# BIOACCUMULATION AND DISTRIBUTION OF $^{137}$Cs IN TOBACCO CULTIVATED UNDER HYDROPONIC CONDITIONS

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**Abstract:** Potential of plants to uptake metals from soil solution can be successfully applied for removal of long-lived radionuclides such as radiostrontium $^{90}$Sr or radiocaesium $^{137}$Cs. This work deals with bioaccumulation of Cs in tobacco plants (*Nicotiana tabacum* L.) hydroponically grown in diluted Hoagland media (HM) spiked with $^{137}$CsCl. Speciation analysis using a program Visual MINTEQ showed, that more than 97% of caesium in HM occurred in the form of $Cs^+$ ions. We found that bioaccumulation of Cs significantly decreased from the value 100% to the value 20% removing of Cs from media after 8 days cultivation of plants with increasing HM concentration. However, the concentration ratio (*CR*) $[Cs]_{shoot} : [Cs]_{root}$ increased with increasing HM concentration from the value 0.10 to the value 0.85. Bioaccumulation of Cs by tobacco plants significantly decreased with increasing CsCl concentration in media from the value 95% found at concentration of CsCl 10 μmol/dm$^3$ to the value 44% at concentration of CsCl 1 000 μmol/dm$^3$. We did not found visual symptoms of Cs toxicity on plants after 8 days cultivation or significant differences in growth rate or transpiration activity at CsCl concentration up to 0.2 mM. However, at > 0.2 mM CsCl concentration the decrease of growth rate and necrosis of young leaves or die-back of leaves (> 2 mM CsCl) were observed. The *CR* ($[Cs]_{shoot} : [Cs]_{root}$) increased with increasing concentration of CsCl (10 – 1 000 μmol/dm$^3$) in media from the value 0.10 to the value 0.40. The obtained data suggest that fast growing plant species with high biomass production like tobacco might be a suitable in phytoextraction or rhizofiltration technologies used for $^{137}$Cs removing from environment.

**Key words:** $^{137}$Cs, radiocaesium, bioaccumulation, tobacco, *Nicotiana tabacum*, phytoremediation

## 1. Introduction

The presence of radionuclides in soil and water exposes the stability of ecosystem and poses serious risk to human health through the food chain. Radionuclides can enter to the environment from natural or artificial sources. The artificial sources of radionuclides in the environment represent: testing of nuclear weapons, nuclear waste disposal, accidents resulting from nuclear power generation as well as manipulation with nuclear fuel (see e.g. HU *et al.*, 2010). Contamination caused by long-lived radionuclides, particularly $^{137}$Cs and $^{90}$Sr, poses a long-term environmental problem.

Caesium can exist in at least 39 isotopes forms, mostly within the range of atomic masses $^{112}$Cs to $^{151}$Cs. Of these, the two Cs isotopes are of environmental concern owing to their rapid incorporation into biological systems as physico-chemical analogue of potassium, their relatively long half-lives and emissions of β- and γ-radiation during decay, are $^{134}$Cs (τ = 2.06 y) and $^{137}$Cs (τ = 30.2 y) (WHITE and BROADLEY, 2000).

From the monograph KABATA-PENDIAS and PENDIAS (2001) results that Cs contents in world soils are in the range from several tenths to tens of mg/kg. In some Canadian soils range from 0.3 to 5 mg/kg, in Bulgarian soils from 2 to 17 mg/kg, in Ukrainian soils the mean value is 3.2 mg/kg. The background values of Cs contents in Slovakian soils are 5 mg/kg, similarly to the other world soils (ČURLÍK and ŠEFČÍK, 1999).

As the result of Chernobyl accident, in Ukraine alone, over 260 000 km$^2$ have received more than 40 GBq/km$^2$ of contamination with $^{137}$Cs (DUSHENKOV, 2003). Median values for the inventory of $^{137}$Cs in topsoil of Belarus, about a decade after the Chernobyl accident, ranged from 103 to 1 500 kBq/m$^2$, and the highest concentration was as much as 600-times higher than the pre-accident value (KAGAN and KADATSKY, 1996). These authors estimated that about 90% of the inventory of $^{137}$Cs is contained in the top 3 to 7 cm soil layer. Nowadays, it can be expected that the radionuclide contamination is lower and significantly decrease with time. KASHPAROV et al. (2003) found that in the year 2000 radionuclide contamination within the 30-km Chernobyl zone was 2 – 3 times lower than the previous estimates. However, the release of new contamination still persists. Also, climate/environmental changes e.g. global warming (DOWDALL et al., 2008) can play a possible role in distribution of radionuclides in soils as well as in soil-to-plant transfer processes.

