Thorium As An Environment Stressor For Plant Growth

Hrdinová A.[1,2], Lhotáková Z.[2], Mihaljevič M.[3,*], Petrová Š. [1], Albrechtová J.[2], Vaněk T. [1] and Soudek P. [1,*]

[1]Laboratory of Plant Biotechnologies, Institute of Experimental Botany AS CR, v.v.i., Rozvojová 263, 165 02 Prague 6 – Lysolaje, Czech Republic

[2]Department of Experimental Plant Biology, Faculty of Science, Charles University, Viničná 5, 128 44 Praha 2, Czech Republic

[3]Institute of Geochemistry, Mineralogy and Mineral Resources, Faculty of Science, Charles University, Albertov 6, 128 43 Prague 2, Czech Republic

*corresponding author: Soudek P.

e-mail: soudek@ueb.cas.cz

Abstract The plants of *Nicotiana glutinosa* (L.) were hydroponically cultivated on Hoagland nutrient media supplemented by thorium, tartaric acid, putrescine and/or phosphates. The accumulation of thorium by tobacco was monitored. The effect of thorium on the photosynthetic apparatus (contents of photosynthetic pigments, rapid fluorescence PSII,) was studied.

Deficiency of phosphorus in the medium caused about 10-times higher Th accumulation in plants. However, the selected species – *N. glutinosa* does not have ability to accumulated Th enough for phytoremediation of contaminated environment. The application of putrescine on leaves lead to higher Th translocation to shoots but the effect of tartaric acid on Th accumulation was not observed. The presence of thorium in plants caused an increase in contents of photosynthetic pigments and a decrease in values of selected fluorescence parameters. Exogenous application of putrescine showed a potential in phytoremediation methods to support translocation of heavy metals to shoots.

Keywords: fluorescence, phytoremediation, putrescine, tartaric acid, thorium

1. Introduction

Despite the current problems in Japan nuclear power plant Fukushima I, nuclear power is an important source of energy for mankind. In majority, nuclear power plants are run on uranium; however, more attention has been lately turned to thorium as a promising fuel of nuclear power plants. India leads in the development of technology using thorium as a fuel source. India's Kakrapar-1 was the first reactor in the world to use thorium rather than depleted uranium to achieve power flattening across the reactor core. Both Kakrapar-1 and -2 units are loaded with 500 kg of thorium fuel to improve their operation at start-up. After India, the main countries driving thorium research are U.S.A. and Russia with recent interest from Norway and Poland (NIRS, 2008). The advantages of using thorium instead of uranium include the high abundance of nuclei ^{232}Th, which represents the major nuclide in natural thorium, production of less long-lived radioactive waste and behavior as α-emitter. This means that thorium cannot be engaged in spontaneous fission, but due to neutron capture may be converted to uranium ^{233}U, which is an excellent nuclear fuel and a strong source of neutrons (IAEA, 1985).

Thorium, like uranium, is a radionuclide with long half-live, and, as a source of radiation, constitutes a potential problem for human health. When an organism is exposed to an inhalation of thorium dust, there is a possibility of increased risk of lung and liver diseases, lung and pancreatic cancer, adverse effect on blood and changes in the genetic material of the human body (ATSDR, 1999).

The understanding of the mechanism of thorium uptake by plants and of the possibilities to influence its entry into the plants can be utilized in phytoremediation studies or for the exclusion of undesirable contaminants in crops.

The present knowledge of thorium uptake by plants is rather limited. The thorium accumulation has been tested mainly in Poaceae plants (Shtangeeva and Ayrault, 2004; Shtangeeva el al., 2005; Pulhani *et al*., 2005; Guo *et al*., 2010). The studies were performed either under greenhouse conditions (Shtangeeva and Ayrault, 2004; Shtangeeva el al., 2005; Knox *et al*., 2008) or in areas affected by mining (Zararziz *et al*., 1997; Blanco Rodríguez *et al*., 2002; Chen *et al*, 2005; Mihucz *et al*., 2008).

Phosphorus is the nutrient essential for plant growth and development. It's involved in converting light energy to chemical energy, may alter the activity of various enzymes and is an important component of DNA, RNA and phospholipids. Its deficiency results in intense green color of leaves, necrosis and plant growth reduction.

The accumulation of thorium (Shishkunova *et al*., 1989, Soudek *et al*., 2013), as well as of uranium (Eapen *et al*., 2003; Soudek *et al*. 2011a; Soudek *et al*., 2011b), is strongly affected by the presence of phosphate ions. For example, the bioavailability of thorium is strongly increased by pyrophosphate, whereas it is decreased by hydroxyapatite (Guo *et al*., 2010).

