DETERMINATION OF LONG DISTANCE TRANSPORT OF Cs^{+}, Co^{2+} AND Zn^{2+} IONS IN VASCULAR PLANTS BY AUTORADIOGRAPHY AND GAMMA-SPECTROMETRY

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Abstract: Heavy metals and radionuclides can enter the food chain *via* cereals and vegetables grown in contaminated soils. In the case of microelements such as zinc, studies have not focused only to assessing its environmental risk, but also to enhancing its uptake by plants as an important growth-limiting factor. In our study, digitalized autoradiograms of whole plants of celery (*Apium graveolens* L.), tobacco (*Nicotiana tabacum* L.) and sunflower (*Helianthus annuus* L.) grown in hydroponic nutrient media spiked with $^{137}CsCl$, $^{60}CoCl_2$ and $^{65}ZnCl_2$ were used for quantitative determination of uptake, long-distance transport and distribution of Cs^{+}, Co^{2+} and Zn^{2+} ions in plant tissues. Results from autoradiography and gamma-spectrometry of plants showed, that cesium was translocated to aboveground part of the plants with the shoot-to-root ratio 1.0 : 0.6. On the contrary, cobalt and zinc were more immobilized in roots, with the shoot-to-root ratio up to 1.0 : 3.8. The highest concentration of cesium, cobalt and zinc, expressed in specific radioactivity per unit of leaf surface (Bq/cm^2) was found in top, rapidly growing leaves, the lowest concentration in the oldest leaves in low position. Detection limits 3, 2 and 14 Bq/cm^2 by using X-ray film for ^{137}Cs, ^{60}Co and ^{65}Zn, respectively were obtained. These data correspond to detection limits 10.5 pg Cs^{+}/cm^2, 7.2 pg Co^{2+}/cm^2 and 785 pg Zn^{2+}/cm^2 at specific radioactivity of commercially available $^{137}CsCl$, $^{60}CoCl_2$ and $^{65}ZnCl_2$. Resolutions 1-2 mm was sufficient for visualization of metal uptake and distribution in roots, stalks, leaves and leaf venation. Obtained data are part of quantitative study of uptake and translocation of both low level-radioactive contamination in plants and microelements applied as fertilizers.

Keywords: cobalt, cesium, zinc, distribution, vascular plants, autoradiography

1. Introduction

Heavy metals are present in soils and aqueous streams as both natural components or as a result of human activity i.e. metal-rich mine tailings, metal smelting, electroplanting, energy and fuel production, intensive agriculture, etc. (RASKIN *et al*., 1994). Radionuclides exist in the environment either naturally or artificially by aboveground nuclear testing, nuclear accidents and nuclear power generation (ZHU and SHAW, 2000).

Cesium ^{137}Cs (τ = 30.17 y) is produced during the fission of actinides or the neutron bombardment of ^{133}Cs or ^{136}Ba (WHITE and BROADLEY, 2000). The behavior of Cs in soils resembles that of K and, even in soils with high clay content, Cs is available for uptake by plants since Cs-fixation tends towards a reversible steady-state.

Cobalt usually occurs in the environment in association with other metals such as copper, nickel, manganese and arsenic. Background soil cobalt levels range from 1.6

to 21.5 mg.kg^{-1} (PEREZ-ESPINOZA *et al*., 2005). Cobalt ^{60}Co (τ = 5.27 y) is also present in low-level radioactive wastes.

Although zinc is an essential nutrient as a trace element for animals, plants, and microorganisms, it is toxic to these organisms when present at supranormal concentrations. Zinc is a trace element essential for cell proliferation and differentiation. It is a structural constituent of many enzymes and proteins, including metabolic enzymes, transcription factors, and cellular signaling proteins.

There is considerable interest in remediation of sites contaminated by these elements using extraction by plants that do not enter the human food chain. Several comprehensive reviews have been written, summarizing many important aspects of this plant-based technology (LASAT, 2002; DUSHENKOV, 2003; SURESH and RAVISHANKAR, 2004; PILON-SMITS, 2005; LEDUC and TERRY, 2005; MARMIROLI *et al*., 2006).

The ecological problems related to heavy metals and radionuclides are not dependent only on their total content and radioactivity in the soil, but rather on their form of bonding and therefore their bioavailability. Therefore, several studies have been conducted using seedlings or adult plant, which have been cultivated in hydroponic conditions (see e.g. PAGE and FELLER, 2005; SOUDEK *et al*., 2006).

