UDC 574.24

S.D. Atabayeva*, A.J. Beysenova, S.S. Kenzhebayeva, S.S. Asrandina

Al-Farabi Kazakh National University, Almaty, Kazakhstan

The peculiarities of lead and cadmium uptake by barley (*Hordeum vulgare* l.) in the presence of edta in the medium

Abstract

Phytoremediation is a cost-effective and safety technology for cleaning contaminated soils. For phytoextraction of toxic metal as lead (Pb) the main limiting factor is the limited solubility and uptake by plant roots. One of the ways to improve phytoavailability of Pb is the use of chelating agents such as EDTA. It was studied the effects of EDTA on Pb uptake by barley plants parts. The anionic form of Pb $[Pb\text{-}EDTA]^{2-}$ is absorbed in lower amounts by excised roots compared with roots of whole plants and compared with the cationic form (Pb^{2+}) of excised roots. It was established that the concentration of Pb in the roots and aerial parts of the whole plant in the presence of EDTA exceeds the content of this metal in excised roots and aboveground organs. Metal ions are absorbed by plant roots and aerial parts to a greater extent as compared with variants with excised roots and aerial parts. It is explained by participation of transpiration in the process of metal translocation. This confirms the significant role of transpiration in the absorption of anionic form of lead. Similar results in variants with cadmium are received.

Key words: barley, heavy metals, phytoremediation, EDTA, phytoextraction.

Introduction

The use of food for many years crops, grown on the soils, that contains dangerous amounts of toxic metals, can have negative consequences for human health due to the constant accumulation of heavy metals in the body. One of the developed areas of biotechnology is the phytoremediation of soils, contaminated by heavy metals. Phytoremediation is defined as the technology of using plants to clean contaminated soils, cost-effective and safety compared with other physical and chemical methods of cleaning. Depending on soil conditions and metal concentrations, the cost of treatment using plants (using only the sun's energy) may be only 5% of the expenditure required for other ways to restore ecosystems contaminated with metals [1]. For the effective development of phytoremediation, each item must be considered separately. It is required the agronomic approach, taking into account the physical and chemical properties of the metal, soil and genetic properties of plants [2]. The level of extraction of heavy metals from the soil depends on the biomass and metal concentrations in aerial parts. The main problem of phytoremediation is that hyperaccumulators have low biomass and small size of the leaves. The success of phytoremediation ultimately depends on the agronomic practices used on site [3]. The use of mineral fertileizers necessary for the greatest accumulation of biomass plants, respectively, to increase the amount of extractable metals [2, 4-6]. Metal uptake by plants may be limited by low solubility of metals in the soil [7, 8]. For toxic metals such as Pb in the main limiting factor is the limited solubility and uptake by plant roots. One way to induce solubility – decrease in pH [9-11]. Strong acidification of soils mobilizes Pb below the root zone. The another way to enhance the phytoextraction is the use of synthetic chelating agents. These components are associated with lead and keep the metal in soluble chelate complexes that are available to plants and transport within them [12].

Adding EDTA stimulated the accumulation of Pb in the above-ground organs of plants up to 1,6% [13]. In other studies with Indian mustard, subjected to the action of lead and EDTA in hydroponic environments, plants accumulate up to 1% of dry biomass [13]. Other synthetic chelators as HEDTA applied at a concentration of 2.0 g/kg at concentra-

*E-mail: sauleat@yandex.ru

tion of Pb in the soil 2.5 g / kg Pb increased the accumulation of lead in the shoots of Indian mustard from 40 to 10 600 g/kg. Blaylock et al. (1997) [14] notes that the addition of chelates is also possible for other metals. EDTA also stimulated Cd-, Ni-, Cu- and Zn-phytoextraction

The use of chelating agents such as EDTA can shed light on the mechanisms of entry of heavy metals in plants. It is assumed that the absorption of lead, copper, zinc and cadmium as two-valent metals is ion-exchange process with the release of calcium or protons in the solution [15]. This initial reaction at the root of the environment is important in the absorption of toxic metals from contaminated soil. In this context, the aim of our work was to study the absorption of lead ions in the presence of EDTA. It was studied the effect of lead and cadmium in the presence of EDTA on the metal content of excised roots and aerial parts in order to determine the effect of metal ions on metal-accumulating ability of plants.