Remediation of areas contaminated with toxic metals or radionuclides represent one of the objects of recent science research. Remediation of contaminated areas by traditional physico-chemical methods e.g. precipitation, coagulation, soil leaching, flocculation, ion-exchange, reverse osmosis, adsorption, ultrafiltration, using of disperse and chelating agents, is financially unbearable and in many cases also ecologically unacceptable.

Bioremediation as a using of biological systems (microorganisms, algae and plants) in remediation processes represent new potential approach to solve the problems with toxic metals and radionuclides contaminations. Phytoremediation technologies involve the processes, which use of plants for remediation of contaminated environment. Several comprehensive reviews (see over the past five years e.g. GARDEA-TORRESDEY et al., 2005; GHOSH and SINGH, 2005; PRASAD et al., 2005; PILON-SMITS, 2005; LeDUC and TERRY, 2005; AUDET and CHAREST, 2006; LEŠTAN et al., 2008; VANGRONSVELD et al., 2009 and WENZEL, 2009) and monographs (RASKIN and ENSLEY, 2000; TERRY and BAÑUELOS, 2000; MACKOVA et al., 2006; MOREL et al., 2006, SINGH and TRIPATHI, 2007; WILLEY, 2007) have been written, summarizing many important aspects of this technology. These reviews also give general guidance and recommendations for applying phytoremediation in practice.

Plants ideal for phytoremediation of contaminated soils should fulfill some requirements: fast growing and high biomass (more than 8 tons/ha for year), extensive root system, easy to harvest, ability to tolerate and accumulate of high amount of metals in the aboveground parts (more than 1 g/kg). However, plants are able to metal uptake only from soil solution.

Tobacco was selected as a model of plant mainly for the reason its fast-growth rate and high biomass production over 12 tons/ha (KELLER et al., 2003). Also, tobacco is

important agricultural plant, which represent important source mainly of Cd exposure from smoking cigarettes (WHO, 1992). In our previous papers we study cobalt [60]Co (HORNÍK *et al.*, 2006; VRTOCH *et al.*, 2007) and caesium [137]Cs (VRTOCH *et al.*, 2006) bioaccumulation by tobacco as well as effect of complexing agents on Cd, Co and Zn bioaccumulation and distribution in tissues of tobacco plants (HORNÍK *et al.*, 2009a; 2009b; 2009c). In this work we present results from studying of effect of macro- and microelements as well as CsCl concentration on bioaccumulation and translocation of caesium in tissues of tobacco (*Nicotiana tabacum* L.) cultivated in nutrient media spiked with [137]CsCl.

## 2. Materials and methods

### 2.1 Plant material

Seeds of tobacco (*Nicotiana tabacum* L.) were germinated and grown at photoperiod 12h light/12 h dark (illumination 2 000 lx) and 22°C in pots filled with granulated perlite as an inert carrier. Plants were watered with diluted solution of Hoagland medium (HOAGLAND, 1920). The composition of the full strength (100%) nutrient solution was (mmol/dm$^3$): $MgSO_4.7H_2O$ – 1.5; $KNO_3$ – 4.0; $CaCl_2$ – 4.0; $NaH_2PO_4.2H_2O$ – 2.1; $Na_2HPO_4.12H_2O$ – 0.13; $FeSO_4.7H_2O$ – $6.4.10^{-2}$; $NaNO_3$ – 4.0; $NH_4Cl$ – 4.0; $NH_4NO_3$ – 2.0; $H_3BO_3$ – 0.14; $Na_2MoO_4.2H_2O$ – $2.5.10^{-4}$; $MnSO_4.5H_2O$ – $2.1.10^{-2}$; $ZnSO_4.7H_2O$ – $2.3.10^{-3}$; $CuSO_4.5H_2O$ – $3.2.10^{-3}$ (pH 6). After 4 weeks pre-cultivation seedlings were gently removed from perlite, roots were washed by deionized water for removing of perlite granules and used in bioaccumulation experiments.

