BIOACCUMULATION OF $^{60}Co^{2+}$ IONS IN TOBACCO PLANTS

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Abstract: Tobacco has previously been used in investigations of metals and radionuclide uptake. This study presents determination of bioaccumulation and translocation of $^{60}Co^{2+}$ ions in tobacco plants (*Nicotiana tabacum* L.) grown in Hoagland's nutrient solution. Cobalt concentration in tobacco plants increased with increasing concentration in nutrient solution. Bioaccumulation from the initial concentration C_0 = 0.96 μM Co reached 90% after 7 day cultivation. Only small amounts of Co accumulated in roots, up to 2 - 4 % were removable from roots by washing with 0.1 M $CoCl_2$, indicating that this portion of Co is bound to the root surface in ion-exchangeable form.

Tobacco roots retained approximately 2/3 of accumulated cobalt and 1/3 was transported to shoots. Autoradiography revealed that ^{60}Co was preferentially localized in younger leaves. Prolongation of cultivation time did not change the $[Co]_{roots}$: $[Co]_{shoots}$ ratio significantly. Relationships between growth rate, transpiration rate, uptake and distribution of cobalt in plant tissue are discussed.

Key words: Cobalt, $^{60}Co^{2+}$, *Nicotiana tabacum* L., accumulation, hydroponics, autoradiography

1. Introduction

Many papers explaining mechanism of uptake, translocation and detoxication of cobalt have been published (BAKKAUS *et al*., 2005; PEREZ-ESPINOZA *et al*., 2005; WOODARD *et al*., 2003; MORENO-CASELLES *et al*., 1997). A number of mechanisms have been proposed on how cobalt is taken up and transported through the plant (WOODARD *et al*., 2003). Early literature suggested that cobalt was present in xylem tissues in tomato as an inorganic cation (TIFFIN, 1967). Others have suggested an organic anion of molecular weight from 1000 to 5000 Da as found in *Ricinus communis* (WIERSMA and VAN GOOR, 1979).

It has been reported previously that cobalt is one of those metals that do not activate phytochelatin synthase (ZENK, 1996). It was therefore suggested that cobalt cannot be detoxified via the phytochelatin system in plant cell (OVEN *et al*., 2002). This is in contrast with results obtained for other metals such as Cd and Zn. On the other hand, OVEN *et al*. (2002) identified cysteine as a compound involved in Co complexation in cobalt hyperaccumulator *Crotalaria cobalticola* suspension cells. Cobalt also induced cysteine increase in non-hyperaccumulator species, suggesting that there are also other cellular mechanisms that enable cobalt tolerance and hyperaccumulation.

Tobacco has previously been used in other investigations of metals and radionuclides uptake (FUHRMANN and LANZIROTTI, 2005; MENCH and MARTIN, 1991). An excellent review on utilization of genetically modified tobacco

and other plants for phytoremediation of toxic metal contaminated soil was published by EAPEN and D´SOUZA (2005). In our paper, the accumulation of ^{60}Co by tobacco plants *Nicotiana tabacum* L. has been studied with the aim to characterize cobalt bioaccumulation and translocation in roots, stems and leaves at hydroponic cultivation. The tobacco was chosen as a model system due to its high biomass production, easy hydroponic cultivation, and as a model of widely cultivated agricultural plant.

2. Material and methods

2.1 Plant material

Seeds of tobacco were germinated and grown in pots filled with granulated moist perlite as an inert carrier in day/night photoperiod 12/12 h (illumination with two fluorescent tubes Fluora 550 lm, L18W/77, Osram; one tube Cool white 1150 lm, F18W/33, Tungsram), temperature 22 ± 1°C, and relative humidity 50%. Plants were irrigated with 25% Hoagland nutrient medium (HOAGLAND, 1920). After 2 months, seedlings were removed from perlite, roots were washed free of any adhering perlite granules by distilled water and pre-cultured for 7 days in 25 % Hoagland nutrient medium in Erlenmeyer flasks and used for experiments. Plants used were about 15 cm tall and about 4.0 – 6.0 g of fresh weight.

2.2 Bioaccumulation experiments

Plants were cultivated in 25 or 50% Hoagland´s medium supplemented with 0.96; 9.6; 25 or 50 µmol.L^{-1} $CoCl_2$ spiked with $^{60}CoCl_2$ (125 kBq.L^{-1}) for 7 or 14 days under the conditions as described in the previous section. Bioaccumulation experiments were replicated three times.