In our study, digitalization of autoradiograms of whole plants of celery (*Apium graveolens* L.), tobacco (*Nicotiana tabacum* L.) and sunflower (*Helianthus annuus* L.) after cultivation in hydroponic nutrient media spiked with $^{137}CsCl$, $^{60}CoCl_2$ and $^{65}ZnCl_2$ was used as the method for quantitative determination of uptake, long-distance transport and distribution of Cs^+, Co^{2+} and Zn^{2+} ions in the plant tissues.

2. Materials and methods

2.1 Plant material

Seeds of celery (*A. graveolens* L.), tobacco (*N. tabacum* L.) and sunflower (*H. annuus* L.) were germinated and grown in pots filled with granulated perlite as an inert carrier and watered with diluted hydroponic medium according to HOAGLAND (1920) in day/night period 12/12 h (illumination with 2 tubes Brilliant daylight - 6000 K, 1 300 lm and Tropic sun – 4 700 K, 1 000 lm, SERA) at 22±2°C. After 5 weeks (for celery and sunflower) or 8 weeks (for tobacco), seedlings were gently removed from perlite and roots were washed free of any adhering perlite fragments by distilled water. Plants were pre-cultivated for one week in vessels with a cover to protect plant roots against lights in 25 % Hoagland medium without perlite support.

2.2 Bioaccumulation experiments

For experiments selected plants were cultivated at 22±2°C in 25 % Hoagland medium supplemented with 10 μmol.L^{-1} CsCl, 10 μmol.L^{-1} $CoCl_2$ or 5 μmol.L^{-1} $ZnCl_2$ and spiked with ^{137}Cs, ^{60}Co or ^{65}Zn at illumination 2 000 lx with 12/12 h day/night cycle. In time intervals aliquot samples of cultivation media were measured for determination of remaining radioactivity by gamma-spectrometry. The amounts of transpired water were determined by measuring the weight changes of cultivation vessels. The amount of water lost by plant transpiration was replenished with fresh 25 % Hoagland medium to initial solution volume. At the end of experiments plants were harvested and roots carefully rinsed in distilled water. In roots, stalks and leaves incorporated radioactivity was measured by gamma-spectrometry and topography visualized by autoradiography as well.

2.3 Autoradiography

Plants were pressed between two filter papers and air-dried for 7 days at 20°C. ^{137}Cs, ^{60}Co and ^{65}Zn in dried plant were detected by autoradiography by exposing X-ray films (HR-GB 100 NIF, FUJIFILM, JP) for 40 days at 20°C. Developed films were scanned and data were converted to colour scale gradient by software Photoshop CS2 ver. 9.0 (ADOBE, USA).

2.4 Radiometric analysis

Gamma-spectrometric scintillation detector 54BP54/2-X with well type crystal NaI(Tl) (Scionix, NL) and data processing software Scintivision32 (ORTEC, USA) were used for ^{137}Cs, ^{60}Co and ^{65}Zn determination in plant and solution. Counting time 600 s allowed obtaining data with measurement error <2 %, which do not reflect other source of errors. Standardized $^{137}CsCl$ (5.723 MBq.mL^{-1}; 20 mg.L^{-1} CsCl + 3 g.L^{-1} HCl), $^{60}CoCl_2$ (5.571 MBq.mL^{-1}; 20 mg.L^{-1} $CoCl_2$ + 3 g.L^{-1} HCl) and $^{65}ZnCl_2$ (0.892 MBq.mL^{-1}; 50 mg.L^{-1} $ZnCl_2$ + 3 g.L^{-1} HCl) were obtained from CMI (CZ).

3. Results and discussion

Kinetics of cesium and cobalt bioaccumulation by celery (*A. graveolens* L.), tobacco (*N. tabacum* L.) and sunflower (*H. annuus* L.) hydroponically growing in nutrient media spiked with $^{137}CsCl$ and $^{60}CoCl_2$ were described in our previous papers (HORNIK *et al*., 2005; BARATOVA *et al*., 2006; VRTOCH *et al*., 2006; VRTOCH *et al*., 2007). We found, that bioaccumulation of ^{137}Cs and ^{60}Co by these plants was proportional to the transpiration rate.

The aim of this paper is to characterize long-distance transport of $^{137}Cs^{+}$, $^{60}Co^{2+}$ and $^{65}Zn^{2+}$ in celery, tobacco and sunflower.