### 2.2 Bioaccumulation experiments

Uniformed plants (approx. 15 cm height and 7 g wet weight) from pre-cultivation phase were transferred into series of 250 mL Erlenmeyer flask with 120 mL of 25% Hoagland medium with the known [133]CsCl concentration and added radioactivity of [137]CsCl as a tracer. The pH of nutrient solutions was adjusted to 6.0 using 1 M NaOH solution. All flasks were covered with black foil to protect plant roots against the lights. Plants were hydroponically cultivated during 8 days in triplicate series under the same conditions as was mentioned in pre-cultivation phase without the addition of radionuclides. In time intervals aliquot samples of nutrient solution were taken, ^{137}Cs radioactivity was measured by gamma-spectrometry and subsequently samples were returned into the cultivation medium. At the same time reduction of nutrient medium volume caused by transpiration of water was recorded and the difference was compensated by glass balls. At the end of the experiments plants were removed from nutrient solutions, roots were carefully washed in deionized water and incorporated ^{137}Cs radioactivity in roots, stems and leaves was measured. Plant parts were dried at 60°C for 48 hours and dry weights were determined.

Growth value (GV) was calculated using the following equation (1):

$$GV = \frac{m_t - m_0}{m_0} \qquad (1)$$

where $m_t$ or $m_0$ are fresh weight of plants at the start or end of experiments, respectively.

## 2.3 Radiometric analysis

For determination of $^{137}$Cs radioactivity in plant parts and nutrient solution gamma-spectrometric scintillation detectors 54BP54/2-X and 76BP76/3 with well type crystal NaI(Tl) (Scionix, NL) and data processing software Scintivision32 (Ortec, USA) were used. A library of radionuclides was built by selecting characteristic γ-ray peaks (88.04 keV for $^{109}$Cd, 661.66 keV for $^{137}$Cs, 834.81 keV for $^{54}$Mn and 1115.52 keV for $^{65}$Zn) for energy and efficiency calibration. Standardized solution of $^{137}$Cs in the form of $^{137}$CsCl (5.723 MBq/cm$^3$, 20 mg/dm$^3$ CsCl in 3 g/dm$^3$ HCl) was provided from Czech Metrological Institute (Prague, CR).

## 2.4 Speciation modelling

The percentage occurrence of individual Cs ionic forms in nutrient solution was determine by equilibrium speciation modelling software Visual MINTEQ (ver. 2.53) obtained from GUSTAFSSON (2010). This speciation modelling program allows the calculation of metals speciation in solutions as a function of total salt concentration, solution pH, ionic strength and temperature.

## 2.5 Statistical analysis

All analytical determinations were performed in triplicate. Statistical significance of differences in calculated values of Cs bioaccumulation in plant tissues were evaluated by Kruskal-Wallis non-parametric test and analysis of variance, respectively. This was followed by multiple range test to ascertain differences between individual groups. The level of significance was 0.05 in all cases. Origin 7.0 (OriginLab Corp., USA) and STATGRAPHICS Centurion ver. 15 (StatPoint, Inc., USA) were used for graphing and statistical analyses, respectively.

# 3. Results and discussion

Necessary assumption for successful remediation of contaminated soils with radiocaesium is ability of plants to accumulate significant amount of radioactivity from soil to aboveground part of plants. However, irreversible incorporation of $^{137}$Cs onto clay minerals is a limiting factor of these processes. Sorption reactions in soil-water boundary line are important phenomena, which determine the behaviour, bioavailability and transport of Cs in environment. Caesium has very low hydratation

energy, thus electrostatic attraction between $Cs^+$ ions and clay particles is high and therefore Cs ions are preferentially bound onto clay particles. However, sesquioxides mainly organically bound aluminium and iron oxides are responsible also for Cs sorption in soils (CHIANG *et al.*, 2010). Despite these facts, phytoremediation of soil could be effective method of soil decontamination during the first years after radiocaesium penetration into the soil (WILLEY, 2007; WILLEY and COLLINS, 2007).

The results of laboratory experiments with plants can not be direct applied for phytoextraction of Cs in real conditions. However, they can be useful for study of three processes take place in phytoextraction independently i.e. solubilization of metals from soil and their transport to root system of plants, uptake of metals by root system of plants and translocation of metals into aboveground biomass. Experiments with hydroponics can be useful for study of two last mentioned processes (see e.g. TANDY *et al.*, 2006). Also, this configuration of experiments allows quantitative study of individual speciation forms of metals under strictly defined conditions. In the case of plant cultivation in soil a complex equilibrium system is formed when the concentration of all substances in soil solution is in equilibrium with concentration of substances bound onto soil particles. Also, inorganic nutrients occurred in soil solution or in synthetic solutions formed a very complex equilibrium system defined by dissociation constants, concentration, temperature and pH value. Experimental determination of all speciation forms of metals is very difficult. Therefore, for description of this system is very useful to use the speciation models.

