extraction of trace elements soluble in aqua regia.

We used atomic absorption spectrometry (ET-AAS) as a method for determination of total Cd content. Analysis was performed on SpectrAA 200 (Varian, Mulgrave, Australia) instrument, equipped with electrothermal atomization and deuterium background correction. For this technique, cadmium hollow cathode lamp operates at a wavelength of 228.8nm with a slit width set to 0.5nm and an electrodeless discharge lamp set at 4mA current with deuterium background corrector. The method was validated and limit of detection and limit of quantitation were $0.0045 mg\cdot kg^{-1}$ and $0.0107 mg\cdot kg^{-1}$, respectively.

Statistical analysis of the results has been done using the analysis of variance with one variable and with Tukey b test.

The bioconcentration factors and translocation factor (Mattina et al. 2003), as defined in equations (1), (2) and (3), were used to express the phytoextraction efficiency. Calculations were performed from Cd concentrations (c) in $mg\cdot kg^{-1}$ on a dry weight basis.

$$BCF_{a/s} = \frac{c_{aerial\ plant\ parts}}{c_{Soil}} \qquad (1)$$

$$BCF_{r/s} = \frac{c_{roots}}{c_{soil}} \qquad (2)$$

$$TF = \frac{c_{aerial\ plant\ parts}}{c_{roots}} \qquad (3)$$

The evaluation of bioconcentration and translocation factors was focused on following of Cd translocation efficiency from growing medium to plant and also on its distribution in plant parts.

RESULTS AND DISCUSSION

Determination of Cd in roots and aerial parts showed that the chelator EGTA had an impact on Cd transfer from soil to plant. Maize roots showed the ability to accumulate Cd in higher concentrations without application of chelating agent. Concentration of 8mgCd·kg-1 was found in maize roots when grown on soil with addition of $5mg_{Cd}\cdot kg^{-1}$. Addition of $10mg_{Cd}\cdot kg^{-1}$ resulted in a $27mg_{Cd}\cdot kg^{-1}$ in roots. Amount of Cd accumulated in aerial parts of the plants equaled a half of that observed in variants with the same Cd addition to soil but with the application of chelator.

Increasing Cd level in growing substrata in combination with EGTA application enhanced the content of Cd in roots and aerial parts of maize as compared to the variants without addition of chelator. Cd content in roots of maize grown on soil with $5mgCd\cdot kg^{-1}$ contamination significantly increased (1.9 times or 90 %) after application of $6mmol_{EGTA}\cdot kg^{-1}$, but the application of $12mmol_{EGTA}\cdot kg^{-1}$ did not result in a statistically significant increase (only 69%) in comparison to the case without EGTA treatment (Table 1).

Cd content in aerial parts of maize, grown in soil contaminated with $5mg_{Cd}\cdot kg^{-1}$, significantly increased (two-fold) after application of $6mmol_{EGTA}\cdot kg^{-1}$ as well as after addition of $12mmol_{EGTA}\cdot kg^{-1}$ (102 % increase). Cd content in roots and in aerial parts of maize, grown on soil with $10mgCd\cdot kg^{-1}$ contamination, significantly increased to the same level (two-fold) after applying of both EGTA concentrations in comparison with variant without EGTA treatment.

Enhanced accumulation of heavy metal in plants in the process of induced phytoextraction was followed in many studies, whose results confirmed that application of chelating agents enhance the mobility of metals and increases their accumulation in plants.

Biomass production

The production of maize biomass (roots and aerials parts: stems and leaves) and the content of its dry mass was followed after EGTA and Cd treatment. Comparison of weights of roots or aerial parts among treated and non-treated variants showed statistically non-significant difference between the variants, which differed in Cd and EGTA treatment.

Bioconcentration factors and translocation factor

Bioconcentration factor (BCF) is the indicator of Cd intake into plant parts. The calculation of the ratio showed that transfer of Cd from contaminated soil (5 and $10mg_{Cd}\cdot kg^{-1}$) to maize roots ($BCF_{r/s}$) and to aerial parts ($BCF_{a/s}$) increased approximately about 100% after application of 6 and $12mmol_{EGTA}\cdot kg^{-1}$, respectively (Table 2). The intensity of Cd transfer from maize roots to aerial parts was similar after treatment with both concentrations of EGTA. The lower concentration of the chelator ($6mmol_{EGTA}\cdot kg^{-1}$) was sufficient for the efficient induction of phytoextraction.

The highest values of Cd bioconcentration factors were observed in roots when the soil was contaminated with $10mg_{Cd}\cdot kg^{-1}$ and treated with EGTA. The values of translocation factor (TF) were lower in comparison to the bioconcentration

Table 2. Bioconcentration factors and translocation factors.

