COMPARISON OF Cd AND Zn ACCUMULATION IN TISSUES OF DIFFERENT VASCULAR PLANTS: A RADIOMETRIC STUDY

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Abstract: The aim of the present work was to compare the accumulation and translocation of Cd and Zn in plants of tobacco (*Nicotiana tabacum* L.), celery (*Apium graveolens* L.), maize (*Zea mays* L.), giant reed (*Arundo donax* L.), and alpine pennycress (*Noccaea caerulescens* L.) under conditions of short-term hydroponic experiments using nutrient solutions spiked with radionuclides ^{109}Cd or ^{65}Zn, and direct gamma-spectrometry. It was found that the time-course of metals accumulation in studied plants was not different in terms of target metal, but it was significantly different on the level of plant species. The highest values of Cd accumulation showed plants of giant reed, whereby the accumulation decreased in the order: giant reed > tobacco > alpine pennycress >> maize and celery. On the basis of concentration ratios (*CR*) [Me]$_{shoot}$ / [Me]$_{root}$ calculation for both metals, it was found that Cd and Zn were in prevailing part accumulated in the root tissues and only partially accumulated in the shoots, where the amount of accumulated Cd and Zn increased from the oldest developed leaves to the youngest developed leaves. The *CR* values corresponding to these facts were calculated in the range 0.06 – 0.27 for Cd and for Zn 0.06 – 0.48. In terms of plant species, the *CR* values obtained for Cd decreased in the order: maize > celery > tobacco and giant reed > alpine pennycress. The similarity between studied objects – individual plant species – on the basis of the obtained variables defining Cd or Zn accumulation at different conditions of the experiments as well as the relationships between obtained variables and conditions of the experiments were subjected to multivariate analysis method – cluster analysis (CA). According to the findings and this analysis, it can be expected that plants of tobacco and giant reed will dispose with similar characteristics as plants of alpine pennycress, which are classified as Zn/Cd hyperaccumulators, in terms of Cd or Zn accumulation and other positive parameters for their utilization in phytoremediation processes and techniques.

Key words: cadmium, zinc, plants, accumulation, translocation, speciation modelling, cluster analysis

1. Introduction

Heavy metals are well-known risk elements for the environment as non-degradable and persistent contaminants, which can be harmful for humans and the environment. The pollution of the environment with heavy metals predominantly comes from anthropogenic sources. Despite the fact that Hg, Pb, and Cd emissions from the non-ferrous metal industry decreased in Europe over the last 50 years, the increasing industrial activities in other continents (Asia, Africa) could significantly affect the global emissions of metals (ETTLER, 2015). The biota may require some of these

elements considered to be essential (e.g. iron, zinc, copper or molybdenum) in trace quantities, but at higher concentrations they may become toxic. However, metals like cadmium, lead or aluminium are non-essential and toxic even at very low levels (IANNONE *et al.*, 2015).

Cadmium (Cd) represents one of the most dangerous of the trace elements not only because of its significant occurrence in the environment together with zinc (Zn) as its chemical analogue, but also because of its negative accumulation, impact on living organisms, and being the fourth most toxic element to vascular plants as well. According to the Agency for Toxic Substances and Disease Registry Cd is actually ranked at the 7th place within the priority list of hazardous substances based on their frequency of occurrence in the environment, toxicity and adverse potential to affect human health (ATSDR, 2013). First, second, and third places in the mentioned priority list belong to arsenic (As), lead (Pb), and mercury (Hg), respectively. Cadmium is also considered as a priority pollutant by the European Community, within the Water Framework Directive (EC, 2001) and is released into the environment mainly by power stations, heating systems, metal working industries, waste incinerators, urban traffic, cement factories, and as a by-product of phosphate fertilisers (HAWRYLAK-NOWAK *et al.*, 2014).

The free ionic form Cd^{2+} is the most bioavailable and consequently more toxic for organisms and plants, but it can complex with oxides and organic compounds, and it is not soluble above pH 7.5. The content of Cd in crop plants varies in a wide range from 5 up to 400 μg/kg of dried weight (KABATA-PENDIAS and MUKHERJEE, 2007). Even at low concentrations, Cd is toxic for most plants, while at concentrations greater than 5 – 10 mg/kg of leaf dry weight it leads to plant death (WHITE and BROWN, 2010). Cd is easily taken up by plant roots and transported to aerial parts, thus entering into the food chain causing health problems in animals and humans (BERNARD, 2008). Cd-induced toxicity in plants can be described by: growth reduction, chlorosis and necrosis of leaves, wilting, red-brown colouration of leaf margins or veins (GILL *et al*., 2013). Moreover, Cd can disturb the water balance, mineral nutrition, photosynthesis, respiration as well as general plant development (WÓJCIK and TUKIENDORF, 2005). However, the biochemical and physiological basis of Cd phytotoxicity is not always clear (HAWRYLAK-NOWAK *et al.*, 2015). Cadmium, like other heavy metals, can accept a pair of electrons from a coordinate covalent bond, react with –S⁻ groups, –OH⁻ groups, amino groups, and carboxylic acid termini, and thus affect sulfhydryl groups and N atoms in proteins causing their inactivation (LAMBERS *et al.*, 2008).

