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EDTA-ASSISTED PHYTOEXTRACTION OF COPPER, CADMIUM AND ZINC USING CHAMOMILE PLANTS

FITOEKSTRAKCJA WSPOMAGANA EDTA KADMU, MIEDZI I CYNKU ZA POMOCĄ RUMIANKU POSPOLITEGO

Summary: The effect of EDTA on Cu, Zn and Cd bioaccumulation in *Matricaria recutita* L. plants (cv. Goral) was studied. Application of 12, 24 and 60 μmol MSO_4 dm^{-3} (M = Cu, Zn or Cd) practically did not affect dry matter of roots and shoots of chamomile plants. The studied plants accumulated more Cd, Zn and Cu in the roots than in the shoots. Metal content in plant organs increased with increasing MSO_4 concentration in hydroponic solution. In the presence of equimolar EDTA concentration a significant decrease of accumulated Cu and Zn amount in plant roots was observed. For Cd this effect was relatively low. On the other hand, application of EDTA caused sharp increase of Cu concentration in the shoots, whereas Cd shoot concentration increased only slightly and Zn concentration showed a moderate decrease. Bioaccumulation (BAF) and translocation factors (TF) also were evaluated. BAF values related to the accumulated metal amount in the shoots ranged were 69–168 for Zn, 3.8–11.5 for Cu and 56–100 for Cd (application without EDTA) and 62–162 for Zn, 34–39 for Cu and 78–129 for Cd (with EDTA application). Plants treated with 12 μmol dm^{-3} $CdSO_4$ accumulated in the shoots 16.9 % from the total Cd amount accumulated by plant and in the presence of EDTA this portion increased to 30.9 %. On the other hand, at the highest applied $CdSO_4$ concentration (60 μmol dm^{-3}) this portion was only little affected by the presence of EDTA (33.5 % and 36.4 %, respectively).

Keywords: bioaccumulation, chelator, *Matricaria recutita*, toxic metals, translocation

Matriacaria recutita plants are known to tolerate relatively high metal concentrations [1, 2]. Chizzola [3] found that 3-month-old chamomile plants treated with both lower (0.4 mg dm^{-3}) and higher (2 mg dm^{-3}) Cd concentration accumulated more Cd in the shoots than in the roots. Grejtovský and Pirč [4] also found no necroses and only small growth inhibition after Cd application despite relatively high values of accumulated Cd in chamomile tissues and from two varieties of *Chamomilla recutita* (L.) Rausch., diploid var. “Novbona” exhibited higher Cd-accumulating ability than tetraploid var. “Lutea”. Addition of 30 mg Zn kg^{-1} soil resulted in 18-fold increase of Zn concentration

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in chamomile shoots where it reached the level of 271.0 mg kg^{-1} dry matter and such a treatment resulted in a 5-fold increase of Zn in chamomile anthodia (chamomile flos drug) reaching a level of 159.8 mg kg^{-1} dry matter [5]. It was shown previously that low copper translocation into chamomile shoots could be enhanced if Cu is supplied in the form of chelates [6].

Ethylenediaminotetraacetic acid (EDTA) is usually persistent in the environment. The presence of EDTA in soil can alter the mobility and transport of Zn, Cd, and Ni in soils because of the formation of water soluble chelates, thus increasing the potential for metal pollution of natural waters. EDTA adding could also increase the bioavailability and uptake of other metals, such as Cu, Cd, Ni, by plants. Blaylock et al. [7] showed that EDTA addition to soil containing Cd, Cu, Ni, Pb and Zn substantially increased transport of those metals into the shoots of *Brassica juncea.* Direct measurement of a complex of Pb and EDTA (Pb-EDTA) in xylem exudate of Indian mustard confirmed that the majority of Pb in these plants is transported in coordination with EDTA [8]. These results demonstrated that coordination of Pb transport by EDTA enhanced the mobility within the plants of this otherwise insoluble metal ion, allowing plants to accumulate high concentrations of Pb in shoots. Luo et al. [9] found that the application of EDTA significantly increased the shoot to root ratios of the concentrations of Cu, Pb, Zn and Cd in *Zea mays* L. and *Phaseolus vulgaris* L. On the other hand, the experiments with tobacco (*Nicotiana tabacum*) plants showed that the uptake of Cu was increased by the addition of EDTA, while no increase was observed in the uptake of Cd and a very low root to shoot translocation capability was observed [10]. Similarly, no increase in Cd accumulation was found in maize plants under the influence of EDTA, but this chelating agent was observed to suppress the effect of Cd toxicity on the plant growth [11]. Chelated metals are taken up via the apoplastic pathway. Disruption of the Casparian band is required to achieve the high shoot concentrations needed for phytoextraction. Therefore, adding chelators to a soil increases not only the total dissolved metal concentration but also changes the primary route of plant metal-uptake from the symplastic to the apoplastic pathway [12].

