THE LEAD PHYTOEXTRACTION AND THE EDTA-INDUCED HYPERACCUMULATION. CYCLE I

FITOEXTRACȚIA PLUMBULUI ŞI HIPERACUMULAREA INDUSĂ DE EDTA

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***Abstract**: Phytoremediation is a new technology used for remediation the agricultural and industrially soils contaminated/polluted with heavy metals.*

One of the phytoremediation techniques, the so-called phytoextraction, is a method to extract heavy metals from contaminated/polluted soils using hyperaccumulators plants and to translocate them to shoots.

This case study presents the effects of ethilene diamine tetraacetic acid (EDTA) application as a potential amendment for an artificial contaminated soil with Pb to enhance the translocation capacity in the harvestable plant parts of maize.

The treatments consisted in the application of some Pb concentrations (1000 $mgPb \cdot kg^{-1}$, 2000 $mgPb \cdot kg^{-1}$, 3000 $mgPb \cdot kg^{-1}$) in combination with different levels of EDTA (expressed as molar ratio between EDTA and Pb).

The aim of EDTA added was to mobilise and increase metal accumulation in maize, Pb being one of the largest immobile heavy metals in soil.

Obtained data revealed the significant differences concerning the biomass and the height of the plants and the Pb contents in the leaves according to the treatment applied.

In the first experimental cycle were established the EDTA:Pb optimal rations soil needed to increase the lead bioaccessibility for each of the three loading degree with (1000 $mgPb \cdot kg^{-1}$, 2000 $mgPb \cdot kg^{-1}$, 3000 $mgPb \cdot kg^{-1}$).

***Rezumat**: Fitoremedierea, o tehnologie de depoluare relativ nouă, se adresează în principal problemelor legate de contaminarea terenurilor agricole sau a zonelor intens poluate datorită activității urbane şi industriale. Fitoremedierea prin fitoextracție este un procedeu de extracție şi acumulare a contaminanților/poluanților în țesuturile plantei considerată hiperacumulatoare – inclusiv în rădăcini şi partea aeriană.*

Lucrarea prezintă teste preliminare efectuate în laborator în scopul stabilirii efectelor aplicării EDTA (etilen diamin tetraacetic acid) ca potențial amendament pe un soil poluat artificial cu Plumb din punctul de vedere al capacității şi gradului de translocare a metalului în partea vegetală aeriană a plantelor.

S-a administrat Plumb în diferite concentrații (1000 $mgPb \cdot kg^{-1}$, 2000 $mgPb \cdot kg^{-1}$, 3000 $mgPb \cdot kg^{-1}$) asociat cu nivele diferite de EDTA (exprimat ca raport molar între EDTA şi Pb). Adaosul de EDTA a avut ca scop creşterea bioaccesibilității Plumbului, cunoscut din literatura de specialitate ca fiind unul din metalele grele, nu foarte accesibile.

În urma experimentelor s-a constatat că există diferențe asigurate statistic în ceea ce priveşte greutatea plantelor la recoltare, înălțimea lor şi conținutul de Plumb din acestea în funcție de tratamentul aplicat.

În ciclul I de experimentare au fost stabilite rapoartele optime EDTA:Pb necesare creşterii bioaccesibilității Plumbului pentru fiecare din cele trei grade de încărcare a soilului cu 1000 $mgPb \cdot kg^{-1}$, 2000 $mgPb \cdot kg^{-1}$ şi 3000 $mgPb \cdot kg^{-1}$.

***Key words:** phytoextraction, soil pollution, Pb, EDTA*
***Cuvinte cheie:** fitoextracție, poluarea* soilului, Pb, EDTA

INTRODUCTION

Soil contamination with heavy metals became a serious problem both in the high affected industrial areas and in the agriculture. This problem requires obligatory the remediation of polluted soils to keep healthy the environment.[1]

Phytoremediation is a new in research developing technology, addressing to the potential problems concerning lands contamination intensively polluted areas because of urban and industrial activities.

Generally, phytoremediation can be defined as the process to use the plants to improve environment quality. The principle of method is the extraction of heavy metals from soil by plants.[2] As a new approach, phytoremediation using phytoextraction, as a new technology to remediate the polluted soils, will be accepted if this processes can be more efficient than classic technologies.