The accumulation of thorium in plants is also highly dependent on climatic conditions, plant species and

especially on the content of thorium in the soil. Oufni *et al*. (2011) tested roots, stems and leaves of medicinal plants in south-eastern Morocco for their ability to accumulate thorium. They found horehound (*Marrubium vulgare*), lemon verbena (*Lippia citriodora*) and harmal peganum (*Peganum harmala*) to be the best thorium accumulators (in roots 3.60, 3.40 and 3.15 Bq kg^{-1}, respectively). Chen *et al*. (2005) reported that Italian ryegrass (*Lolium multiflorum*) and red clover (*Trifolium pretense*) were high thorium accumulators (in roots 8.82 and 5.90 Bq kg^{-1}, respectively). These authors also tested tobacco (*Nicotiana tabacum*) plants and found relatively high thorium uptake in shoots and roots (0.19 and 2.36 Bq kg^{-1}, respectively). Linsalata *et al*. (1987) published the contents of thorium in vegetables grown in Brazil on soils with high thorium content. The highest contents were measured in zucchini (*Cucurbita pepo*) and brown beans (*Phaseolus vulgaris*) (both 0.011 mg g^{-1} DW), no significant transport of thorium being observed from soil to edible parts of plants.

The aim of our study has been to evaluate the possibility of thorium accumulation by plants under hydroponic conditions for the potential phytoremediation purposes. Tobacco (*Nicotiana* sp.) plants in hydroponic culture were selected as a model system. Potential effect of medium amendments (organic acids, polyamines, phosphates) on thorium uptake and its distribution in the plant was tested.

2. Material and methods

2.1. Plant material and cultivation conditions

Tobacco seeds (*Nicotiana glutinosa*) were sown in Perlite and cultivated for two months. All seedlings were watered by modified Hoagland medium every three days. The hydroponic medium with pH adjusted to 5.0 contained 4 mM $CaCl_2$, 2 mM K_2SO_4, 2 mM NH_4NO_3, 2 mM NaH_2PO_4, 1.5 mM $MgSO_4$, 4 mM $NaNO_3$, 4 mM NH_4Cl, 0.2 mM $FeSO_4$, 138.8 µM H_3BO_3, 20.8 µM $MnSO_4$, 2.3 µM $ZnSO_4$, 3.3 µM $CuSO_4$ and 0.2 µM Na_2MoO_4. The plants were kept at 23°C and with relative humidity about 60% and they were irradiated with a 16 h light (average irradiation of 72 µmol/m^2 s1 at the plants' surfaces, with horizontal differences in irradiation less than 20%, sodium discharge lamps - 400 W, (Thorn Radbay). Eight-week old plants were used for the experiments.

2.2. Sampling and sample preparation

The seedlings of tobacco plants (*Nicotiana glutinosa*) were cultivated (each plant per one pot) in Perlite. Hydroponic medium was supplemented with thorium. Thorium ions were obtained from salts $Th(NO_3)_4$ (Lachema n.p., Brno, Czech Republic). Tested concentration of thorium was 0.1 mM. Samples for thorium uptake determination were harvested after 14 days of cultivation.

2.3. Sampling and sample preparation

Five replications for each treatment, each concentration and each harvest time were used. Roots were washed with distilled water, 0.1 M EDTA and distilled water again. The samples were frozen in liquid nitrogen and stored at -80 ºC. The samples were freeze-dried at -50 °C until constant weight.

Approximately 0.25 g of dry plant sample was predigested in 5 mL of mixture $HNO_3/HClO_4$ in ratio 7:1 (v/v) overnight at lab temperature. Then 3 mL of acid mixture were added to clean the walls of tube and the contents of the closed Teflon vessel digested in gradient to 100% power after 15 min. and at 100% power for further 25 min. Digestion was accomplished in a Multiwave reaction system (Multi-wave PRO, Anthon Paar GmbH, Austria). The cooling was made another 20 min. The samples were filled up to 10 mL volume and analyzed.

2.4. Thorium content determination

The thorium concentration in the solutions was determined by quadrupole based inductively coupled plasma mass spectrometry (ICP MS, X Series 2, Thermoscientific, USA). For solution introduction, a Meinhard nebulizer with spray chamber cooled to 3 °C was used. A flow rates of argon nebulizer gas 0.9 mL L^{-1}, Ar plasma gas 13 L ml^{-1} and Ar auxiliary gas 1.3 L ml^{-1} was used. The RF power of the plasma generator was 1400 W. The ICP-MS instrument was tuned with a 10 µg mL^{-1} Th standard, focusing on isotope ^{232}Th. A Bi solution of 10 µg mL^{-1} in 2% v/v HNO_3 was used as internal standard with the formation of oxides Me/MeO < 0.5%. The concentration of thorium in the sample was determined using calibration curve (5, 10, 20 µg mL^{-1}) Th. The concentration values represent the arithmetic mean of three repetitions.