In 24 h intervals aliquot samples of nutrient solution were taken and ^{60}Co radioactivity measured by gamma spectrometry. At the end of experiments plants were harvested and roots carefully rinsed in 0.1 M $CoCl_2$ and distilled water. Growth value (GV) was calculated following the equation $GV = (m - m_0) / m_0$, where m and m_0 is plant fresh weight at the end and at the beginning of experiments respectively (SOUDEK *et al*., 2006). Radioactivity incorporated in roots, stems and leaves was measured by gamma spectrometry. Material was dried in oven at 60 °C to a constant weight.

After cultivation in the presence and in the absence of Co^{2+} ions, tobacco leaves were observed with Jenatech light microscope (Carl Zeiss). Micrographs were taken with digital camera Olympus Camedia 4000.

2.3 Radiometric analysis

A gamma spectrometric assembly using the well type scintillation detector 54BP54/2-X, NaI(Tl) (Scionix, the Netherlands) and data processing software Scintivision32 (ORTEC, USA) were used for ^{60}Co determination in plant and solution. Counting time 600s allowed obtaining data with relative measurement error <2%,

which do not reflect other source of errors. Standardized $^{60}CoCl_2$ solution (20 mg/L $CoCl_2$ in 3 g/L HCl) with specific radioactivity 5.181 MBq/mL was obtained from The Czech Institute of Metrology, Prague, Czech Republic).

Before autoradiography, roots were rinsed with distilled water, drops were wiped off; plants were pressed between two layers of filter papers and dried at laboratory temperature. Dried plants were mounted on the X-ray film (HR-GB 100 NIF, FUJIFILM, Japan), and developed after 40 days. Developed films were scanned and black and white film was converted to color scale gradient by software Photoshop CS2 ver. 9.0 (ADOBE, USA).

2.4 Transpiration rate

Transpiration rates (TR) were calculated from the daily weight losses of cultivation vessels containing plants, corrected for water evaporation of vessels media without plants. Transpired water was daily replenished with distilled water to the initial volume. Leaf area (m^2) was calculated from weights of leaf copies (g) made from the paper sheet of known specific weight (g/m^2).

3. Results and discussion

3.1 Cobalt uptake

Tobacco plants (*Nicotiana tabacum* L.) cultivated in 25 % Hoagland's medium were tested to the ability accumulate Co^{2+} ions from nutrient solutions. Bioaccumulation at the initial cobalt concentration C_0 = 0.96 μM reached 90 % within 7 days (Fig. 1). The same kinetics (data not shown) and comparable uptake values 89.5±1.9 and 86.8±3.3% were observed for C_0 = 9.6 and 25 μM respectively. At C_0 = 50 μM $CoCl_2$, toxic effects appeared and total uptake decreased to 54.2±5.5% (Table 1). Transpiration rate (mL/day) at 22 ± 1°C and 50 % humidity was linear within 7 days (Fig. 1). Only small amount of cobalt, up to 2-4 % was removable from roots at the end of the experiment by washing with 0.1 M $CoCl_2$, indicating that this portion of cobalt was bound to the surface of the roots in ion-exchangeable form.

At concentrations $C_0 \geq 50\mu M$ $CoCl_2$, significant decrease in biomass production expressed by growth values (Table 1), decrease in Co uptake and transpiration rates (Table 3) and chlorosis localized in interveinal areas of younger and new leaves were observed. The cobalt treatments within concentration range C_0 = 0.96 - 25 μM had no significant effect on transpiration rates and therefore induced no toxic effect. The average transpiration rates 11.5, 11.6 and 11.9 $ml.m^{-2}.h^{-1}$ at 0.96, 9.6 and 25 μM respectively were obtained. The transpiration rate of tobacco plants was reduced to 8.3 $ml.m^{-2}.h^{-1}$ under higher Co exposure (50 μM) (Table 3). On the other hand, increasing amounts of Co in the shoots was observed (Table 1), indicating that under higher Co exposure, the Co transport mainly through the path of phloem and driven by the concentration gradient of Co in the roots. Similar Cu transport in rice seedlings under extreme Cu exposure observed CHEN *et al*., (2004).

Uptake of cations by root system is influenced by other ions present in solution. The increase of Hoagland´s strength from 25 % to 50 % caused decrease of cobalt uptake from 90 % to 54 % at C_o = 9.6 µM $CoCl_2$ (Table 2). This fact can be explained by competitive effects of bivalent cations. In more concentrated Hoagland's solution such as 50 %, also suppression of transpiration rate and lower GV values were also observed (Table 2 and 3). Inhibition of cobalt uptake by sunflower from 50 % Hoagland´s medium was described in our previous paper (HORNIK *et al*., 2005). Inhibition of transpiration by higher nutrient concentrations was described by KANG and VAN IERSEL (2004).

