Bioaccumulation of ^{137}Cs and ^{60}Co by *Helianthus annuus*

Miroslav Horník, Martin Pipíška, L'uboš Vrtoch, Jozef Augustín, Juraj Lesný

Abstract The ^{60}Co and ^{137}Cs bioaccumulation by *Helianthus annuus* L. was measured during 9 day cultivation at 20 ± 2°C in hydroponic Hoagland medium. Previous starvation for K^+ and for NH_4^+ 2.2 and 2.7 times, respectively, enhanced ^{137}Cs uptake rate. Previous cultivation in surplus of K^+ ions 50 mmol·l^{-1} has no effect on ^{137}Cs bioaccumulation rate. Both ^{137}Cs and ^{60}Co bioaccumulation significantly increase with dilution of basic Hoagland medium up to 1:7 for caesium and up to 1:3 for cobalt followed by mild decrease at higher dilutions. Root to shoot specific ^{137}Cs radioactivity ratio (Bq·g^{-1}/Bq·g^{-1}, fresh wt.) increased with dilution from 1.46 to 9.6–9.8. The values root to shoot specific radioactivity ratio for ^{60}Co were less dependent on the nutrient concentrations and were within the range 5.7 to 8.5. ^{137}Cs was localized mainly in young leaves (30%) and roots (39%) and ^{60}Co mainly in roots (67%) and leaves (20%). Obtained data showed less sensitivity of ^{60}Co uptake by sunflower on nutrient concentration in hydroponic media.

Key words *Helianthus annuus* • ^{60}Co • ^{137}Cs • bioaccumulation • starvation • autoradiography

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Introduction

Radionuclides exist in the environment either naturally or artificially by aboveground nuclear testing, nuclear accidents, and nuclear power generation [11]. Radionuclides characteristic of nuclear fission, such as ^{137}Cs, ^{90}Sr and ^{60}Co, are of environmental concern due to their relatively long half-life, emission of γ-radiation during decay and rapid incorporation into living organisms. There is a great need for reliable and inexpensive technologies that can reduce toxic metal concentrations to environmentally acceptable levels [3]. Recently, scientists and engineers have started to generate cost-effective technologies that include the use of microorganisms, biomass and live plants in the cleaning process of polluted areas [1, 2, 10]. There is considerable interest in remediation of sites contaminated by these isotopes using extraction by plants (phytoextraction) that do not enter the human food chain [7]. 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 [4, 12]. Hydroponic growing plants have been also used as a model for bioaccumulation and translocation of radionuclides in aboveground parts of plants [9].

Our study presents data characterizing bioaccumulation of ^{137}Cs and ^{60}Co by sunflowers (*Helianthus annuus* L.) growing in hydroponic media and the influence of previous starvation for NH_4^+ and K^+ ions on these processes. Distribution and translocation of ^{137}Cs and ^{60}Co in plant tissues was evaluated by autoradiographic procedure. Caesium has unknown role in plant nutrition, however it can compete with potassium transport. Cobalt is necessary as a trace element for all cells but is toxic at higher concentrations.

Material and methods

Plant material

Seeds of sunflower (*Helianthus annuus* L.) were sterilized in 10% H_2O_2 solution for 20 min, germinated and grown in pots filled with granulated moist perlite as an inert carrier in day/night period 16/8 h at 20 ± 2°C. Hydroponic medium according to Hoagland [5] was used as a nutrient. After 21 days, seedlings were gently removed from perlite and roots were washed free of any adhering perlite fragments by distilled water. For experiments, plants of comparable weight and height were selected. Plants were then transferred to fresh hydroponic medium without perlite support. The following molar concentrations of salts were present in non-diluted Hoagland medium: 1.5 mM $MgSO_4 \cdot 7H_2O$, 4.0 mM KNO_3, 4.0 mM $CaCl_2$, 1.87 mM $NaH_2PO_4 \cdot 2H_2O$, 0.13 mM $Na_2HPO_4 \cdot 12H_2O$, 0.06 mM $FeSO_4 \cdot 7H_2O$, 4.0 mM $NaNO_3$, 3.17 mM NH_4Cl, 2.0 mM NH_4NO_3, 1.39 mM H_3BO_3, 0.0025 mM $Na_2MoO_4 \cdot 2H_2O$, 0.21 mM $MnSO_4 \cdot 5H_2O$, 0.023 mM $ZnSO_4 \cdot 7H_2O$, 0.033 mM $CuSO_4 \cdot 5H_2O$.