Soil-to-plant transfer of Cd is the major exposure pathway for humans with regards to soil contamination (LIANG *et al.*, 2013). Food chain contamination is the main source of Cd entry to human, especially non-smoking general population, and is the main constraint for food safety and agricultural land quality (ATAFAR *et al.*, 2010). Excessive dietary intake of Cd is accumulated in the body throughout life, and can lead to kidney malfunction and other diseases (MCLAUGHLIN *et al.*, 1999).

Besides the ability to take up essential elements, plants are able to absorb and accumulate other metals, even those of a toxic character or with unidentified metabolic functions (HAWRYLAK-NOWAK *et al.*, 2014). Toxic trace heavy metals,

such as Cd, have chemical properties similar to essential micronutrients, such as Zn, and are generally transported in plants by the same transporters as those essential micronutrients (TAVAREZ *et al.*, 2015). These mechanisms responsible for toxic metals uptake, translocation and accumulation in plant tissues represent serious risks related to their entering into the food chain. On the other hand, these mechanisms can be utilized for removal of heavy metals and radionuclides from contaminated environment, thus for remediation of contaminated environment by plants.

Phytoremediation, the use of plant systems to remove the contaminants from the soils and water has recently attracted a great deal of attention as an alternative means of soil decontamination, since it is a cost-effective, environment-friendly approach, applicable to large areas (BAUDDH *et al.*, 2015). The capability of the plants to uptake contaminants, survive in contaminated soil and the low bioavailability of the contaminants in the soil are some of the limiting factors that influence phytoremediation efficiency (CHIRAKKARA and REDDY, 2015). The plenty of plant species (including agricultural crops, grasses, shrubs, water plants, alpine plants a.o.) were subjected to characterization their potential for phytoremediation techniques. Some of them showed an ability to tolerate and accumulate high concentrations of heavy metals and therefore were defined as hyperaccumulators. In order to fully utilize the contaminated sites and to overcome the disadvantage of phytoremediation, a new strategy of combining phytoremediation with energy crops cultivation, with a view to achieving low cost decontamination of soil through the production of biofuels (BAUDDH *et al.*, 2015; PANDEY *et al.*, 2016). A further current area of interest, at least for those metal ions essential for the human diet, also lies in the prospect of transferring the trait to crop plants as a way of combatting dietary microelements deficiencies (VISIOLI and MARMIROLI, 2013).

Our previous works studied the accumulation of Zn, Co, Cd or Cs from nutrient solutions by roots of hydroponically cultivated plants of sunflower (*Helianthus annuus* L.), tobacco (*Nicotiana tabacum* L.), celery (*Apium graveolens* L.), lettuce (*Lactuca sativa* L.), and giant reed (*Arundo donax* L.) plants (HORNÍK *et al.*, 2005; HORNÍK *et al.*, 2007; HORNÍK *et al.*, 2008; HORNÍK *et al.*, 2009; GULDANOVÁ *et al.*, 2010; ŠUŇOVSKÁ *et al.*, 2012; DÜREŠOVÁ *et al.*, 2014). The aim of this work was to compare the uptake, translocation, and accumulation of heavy metals Cd and Zn in various plants including tobacco (*N. tabacum* L.), celery (*A. graveolens* L.), maize (*Zea mays* L.), giant reed (*Arundo donax* L.), and alpine pennycress (*Noccaea caerulescens* L.) under conditions of short-term hydroponic experiments. A quantitative description of Cd and Zn accumulation was carried out using nutrient solutions spiked with radionuclides ^{109}Cd or ^{65}Zn, and direct gamma-spectrometry. The selection of the chosen plants included a typical model plant (tobacco), root crop (celery), agricultural crop (maize), potential energy crop (giant reed), and alpine plant (alpine pennycress) classified as a hyperaccumulator of heavy metals. In addition, the similarity between individual plant species on the basis of obtained variables defining the studied metals accumulation in plants at different conditions of the experiments, and the relationships between obtained variables and conditions of the experiments, were evaluated applying multivariate analysis method – cluster analysis (CA).

2. Materials and methods

2.1 Plant material

Seeds of tobacco (*Nicotiana tabacum* L.) were obtained from Plant Production Research Institute in Piešťany (Slovak Republic). Seeds of celery (*Apium graveolens* var. *rapaceum* Mill.) and maize (*Zea mays* convar. *saccharata* Koern.) were used as commercially available seeds from the company SEMO, Inc. (Czech Republic). Seeds of alpine pennycress (*Noccaea caerulescens* L.) were purchased from B&T World Seeds (France). All mentioned seeds were germinated and grown in pots filled with granulated perlite as an inert carrier and watered with 25 % or 50 % strength nutrient medium according to HOAGLAND (1920). The composition of the full strength Hoagland medium (100 % HM) was (in mg/dm^3) $MgSO_4.7H_2O$ – 370; KNO_3 – 404; $CaCl_2$ – 444; $NaH_2PO_4.2H_2O$ – 292; $Na_2HPO_4.12H_2O$ – 46.5; $FeSO_4.7H_2O$ – 17.9; $NaNO_3$ – 340; NH_4Cl – 214; NH_4NO_3 – 160; H_3BO_3 – 8.5; $Na_2MoO_4.2H_2O$ – 0.06; $MnSO_4.5H_2O$ – 5.0; $ZnSO_4.7H_2O$ – 0.66; $CuSO_4.5H_2O$ – 0.8.