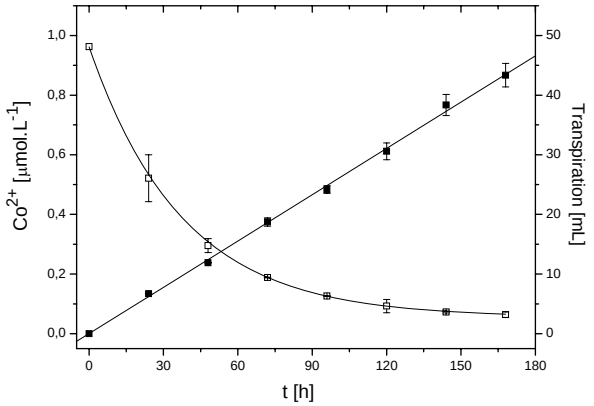

Fig. 1. Kinetic of Co^{2+} bioaccumulation (□-□) and transpiration (■-■) by tobacco plants grown in 25% Hoagland medium at 22±1°C; C_0 = 0.96 µM Co^{2+}. Each point is the mean of three replicates. Error bars represent standard deviation (SD) of the mean.

Tab. 1. Uptake and distribution of Co^{2+} ions in roots and aboveground parts of tobacco plants cultivated 7 days in 25 % Hoagland's hydroponic medium.

Co^{2+} [µmol.L^{-1}]	GV	Co uptake [%]	$[Co]_{root}$ / $[Co]_{shoot}$	Co^{2+} [µg.g^{-1} DW ± SD]		
				Root	Stem	leaves
0.96	0.26±0.06	90.5±0.2	9.3	27.2±12.0	1.8±1.6	1.1±0.6
9.6	0.25±0.07	89.5±1.9	11.8	582±97	27±11	22±3
25	0.23±0.06	86.8±3.3	10.7	1077±113	51±10	50±14
50	0.12±0.01	54.2±5.5	3.5	711±119	124±9	80±20

3.2 Translocation of cobalt

Tobacco roots retained approx. 2/3 of bioaccummulated cobalt and only 1/3 was translocated into shoots. These data expressed in term of the cobalt ratio in root to shoot (65:35) changed only slightly with the increase of the initial cobalt concentration within the range C_0 = 0.96 to 25 µM. More pronounced translocation of cobalt to shoots was observed at 50 µM cobalt in medium where cobalt ratio in root to shoot has changed to 44:56 (Fig. 2).

Autoradiography also demonstrated that ^{60}Co is preferentially localized in the roots and in young leaves. In leaves ^{60}Co is distributed around the veins and significant amount is also localized in leafstalks (Fig. 4). Gamma spectrometry showed the following cobalt ^{60}Co distribution: root 68 %; young leaves (leaf No. 4, 5, 6) approx. 20 %. Stem and older leaves (leaf No 1, 2, 3) contained 8.7 % and 3.2 % ^{60}Co respectively. However, higher concentrations of nutrient salts in cultivation medium did not influence cobalt translocation in tobacco plants significantly (Fig. 3A).

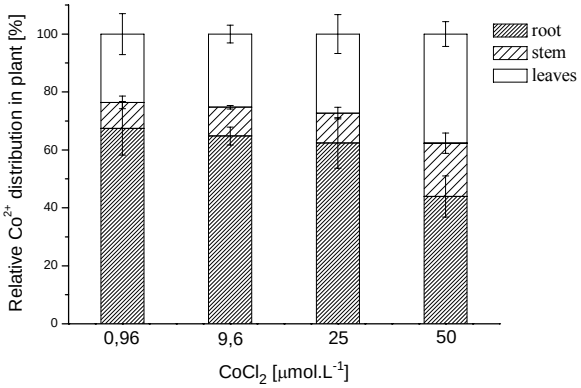

Fig. 2. Relative distribution of Co^{2+} ions in tobacco roots, stems and leaves after 7 days cultivation in 25% Hoagland medium with initial concentration C_0 = 0.96, 9.6, 25 and 50 µmol/dm^3 $CoCl_2$ at 20 ± 2°C. Each column is the mean of three replicates. Error bars represent standard deviation (SD) of the mean.