2.5. Fluorescence induction measurement

After 14 days of plant cultivation, before the harvest, the plants were adapted to darkness in a darkroom. After approximately 30 minutes of dark adaptation, the rapid onset of induced fluorescence by the FluorPen 2 (PSI) was measured. The measurement was always carried out on the second leave from a shoot apex. The measurements were carried out at five places in the leve. The data was processed by FluorPen software (Strasser *et al*., 2000).

2.6. Statistical analyses

All statistical analyses were carried out using Statistica (StatSoft, Inc., Tulsa, Oclahoma, USA). Analysis of variance was performed using one-way ANOVA analysis and taking $P < 0.05$ as significant to determine the effect of medium supplements and to test thorium uptake in a range of plant species. Statistically insignificant values on the level of probability $P < 0.05$ are indicated by the same symbol above columns in figures. The error bars indicate the standard deviation of analyzed contents

.

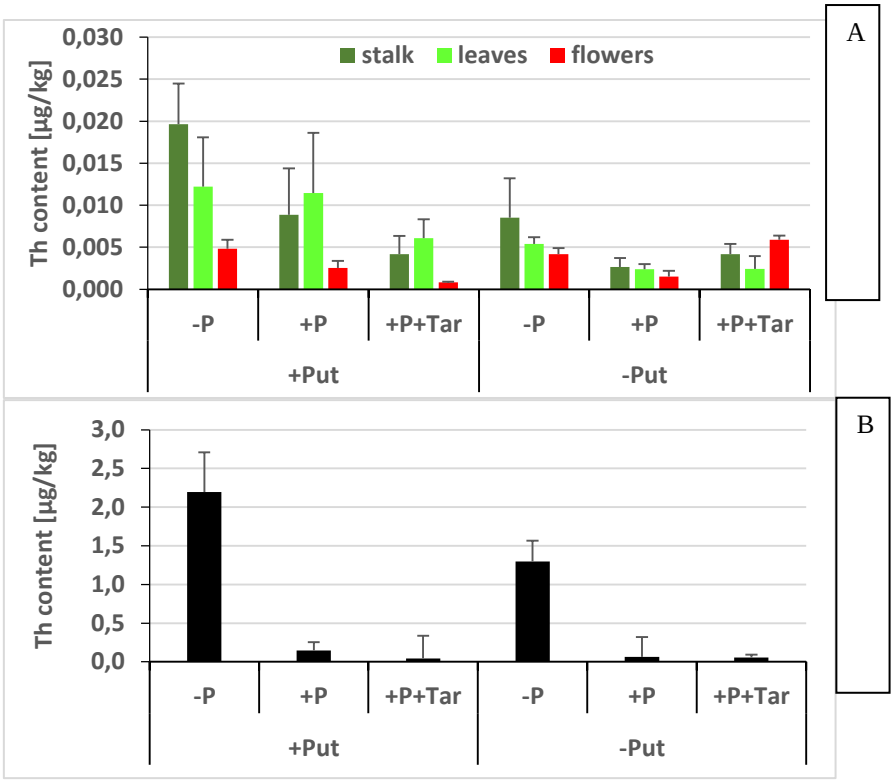

Figure 1. Effect of medium composition on the accumulation of thorium in different parts of tobacco plants. (A) uptake in roots, (B) uptake in stalk, leaves and flowers. Standard deviation is represented as ±SD (n = 5).

3. Results and discussion

On the base of previous experiment (Soudek *et al*., 2013) we select only some potential efficient modification of the media. Thorium content after cultivation significantly increased especially in the roots of the plants. Thorium accumulation was positively affected by the absence of phosphates in the medium in the both cases, presence and absence of putrescine (Fig. 1). The highest content was observed in the case of non-phosphate medium with putrescine spraying (2.20 µg kg^{-1} DW), whereas in case of non-phosphate medium without putrescine spraying was only 1.30 µg kg^{-1} DW. In the both cases of phosphate medium, with and without putrescine spraying, the content was significantly lower (15-times reduction), only 0.15 and 0.07 µg kg^{-1} DW, respectively. Significantly lower thorium content was found in stems, leaves and flowers. The highest thorium content (0.02 µg kg^{-1} DW) was found in the stalks of plants cultivated on non-phosphate medium with putrescine spraying. In comparison with experiments with *N. tabacum* (Soudek *et al*., 2013), the thorium content in roots of *N. glutinosa* was 10-times lower. The transfer to upper part of plants was 100-times lower than in previous publication. The differences can be due a different cultivation conditions (hydroponic against semihydroponic) and different plant species. It can be assumed that the differences in the thorium uptake was due to the higher availability of thorium ions under hydroponic conditions. Phosphate ions otherwise creates an insoluble complex, thorium$^{(IV)}$phosphate (Shtangeeva and Ayrault, 2003) which is less mobile and available for plants grown in Perlite (semihydroponic conditions). Even though phosphorus is essential element for plants, there was no visible negative effect on plant growth and development during the 14-day cultivation in phosphate-free medium.