Radiometric analysis showed, that cesium accumulated by celery and tobacco from nutrient solution containing sub-inhibitory concentrations 10 µmol.L^{-1} and 2.4 µmol.L^{-1}CsCl was transported from roots to shoots. The highest cesium concentration was found in top leaves. (Fig. 1, Fig. 2). Similar pattern of cesium transport in sunflower was observed in our previous paper (HORNIK *et al*., 2005). Shoot-to-root concentration ratio was within the range 1: 0.60 – 1: 0.88 (Tab. 1). Concentration of cesium expressed in specific radioactivity per unit of leaf surface (Bq/cm^2) was 119-

times higher in top, rapidly growing celery leaves than in the oldest celery leaves and 12-times higher in top tobacco leaves (Fig. 3).

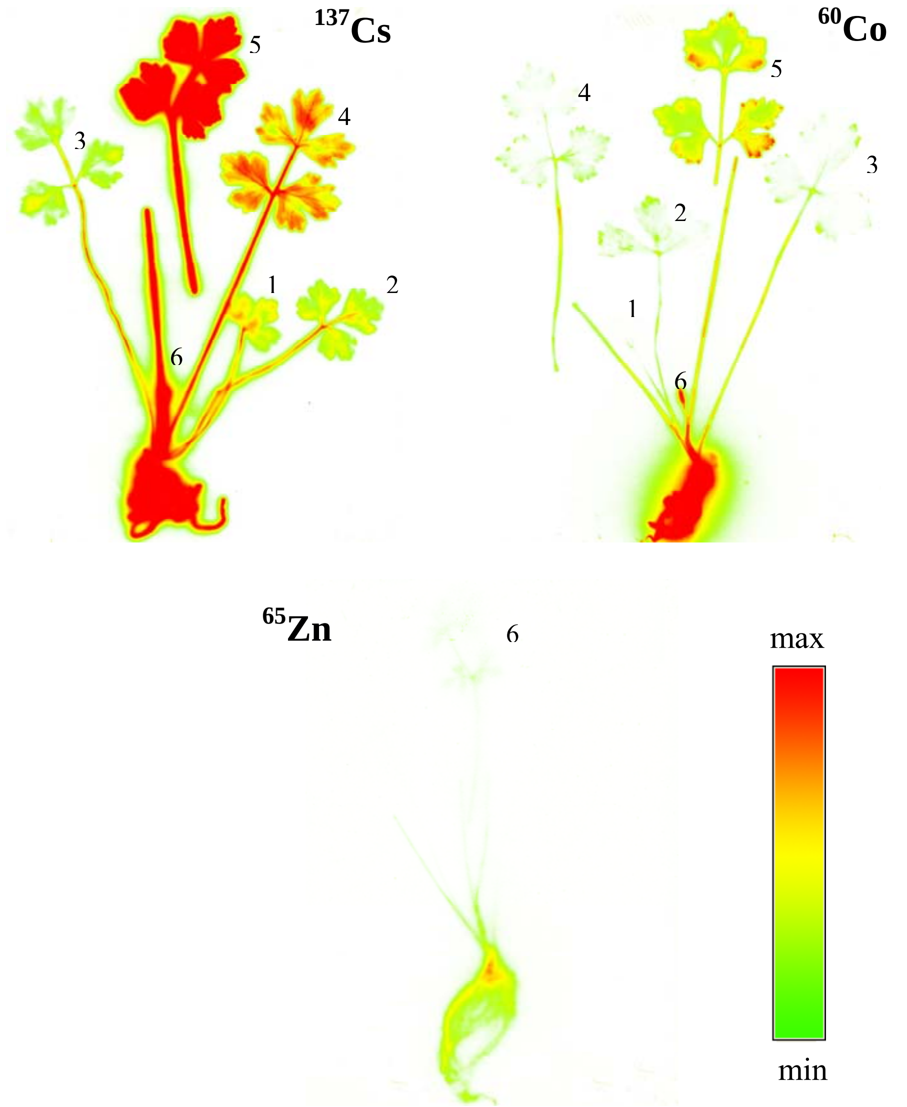

Fig. 1: Autoradiographic visualization of ^{137}Cs, ^{65}Co and ^{65}Zn distribution in celery (*A. graveolens* L.) after 8 days cultivation in 25 % Hoagland medium. Initial concentrations C_0 = 10 μmol.L^{-1} CsCl, 10 μmol.L^{-1} $CoCl_2$ or 5 μmol.L^{-1} $ZnCl_2$, spiked with 167.6 kBq.L^{-1} ^{137}CsCl, 91 kBq.L^{-1} $^{60}CoCl_2$ or 68 kBq.L^{-1} $^{65}ZnCl_2$. Uptake: Cs – 37 %; Co – 97 %; Zn – 40 %.
Numbering of leaves in the order: 1. oldest leaves, 6. youngest leaves.