In our work we used the speciation modelling program Visual MINTEQ ver. 2.53 for calculation of individual ionic forms of Cs in Hoagland media (HM). We found that caesium at pH 6.0 and 22°C was occurred practically only in the form of $Cs^+$ ions ($\geq$ 97%) in the case of all nutrient media containing different concentration of macro- and microelements (8.3% – 100% HM) as well as concentrations of CsCl (10 – 50 000 µmol/$dm^3$).
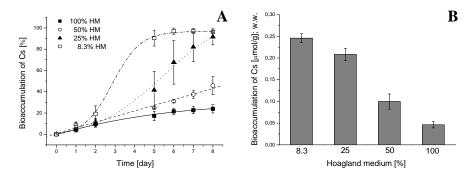


Fig. 1. Influence of nutrient salts concentration in HM on Cs bioaccumulation by roots of tobacco plants (*N. tabacum* L.) during 8 days hydroponic cultivation in 100%, 50%, 25% or 8.3% HM containing 10 µmol/$dm^3$ CsCl (34.0 kBq/$dm^3$ $^{137}$CsCl), pH 6.0, at photoperiod 12h light/12h dark (2 000 lx) and 22°C. Bioaccumulation of Cs expressed in per cent of the total amount of CsCl in medium (A; kinetic) and in µmol/g (wet weight) (B; after 8 days cultivation). Error bars represent standard deviation of the mean ($n = 3$). All values of means of Cs bioaccumulation were significantly different at the $p < 0.05$ level based on multiple range test (Fig. 1B).

Fig. 1 A depicts Cs bioaccumulation by tobacco hydroponics during 8 days at different concentration of HM containing 10 μmol/dm$^3$ CsCl. We found that at the lowest concentration of 8.3% HM (0.83 mM K$^+$ and NH$_4^+$ ions) the bioaccumulation reached 100% already after 5 days of cultivation. In the case of 25% HM (2.5 mM K$^+$ and NH$_4^+$ ions) the total removing of Cs from medium was reached after 8 days of plants cultivation and in the case of 50% (5 mM K$^+$ and NH$_4^+$ ions) or 100% HM (10 mM K$^+$ and NH$_4^+$ ions) at this time was observed only 40% or 20% bioaccumulation of Cs, respectively. Similar effect can be seen on the Fig. 1 B, where bioaccumulation of Cs in μmol/g (wet weight) decreased with increasing HM concentration after 8 days plants cultivation. A Kruskal-Wallis test confirmed significant effects (at $p < 0.05$) of the cultivation conditions on Cs bioaccumulation. On the basis of results of authors SOUDEK *et al.* (2004; 2006) it can be expect that differences in Cs bioaccumulation at different concentration of HM will be caused mainly in change of K$^+$ and NH$_4^+$ concentration in media as competitive ions for Cs uptake by plants. SANDEEP and MANJAIAH (2008) in the case of mustard, spinach and wheat plants cultivated in contaminated soil with $^{134}$Cs observed that Cs bioaccumulation decreased with increasing concentration of K in soil. Similar result found also SINGH *et al.* (2008).
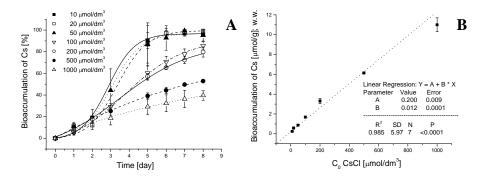


Fig. 2. Influence of CsCl concentration on Cs bioaccumulation by roots of tobacco plants (*N. tabacum* L.) hydroponically cultivated in 8.3% HM containing 10, 20, 50, 100, 200, 500 or 1 000 μmol/dm$^3$ CsCl (33.5 kBq/dm$^3$ $^{137}$CsCl), pH 6.0, at photoperiod 12h light/12h dark (2 000 lx) and 22°C. Bioaccumulation of Cs expressed in per cent of the total amount of CsCl in medium (A; during 8 days cultivation) and in μmol/g (wet weight) (B; after 8 days cultivation). Error bars represent standard deviation of the mean ($n = 3$).