This study is aimed to investigate the effect of EDTA on cadmium, zinc and copper bioaccumulation in roots and shoots of *Matricaria recutita* plants.

Material and methods

For the experiments $(CdSO_4)_3(H_2O)_8$, $ZnSO_4 \cdot 7H_2O$ and $CuSO_4 \cdot 5H_2O$ and EDTA were used. Analytical reagent-grade chemicals purchased from Lachema (Brno, Czech Republic) were employed for the preparation of all solutions. Freshly distilled water was used in all experiments.

For cultivation of experimental plants the seeds of chamomile (*Matricaria recutita* L.), cv. Goral were used (Research Institute of Agroecology in Michalovce, Slovakia). The seeds were sown on May 22, 2007. Two-month-old plants were exposed in hydroponia for seven days in controlled conditions (mean air temperature: 25 ± 0.5 °C, relative air humidity: 80 % and photosynthetic active radiation: 80 μmol m^{-2} s^{-1}): control variant in Hoagland solution and metal treated variants in Hoagland solution containing 12, 24 and 60 μmol dm^{-3} of the studied compounds without and with equimolar concentrations of EDTA. Then the length and dry mass of shoots and roots were determined. Presented results are mean values of 6 repetitions in each variant. The influence of increasing metal concentration on the length of root and shoot was evaluated by multifactorial ANOVA ($p \leq 0.05$), the multiple comparison of means was based on the method of Tukey-contrast.

Plants were processed for metal analysis – they were harvested and thoroughly washed under running water to remove the test solution from the exterior of the roots. Then plant samples were dried at 70 °C, dried powdered samples (separately roots and shoots, respectively) were digested with HNO_3, HF and H_3BO_3 and flame atomic absorption spectroscopy (AAS Perkin Elmer Model 1100, USA) was used for determination of Cd, Zn and Cu contents in shoots and roots of studied species.

Results and discussion

Treatment with 12, 24 and 60 μmol dm^{-3} of the studied compounds practically did affect neither the length nor the dry matter of roots and shoots of *M. recutita* (Table 1). It is necessary to stress that in natural conditions the concentration of 60 μmol Cd dm^{-3} corresponds to very high Cd contamination.

Table 1

Dry mass of roots and shoots of chamomile plants (means ± standard errors, n = 6) treated with MSO_4 and MSO_4-EDTA (1:1).
The values indicated by the same letter do not differ statistically ($p > 0.05$)