Taking into account the utilization strategy of heavy metals phytoextraction from soils, there are two systems: the utilization of plants with natural capacity for heavy metals accumulation, so called „hiperaccumulators” and utilization of plants with high biomass such as: maize, wheat, bean, barley, rice, Indian mustard having a high phytoextraction capacity from the chemical point of view.[3]

Some extraction techniques of heavy metals from contaminated/polluted soils use chelating agents and their selection depends on many factors such as soils characteristics, type of pollutant, loading degree, stability coefficient of chelate-metal etc.[4]

Citric acid, EDTA, CDTA, DTPA, NTA etc. are the chelating agents studied for their ability to mobilize heavy metals and to increase accumulation in different plants.[5]

MATERIALS AND METHOD

The study represents preliminary tests concerning the elaboration of phytoextraction techniques.

The experiments have been set up in the laboratory using a Pb artificial contaminated soil (Chernozem) in 1kg soil/pot.

Maize has been used as test crop.

The treatments: Pb as $Pb(NO_3)_2$ (1000 $mg \cdot kg^{-1}$, 2000 $mg \cdot kg^{-1}$, 3000 $mg \cdot kg^{-1}$) and EDTA (etilen diamino tetraacetic acid) were applied before seeding, at the beginning of the experiments.

EDTA was applied into the soil in order to increase the bioavailability of Pb in the aboveground biomass of maize.

Chemical and physical indicators of soil samples are presented in Table 1 and Table 2.

These values are corresponding to a fertile soil, chernozem, with a normal content in heavy metals for this type of soil. Both the Pb content from soil maize shoots and from have been analysed by atomic absorption spectrometry. [6], [7] the laboratory experiments were achieved in 2 time cycles.

Cycle I represented the first stage consisting in 3 Pb concentration levels, 5 EDTA:Pb ratios (0; 0,5; 1; 2; 10) and 3 replicates in 16 variants (V17-V32).

Table 1

Chemical properties of the soil material (n=3)

	pH H_2O	C %	Nt %	P_{AL} $mg \cdot kg^{-1}$	K_{AL} $mg \cdot kg^{-1}$	Cu	Zn	Pb	Cd	Ni	Co	Mn
						$mg \cdot kg^{-1}$						
$\bar{x}$	6.84	2.30	0.255	17	140	27	83	25	0.3	34	10	761
DS	0.1227	0.0816	0.0367	2.449	24.49	2.160	7.257	2.160	0.0816	2.943	2.160	21.46
CV %	1.79	3.54	14.39	14.40	17.49	8.00	8.74	8.64	27.2	8.65	21.6	2.81

S_E	0.0709	0.0471	0.0212	1.4156	14.156	1.248	4.194	1.248	0.0471	1.701	1.248	12.40

$\bar{x}$ – mean; DS – standard deviation; CV – coefficient of variation; S_E – standard error.

Table 2

Physical properties of the soil material (n=3)

	Coarse sand (2,0–0,2 mm) % g/g	Fine sand (0,2–0,02 mm) % g/g	Silt (0,02–0,002 mm) % g/g	Clay (<0,002 mm) % g/g
$\bar{x}$	0.3`	33.1	30.7	35.9
DS	0.0816	2.222	0.5715	3.047
CV %	27.2	6.71	1.86	8.48
S_E	0.0471	1.284	0.3303	1.761

$\bar{x}$ – mean; DS – standard deviation; CV – coefficient of variation; S_E – standard error.

Cycle II represented the remnant effects using the same test plant (maize) and the conditions established at the end of Cycle I.

The duration of crop vegetation was 7 weeks for each experiment.

RESULTS AND DISCUSSIONS

Measurements of the heights of shoots, the biomass and the Pb accumulations in the leaves have been achieved after harvesting (Cycle I).

The maize plants did not rise at the ratio EDTA:Pb=10 for all Pb treatments, probably due to the high EDTA quantity.

Statistical data revealed differences concerning the weight of the leaves, the height of the shoots and the Pb content in the harvested plants related to the treatment applied.