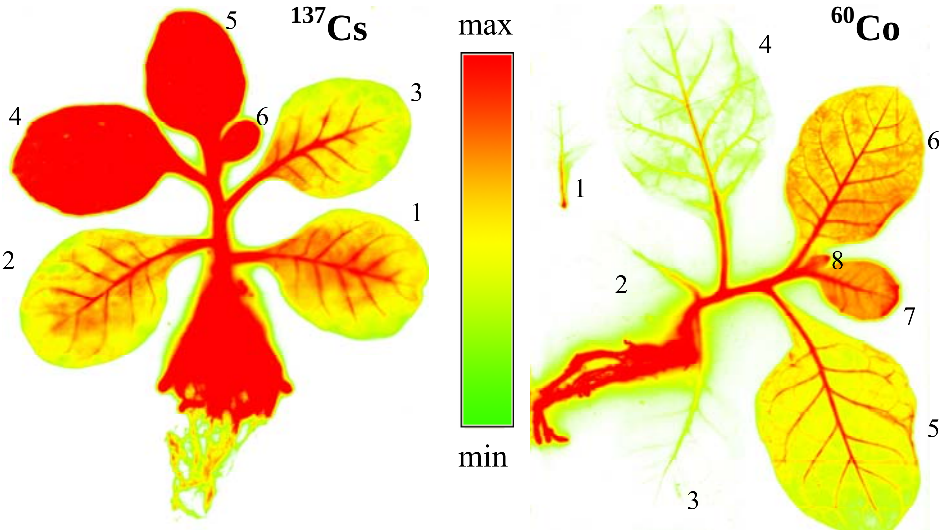

Fig. 2: Autoradiographic visualization of ^{137}Cs and ^{65}Co distribution in tobacco (*N. tabacum* L.) after 9 days cultivation in 25 % Hoagland medium. Initial concentrations C_0 = 2.4 µmol.L^{-1} CsCl or 25 µmol.L^{-1} $CoCl_2$, spiked with 283 kBq.L^{-1} ^{137}CsCl or 29.7 kBq.L^{-1} $^{60}CoCl_2$. Uptake: Cs – 65 %; Co – 65 %.
Numbering of leaves in the order: 1. oldest leaves, 8. youngest leaves.

Long-distance transport of both cobalt and zinc in celery, tobacco and sunflower cultivated in nutrient media in sub-inhibitory concentration 0.385 – 25 µmol.L^{-1} $CoCl_2$ or 5 µmol.L^{-1} $ZnCl_2$ was significantly lower than in the case of cesium. Cobalt and zinc were mainly trapped in roots. Shoot-to-root concentration ratio up to 1 : 3.78 was observed (Tab. 1). Concentration of cobalt and zinc, expressed in specific radioactivity per unit of leaf surface (Bq/cm^2) of celery, tobacco and sunflower was approx. 50-times higher in top, younger leaves than in the oldest leaves (Fig. 3).

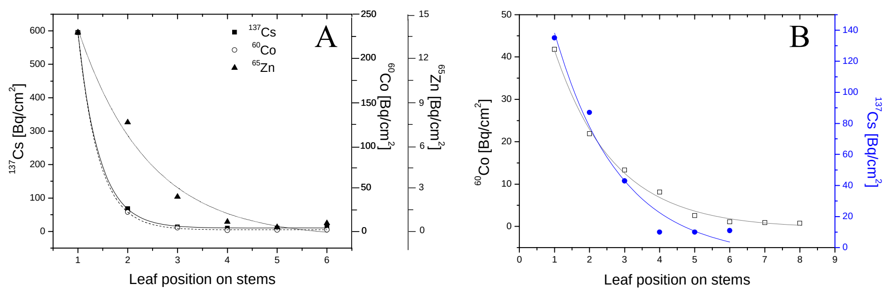

Fig. 3: Specific radioactivity of ^{137}Cs, ^{60}Co and ^{75}Zn accumulated in leaves of celery (A) and tobacco (B), calculated for leaf surface (Bq/cm^2). Numbering of leaves from top to root.

Mobility of monovalent cesium in tobacco, celery and sunflower plants is higher than mobility of bivalent zinc and cobalt. This fact is in an agreement with well described transport mechanisms of cesium, comparable with transport mechanisms of essential potassium K^+ ions (WHITE and BROADLEY, 2000). Cesium, with the exception of some synthetic compounds such as crown ethers, does not form stable organic complexes (DOZOL *et al*., 2004).