The objects of our research were seedlings of barley (*Hordeum vulgare* L.). It is often used as a model object to study of metal accumulation capacity of plants in the presence of EDTA in the growth medium. The aim of our study was to reveal the differences between metal accumulation ability of plant roots and shoots in the presence of EDTA.

Materials and methods

Barley plants were grown in tap water in plastic bottles in volume of 5 L during 7 days. On 8-th day, plants were placed in solutions containing Cd-60 mg/L, Pb – 1000 mg /L. The experiments were conducted at several variants for each metal separately for determination of heavy metals content in the shoots – variant 1 – whole plants in a medium without the metal (control), variant 2 – whole plants in a medium, containing Cd-60 mg/L (for lead – Pb – 1000 mg /l), variant 3 – with excised roots (shoots without roots) in a solution containing Cd-60 mg / l (for the variant with lead – Pb – 1000 mg /L), variant 4 – plants with excised roots in a solution containing Cd-60 mg /L+ EDTA- 1 g/L (for the variant with lead- Pb-1000 mg /L); for roots – variant 1 – whole plants in a medium without the metal (control), variant 2 – whole plants in a medium, containing Cd-60 mg/L (for lead – Pb – 1000 mg/L), variant 3 – with excised shoots (roots without shoots) in a solution containing Cd-60 mg /L (for the variant with lead – Pb – 1000 mg /L), variant 4 – plants with excised shoots in a solution containing Cd-60 mg /L+ EDTA-1 g/L (for the variant with lead- Pb-1000 mg/L). At the 14-day plants were removed, in the plant organs contents of heavy metals (cadmium and lead) were determined.

To determine heavy metals in plant organs, plant samples were separated into aboveground organs and roots, dried for 3 h at t-105^0 C. The samples of plants were subjected to digestion in a mixture of nitric and sulfuric acids and analyzed for content of heavy metals on an atomic absorption spectrophotometer (AAS-1).

The contents of trace metals in shoots and roots were determined as described next. Plant samples (0.5 g) were digested in a mixture of 5 ml of 50% HNO_3 and 0.5 ml HCl at 95±5°C according to standards for operation procedures [16]. Samples were transferred to digestion block (section) at temperature 90±5^{o}C, closed by glass and heated without bringing to a boil for 10-15 minutes. Then they were cooled and added 5 ml of concentrated HNO_3, moved-in digestion block with 90±5^{o}C, closed by glass and heated without bringing to a boil for 30 minutes before the disappearance of brown fumes. Then the samples were cooled and added 2 ml of water and 3 ml of H_2O_2, continued heating up until the volume has been reduced to about 5 ml, removed from digestion blocks, allowed to cool, filtered, washed filter and added deionized water up to final volume to 50 ml. Proceeded to the analysis of samples, using the appropriate SOP.

The concentrations of metals in plants and soils were measured by atomic absorbtion spectrophotometry using an AAnalyst 300 (Perkin Elmer, Germany) [17].

Results and discussion

The effects of lead and cadmium in the presence of EDTA on the metal content in the excised roots and aerial parts in order to reveal the influence of type of metal ions on the metal-accumulating ability of plants was studied.

It was found that the lead content in the roots of whole plants in the variant without chelating agents ("Whole plant+Pb") is 1,6 times lower than in the variant with EDTA ("Whole plant +Pb+EDTA"). This is consistent with the literature that EDTA promotes greater availability of poorly soluble metals to plants and a greater accumulation of heavy metals by plants [9].

In the study of lead content in the variant with clipping aboveground organs revealed, that the highest lead content was in the roots of plants in the variant "Whole plant +Pb+EDTA" (Fig. 1). In this variant the content of lead in the roots was 40% more, than in the variant without the above-ground organs ("-" shoots) with Pb without EDTA (Roots+Pb) and 58% more in comparison with a variant without above-ground organs with Pb+EDTA . The lowest lead content was observed in the roots of barley plants in the variant "Pb+EDTA" (Fig.1).