1. *Experiment: maize – soil with one Pb concentration level (1000 mg Pb·kg^{-1}) and some EDTA contents (molar ratio 0; 0,5; 1; 2; 10)*.

Table 3 presents the aboveground biomass, the height of the maize shoots and the Pb contents on an artificial polluted soil with 1000 mg Pb·kg^{-1} and some contents of Chelating Agent (CA) in a molar ratio between 0 and 10.

Comparing the values of these parameters with the treatments, we can say that only up to EDTA:Pb=0,5 ratio the biomass did not significant decrease. In the same time, the Pb content in the leaves significant increased in all treatments.

Table 3

Biomass, height and Pb content of maize on a polluted soil with 1000 mg Pb·kg^{-1} and some EDTA contents

Treatment	Shoots weight (g)	Height (cm)	Pb (mg·kg^{-1} d.w.)
V17 – Control (Chernozem Fundulea)	5,4	28,0	6
V18: Soil + [1000 mgPb·kg^{-1} – EDTA (CA:Pb=0)]	9,2	30,3	27
V19: Soil + [1000 mgPb·kg^{-1}+EDTA (CA:Pb=0,5)]	3,5	24,3	200
V20: Soil + [1000 mgPb·kg^{-1} + EDTA (CA:Pb=1)]	1,2	17,3	375
V21: Soil + [1000 mgPb·kg^{-1} + EDTA (CA:Pb=2)]	0,7	7,7	990
DL 5% (Tukey Test)	**3,0**	**4,04**	**65**
Fisher Test	**	**	**

2. Experiment: maize-soil with one Pb concentration level (2000 mg Pb·kg^{-1}) and some EDTA quantities (molar ratio 0; 0,5; 1; 2,10)

On a background of 2000 mg Pb·kg^{-1} soil and some EDTA contents, the aboveground biomass distinct significantly decreased inclusively in the variant with EDTA:Pb=0,5 ratio simultaneously with the increasing of Pb concentration in the leaves for all variants (Table 4).

Table 4

Biomass, height and Pb content of maize on a polluted soil with 2000 mg Pb·kg^{-1} and some EDTA contents

Treatment	Shoots weight (g)	Height (cm)	Pb (mg·kg^{-1} d.w.)
V17 – Control (Chernozem Fundulea)	5.40	28.000	6
V23: Soil + [2000 mg Pb·kg^{-1} –EDTA (CA:Pb=0)]	5.03	30.000	44
V24: Soil + [2000 mg Pb·kg^{-1} +EDTA (CA:Pb=0.5)]	1.97	15.333	402
V25: Soil + [2000 mg Pb·kg^{-1} +EDTA (CA:Pb=1)]	0.80	3.000	564
V26: Soil + [2000 mg Pb·kg^{-1} +EDTA (CA:Pb=2)]	0.17	2.000	2774
DL 5% (Tukey Test)	**2.48**	**2.773**	**450**
Fisher Test	**	**	**

These conclusions can be used in an advanced experiment on a soil material loaded with 2000 mg Pb·kg^{-1} and with EDTA:Pb ratio lower than 0,5 and with maize as test crop.

3. *Experiment: maize-soil with one Pb concentration level (3000 mg Pb·kg^{-1}) and some EDTA quantities (molar ratio 0; 0,5; 1; 2,10).*

Table 5 presents a distinct significant decrease both of biomass (Fisher Test) and heights of the plants. Pb concentration maize shoots distinct significantly increased for all variants compared with control.

The biomass significantly decreased even at V29 variant (EDTA:Pb=0,5) and the Pb concentration significantly increased (>1053) from V3 variant (EDTA:Pb=1).

Considering this treatment and the results obtained the conclusion is that EDTA application can not influence the hyperaccumulation. The toxicity of 3000 mg Pb·kg^{-1} is too high and plants of maize do not tolerate this toxicity.