Influence of starvation for NH_4^+ and K^+ ions on ^{137}Cs bioaccumulation

Sunflower seedlings were pre-cultivated for 7 days at 20 ± 2°C in 1/4 diluted complete Hoagland medium and in the same medium where NH_4NO_3 and NH_4Cl or KNO_3 were omitted in order to obtain condition for NH_4^+ or K^+ starvation and in medium supplemented with 50 mmol·l^{-1} KCl. Cultivations were carried out in day/night period 16/8 h in periodically weighed Erlenmeyer flasks. Transpired water was refilled with distilled water. After pre-cultivation, plants were cultivated for 16 days in fresh four times diluted Hoagland medium labeled with ^{137}CsCl (50 kBq·l^{-1}, 2.5 μmol·l^{-1} CsCl). In time intervals aliquot medium samples were taken and ^{137}Cs radioactivity measured. Gamma spectrometer scintillation detector 54BP54/2-X with well type crystal NaI(Tl), (Scionix, The Netherlands) and data processing software Scintivision32 (Ortec, USA) were used. ^{137}CsCl was obtained from Research Institute of Nuclear Energy, Trnava, Slovak Republic and $^{60}CoCl_2$ from Alldeco Inc., Slovak Republic.

Influence of total nutrient content on bioaccumulation and translocation of ^{137}Cs and ^{60}Co

Cultivation media differing in total nutrient contents were prepared by dilution of Hoagland medium with distilled water in the ratio 1:1, 1:3, 1:7 and 1:11. Diluted media were supplemented with ^{137}Cs (25 kBq·l^{-1}, 1.25 μmol·l^{-1} CsCl) and ^{60}Co (25 kBq·l^{-1}, 0.385 μmol·l^{-1} $CoCl_2$), respectively. Plants were cultivated in prepared media for 9 days under similar conditions as described in the previous paragraph. Erlenmeyer flasks were periodically weighed and nutrient solution lost through transpiration was refilled with distilled water throughout the experiment. In time intervals aliquot samples of cultivation media were measured for determination of remaining radioactivity. At the end of experiments, roots were separated from shoots, blotted on filter paper and fresh weights recorded and radioactivity measured.

Distribution and translocation of ^{137}Cs and ^{60}Co in plant tissues

Plants from bioaccumulation experiments were evaluated for ^{137}Cs and ^{60}Co distribution by autoradiography. Roots were rinsed with distilled water and plants were pressed between two filter papers and air-dried for 5 days at 20°C. ^{137}Cs and ^{60}Co in dried plant were detected by autoradiography by exposing X-ray films (HR-GB 100 NIF, FUJIFILM, Japan) for 60 days at room temperature.

Results and discussion

Bioaccumulation of ^{137}Cs by sunflower from hydroponic medium was proportional to the transpiration rate. Results of typical experiment are shown in Fig. 1A,B. Significant, approx. 2.2 fold increase in ^{137}Cs bioaccumulation rate was observed when sunflower plants were pre-cultivated in conditions of K^+ ion starvation and 2.7 fold increase in conditions of or NH_4^+ starvation. Starvation has no significant effect on transpiration rates. Pre-cultivation in surplus of K^+ ions 50 mmol·l^{-1} has no effect on ^{137}Cs bioaccumulation rate. Our data are in agreement with data published by Zhu *et al.* [13]. They observed significant increase in radiocaesium uptake by wheat seedlings after 3-day potassium starvation.