On the other hand, cobalt and zinc can react with low and high molecular organic components of plants (OWEN *et al*., 2002). SALT *et al*. (1999) found, that Zn was mostly complexed to histidine in roots, transported as Zn^{2+} in the xylem sap, and complexed to organic acids in leaves. In dependence on pH value, zinc and cobalt can form charged species by the reaction with CO_2 and OH^- ions (DANG *et al*., 1996; GÜNTHER and KASTENHOLZ, 2005).

PAGE and FELLER (2005) showed that zinc was translocated from root to young wheat leaves within 50 days nearly uniformly in all leaves of the shoot. On the contrary, prevailing part of cobalt even after 50 days remained in root and transport to all leaves including apex leaves was negligible.

Mobility of zinc and cobalt in vascular plants within time intervals, comparable with duration of vegetation period, will be the topic of our next study.

Tab. 1: Distribution of cesium, cobalt and zinc in shoots and roots of celery (*A. graveolens* L.), tobacco (*N. tabacum* L.) and sunflower (*H. annuus* L.) cultivated in 25 % Hoagland medium spiked with $^{137}CsCl$ $^{60}CoCl_2$, or $^{65}ZnCl_2$.

Nuclide	Shoot-to-root ratio		
	Celery [1]	Tobacco [2]	Sunflower [3]
^{137}Cs	1.0 : 0.60	1.0 : 0.88	1.0 : 0.64
^{60}Co	1.0 : 3.78	1.0 : 1.02	1.0 : 2.03
^{65}Zn	1.0 : 1.29	–	–

1 - After 8 day cultivation, C_0 = 10 μmol.L^{-1} CsCl (168 kBq.L^{-1}), C_0 = 10 μmol.L^{-1} $CoCl_2$ (91 kBq.L^{-1}) or C_0 = 5 μmol.L^{-1} $ZnCl_2$ (68 kBq.L^{-1})
2 - After 9 day cultivation, C_0 = 2.4 μmol.L^{-1} CsCl (283 kBq.L^{-1}) or C_0 = 25 μmol.L^{-1} $CoCl_2$ (29.7 kBq.L^{-1})
3 - After 9 day cultivation, C_0 = 1.25 μmol.L^{-1} CsCl (25 kBq.L^{-1}) or C_0 = 0.385 μmol.L^{-1} $CoCl_2$ (25 kBq.L^{-1}). See also HORNIK *et al*., (2005).

Detection limits obtained by autoradiography of the whole plants was 3, 2 and 14 Bq/cm^2 for ^{137}Cs, ^{60}Co and ^{65}Zn, respectively. It corresponds to detection limits 10.5 pg Cs/cm^2, 7.2 pg Co/cm^2 and 785 pg Zn/cm^2 at specific radioactivities of commercially available $^{137}CsCl$, $^{60}CoCl_2$ and $^{65}ZnCl_2$. Resolution 1-2 mm was sufficient for visualization of distribution in roots, stalks, leaves and leaf venation.

4. Conclusions

Autoradiography and gamma-spectrometry of celery (*Apium graveolens* L.), tobacco (*Nicotiana tabacum* L.) and sunflower (*Helianthus annuus* L.) after cultivation in hydroponic nutrient media spiked with $^{137}CsCl$, $^{60}CoCl_2$ and $^{65}ZnCl_2$ was used for quantitative determination of uptake and long-distance transport of Cs^+, Co^{2+} and Zn^{2+} ions in the plant tissues.

Cesium was traslocated to the aboveground part of the plants with the shoot-to-root ratio 1.0: 0.6. On the contrary, cobalt and zinc was more immobilized in roots, with the shoot-to-root ratio up to 1.0: 3.8.

The highest concentration of cesium, cobalt and zinc expressed in specific radioactivity per unit of leaf surface (Bq/cm^2) was found in top, rapidly growing leaves, the lowest concentration in the oldest leaves in low position.

Detection limits 3, 2 and 14 Bq/cm^2 by using X-ray film for ^{137}Cs, ^{60}Co and ^{65}Zn, respectively were obtained. It corresponds to detection limits 10.5 pg Cs^+/cm^2, 7.2 pg Co^{2+}/cm^2 and 785 pg Zn^{2+}/cm^2 at specific radioactivity of commercially available $^{137}CsCl$, $^{60}CoCl_2$ and $^{65}ZnCl_2$. Resolution 1-2 mm was sufficient for visualization of distribution in roots, stalks, leaves and leaf venation.

Obtained data are part of quantitative study of uptake and translocation of both low-level radioactive contamination in plants and microelements applied as fertilizers.

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