MSO_4	c [μmol dm^{-3}]	MSO_4		MSO_4-EDTA (1:1)	
		shoot d.m. [mg]	root d.m. [mg]	shoot d.m. [mg]	root d.m. [mg]
$ZnSO_4$	0	308.5 ± 8.3^{a}	68.5 ± 8.7^{a}	308.5 ± 8.3^{a}	68.5 ± 8.76^{a}
	12	245.5 ± 6.4^{a}	53.4 ± 8.3^{a}	271.0 ± 13.2^{a}	52.0 ± 5.4^{a}
	24	246.3 ± 13.1^{a}	58.1 ± 8.3^{a}	293.3 ± 22.4^{a}	68.5 ± 9.6^{a}
	60	271.3 ± 11.3^{a}	54.2 ± 5.9^{a}	282.1 ± 11.4^{a}	55.4 ± 7.2^{a}
$CuSO_4$	0	282.2 ± 28.9^{a}	68.6 ± 15.2^{a}	282.2 ± 28.9^{a}	68.6 ± 15.2^{a}
	12	307.0 ± 25.9^{a}	43.3 ± 10.5^{a}	297.4 ± 38.7^{a}	72.6 ± 17.3^{a}
	24	313.8 ± 33.7^{a}	75.6 ± 13.6^{a}	308.4 ± 67.9^{a}	63.5 ± 9.4^{a}
	60	360.5 ± 36.2^{a}	68.8 ± 8.9^{a}	332.3 ± 48.0^{a}	71.8 ± 14.1^{a}
$CdSO_4$	0	282.2 ± 28.9^{a}	68.8 ± 15.2^{a}	282.2 ± 28.9^{a}	68.8 ± 15.2^{a}
	12	341.6 ± 35.6^{ab}	48.8 ± 10.3^{a}	306.8 ± 33.8^{ab}	50.2 ± 7.5^{a}
	24	418.1 ± 81.7^{b}	76.0 ± 14.9^{a}	350.5 ± 21.7^{ab}	49.3 ± 4.2^{a}
	60	270.7 ± 27.3^{a}	45.7 ± 8.8^{a}	263.9 ± 37.8^{a}	53.8 ± 28.5^{a}

Chamomile plants accumulated more Cd, Zn and Cu in the roots than in the shoots. In general, metal content in plant organs increased with the increasing concentration of