Both ^{137}Cs and ^{60}Co bioaccumulation rates increase with dilution of basic Hoagland medium up to 1:7 for caesium and up to 1:3 for cobalt followed by mild decrease at higher dilutions (Fig. 2). This effect can be explained by competition effect of mono or bivalent cations in more concentrated media and by limitation of metabolic activity by low nutrient concentration in diluted media. Among all alkaline metals and NH_4^+ it appears that K^+ is the most important cation that competes with Cs^+ uptake by wheat [8].

Data presented in Fig. 2 represent total radionuclide uptake calculated from decrease of radioactivity form cultivation media. Distribution of radioactivity found in roots and shoots of sunflower grown in diluted media is shown in Fig. 3. The decrease of nutrient concentrations caused by dilution of media has little effect on ^{60}Co transport from roots to shoots. Observed root to shoot radioactivity ratio was within 1.54 to 1.85. On the contrary root to shoot ^{137}Cs ration increased with dilution from 0.22 to 2.16–2.47. It means, that caesium

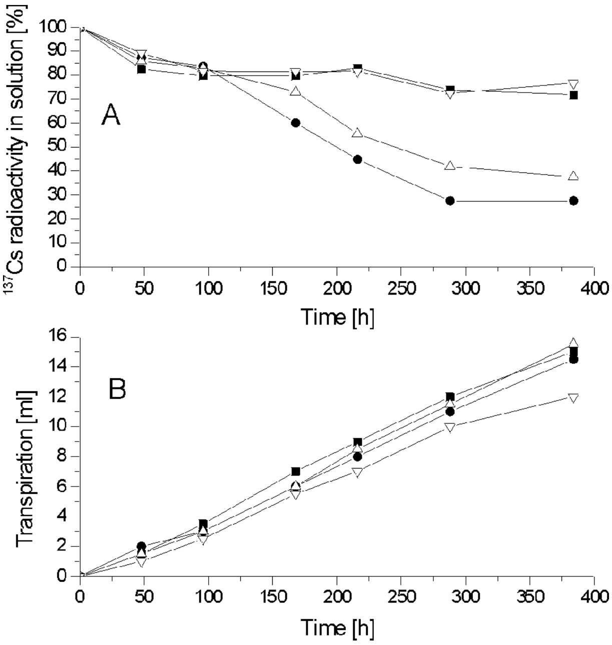

Fig. 1. Influence of starvation for NH_4^+ and K^+ ions in pre-cultivation phase on ^{137}Cs bioaccumulation (A) and transpiration activity (B) of sunflower (*Helianthus annuus* L.). Pre-cultivation: Sunflower seedlings cultivated for 7 days at 20 ± 2°C in 1/4 diluted complete Hoagland medium HM (■-■-■), and in 1/4 HM without NH_4^+ (●-●-●) or without K^+ (△-△-△) ions and 1/4 HM supplemented with 50 mmol·l^{-1} K^+ (▽-▽-▽). Bioaccumulation: Sunflower seedlings cultivated for 16 days at 20 ± 2°C in 1 complete HM, supplemented with 2.5 μmol·l^{-1} CsCl (50 kBq·l^{-1}). The fresh weight of individual plants at the end of experiments was 1.47 ± 0.10 g (SD, n = 8).

is preferentially accumulated in root tissue and its transport to shoot organs is limited in media with low nutrient contents.