Table 1. Conditions of the experiments evaluating the accumulation of Cd and Zn in plants of tobacco (*N. tabacum* L.), celery (*A. graveolens* L.), maize (*Z. mays* L.), giant reed (*A. donax* L.), and alpine pennycress (*N. caerulescens* L.).

Plant	Metal	Conditions of the experiments
Tobacco	Cd	25 % HM; pH 6.0; 10 µmol/dm^3 $CdCl_2$; 40.5 kBq/dm^3 [109]$CdCl_2$; 12h/12h day/night (2 000 lx); 60 % relative humidity; 22±2 °C
	Zn	25 % HM; pH 6.0; 10 µmol/dm^3 $ZnCl_2$; 36.4 kBq/dm^3 [65]$ZnCl_2$; 12h/12h day/night (2 000 lx); 60 % relative humidity; 22±2 °C
Celery	Cd	50 % HM; pH 5.5; 10 µmol/dm^3 $CdCl_2$; 45.0 kBq/dm^3 [109]$CdCl_2$; 12h/12h day/night (2 000 lx); 60 % relative humidity; 22±2 °C
	Zn	25 % HM; pH 5.5; 10 µmol/dm^3 $ZnCl_2$; 50.0 kBq/dm^3 [65]$ZnCl_2$; 12h/12h day/night (2 000 lx); 60 % relative humidity; 22±2 °C
Maize	Cd	50 % HM; pH 5.5; 10 µmol/dm^3 $CdCl_2$; 45.0 kBq/dm^3 [109]$CdCl_2$; 12h/12h day/night (2 000 lx); 60 % relative humidity; 22±2 °C
	Zn	50 % HM; pH 5.5; 5.9 µmol/dm^3 $ZnCl_2$; 49.0 kBq/dm^3 [65]$ZnCl_2$; 12h/12h day/night (2 000 lx); 60 % relative humidity; 22±2 °C
Giant reed	Cd	25 % HM; pH 6.0; 10 µmol/dm^3 $CdCl_2$; 95.5 kBq/dm^3 [109]$CdCl_2$; 12h/12h day/night (4 000 lx); 60 % relative humidity; 25/22 °C
	Zn	25 % HM; pH 6.0; 1 µmol/dm^3 $ZnCl_2$; 74.4 kBq/dm^3 [65]$ZnCl_2$; 12h/12h day/night (4 000 lx); 60 % relative humidity; 25/22 °C
Alpine pennycress	Cd	25 % HM; pH 6.0; 10 µmol/dm^3 $CdCl_2$; 168 kBq/dm^3 [109]$CdCl_2$; 16h/8h day/night (11 450 lx); 60 – 80 % relative humidity; 28/15 °C *
	Zn	25 % HM; pH 6.0; 10 µmol/dm^3 $ZnCl_2$; 164 kBq/dm^3 [65]$ZnCl_2$; 16h/8h day/night (11 450 lx); 60 – 80 % relative humidity; 28/15 °C *

* Conditions provided by a plant growth chamber (KBWF 720, Binder, Germany).

Plants of giant reed (*Arundo donax* L. var. *versicolor*) were obtained by vegetative propagation under *in vitro* conditions. Shoots development from lateral buds was induced in an MS medium (MURASHIGE and SKOOG, 1962) with an addition of 2–

4 mg/dm^3 of BAP (6-benzylaminopurine) as a cytokinin-type growth regulator. For rooting of regenerated shoots and *in vitro* storage of giant reed plants, MS medium without an addition of growth regulators was used. Subsequently, giant reed plants were transferred and pre-cultivated in a 25 % HM.

After 4 – 8 weeks of plant pre-cultivation, seedlings were gently removed from perlite and roots were washed free of any adhering perlite granules by deionized water and used in the accumulation experiments.

2.2 Accumulation experiments and conditions of the experiments

Plants from the pre-cultivation phase were transferred into Erlenmeyer flasks (100 – 250 cm^3) with a cover to protect plant roots against lights and cultivated for 8 days in a 50 – 150 cm^3 (according to plant) Hoagland medium (HM) containing Cd (in the form of CdCl$_2$) or Zn (in the form of ZnCl$_2$ or ZnSO$_4$) and spiked with ^{109}CdCl$_2$ or ^{65}ZnCl$_2$. The conditions of the individual experiments are detailed in the Table 1. In time intervals, samples of nutrient solution were taken and ^{109}Cd or ^{65}Zn radioactivities were measured by gamma-spectrometry. At the end of the experiments, plants were harvested, roots were carefully rinsed in deionized water and incorporated radioactivity in roots, stems or leaves was measured. Individual parts of plant were then oven dried (at 60 °C for 24 hours) and dry weights were determined.