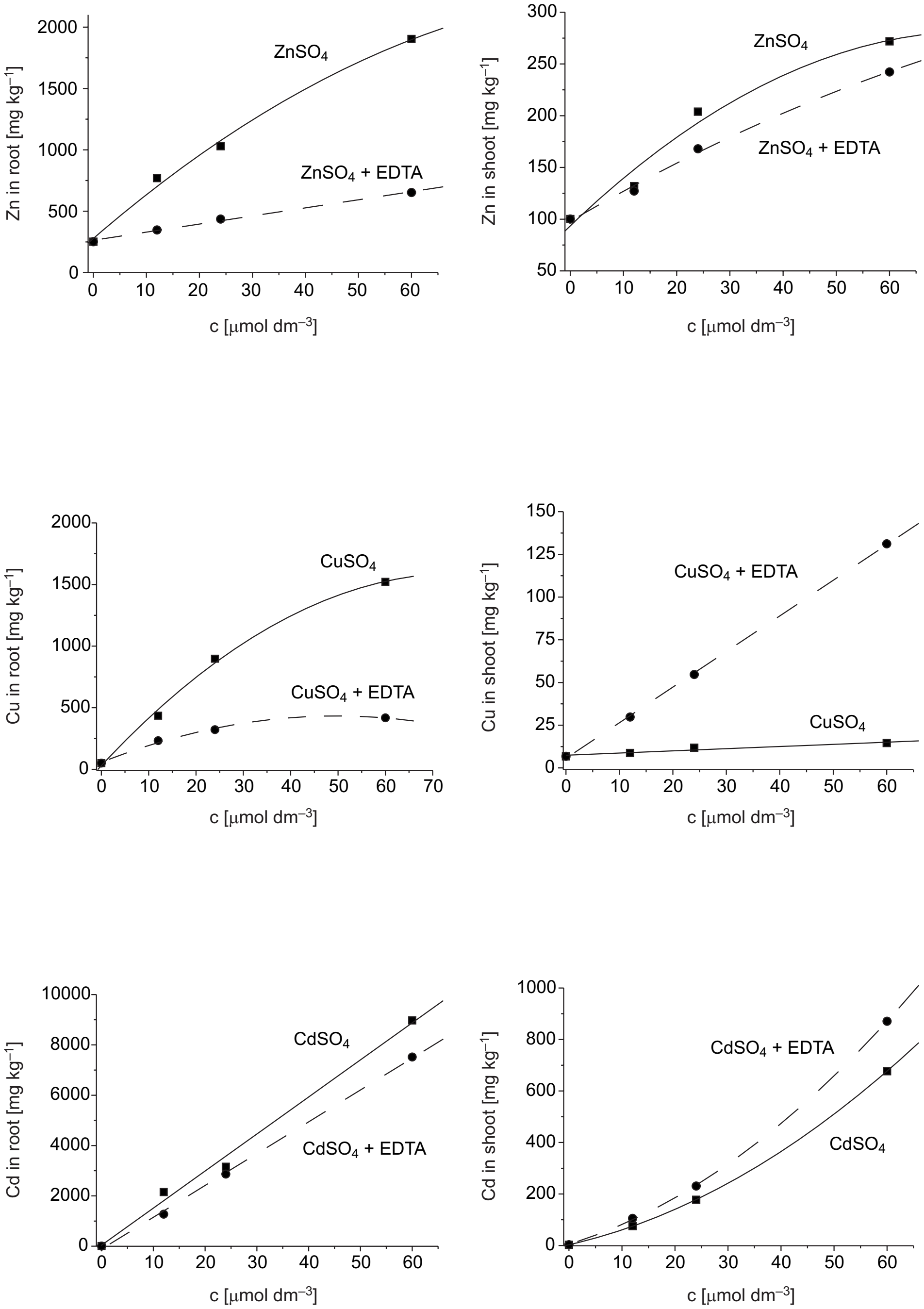

Fig. 1. Dependence of Cd, Zn and Cu bioaccumulation in the roots and shoots of *M. recutita* L. plants on the concentration of metal sulphates applied without and with equimolar EDTA concentration

studied compounds in the hydroponic solution. In the presence of equimolar EDTA concentration significant decrease of bioaccumulated Cu and Zn amount in plant roots was observed. For Cd this effect was relatively low. On the other hand, application of EDTA led to expressive increase of Cu shoot concentration whereas Cd concentration in the shoot was elevated only slightly and Zn concentration showed even a moderate decrease (Fig. 1).

Bioaccumulation factors (BAF) expressing the ratio of the metal concentration in the biological material (in μmol or μg g^{-1} dry matter) to the metal concentration in external solution (in μmol or μg dm^{-3}) were evaluated. Bioaccumulation factors (BAF) related to tested metal accumulation in roots and shoots of chamomile plants treated with MSO_4 and MSO_4-EDTA (1:1) are shown in Table 2.

Table 2

Bioaccumulation factors (BAF) related to tested metal accumulation in roots and shoots of chamomile plants treated with MSO_4 and MSO_4-EDTA (1:1)

MSO_4	c [μmol dm^{-3}]	BAF			
		MSO_4		MSO_4-EDTA (1:1)	
		shoot	root	shoot	root
$ZnSO_4$	12	168	981	162	442
	24	130	656	107	278
	60	69	485	62	166
$CuSO_4$	12	11.5	570	39	305
	24	7.7	589	36	211
	60	3.8	399	34	110
$CdSO_4$	12	56	1596	78	944
	24	66	1171	86	1063
	60	100	1331	129	1115

BAF related to accumulated metal amount in roots varied in the range 485–981 for Zn, 399–570 for Cu and 1170–1596 for Cd (treatment without EDTA) and 166–442 for Zn, 110–305 for Cu and 944–1115 for Cd (treatment with EDTA). Thus it is evident that the most efficient metal phytoextraction by chamomile roots was obtained for Cd, the lowest one for Zn. On the other hand, the corresponding BAF related to accumulated metal amount in shoots varied in the range 69–168 for Zn, 3.8–11.5 for Cu and 56–100 for Cd (treatment without EDTA) and 62–162 for Zn, 34–39 for Cu and 78–129 for Cd (treatment with EDTA).

The translocation factor (TF) corresponds to the ratio of accumulated metal amount in shoots and roots. This parameter depends (similarly like the portion from the total accumulated metal amount by the plant occurring in the shoots) on the actual dry matter of plant organs. TF values related to tested metals for chamomile plants treated with MSO_4 and MSO_4-EDTA (1:1) are shown in Table 3.