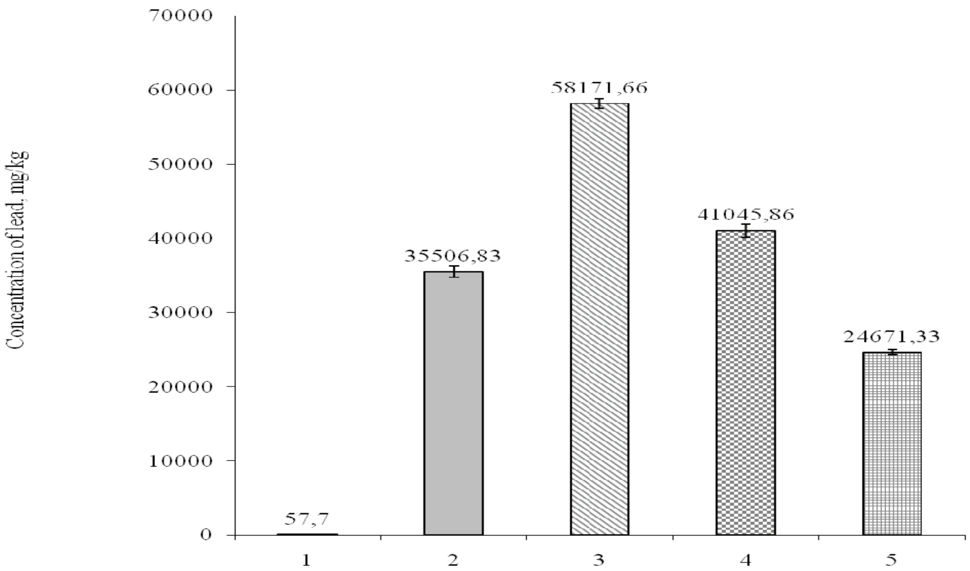

Fig.1 – Concentration of lead in the roots of *Hordeum vulgare* L. Note: 1-Control, 2-Whole plant+Pb, 3 – Whole plant +Pb+EDTA, 4 – Roots+Pb ("-" shoots), 5-Roots+Pb+EDTA

The study of lead content in plants with aboveground organs without roots showed the highest content of in the aboveground organs in the variant "Pb+EDTA" (Fig. 2), the least – in the shoots of variant " Whole plant + Pb" (Fig. 2).

The lead concentration in the aerial parts in the variant with Pb without EDTA was 5 times greater than in the shoots of the whole plant, growing in the same conditions (variant "Whole plant+Pb"). It is thought, that is because the lead is concentrated in the aboveground organs, bypassing the roots of plants, and in the variant with the whole plant it is a redistribution of lead between shoots and roots.

In the variant with aboveground organs without roots ("Shoots+Pb+EDTA") the lead content is exceeding 1,6 times compared with the lead content in the variant without EDTA ("Shoots+Pb" ("-" roots)) (59%) (Fig.2). This implies that the presence of EDTA enhances the absorption of lead by the aboveground organs.

Thus, the study of lead concentration in plants with excised roots and aerial parts showed that the mean of lead concentration in the presence of EDTA in the roots and aerial parts of the whole plant exceeds the concentration of this metal in excised roots and aboveground organs. In the presence of EDTA Pb is absorbed in lower amounts by excised roots compared with roots of a whole plant (24671.3 < 58171.66) and compared with the variant excised roots without EDTA (24671.3 < 41 015.86). It is known from the literature that the flow of divalent metal ions (cations) such as lead, copper, zinc and cadmium occurs in exchange for calcium ions and protons [15]. According to Crist et al. (2004) in the presence of EDTA metals in the medium are in the anionic form [metal-EDTA]$^-$ [15]. Metal-EDTA complexes are absorbed mainly through nonselective apoplasmic way [18]. The transpiration of water from leaves which is the main driving force for the movement of water in xylem. Water and dissolved substances move from the xylem across the leaf to the air spaces by the apoplast and symplast and then evaporates through the stomata (transpiration) [19].