2.3 Radiometric analysis

All nutrient solutions used in the experiments were spiked with ^{109}CdCl$_2$ and ^{65}ZnCl$_2$ radioactive solutions obtained from the Czech Metrological Institute (Czech Republic). The radiometric analysis in terms of volume or specific radioactivity of ^{109}Cd and ^{65}Zn in the samples of nutrient solutions or individual parts of plant was realized using a well type NaI(Tl) scintillation gamma-spectrometer 54BP54/2-X or 76BP76/3 (Scionix, The Netherlands or Envinet, Czech Republic) and the data processing software ScintiVision-32 (Ortec, USA). For energy and efficiency calibration, a library of radionuclides was built by selecting characteristic γ-ray peaks for ^{109}Cd (E_γ = 88.04 keV), ^{137}Cs (E_γ = 661.66 keV) or ^{65}Zn (E_γ = 1115.52 keV), and for the calibration, standard solutions of ^{109}CdCl$_2$, ^{137}CsCl or ^{65}ZnCl$_2$ with known radioactivity including the half-life of radionuclides (^{109}Cd: $T_{1/2}$ = 462.6 d; ^{137}Cs: $T_{1/2}$ = 11 019 d, and ^{65}Zn: $T_{1/2}$ = 243.9 d) were used.

2.4 Speciation modelling

The qualitative and quantitative proportion of the ion forms of Cd and Zn in nutrient solutions were calculated by the software MINEQL+ ver. 4.62 (Environmental Research Software, USA). This software employs chemical equilibrium models to calculate metals speciation and solubility equilibria in the laboratory or natural aqueous systems under a variety of conditions including

a gas phase with constant partial pressure, pH, temperature, ionic strength, and concentrations of cations and anions.

2.5 Statistical analysis

All analytical determinations were performed in triplicate. The statistical significance of differences in calculated values of Cd or Zn accumulation in plant tissues were evaluated by a multiple range test to ascertain differences between individual groups. The level of significance p was 0.05 in all cases.

To evaluate the similarity degree between the objects – individual plant species on the basis of obtained variables and conditions of the experiments as well as to evaluate the relationships between the obtained variables and conditions of the experiments, the cluster analysis (CA) was applied. Parameters describing the obtained variables, such as Cd or Zn accumulation in plants (in μmol/g; fresh wt.) or concentration ratio (*CR*) defined as the ratio of the concentration of Cd or Zn accumulated in shoots to concentration of Cd or Zn accumulated in roots ($[Me]_{shoot}$ / $[Me]_{root}$), and the conditions of the experiments, such as the initial concentrations of Cd^{2+} or Zn^{2+} ions (in $μmol/dm^3$) predicted by the speciation modelling, the pH value of the nutrient solutions, the strength of Hoagland medium (25 % or 50 % HM) or conditions of the plant cultivation (temperature, relative humidity, day/night photoperiod or intensity of illumination) were utilized in this analysis.

Statistical analysis, evaluation, and graphical interpretation of the obtained data were carried out using programs OriginPro ver. 8.5 (OriginLab Corp., USA), STATGRAPHICS Centurion ver. 15 (StatPoint Technologies Inc., USA), SYSTAT ver. 13 (Systat Software Inc., USA), and SigmaPlot ver. 12 (Systat Software Inc., USA).
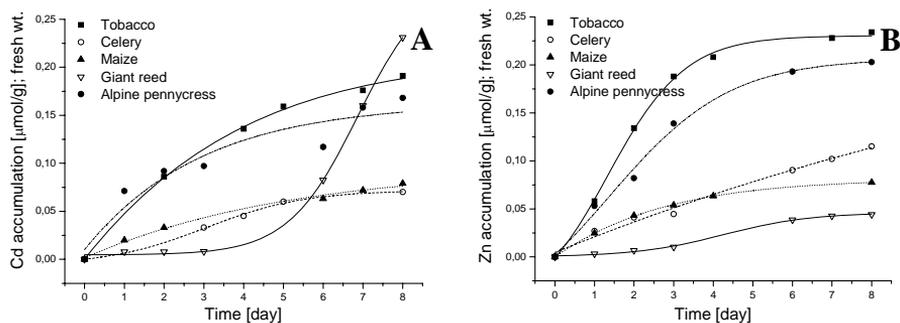
3. Results and discussion

Fig. 1 depicts the kinetics of Cd and Zn accumulation in plants of tobacco (*N. tabacum* L.), celery (*A. graveolens* L.), maize (*Z. mays* L.), giant reed (*A. donax* L.), and alpine pennycress (*N. caerulescens* L.) during 8 days of plant cultivation under hydroponic conditions. The conditions of the experiments were partly different (see Table 1) in terms of the initial concentrations of Cd (in the form of $CdCl_2$) and Zn (in the form of $ZnCl_2$ or $ZnSO_4$), the pH of nutrient solutions and the strength of Hoagland medium (HM) as well as from the point of view of conditions of plant cultivation (temperature, relative humidity, day/night photoperiod or intensity of illumination). To carry out a calculation of free Cd^{2+} and Zn^{2+} ionic forms proportion and concentration in nutrient solutions as the most bioavailable metal forms for plants, the program MINEQL+ was used. However, this prediction has to be considered with caution, because the modelling program does not include the participation of the plant. Therefore, it can be used for the evaluation of initial conditions of the experiments (without plants).