Table 3

Translocation factors (TF) related to tested metal for chamomile plants treated with MSO_4 and MSO_4-EDTA (1:1)

MSO_4	c [μmol dm^{-3}]	TF	
		MSO_4	MSO_4-EDTA
$ZnSO_4$	12	0.788	1.909
	24	0.839	1.649
	60	0.715	1.890
$CdSO_4$	12	0.204	0.505
	24	0.309	0.572
	60	0.447	0.568
$CuSO_4$	12	0.116	0.525
	24	0.055	0.825
	60	0.051	1.453

The portion from the total accumulated metal amount by the plant occurring in the shoots of *M. recutita* plants for MSO_4 and MSO_4-EDTA (1:1) treatments (M = Zn, Cd or Cu) is shown in Fig. 2. Plants treated with 12 μmol dm^{-3} $CdSO_4$ accumulated in

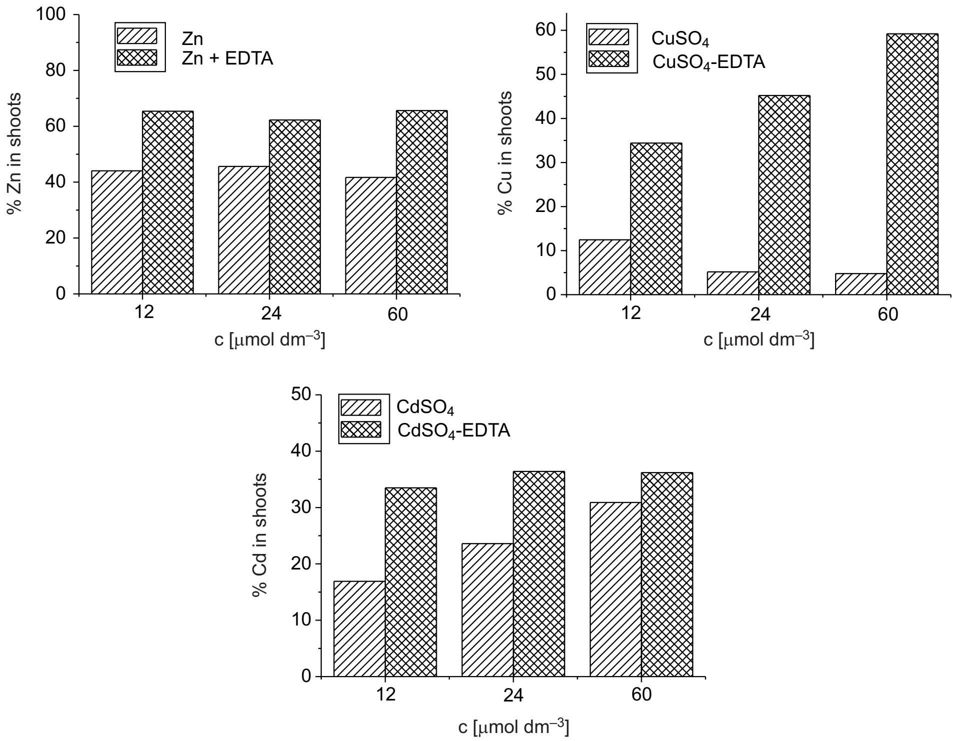

Fig. 2. The portion from the total accumulated metal amount by the plant occurring in the shoots of *M. recutita* plants for MSO_4 and MSO_4-EDTA (1:1) treatments; M = Zn, Cu and Cd

shoots 16.9 % of Cd accumulated by total plant and this portion was enhanced to 30.9 % in the presence of EDTA, whereas for the treatment with 60 μmol dm^{-3} $CdSO_4$ the presence of EDTA this portion affected only to a little extent (33.5 % and 36.4 %, respectively). In the presence of equimolar EDTA concentration more than 60 % Zn accumulated by chamomile plants was allocated in the shoots, independently on the applied metal concentration (Fig. 2). EDTA also promoted very effectively Cu translocation into the shoots which was reflected in the fact that at the treatment with higher metal concentrations (24 and 60 μmol dm^{-3}) even 45% and 59% Cu accumulated by *M. recutita* plants was allocated in the shoots. In the absence of chelator this portion reached only 5.2 and 4.8 %, respectively.