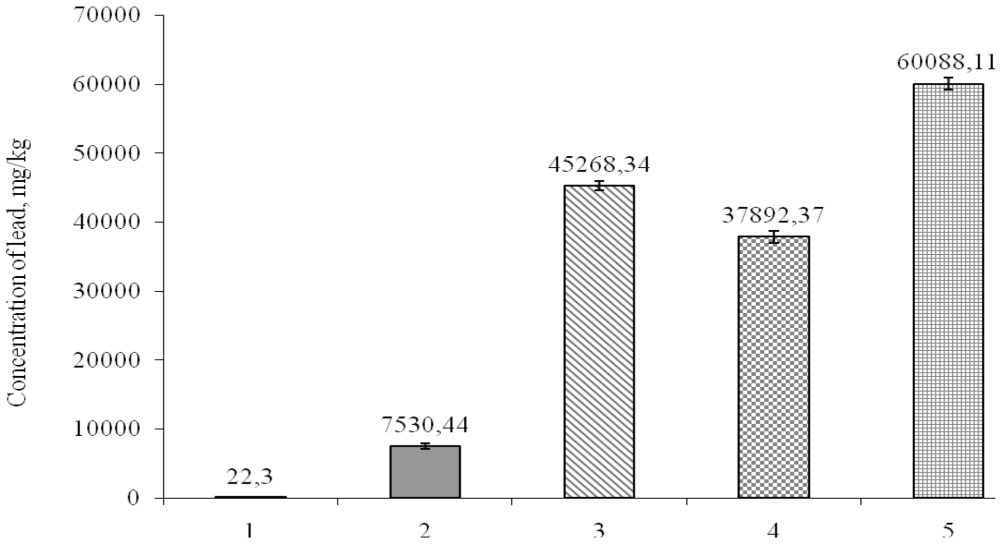

Fig.2 – Concentration of lead in the shoots of *Hordeum vulgare* L. Note: 1 – Control, 2 – whole plant+Pb, 3 – whole plant +Pb+EDTA, 4 – shoots+Pb ("-" roots), 5- shoots+Pb+EDTA ("-" roots)

Uptake of lead in the form of $[Pb\text{-}EDTA]_2$ is promoted by transpiration. In the absence of transpiration (in the variants without shoots), which facilitates mainly the absorption of anionic form of metal, lead is accumulated in the roots to a lesser extent than in the cationic form, which is mainly absorbed by ion exchange process [15].

In general, metal ions are absorbed by whole plant roots and aerial parts to a greater extent as compared with variants with excised roots and aerial parts. It might be explained by participation of transpiration in the process of metal translocation.

This is evidenced by the fact that in the variant "shoots+Pb+EDTA ("-" roots)" lead was absorbed to the greatest extent. This confirms the significant role of transpiration in the absorption of anionic form of lead. In variants with cadmium are received the similar results. In the study of cadmium content in plant parts in variants with cadmium were obtained similar results. The highest content of cadmium was observed in the roots of the variant "Roots+Cd ("-" roots)", the lowest – in the roots in the variant "Whole plant+Cd".

Anionic form of cadmium as well as lead, is less absorbed by excised roots (variant "Roots+Cd+EDTA") compared with the roots of whole plants (variant "Whole plant +Cd+EDTA") (in 1.7 times) and compared with the cationic form (Cd^{2+}) in the variant "roots+Cd ("-" roots)" without the above-ground organs (in 5,5 times). This indicates that the uptake of cadmium ions also has the ion-exchange characteristics.

In living plants in the presence of EDTA, cadmium content in the roots and in the shoots significantly more than those without EDTA (at the roots – 15 505.27> 76.3, in the aboveground organs – 15569.46 > 25.81).

In the presence of EDTA in the whole plant cadmium is accumulated in large amounts under the influence of transpiration.

According to Crist et al. [15] cadmium in the presence of EDTA, in the medium is in the anionic form $[Cd\text{-}EDTA]^{2-}$.

In plants without roots cadmium content in the anionic and cationic forms in the aboveground organs was approximately in the same level (27 267.66 and 29698.45, respectively).

This indicates that the in the absorption of cadmium in both forms significant role is played transpiration. Thus, the results of our experiments confirm the assumption that the roots of divalent metal ions are absorbed by the ion-exchange processes and uptake of metals in the presence of EDTA aboveground organs and roots is due to transpiration, and uptake of metals in the form of cations occurs with help ion exchange processes with the participation of transport ATPases [20].