According to *in silico* speciation analysis by mentioned equilibrium modelling software, it was found that Cd present in a 25 % or 50 % HM representing the model

of soil solution can occur in 15 different inorganic chemical forms within the range of pH 3 – 12. In the case of Zn, 12 different inorganic chemical forms were calculated under the same conditions. At the pH 5.5 or 6.0, 25 % or 50 % HM, and initial concentration of $CdCl_2$ 10 μmol/dm^3, which were included in the experiments, the proportion of Cd^{2+} form from the total amount of Cd was in the range 71.7 % to 77.1 %. In the case of Zn and the given initial conditions, the proportion of Zn^{2+} form represented 92.2 % – 94.8 %.

Fig. 1. Kinetics of Cd (A) and Zn (B) accumulation in plants of tobacco (*N. tabacum* L.), celery (*A. graveolens* L.), maize (*Z. mays* L.), giant reed (*A. donax* L.), and alpine pennycress (*N. caerulescens* L.) during 8 days of plant cultivation under hydroponic conditions. Conditions are detailed in Table 1. Data are presented as arithmetic means of triplicate experiment.

From the Fig. 1, it is evident that the curves describing the Cd accumulation (Fig. 1A) in tobacco, celery, maize, giant reed, and alpine pennycress plants show a relatively similar trend with the curves obtained for Zn (Fig. 1B). This result corresponds with the fact that Cd and Zn are mutual physico-chemical analogues as well as that Cd is transported within the plants *via* the Zn pathways, but the mechanisms responsible for cellular Zn tolerance cannot detoxify Cd effectively (ZHAO *et al*., 2006). However, there were significant differences between plant species in time-course of Cd and Zn accumulation and in the obtained values of accumulation (μmol/g; fresh wt.). The most different trend of the curve describing the Cd and Zn accumulation showed plants of giant reed, when the two-phase process given by a slow Cd or Zn accumulation within the first 3 days of plant cultivation followed by exponential increase of accumulation within the next 5 days was observed. At the other studied plant species, the typical curves with gradual increasing the Cd or Zn accumulation in plants were found. The phytotoxic symptoms at used Cd concentrations were not observed in either case. The Fig. 2 compares the differences in the obtained values of Cd and Zn accumulation in individual plant species on the basis of multiple range test at the level of $p < 0.05$. It was found that the highest value of Cd accumulation showed plants of giant reed and the accumulation decreased in the order: giant reed > tobacco > alpine pennycress >> maize and celery. QUEZADA-HINOJOSA *et al.* (2015) also found the variability in Cd contents among the six different perennial plant species growing in a wooded pasture of the Swiss Jura Mountains, where the soils are geogenically enriched in Cd. In the case of Zn, when

the different initial concentrations of Zn (see Table 1) were used, the order of plants for Zn accumulation was: tobacco and alpine pennycress > celery > maize >> giant reed. It should be mentioned that the nutrient solution for giant reed contained a 10-fold lower concentration of Zn in comparison with plants of tobacco and alpine pennycress. From these results, it can be concluded that tobacco and giant reed showed a similar Cd accumulation efficiency as plants of alpine pennycress, which are classified as Zn/Cd hyperaccumulators. In the case of celery and maize, as typical agricultural crops, the Cd and Zn accumulation reached significantly lower levels.
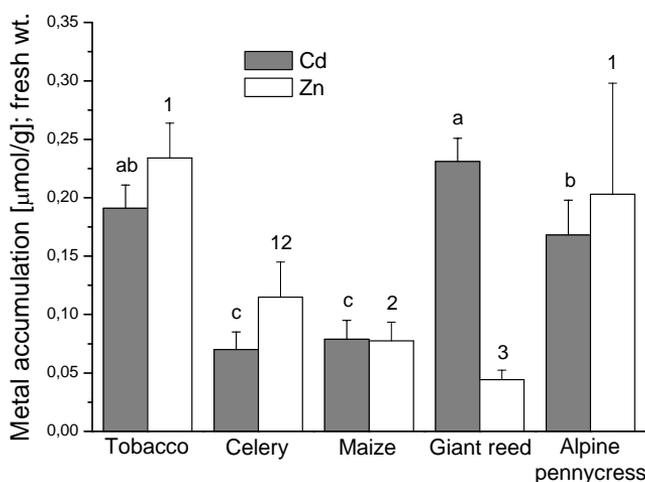

Fig. 2. Comparison of Cd (A) and Zn (B) accumulation in plants of tobacco (*N. tabacum* L.), celery (*A. graveolens* L.), maize (*Z. mays* L.), giant reed (*A. donax* L.), and alpine pennycress (*N. caerulescens* L.) after 8 days of plant cultivation under hydroponic conditions. Conditions are detailed in Table 1. Mean values from triplicate experiments with the same letter (for Cd) or number (for Zn) above columns are not significantly different at the $p < 0.05$ level based on multiple range test.