Tab. 2. Influence of strength of Hoagland medium and cultivation time on uptake and translocation of Co^{2+} ions by tobacco plants. C_0 = 9.6 umol/L $CoCl_2$.

Time [day]	GV	Hoagland´s strength [%]	Co uptake [%]	Co (root/shoot) ratio [µg.g^{-1} DW] /[µg.g^{-1} DW]
7	0.25±0.07	25	89.5±1.9	11.8
7	0.15±0.04	50	54.8±4.1	4.7
14	0.46±0.04	25	85.8±0.8	8.8

Tab. 3. Relation between Co^{2+} uptake and transpiration rate (TR) of tobacco at different initial Co^{2+} concentration in cultivation medium after 7 days cultivation.

Co^{2+} [µmol.L^{-1}]	Co Uptake [%]	TR [ml.m^{-2}.h^{-1}]	Hoagland´s strength [%]
0.96	90.5±0.2	11.5±1.5	25
9.6	89.5±1.9	11.8±0.6	25
9.6	54.8±4.1	8.8±0.9	50
25	86.8±3.3	11.9±0.8	25
50	54.2±5.5	8.3±0.9	25

In our experiments cobalt was added to the nutrient solution as a single dose in such amounts that more than 90 % uptake was reached within 7 days of cultivation. We expected that during prolonged cultivation in the same cultivation medium, where

cobalt was depleted, would result in additional cobalt translocation from roots to shoots by transpiration stream. This hypothesis was not confirmed. Prolongation of cultivation from 7 to 14 days did not change root to shoot cobalt ratio significantly. As can be seen in Fig. 3B, additional translocation of cobalt primarily localized in roots into shoots was not observed. The only cobalt movement was slight translocation from stems to leaves. On the contrary, PAGE and FELLER (2005) observed negligible mobility of cobalt in wheat. Cobalt ^{57}Co was trapped mainly in roots and bound so tightly, that was not significantly translocated to wheat shoots during the next 50 days of cultivation. Differences in cobalt mobility in plant organs described in literature are explained by differences in biochemical composition of plant organs and differences in transport systems.

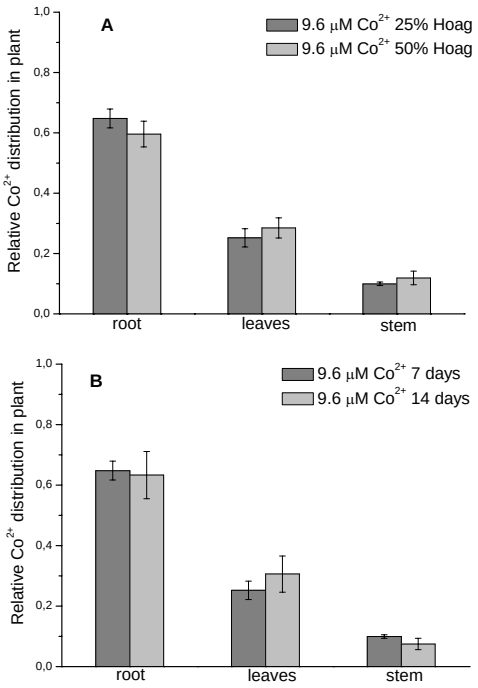

Fig. 3. Relative distribution of Co^{2+} ions in tobacco roots, stems and leaves in dependence on the concentration of Hoagland nutrient medium (A), and on the cultivation time (B). Each column is the mean of three replicates. Error bars represent standard deviation (SD) of the mean.

3.3 Phytotoxicity of cobalt

It has been established that Co like other pollutant elements are relatively toxic to plants when given in supranormal doses (CHATTERJEE and CHATTERJEE 2000; PANDEY *et al*., 2002; GOPAL *et al*., 2003). After 50 μM Co treatment, tobacco showed interveinal chlorosis on young leaves and significant suppression of growth (Fig. 5, Table 1). Similar toxic effect of cobalt on tomato plants was described by GOPAL *et al*., (2003). Chlorosis is considered as indirect effect caused by: alterations