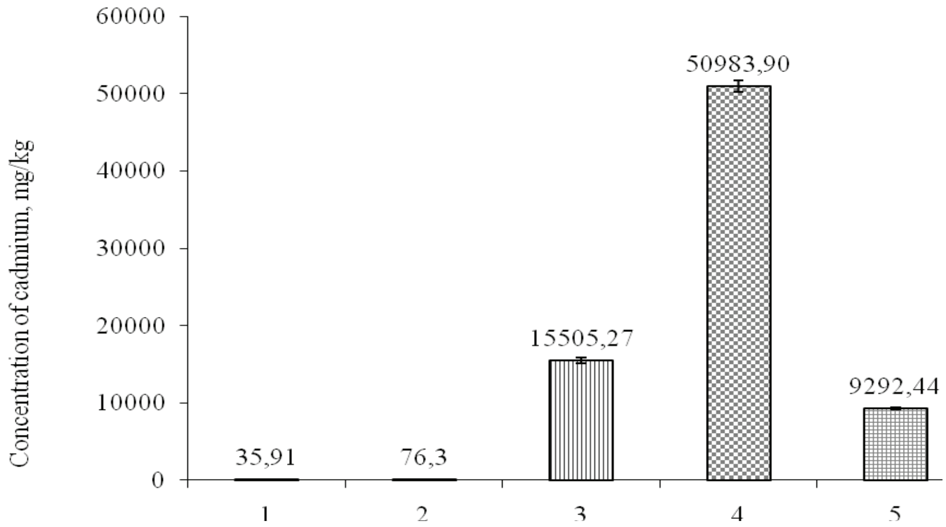

Fig. 3 – Concentration of cadmium in the roots of *Hordeum vulgare* L. Note: 1-Control, 2 – whole plant+Cd, 3 – whole plant +Cd+EDTA, 4 – roots+Cd ("-" roots), 5- roots+Cd+EDTA

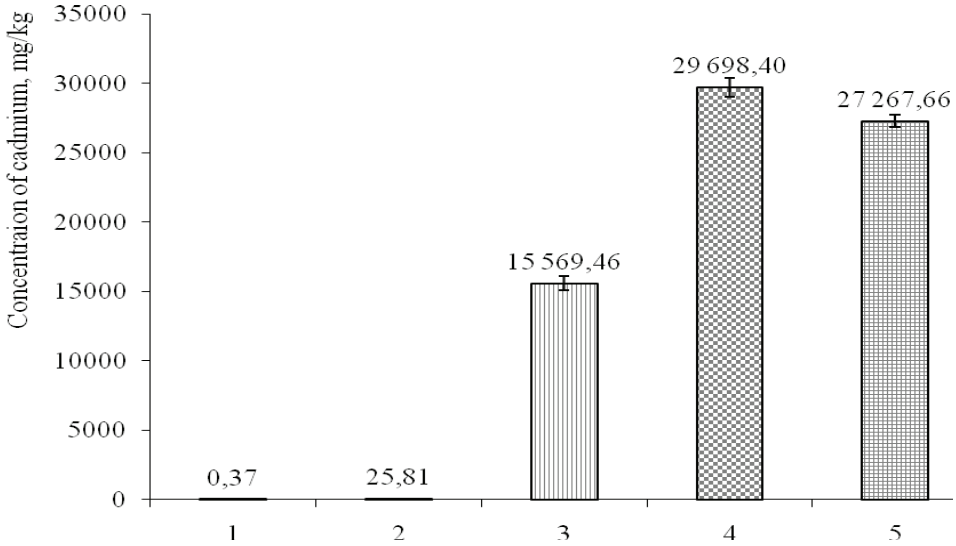

Fig. 4 – Concentration of cadmium in the shoots of *Hordeum vulgare* L. Note:
1-Control, 2 – whole plant+Cd, 3 – whole plant +Cd+EDTA, 4 – shoots+Cd ("-" roots), 5- shoots+Cd+EDTA

References

1 M.N.V. Prasad,The practical use of plants to remediate of ecosystems, contaminated by metals, Plant Physiology 5 (50) (2003) 764-780 (in Russian with English abstract)

2 N.S. Bolan, D.C.Adriano, P.Duraisamy, A.Mani, K.Arulmozhiselvan, Immobilization and phytoavailability of cadmium in variable charge soils. I. Effect of phosphate addition, Plant and Soil 250(1) (2003) 83-94.

3 R.Chaney, S. Brown, Y-M. Li, S.Angle, F.Horner, C.Green, Potential use of hyperaccumulator plant species to decontami-nate metal polluted soils, Mining Environmental Management 3 (3) (1995) 9-11.