In terms of phytoremediation technologies, it is important to mention that toxic metals or radionuclides have to be accumulated mainly in above-ground parts of plants. In general, significant differences in distribution of Cd and Zn within shoots and roots of studied plant species and higher percentage distribution of Cd into the shoots from the total accumulated amount of Cd in comparison with the data obtained for Zn were found as well (Fig. 3). The highest proportion of translocated Cd into shoots was observed in plants of tobacco, celery and alpine pennycress, whereby the distribution of Cd for account of shoots decreased in the order: tobacco, celery and alpine pennycress > maize > giant reed. In the case of Zn, when the different initial concentrations of Zn (see Table 1) were used, the percentage distribution into shoots decreased in the order: giant reed >> alpine pennycress > celery > tobacco > maize. Within the shoots, the direct gammaspectrometric determinations revealed that the amount of accumulated Cd and Zn (in specific radioactivities Bq/g of dry wt. or Bq/cm^2) increased from the oldest developed leaves to the youngest developed leaves (data not shown). This result was observed in all studied plant species. ZHANG

et al. (2015) reported that Cd accumulation within the plants of two castor cultivars (*Ricinus communis* L.) decreased in the order: root > stem > young leaf > old leaf.
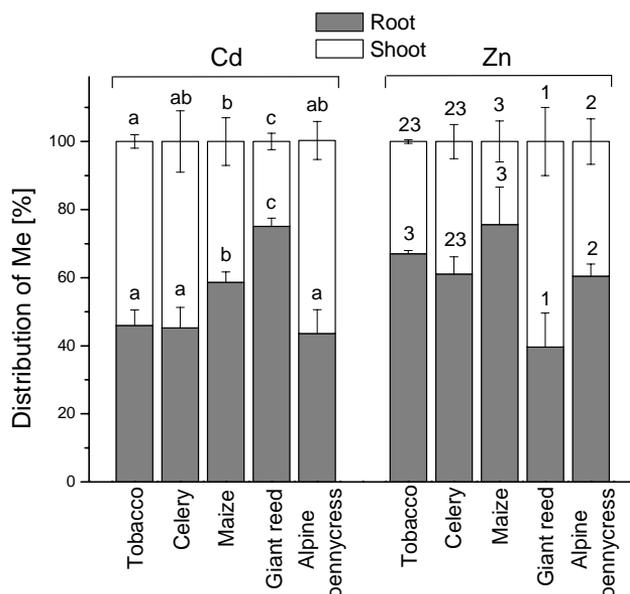

Fig. 3. Distribution of Cd and Zn in roots and shoots of tobacco (*N. tabacum* L.), celery (*A. graveolens* L.), maize (*Z. mays* L.), giant reed (*A. donax* L.), and alpine pennycress (*N. caerulescens* L.) after 8 days of plant cultivation under hydroponic conditions. Conditions are detailed in Table 1. Mean values from triplicate experiments with the same letter (for Cd) or number (for Zn) above columns are not significantly different at the $p < 0.05$ level based on multiple range test.

Also, for the evaluation of Cd and Zn mobility in conductive tissues of studied plants in the term of their translocation efficiency it was established a non-dimensional concentration ratio (*CR*) which represents the ratio of metal concentration in the above-ground part of plants $[Me]_{shoot}$ (μmol/g; dry wt.) to metal concentration in roots $[Me]_{root}$ (μmol/g; dry wt.). From the data mentioned in Table 2, it is evident that on the basis of these concentration ratios $[Me]_{shoot}$ / $[Me]_{root}$ Cd and Zn were in prevailing part accumulated in the root tissues and only partially accumulated in shoots. In general, the obtained values of *CR* were very similar for both target metals Cd and Zn. This result is in agreement with the above mentioned fact that Cd and Zn are mutual physico-chemical analogues and that Cd is transported *via* the Zn pathway as well. Some present differences in the obtained *CR* values between Cd and Zn were probably caused by lower concentrations of Zn in nutrient solutions. The *CR* values were calculated in the range 0.06 – 0.27 for Cd and for Zn 0.06 – 0.48. Similar concentration ratios found POŁEĆ-PAWLAK *et al.* (2005) for plants of *Arabidopsis thaliana* cultivated under hydroponic conditions during 14 days in HM containing 25 or 50 μmol/dm^3 of Cd^{2+}. From the point of view of plant species, the *CR* values obtained for Cd decreased in the order: maize > celery > tobacco and giant reed

> alpine pennycress. These results suggest that plants of celery and maize with a lower ability to accumulate Cd and Zn from nutrient solutions showed higher values of *CR*. On the other side, plants, such as tobacco, giant reed and alpine pennycress, with a higher ability to accumulate Cd and Zn from nutrient solutions showed lower values of *CR*. This fact can be related to ability of these plants to bind and detoxicate of metals, such as Cd and Zn, in root cells and cellular compartments (e.g. vacuoles). Thus, in this case the root system acts as a protective barrier to further metals movement into the above-ground parts of plants. This character results in a high tolerance to metals and metals accumulation in plants or in given plant tissues. SALT *et al.* (1999) reported that Zn was mostly complexed to histidine in roots, transported as Zn^{2+} in the xylem sap, and complexed to organic acids in leaves. It can be expected that mentioned processes are also involved in Cd accumulation. Some works reported that the application of chelating agents, such as ethylenediaminetetraacetate (EDTA) or citric acid (CA), can increase not only the concentration of metals in soil solutions, but also the translocation of metals within the plants (HORNÍK *et al.* 2008; HORNÍK *et al.* 2009; GULDANOVÁ *et al.*, 2012; EHSAN *et al.*, 2014).

Table 2. Comparison of the values of non-dimensional concentration ratio (*CR*) calculated for Cd and Zn after 8 days cultivation of plants of tobacco (*N. tabacum* L.), celery (*A. graveolens* L.), maize (*Z. mays* L.), giant reed (*A. donax* L.), and alpine pennycress (*N. caerulescens* L.) under hydroponic conditions. Conditions are detailed in Table 1. Mean values from triplicate experiments with the same letter (for Cd) or number (for Zn) above columns are not significantly different at the $p < 0.05$ level based on multiple range test.

