

## PHYTOREMEDIATION OF SOILS BY CEREALS

Sorochan O.O., Shtemenko N.I.

Dniepropetrovsk National University, Board of Biophysics and Biochemistry  
13 Naukoviyy by-str., 49050 Dniepropetrovsk, Ukraine, phone: 8 0562 469280,  
e-mail: ashtem@a-teleport.com

### Abstract

Seeds of *Lolium perenne* L. (1) and *Festuca rubra* L.(2) were treated with growth regulator and planted on soils contaminated with salts of Zn, Pb, Cd and some salt mixtures. It was shown that during 15 days of germination quantity of Zn in primary leaves of 1 increased from to 243,75 % in comparison to control experiments and decreased in soil: in upper layer from 95,74 to 85,07, middle layer from 95,83 to 82,04, lower layer from 85,72 to 72,74 mkg/kg correspondingly. Approximately the same was shown for 2 and for other metals. Absorption of heavy metals by a plant was shown to be depended from growth stimulator pretreatment procedure and is connected with exudative activity of the roots of a plant. Specificity of the process of phytoextraction for each metal and for the type of a plant are speculated. Thus, it was shown that stimulation of root exudative activity by pretreatment with a growth regulator may be succesful in cleaning of soils.

### Introduction

Our previous works [1 - 3] showed that pretreatment of seeds of cultural and wild cereals with the growth stimulator – (unstechiometric mixture of Fe-succinate - FeSuc) before planting enhanced Zn, Pb and Cd ions phytoextraction, plant uptake and translocation of the metal ions from roots to green parts of tested plants. Detailed analysis of amino acid content of root exudates of different cereals planted in sterile conditions showed that wild specie differed much from that of of cultural species [4, 5]. In these works it was shown that during 15 days of germination quantity of some metal ions in primary leaves increased approximately in twice and decreased in soil, especially in upper layers. Stimulation of root exudative activity by pretreatment with a growth regulator was shown to be succesful in cleaning of soils that might be a good instrument for phytoremediation. This work tries to unite some data and to answer why wild cereals are good species to clean Zn-, Cd and Pb-rich soils due to the process amino acid – metal ions interaction.

### Materials and methods

Soil experiments. Seeds of *Festuca rubra* L. and *Lolium perenne* L. were treated with strong water solution of FeSuc before planting in soil of black earth type and in the same soil, which was previously treated with solutions of ZnSO<sub>4</sub> (650 mg per 1 kg of soil), PbNO<sub>3</sub> (360 mg per 1 kg of soil) and CdCO<sub>3</sub> (35 mg per 1 kg of soil). Control experiments with and without FeSuc and salts of metals were done in the same conditions. Cd, Zn and Pb in plants and in soil were measured by ICP-AES metod on spectrophotometer AAS-30 (Germany).

Sterile experiments. Seeds of several inbred lines, hybrid of *Zea mays* L., *Festuca rubra* L. and *Lolium perenne* L. were germinated in vessels with sterile sand during 15 days with following collection of root exudates on ion exchange resin column according to [1].

Amino acid analysis of primary leaves and exudates was performed on automatic amino acids analyzer AAA-339 (Czech) [4].

### Results

Optimal concentrations, conditions of pretreatment of seeds by solutions of FeSuc was experimentally chosen and led to increase of ability to germinate (energy of germination of treated seeds was higher). It is very important fact in the case of highly contaminated soils where remediants may not germinate at all. It is possible to demonstrate the process of plant uptake of metal ions enhanced by application of the growth regulator in soil experiments (Table 1).

Table 1: Quantity of metal ions in primary leaves of *Lolium perenne* L during growing on clean and contaminated with metal salts soils with application of FeSuc in % to control\*

| Conditions of experiments                | Quantity of metal ions in % to control |              |              |
|--|--|--------------|--------------|
|  | Zn                                     | Pb           | Cd           |
| 1. -                                     | 121,46±10,63                           | 141,85±13,34 | 122,53±10,99 |
| 2. ZnSO <sub>4</sub>                     | 243,75±19,35                           | 134,78±12,41 | 160,53±15,03 |
| 3. Pb(NO <sub>3</sub> )                  | 117,88±9,48                            | 121,76±10,72 | 151,85±14,62 |
| 4. ?d CO <sub>3</sub>                    | 109,33±7,99                            | 124,87±11,83 | 124,76±11,66 |
| 5. CdCO <sub>3</sub> + ZnSO <sub>4</sub> | 111,41±8,31                            | 108,68±7,01  | 139,01±12,98 |

?? As a control (100%) quantities of the certain metal ion in experiments without FeSuc were taken.