4 G.S. Dheri, M.S. Brar, S.S. Malhi, Influence of phosphorus application on growth and cadmium uptake of spinach in two cadmium-contaminated soils, Journal of Plant Nutrition and Soil Science, 170(4) (2007) 495-499.

5 D.Claus, H. Dietze, A.Gerth, W. Grosser, A. Hebner, Application of agronomic practice improves phytoextraction on a multipolluted site. Journal of Environmental Engineering and Landscape Management 15(4) (2007) 208-212.

6 B.M. Zadeh, G.R. Savaghebi-Firozabadi, H. A. Alikhani, H.M. Hosseini, Effect of sunflower and amaranthus culture and application of in-oculants on phytoremediation of the soils con-taminated with cadmium, American-Eurasian J. Agric. & Environ. Sci. 4 (1) (2008) 93-103.

7 J.-M. Lim, A.L. Salido, Phytoremedia-tion of lead using Indian mustard (Brassica juncea) with EDTA and electrodics, Micro-chemical journal 1-2 (76) (2004) 3-9

8 J. Li, Y. Sun, R. Ji, Y. Yin, J. Wu, X. Wang, H. Guo, Ethyl lactate-EDTA composite system enhances the remediation of the cadmium-contaminated soil by willows,Journal of Hazardous Materials 1-3 (181), (2010) 673-678

9 J. Lia, Y.Sunb, Y.Yina, R.Jia, J. Wub, X. Wanga, H. Guoa, G. Hongyan, Ethyl lactate-EDTA composite system enhances the remedia-tion of the cadmium-contaminated soil by Au-tochthonous Willow (Salix × aureo-pendula CL ‘J1011’) in the lower reaches of the Yangtze River, Journal of Hazardous Materials, 1-3 (181), (2010) 673-678

10 E. Saifullah, M. Meers, P.Qadir, F.M.G. de Caritat, G.Tack, L.Du, M.H. Zia, EDTA as-sisted Pb-phytoextraction,Chemosphere 10 (74) (2009) 1279-1291

11 M.McBride. Environmental chemistry of soils,. Oxford University. Press. New York, NY. (1994) 336-337

12 M.J. Blaylock, D.E. Salt, S. Dushenkov, O. Zakharova, C. Gussman, Enchanced accumulation of Pb in Indian mustard by soil-applied chelating agents, Environ Sci Technol. 31 (1997) 860-865

13 M. Puschenreiter, G. Stöger, E. Lombi, O.Horak, W.W.Wenzel, Phytoextraction of heavy metal contaminated soils with Thlaspi goesingense and Amaranthus hybridus: Rhizosphere manipulation using EDTA and ammonium sulfate, J.Plant Nutr. Soil Sci., 164 (2001) 615–621.

14 A.Vassil, Y. Kapulnik, I. Raskin, D.E. Salt, The role of EDTA in leadtransport and accumulaation by Indian mustard, Plant Physiol. 117 (1998). 447-453.

15 M.J. Blaylock, D.E. Salt, S., Dushenkov, O. Zakharova, C. Gussman 1997. Enchanced accumulation of Pb in Indian mustard by soil-applied chelating agents, Environ Sci Technol. 31(1997)860-865

16 R.Crist, J. Martin, C.DeLanson Crist, Ion-exchange aspects of toxic metal uptake by Indian mustard, International Journal of Phytoremediation, 1(6) (2004) 85-94.

17 LMN/SOP-06 Digestion of hard samples for heavy metal determination in the flame (2001) 10 p.

18 LMN/SOP-08 (FLAA). Work process of Analyst 300 Perkin Elmer (in the flame) (2001), 14 p.

19 J. Hernandez-Alica, C. Garbisu, O. Barrutia, J.M. Becerril, EDTA-induces heavy metal accumulation and phytotoxity in cardoon plants, Environmental and Experimental Botany 1(60) (2007) 26-32.

20 Schulz, Living sieve cells of conifers as visualized by confocal, laser-scanning fluores-cence microscopy, Protoplasma 166 (1992)153-164.

21 E. Eren, J.V. Agrűello, Arabidopsis HMA2, a divalent heavy metal-transporting P-type AT-Pase, is involved in cytoplasmic Zn2+ homeosta-sis, Plant Physiology 136 (2004) 3712-3723.