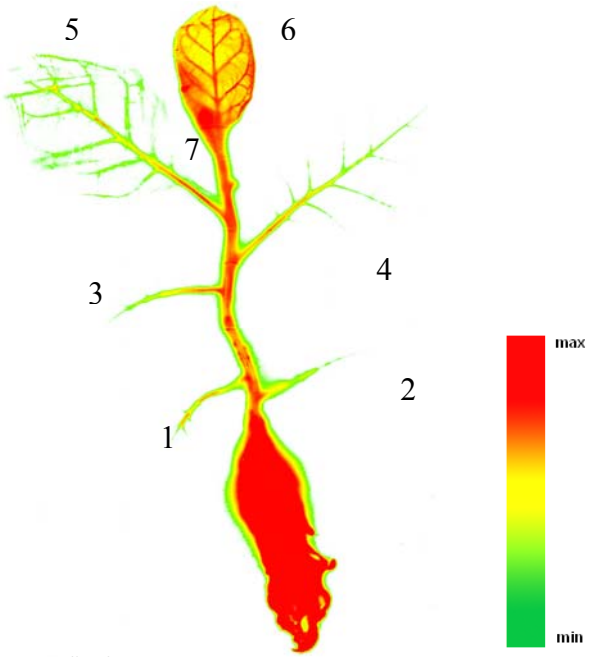

Fig. 4. Autoradiography of $^{60}Co^{2+}$ distribution in root, stem and leaves of tobacco after 7 days cultivation in 25% Hoagland medium with initial concentration C_0 = 1,9 µmol/L $CoCl_2$ (263 kBq/L $^{60}CoCl_2$) at 20 ± 2°C. Autoradiogram was converted to color scale gradient by software Photoshop CS2. Single leaves are numbered as: 1 – the oldest (lower), 7 – the youngest (upper). Relative distribution of ^{60}Co in plant parts calculated from gama-spectrometric data: 68.0% root; 8.7% stem; 0.8% leaf 1; 1.2% leaf 2; 1.4% leaf 3; 2.8% leaf 4; 5.4% leaf 5; 11.7% leaf 6+7.

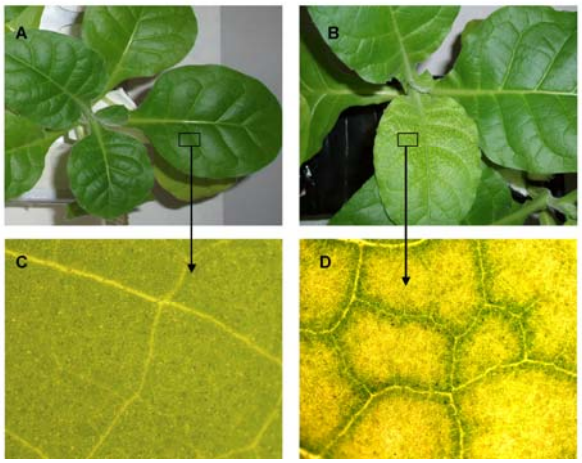

Fig. 5. Visible symptoms of tobacco plants to excess supply of Co^{2+} (50 µM) after 7 day cultivation in nutrient solution: A-plant cultivated in the absence of Co^{2+} (magnification 0,4 ×), B – plant cultivated in the presence of 50 µM Co^{2+} (magnification 0,5 ×), C – leaf of Co^{2+} non-treated plant (magnification 30 ×), D – leaf of 50 µM Co^{2+} treated plant with developed interveinal chlorosis (magnification 10 ×).

in the concentrations of essential mineral nutrients, a decrease in net photosynthesis as a consequence of stomatal closure, reduced intercellular spaces and by alterations within chloroplasts (CHATTERJEE and CHATTERJEE 2000; VAZQUEZ *et al*., 1987). On molecular level it was found, that Co in supranormal concentrations caused inhibition of catalase activity, decrease of chlorophyll content connected with chlorosis (CHATTERJEE and CHATTERJEE, 2000; PANDEY and SHARMA, 2002). Toxicity of cobalt at C_0 50 μM resulted in both decrease of tobacco transpiration rates and cobalt uptake.

4. Conclusion

Uptake of Co^{2+} ions from nutrient hydroponic solution by tobacco plants is a process dependent on transpiration rate. Cobalt is translocated from root to shot biomass in the ratio 70: 30. At concentrations $C_0 \geq 50$ μM Co^{2+}, phytotoxic effects such as intervenial chlorosis was observed. Uptake of Co^{2+} ions by tobacco roots and translocation to shoots is influenced by the presence of other bivalent metal ions. Cobalt can be considered as a next toxic agents accumulating in tobacco grown in contaminated soils. On the other hand, roots contain cobalt in concentrations 27 times higher than in the aboveground part calculated on dry weight biomass, what have to be taken in consideration in calculations of cobalt and radiocobalt persistence in soil/plant system.

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