Table 5

Biomass, height and Pb content of maize on a polluted soil with 3000 mg Pb·kg^{-1} and some EDTA contents

Treatment	Shoots weight (g)	Height (cm)	Pb (mg·kg^{-1} d.w.)
V17 – Control (Chernozem Fundulea)	5.400	28.000	6
V28: Soil + [3000 mg Pb·kg^{-1} – EDTA (CA:Pb=0)]	5.533	29.667	75
V29: Soil + [3000 mg Pb·kg^{-1} + EDTA (CA:Pb=0.5)]	1.433	10.000	848
V30: Soil + [3000 mg Pb·kg^{-1} + EDTA (CA:Pb=1)]	0.466	3.333	1616
V31: Soil + [3000 mg Pb·kg^{-1} + EDTA (CA:Pb=2)]	0.133	1.000	2259
DL 5% (Tukey Test)	**3.041**	**2.858**	**1053**
Fisher Test	**	**	**

4. *Considering the experiment with 3 Pb concentrations at the same EDTA:Pb ratio (0,5)*, the experimental data indicated a significantly decrease of biomass and a distinct significantly decrease of maize shoots height (Table 6). Compared with control both the decrease of biomass and the decrease of shoots height are lower at 1000 mg Pb·kg^{-1} treatment than the other two treatments (2000 mg Pb·kg^{-1}, 3000 mg Pb·kg^{-1}). This is a good reason to

choose the treatment with 1000 mg Pb·kg^{-1} for the next step in our experiment to optimise the phytoextraction test.

Table 6

Biomass, height and Pb content of maize on a polluted soil with 1000, 2000 and 3000 mg Pb·kg^{-1} soil and the same ratio (EDTA:Pb=0,5)

Treatment	Shoots weight (g)	Height (cm)	Pb (mg·kg^{-1} d.w.)
V17 – Control (Chernozem Fundulea)	5.400	28.000	6
V19: Soil + [1000 mg Pb·kg^{-1} + EDTA (CA:Pb=0.5)]	3.533	24.333	200
V24: Soil + [2000 mg Pb·kg^{-1} + EDTA (CA:Pb=0.5)]	1.967	15.333	402
V29: Soil + [3000 mg Pb·kg^{-1} + EDTA (CA:Pb=0.5)]	1.433	10.000	848
DL 5% (Tukey Test)	**3.058**	**3.113**	**368**
Fisher Test	*	**	**

5. *At EDTA:Pb=1 ratio and 3 Pb concentrations (1000, 2000 and 3000 mg Pb·kg^{-1})*, Fisher Test showed a distinct significantly decrease both for biomass and shoots height (Table 7). The Pb concentrations in the shoots significantly increased for all treatments but the biomass distinct significantly decreased for all treatments compared with control. This is the reason to not consider the EDTA:Pb=1 ratio in the next test experiments.

Table 7

Biomass, height and Pb content of maize on a polluted soil with 1000, 2000 and 3000 mg Pb·kg^{-1} soil and the same ratio (EDTA:Pb=1)

Treatment	Shoots weight (g)	Height (cm)	Pb (mg·kg^{-1} d.w.
V17 – Control (Chernozem Fundulea)	5.400	28.000	6
V20: Soil + [1000 mg Pb·kg^{-1} + EDTA (CA:Pb=1)]	1.167	17.333	375
V25: Soil + [2000 mg Pb·kg^{-1} + EDTA (CA:Pb=1)]	0.800	3.000	563
V30: Soil + [3000 mg Pb·kg^{-1} + EDTA (CA:Pb=1)]	0.467	3.333	1615
DL 5% (Tukey Test)	**2.714**	**4.467**	**158**
Fisher Test	**	**	**

CONCLUSIONS

1. The statistical data reveale significant differences concerning crop development and the Pb accumulation in the maize shoots related to the treatment applied.

2. The Pb concentrations in the harvested shoots increased with EDTA concentrations due to the capacity of Chelating Agent to increase the solubility of Pb in soil and its absorption by plants.

3. For the next test experiment, on a chernozem with 1000 mg Pb·kg^{-1} loading degree, the molar EDTA:Pb ratio do not exceed 0,5 value. If the Pb loading degree of soil is about 2000 mg Pb·kg^{-1}, the molar EDTA:Pb ratio has to be less than 0,5.

4. The EDTA application does not influence hiperacumulation on a Chernozem with 3000 mg Pb·kg^{-1}. This high level concentration of Pb achieves negative effects on the plants.

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