It is known that the uptake of Cs is operated mainly *via* two transport pathways on plant root cell membranes, namely K$^+$ transporters or K$^+$ channels (ZHU and SMOLDERS, 2000). However the role of Cs$^+$ in plant nutrition is no known (MARSCHNER, 1995; WHITE and BROADLEY, 2000). Cs$^+$ is nontoxic to plants at external Cs$^+$ concentrations below approx. 200 μmol/dm$^3$, although this limit depends critically on the concentrations of other ions (K$^+$, NH$_4^+$) in the substrate. The toxicity symptoms induced by unnaturally high Cs concentrations include necrosis of shoot and root tissues (KORDAN, 1987; WHITE and BROADLEY, 2000).

In the next experiments we observed influence of CsCl concentration in the range $10 - 50\ 000$ μmol/dm$^3$ on plant growth as well as on Cs bioaccumulation by tobacco roots. For evaluation of phytotoxic effects on tobacco plants the calculation of growth value (the ratio of fresh weight of plants at the start or end of experiments difference to fresh weight of plants at the start of experiments) and macroscopic observation of phytotoxic symptoms on leaves were used. We did not found visual symptoms of Cs toxicity on plants after 8 days cultivation or significant differences in growth rate at CsCl concentration up to 0.2 mM. However, at CsCl concentration above 0.2 mM CsCl the decrease of growth rate and necrosis of young leaves or die-back of leaves (> 2 mM CsCl) were observed (data not shown).

The results in Fig. 2 A show that Cs bioaccumulation by tobacco plants significantly decreased with increasing CsCl concentration in media from the value 95% found at concentration of CsCl 10 μmol/dm$^3$ to the value 44% at concentration of CsCl 1 000 μmol/dm$^3$. After conversion to μmol Cs per gram (wet weight), we found linear correlation ($R^2 = 0.985$) between Cs bioaccumulation by tobacco roots and CsCl concentration in nutrient media containing 0.33 mM K$^+$ ions (Fig. 2 B). It can be assumed, that this correlation corresponds with correlation between K uptake in plant and K concentration in soil solution. It means, that at higher Cs concentration than concentration of K in cells of root system will be this correlation affected not only with specific uptake of K or Cs ions by K$^+$ transporters (often $\leq 0.3$ mM), but also with low specific uptake *via* ion channels (for K$^+$ ions). The K$^+$ transporters operating at low K concentrations can transport Cs$^+$ efficiently whereas Cs$^+$ permeates only slowly in K$^+$ channels operating at K concentrations above $0.5 - 1$ mM (ZHU and SMOLDERS, 2000). However, we did not found saturation of the first mentioned system and beginning the second system, what should be reflect to form a characteristic double phase isotherm relationship between Cs$^+$ influx to roots and external Cs$^+$ concentrations.
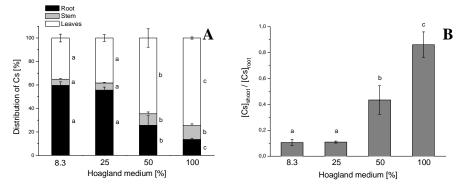


Fig. 3. Influence of nutrient salts concentration in HM on Cs translocation from roots to shoots of tobacco plants (*N. tabacum* L.) after 8 days hydroponic cultivation in 100%, 50%, 25% or 8.3% HM containing 10 μmol/dm$^3$ CsCl (34.0 kBq/dm$^3$ $^{137}$CsCl), pH 6.0, at photoperiod 12h light/12h dark (2 000 lx) and 22°C. Distribution expressed in per cent of the total Cs uptake by plant (A) and as concentration ratio [Cs]$_{shoot}$ : [Cs]$_{root}$ (Bq/g; dry weight) (B). Error bars represent standard deviation of the mean ($n = 3$). Means with the same letter at columns are not significantly different at the $p < 0.05$ level based on multiple range test.

The reviews of authors ZHU and SMOLDERS (2000) or WHITE and BROADLEY (2000) describe the studies demonstrated that $K^+$ and $Cs^+$ competed for influx to excised roots, suggesting that the influx of these cations to root cells is mediated by the same molecular mechanism. The molecular identity and electrophysiological signature of many $K^+$ transporters expressed in the plasma membrane of root cells have been also described. The inward-rectifying $K^+$ (KIR), outward-rectifying $K^+$ (KOR) and voltage-insensitive cation (VIC) channels are all permeable also to $Cs^+$. By modelling cation fluxes through these transporters into a stereotypical root cell, it can be predicted that VIC channels mediate most (30 – 90%) of the $Cs^+$ influx under physiological conditions and that the KUP transporters mediate the bulk of the remainder. Cation influx through KIR channels is likely to be blocked by extracellular $Cs^+$ under typical ionic conditions in the soil.