Plant	CR ([Me]$_{shoot}$ / [Me]$_{root}$) $\pm$ S.D.	
	Cd	Zn
Tobacco	0.12±0.02[c]	0.06±0.01[4]
Celery	0.16±0.02[b]	0.14±0.01[3]
Maize	0.27±0.03[a]	0.18±0.02[2; *]
Giant reed	0.11±0.01[c]	0.48±0.20[1; **]
Alpine pennycress	0.06±0.01[d]	0.06±0.01[4]

* Initial concentration of Zn 5.9 μmol/dm^3 in a nutrient solution;
** Initial concentration of Zn 1.0 μmol/dm^3 in a nutrient solution.

The realized experiments were deliberately designed for different initial conditions of the experiments and conditions of the plant cultivation (see Table 1). This fact allows to evaluate the relationships between variables obtained at the end of experiments and these conditions as well as to find the similarity between objects – individual plant species. For these purposes, the method of multivariate analysis – cluster analysis (CA) was applied and parameters describing the obtained variables, such as Cd or Zn accumulation in plants (in μmol/g; fresh wt.) and concentration ratio (*CR*) defined as the ratio of the concentration of Cd or Zn accumulated in shoots to concentration of Cd or Zn accumulated in roots ([Me]$_{shoot}$ / [Me]$_{root}$) and the conditions of the experiments, such as the initial concentrations of Cd^{2+} or Zn^{2+} ions (C_0 Cd^{2+} or C_0 Zn^{2+}; in μmol/dm^3) predicted by the speciation modelling, the pH value of nutrient solutions, the strength of Hoagland medium (25 % or 50 % HM) or conditions of the plant cultivation (temperature, relative humidity, day/night photoperiod or intensity of illumination) were utilized in this analysis.
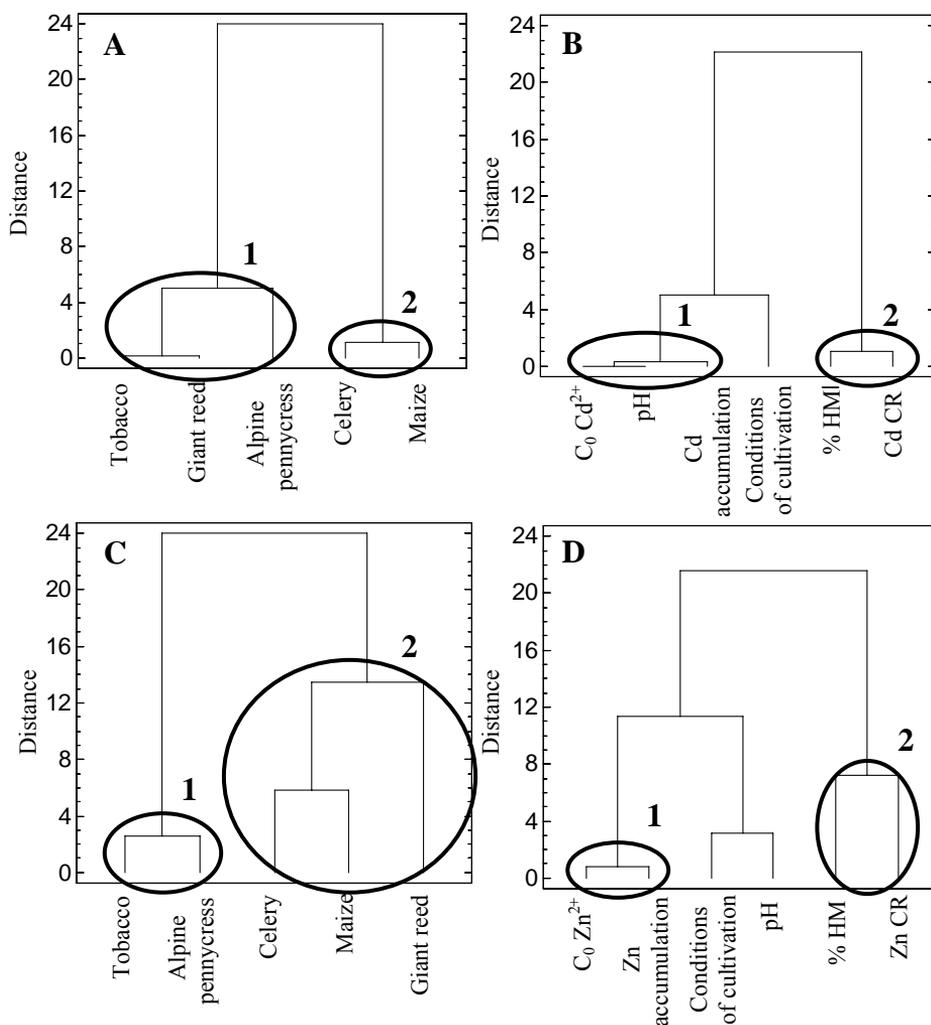
Fig. 4. Dendrogram of cluster analysis including squared Euclidean distance and Ward's method as the aggregation criterion for the evaluation of the similarity degree between objects – individual plant species on basis of obtained variables and conditions of the experiments (A, C) as well as the relationships between obtained variables and conditions of the experiments (B, D) for Cd (A, B) and Zn (C, D). Conditions of the experiments are detailed in Table 1.