Variant	$BCF_{r/s}$	$BCF_{a/s}$	TF
5/0	1.62	1.11	0.68
5/6	3.08	2.24	0.73
5/12	2.74	2.23	0.82
10/0	2.72	0.90	0.33
10/6	6.00	1.86	0.31
10/12	6.14	1.76	0.29

$BCF_{r/s}$ – bioconcentration factor root-to-soil, $BCF_{a/s}$– bioconcentration factor aerial parts-to-soil, TF – translocation factor aerial parts-to-root.

Table 3. Content of cadmium in soil after harvesting of maize.

Variant	Content of Cd in soil ($\mu g \cdot kg^{-1}$)	
	x_m	s_d
Control	0.04	0.019
5/0	2.58	0.183
5/6	2.14	0.241
5/12	1.98	0.215
10/0	6.98	1.946
10/6	6.34	1.082
10/12	5.98	1.166

x_m – Arithmetic mean; s_d – standard deviation.

Table 4. Cadmium distribution in maize.

Variant	Cadmium distribution in the maize yield*			
	roots		aerial parts	
	μg_{Cd}**	%	μg_{Cd}**	%
Control	1.10	68.75	0.50	31.25
5/0	47.10	85.00	8.30	15.00
5/6	102.20	84.95	18.10	15.05
5/12	83.80	88.30	11.10	11.70
10/0	213.50	94.50	12.90	5.50
10/6	370.70	95.00	19.50	5.00
10/12	545.10	95.40	26.00	4.60

* Calculated on yield per one experimental pot; ** – total Cd in yield per experimental pot. Values were calculated from achieved concentration of Cd in plant material and distribution of total Cd was expressed in percentage.

factors. The calculations of translocation factor showed that the concentration of $12 mmol_{EGTA} \cdot kg^{-1}$ enhanced Cd transport to aerial parts at about 20% after Cd treatment by $5 mg_{Cd} \cdot kg^{-1}$. Moreover, translocation factors were the lowest at the contamination level of $10 mg_{Cd} \cdot kg^{-1}$ (0.31 and 0.29).

Cadmium distribution in the plant

Considering the biomass produced in each experimental pot, higher Cd levels (approximately 85% of all uptake of Cd) were found in roots of variants with contamination level of

$5mg_{Cd} \cdot kg^{-1}$. In variants with contamination level of $10mg_{Cd} \cdot kg^{-1}$ the achieved Cd accumulation in roots was about 95% of the total uptake of Cd.

However, when the EGTA was over-supplied, all amounts of the applied cadmium transfer did not transfer to the plant but stayed in soil. The results of total cadmium content in soil after performing the experiments are in Table 3.

Cadmium probably did not transfer to aerial parts of maize, therefore green parts of plants accumulated about 15% of Cd in variants with $5mg_{Cd} \cdot kg^{-1}$ and only 5% of Cd after addition of $10mg_{Cd} \cdot kg^{-1}$ (Table 4).

EGTA was applied two weeks before harvesting of yield, at the time when root system was well developed. The addition of total amount of chelator was performed in two steps. The solution was applied in the amounts which infiltrated only into uppermost soil layer, which represents arable layer of agricultural soils.

The reason of low accumulation of Cd in the aerial parts could be in growth period. Under controlled conditions in growth chamber this period was considerably shorter than the growth in field conditions.

CONCLUSIONS

Despite the fact that in recent years the amount of emissions in Slovakia decreased as a result of system precautions, the problem of heavy metal pollution of the environment still exists. Hygienic safety of agricultural soils was the basic assumption for growth of safe foodstuffs.

The results from the study of induced phytoextraction process on maize plants (*Zea mays* L.) using the chelating agent EGTA allowed us to conclude the following:

- content of cadmium in maize plants (root and aerial biomass) in experimental variants with Cd addition increased after application of chelating agent. Uptake of cadmium to maize doubled in roots as well as in aerial parts after EGTA treatment in concentration levels of 6 and $12mmol_{EGTA} \cdot kg^{-1}$ and the increase of the Cd contents was significant. However, the application of $12mmol_{EGTA} \cdot kg^{-1}$ did not result in a significant effect on Cd content in plant in comparison with $6mmol_{EGTA} \cdot kg^{-1}$ treatment,
- leaching of synthetic chelator complexes of heavy metals into ground water can be avoided by application of appropriate amount of solution volume in at least two steps. By this simple stroke the most of chelator remains in arable layer of soil, where also the majority of plant root systems is located,
- our results showed that if the level of cadmium in soil does not exceed its indication level, the described method of phytoremediation could help to decrease Cd content in the soil to the level that is safe for growing of vegetables,
- the biomass obtained during the phytoremediation can be disposed using several techniques (e.g. biomass from industrial plants could be used as fuel (Kos et al. 2003) or it may be composted to reduce its volume (Chaney et al. 2007).

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