During growth of seeds of wild grasses in conditions of soil experiments, described above, quantity of amino acids in primary leaves increased in the most of experiments under influence of FeSuc and metal ions, that depended from a specie. It is impossible to study real exudation of amino acids in that experiments due to complexity of a natural soil (presence of microorganisms), that's why only results from sterile experiments will be speculated. Total dentativity (TD) of amino acids in exudates of cereals were calculated as a quantity of ionized groups in the molecule of each amino acid (2 – for Ala, 3 – for Asp, for example), multiplied on its quantity in % in the mixture, obtained in sterile experiments (Table 2).

Table 2: Total dentativity (TD) of amino acid in root exudates of cereals.

| Type of Poaceae           | TD           |
|---------------------------|--------------|
| Maize Pioneer hybrid 3978 | 243,09 ± 9,2 |
| Maize line DL 203 HL      | 238,42 ± 6,7 |
| Maize line DL 293 HL      | 245,71 ± 6,1 |
| Festuca rubra L.          | 272,30 ± 2,4 |
| Lolium perenne L          | 274,80 ± 3,6 |

## Discussion

Application of the growth regulator was very effective in the process of absorption of metal ions. Especially significantly the uptake of Zn was increased (table 1). It is well-known fact that wild cereals may accumulate Zn in quantities that exceed the quantity of the metal in soil, i.e. biological coefficient of absorption (BCA) for Zn by some types of wild cereal plants was >1 and reaches in some cases 1,8 – 1,9 [6, 7]. On highly contaminated with Zn salts soils under influence of FeSuc uptake of Zn by plant in our experiments (N 2) increased in ~ 2,5 times. Cd is the second and Pb the third metals in ability to accumulate in plants. But, their BCA are only 0,66 and 0,47 maximum and are highly dependent from type of soil, its pH and quantity of humic acids. Application of the growth regulator changes these values very much and made uptake of Cd more preferential and Pb nearer to that of Zn (N 5). Even more, in these experiments absorption of Cd is more than Zn. Thus, influence of FeSuc led not only to increase of metal ions but developed a mechanism that is not the same that in natural conditions of growth. It is impossible to explain these data only by activity of metallothioneins, but it seems to occur due to better ability of Cd and Pb to formate complexes with amino acids. Amino acids have functional groups that can act as sites of interaction with heavy metal ions in soil. Wild cereals had more polyfunctional amino acids in the exudates and larger values of TD. During growing on sterile sand application of FeSuc led to increase of total quantities of exudated amino acids in 1,5 - 2 times [1]. We guess that similar effect occurs in natural conditions too. Overexudation

of amino acids that is a very important fact which could explain altering of rhizosphere by in conditions of FeSuc application. As more polyfunctional substances are exudated, as more intensive is the process of modification of rhizosphere by enhancing mobility and bioavailability of metals [8].

The origin of amino acid incoming to soil from seeds during germination is not almost clear. Even physiological role of this process is an object of discussion. One point of view is that root exudation has an allelopathic role and that physiological function of a releasing pool is to have a place in ecological low [9]. It is very interesting to note, that we found species of cereals, that differs so much in this process and are so active in the exudation of amino acids under influence of growth regulation.

### Conclusions

Stimulation of growth by pretreatment of cereal seeds with the synthetic substance led to increase of amino acids exudation, changed mechanism of uptake of metal ions and clean the soil. Thus, phytoremediation holds great potential especially promising with elaboration of growth regulators application.

### References

1. N. Shtemenko, 1270, Free amino acids in root exudates of cereals during the process of growth stimulation and phytoremediation, Seventh International FZK/TNO Conference on Contaminated Soil, Leipzig, Germany, (September 18, 2000).
2. N. Shtemenko, 126, Free amino acids of primary leaves of Lolium perenne L. during growing on Zn-rich soils, ISEB 2001 Meeting Phytoremediation, Leipzig, Germany, (May 15, 2001).
3. O. Sorochan, 279, Metal phytoextraction by cereals, 7 International Symposium "Metal Ions in Biology and Medicine", Saint Petersburg, Russia (April 30, 2002) John Libbey Eurotext.
4. N. Shtemenko, Free amino acids on the early stages of maize grain germination, Physiology and biochemistry of cultural plants, **33/ 5**, 441, (2001).
5. N. Shtemenko, Free and bound amino acids in the grain of different genetic form maize, Physiology and biochemistry of cultural plants, **31/ 4**, 270, (1999).
6. A.Cabata-Pendias, Microelements in soil and plants, Mir, Moscow, USSR, (1986).
7. S.Kostishin, Agropyron perens L. as a model for metal absorption by plants, Physiology and biochemistry of cultural plants, **27/ 1**, 65, (1995).
8. D. Salt, Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants, Biotechnology, **13**, 468, (1995).
9. A. Grodzinsky, Basic of chemical interaction between plants. Nukova dymka, Kiev, (1975).