From point of view of phytoremediation technologies it is important that toxic metals and radionuclides have to be accumulated mainly in aboveground parts of plants. For evaluation of caesium mobility in conductive tissues of plants in the term of caesium translocation efficiency we established non-dimensional concentration ratio (*CR*), which represent the ratio of caesium concentration in aboveground part of plants $[Cs]_{shoot}$ to caesium concentration in root system of plants $[Cs]_{root}$.
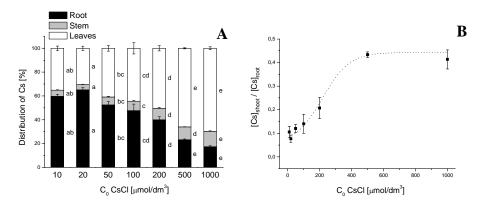


Fig. 4. Influence of CsCl concentration on Cs translocation from roots to shoots of tobacco plants (*N. tabacum* L.) after 8 days hydroponic cultivation in 8.3% HM containing 10, 20, 50, 100, 200, 500 or 1 000 μmol/dm³ CsCl (33.5 kBq/dm³ ¹³⁷CsCl), pH 6.0, at photoperiod 12h light/12h dark (2 000 lx) and 22°C. Distribution expressed: A. in per cent of the total Cs uptake by plant; B. as concentration ratio $[Cs]_{shoot}$ : $[Cs]_{root}$ (Bq/g; dry weight). Error bars represent standard deviation of the mean ($n = 3$). Means with the same letter at columns are not significantly different at the $p < 0.05$ level based on multiple range test.

Our previous results obtained by direct analyzing of radioactivity in individual plant tissues as well as from autoradiography of whole plants showed that caesium was localized mainly in root system and young leaves (VRTOCH *et al.*, 2006). In this work we found that percentual content of Cs in root, stem and leaves are affected with HM concentration i.e. macro- and microelements concentrations (significant differences are shown in Fig. 3A at the $p < 0.05$ level based on multiple range test). The concentration

ratio (*CR*) $[Cs]_{shoot} : [Cs]_{root}$ increased with increasing HM concentration from the value 0.10 to the value 0.85, particularly at 50% and 100% HM, when concentration of monovalent cations ($K^+$ and $NH_4^+$; $[K^+] : [NH_4^+] = 2 : 3$) were 5 mM and 10 mM, respectively (significant differences are shown in Fig. 3B at the $p < 0.05$ level based on multiple range test). Similar effect was observed in our previous work with sunflower hydroponics, when shoot-to-root specific $^{137}Cs$ radioactivity ratio (Bq/g : Bq/g; wet weight) increased with increasing concentration of HM from the value 0.10 to the value 0.69 (HORNÍK *et al*., 2005). BUYSSE *et al*. (1996) and SMOLDERS *et al*. (1996) found that shoot-to-root Cs concentration ratio decreased with decreasing K supply. It can therefore be assumed that the main influence in this respect will be concentration of $K^+$ ions in media.

Fig. 4 depicts influence of CsCl concentration in nutrient media on Cs distribution in tissues of tobacco plants cultivated in 8.3% HM. There is a significant change in Cs distribution in root, stem and leaves of tobacco. The percentual content of Cs in aboveground part of tobacco increased with increasing CsCl concentration in media (significant differences are shown in Fig. 4A at the $p < 0.05$ level based on multiple range test). Also, the *CR* ($[Cs]_{shoot} : [Cs]_{root}$) increased with increasing concentration of CsCl (10 – 1 000 $\mu mol/dm^3$) from the value 0.1 to the value 0.4. However, at concentration of CsCl in media > 500 $\mu mol/dm^3$ the *CR* was not changed (Fig. 4B).

## 4. Conclusions

The results from hydroponic cultivation of tobacco plants (*N. tabacum*) indicate that bioaccumulation of Cs was significantly affected by concentration of Hoagland medium particularly monovalent cations as well as concentration of CsCl in media. On the other side, higher concentration of Hoagland medium and concentration of CsCl in media have a positively effect on translocation of Cs into aboveground parts of tobacco plants. It may be concluded that fast growing plant species with high biomass production like tobacco might be a suitable in phytoextraction or rhizofiltration technologies used for $^{137}Cs$ removing from environment. However, it should be proven by field experiments, because hydroponic experiments are only a principal model to study metals uptake but do not give information on soil-plant relations and metals uptake under the real conditions.

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