From CA including squared Euclidean distance and Ward's method as the aggregation criterion for the evaluation of the similarity degree between objects represented by studied plant species, it was found that in the case of Cd plants of tobacco, giant reed, and alpine pennycress, these formed separate cluster (Fig. 4A, No. 1). It means that these plants showed similar characteristics from the point of view of Cd accumulation and relationships between conditions of the experiments and obtained data describing Cd accumulation. Thus, it can be expected that plants

of tobacco and giant reed will dispose with similar characteristics as plants of alpine pennycress, which are defined as Zn/Cd hyperaccumulators, in terms of Cd accumulation and other positive parameters for their utilization in phytoremediation processes and techniques. LIU *et al.* (2016) also classified the plants of tobacco (*N. tabacum* L.) as hyperaccumulators of Cd on the basis of realized experiments under hydroponic conditions and characterized the effect of conditions of the experiments (chemical inhibitors, temperature or illumination) on Cd accumulation. On the other side, plants of celery and maize as agricultural crops are significantly different from this group, because they formed a second separate cluster (Fig. 4A, No. 2). Within the evaluation of relationships between the conditions of the experiments and obtained values for Cd accumulation (Cd accumulation and *CR* for Cd), the correlations between $C_0 Cd^{2+}$, pH and Cd accumulation (Fig. 4B, No. 1) as well as between the strength of HM (representing the different concentrations of macro- and microelements and ionic strength) and *CR* for Cd (Fig. 4B, No. 2) were found. From these results, it can be concluded that Cd accumulation through the root system of studied plants is strongly dependent on initial concentration of free Cd^{2+} ions and the value of pH. On the other hand, the concentration ratio *CR* describing the Cd translocation from roots-to-shoots is primarily affected by the strength of HM corresponding with concentrations of macro- and microelements in nutrient (soil) solutions. In the case of Zn, on the basis of the similarity degree, two separate clusters were also formed. The first cluster was represented by plants of tobacco and alpine pennycress (Fig. 4C, No. 1) and the second cluster formed plants of celery, maize, and giant reed (Fig. 4C, No. 2). Similarly, as in the case of Cd, the two logical correlations between $C_0 Zn^{2+}$ and Zn accumulation (Fig. 4D, No. 1) and between the strength of HM and *CR* for Zn (Fig. 4D, No. 2) were found.

4. Conclusions

The obtained results showed that the time-course of Cd accumulation in plants of tobacco, celery, maize, giant reed, and alpine pennycress cultivated under conditions of short-term hydroponic experiments had a relatively similar trend with curves obtained for Zn. This result supports the fact that Cd and Zn are mutual physico-chemical analogues as well as that Cd is transported within the plants *via* the Zn pathways. On the other side, a significant difference in time-course of Cd and Zn accumulation and in the obtained values of accumulation (μmol/g; fresh wt.) between plant species existed as well. The differences in the obtained values of Cd and Zn accumulation in individual plant species were evaluated by multiple range test at the level of $p < 0.05$. The highest values of Cd accumulation showed plants of giant reed and the accumulation decreased in the order: giant reed > tobacco > alpine pennycress >> maize and celery.

From the point of view of Cd and Zn distribution within the plants, the highest proportion of translocated Cd into shoots was observed in plants of tobacco, celery, and alpine pennycress. A similar result was also found in the case of Zn. Within the shoots, the amount of accumulated Cd and Zn increased from the oldest developed leaves to the youngest developed leaves. On the basis of concentration

ratios (*CR*) [Me]$_{shoot}$ / [Me]$_{root}$ calculation for both metals, it was found that Cd and Zn were in prevailing part accumulated in the root tissues and only partially accumulated in shoots. The *CR* values corresponding to these facts were calculated in the range 0.06 – 0.27 for Cd and for Zn 0.06 – 0.48. From the point of view of plant species, the *CR* values obtained for Cd decreased in the order: maize > celery > tobacco and giant reed > alpine pennycress. This result suggests that plants of tobacco, giant reed, and alpine pennycress showed the ability to bind and detoxicate of metals, such as Cd and Zn, in root cells and cellular compartments in larger extent than studied agricultural crops (maize and celery).

The similarity between studied objects – individual plant species on the basis of obtained variables and conditions of the experiments as well as the relationships between obtained variables and conditions of the experiments were subjected to multivariate analysis method – cluster analysis (CA). According to this analysis, it can be expected that plants of tobacco and giant reed will dispose with similar characteristics as plants of alpine pennycress, which are defined as Zn/Cd hyperaccumulators, in terms of Cd accumulation and other positive parameters for their utilization in phytoremediation processes and techniques. Also, it was found that Cd and Zn accumulation through the root system of studied plants is strongly dependent on initial concentration of free Me^{2+} ions and the value of pH. On the other hand, the concentration ratios *CR* describing the Cd or Zn translocation from roots-to-shoots are primarily affected by concentrations of macro- and microelements in the nutrient (soil) solutions.

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