From presented results it is evident that effect of EDTA on metal bioaccumulation in roots and shoots of chamomile plants depends on the applied metal (Cd, Zn or Cu). Logarithms of stability constants (log K_1) for the studied metal chelates with EDTA are as follows: 18.8 (Cu), 16.5 (Zn) and 16.36 (Cd) [13, 14]. This means that the stability constant for Cu-EDTA chelates is more than 100 times higher than the corresponding stability constants for Cd-EDTA as well as Zn-EDTA chelates. Whereas chelate formation between EDTA and Zn or Cu resulted in significantly decreased metal uptake into chamomile roots, the decrease of Cd uptake due to chelate formation was very low. These results supported findings of Nowack et al. [12] and Wojcik and Tukendorf [11]. According to Nowack et al. [12] it could be assumed that metal chelates with EDTA are taken up via the apoplastic pathway and disruption of the Casparian band is necessary to achieve the high metal concentrations in shoots. Schaider et al. [15] found that in *B. juncea* plants metal-EDTA complexes were found to dominate xylem sap metal speciation and the fraction of metal in xylem sap present as metal–EDTA was greater for non-nutrient metals (Pb, Cd) than for the nutrient metal (Fe). Very efficient translocation of copper into the shoots observed at the presence of EDTA could be connected with the largest value of Cu-EDTA stability constant.

In general it can be confirmed that EDTA behaves as a persistent pollutant in the environment, enhancing mobility and bioavailability of heavy metals. The effects of EDTA vary according to the type of organism studied, the concentration of EDTA and the metal analysed [16]. Therefore, it is necessary to study the potential risk of increased bioavailability of heavy metals by edible plant species (including medicinal plants) exposed to metal-EDTA complexes.

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FITOEKSTRAKCJA WSPOMAGANA EDTA KADMU, MIEDZI I CYNKU ZA POMOCĄ RUMIANKU POSPOLITEGO

S t r e s z c z e n i e

Badano wpływ EDTA na bioakumulację Cu, Zn i Cd w roślinach *Matricaria recutita* L. (cv. Goral). Stosowane stężenia soli metali ciężkich: 12, 24 i 60 μmol MSO_4 dm^{-3} (M = Cu, Zn lub Cd) praktycznie nie miały wpływu na suchą masę korzeni i pędów roślin rumianku. Badane rośliny akumulowały więcej Cd, Zn i Cu w korzeniach niż w pędach. Stężenie metalu w organach rośliny zwiększało się wraz ze zwiększeniem stężenia MSO_4 w roztworze hydroponicznym. W obecności równomolowego stężenia EDTA stwierdzono znaczące zmniejszenie zawartości Cu i Zn w korzeniach badanych roślin. Dla Cd ten wpływ był stosunkowo mniejszy. Z drugiej strony, stosowanie EDTA spowodowało gwałtowny wzrost zawartości Cu w pędach, podczas gdy zawartość Cd wzrosła tylko nieznacznie, a zawartość Zn nieznacznie zmniejszyła się. Wyznaczono poziom bioakumulacji (BAF) i wartość czynnika translokacji (TF). Wartości BAF badanych metali w pędach wynosiły: 69–168 dla Zn, 3,8–11,5 dla Cu i 56–100 dla Cd (próbki bez EDTA) i 62–162 dla Zn, 34–39 dla Cu i 78–129 dla Cd (próbki z EDTA). W pędach roślin zakumulowało się z roztworu $CdSO_4$ o stężeniu równym 12 μmol · dm^{-3} aż 16,9 % całkowitej ilości kadmu zaakumulowanej w całej roślinie, natomiast w obecności EDTA ten udział wzrósł do 30,9 %. Z drugiej strony, przy największym stosowanym stężeniu (60 μmol · dm^{-3}) $CdSO_4$ w roślinach zaakumulowało się 33,5 % Cd, a w obecności EDTA wartość ta wzrosła tylko do 36,4 %.

Słowa kluczowe: bioakumulacja, chelator, *Matricaria recutita,* metale toksyczne, translokacja