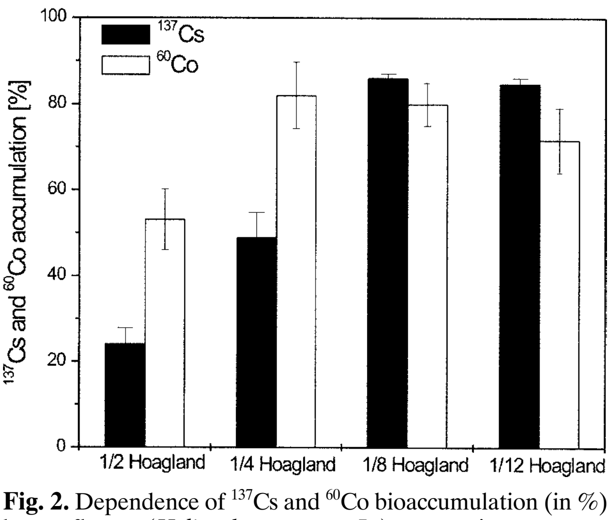

Fig. 2. Dependence of ^{137}Cs and ^{60}Co bioaccumulation (in %) by sunflower (*Helianthus annuus* L.) on nutrient concentration. Sunflower seedlings cultivated for 9 days at 20 ± 2°C in diluted Hoagland medium (1/2, 1/4, 1/8, and 1/12), supplemented both with 1.25 μmol·l^{-1} CsCl (25 kBq·l^{-1}) and 0.385 μmol·l^{-1} $CoCl_2$ (25 kBq·l^{-1}). The fresh weight of individual plants at the end of experiments was 1.58 ± 0.18 g (SD, n = 11). Root to shoot biomass ratio (fresh weight basis): 0.20.

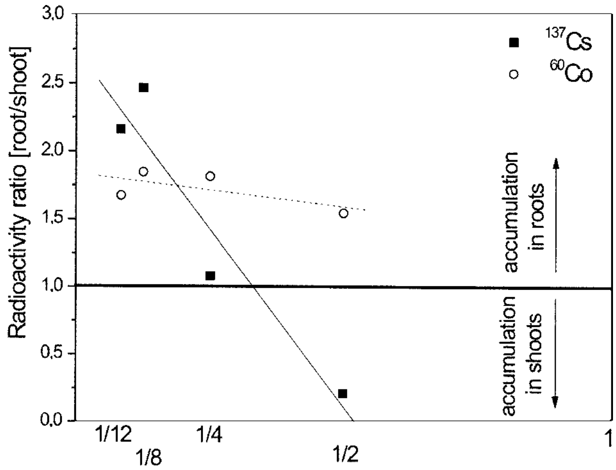

Fig. 3. The effect of dilution of Hoagland cultivation media on ^{137}Cs and ^{60}Co distribution between root and shoot parts of sunflower (*Helianthus annuus* L.). Data and description in Fig. 2.

Root to shoot specific ^{137}Cs radioactivity ratio (Bq·g^{-1}/Bq·g^{-1}, fresh wt.) increased with dilution from 1.46 to 9.6–9.8. The values root to shoot specific radioactivity ratio for ^{60}Co were less dependent on the nutrient concentrations and were within the range 5.7 to 8.5.

Efficient translocation of ^{137}Cs into young sunflower leaves is evident from autoradiographic presentation in Fig. 4A and tendency to accumulate ^{60}Co in root in Fig. 4B. Direct measurements showed that in the case of radiocaesium young leaves accumulated 30% and root 39% of the total amount of ^{137}Cs accumulated by

Fig. 4. Autoradiographic visualization of ^{137}Cs (A) and ^{60}Co (B) distribution in sunflowers (*Helianthus annuus* L.) after 9 days cultivation at 20 ± 2°C in 1/4 Hoagland medium traced with 25 kBq·l^{-1} ^{137}Cs (1.25 μmol·l^{-1} CsCl) and 25 kBq·l^{-1} ^{60}Co (0.385 μmol·l^{-1} $CoCl_2$), respectively.

sunflower plants. Radiocobalt was predominantly accumulated in roots (67%) and only 20% was translocated in leaves.

Diversity of participation of caesium in metabolic pathways is rather limited and transport routes will be similar in many environments. Radiocaesium as contaminant will be mainly fixed in aluminosilicates and therefore not available for plant roots in clay soils. Cobalt ^{60}Co as a potential contaminant from the places of radioactive waste storage represents more complicated problem. Cobalt can exist in Co(III) or Co(II) form in ecosystems and can undergo oxido-reduction conversions. Co(III) is especially mobile when complexed in organic complexes [6]. Microorganisms in soil and root systems will change chemical forms and of cobalt and markedly influence mobility and bioavailability. However, limited data were published and therefore the next research has to be done mainly for explanation of the role of bacteria with pronounced oxido-reduction activities.

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