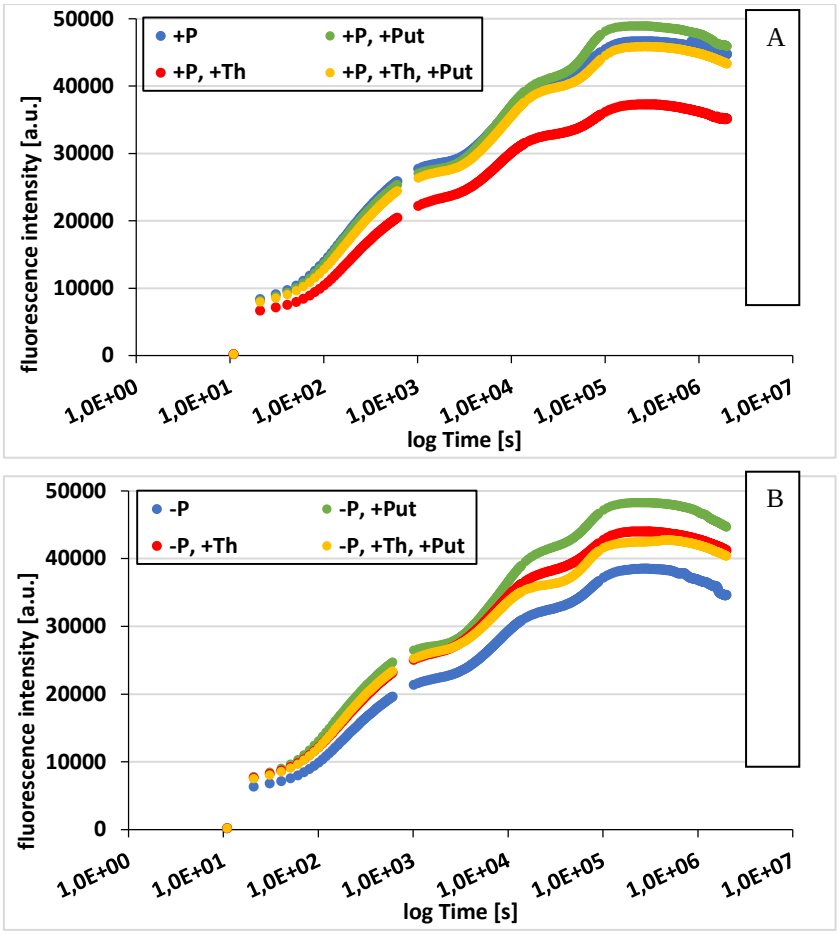

Figure 2. Effect of medium composition on OJIP curve of tobacco plants. (A) plants cultivated on phosphate medium, (B) plants cultivated on medium without phosphate. (n=5)

Our results show (Fig. 2) that thorium exposure show a decrease of maximum fluorescence Fm for the medium with phosphates (+P), so the system of electron transport between QA and the PQ is affected. On the other hand, after putrescine spraying (+Put), the thorium have no-effect on Fm. In case of thorium exposure in medium without phosphates (-P), the maximum fluorescence Fm was increased compare to plants grown on the medium without phosphates and without thorium. Putrescine have no-effect in presence of thorium but increase Fm in case of medium without thorium. It show that PS II is much more affected by phosphate deficiency than thorium. Putrescin has proven to be a protective factor that reduces the impact of stress on PS II.

4. Conclusions

Higher thorium concentrations was found in the roots and translocation to the shoots was very limited. Phosphate deficiency and putrescine sprying positively influenced the thorium uptake. Fluorescence of PS II is affected by thorium and phosphate deficiency too. Putrescin reduces symptoms of stress conditions on PSII.

Acknowledgments

This work was supported by project COST ES1407 (project No. LTC17046).

References

ATSDR (Agency for Toxic Substances and Disease Registry) (1999), Thorium.

http://www.atsdr.cdc.gov/toxfaqs/tf.asp?id=659&tid=121, on line: 24.5.2017

Blanco Rodríguez P., Vera Tomé F. and Lozano J.C. (2002), About the assumption of linearity in soil-to-plant transfer factors for uranium and thorium isotopes and ^{226}Ra. *Sci. Total Environ.*, **284**, 167-175.

Chen S.B., Zhu Y.G. and Hu Q.H. (2005), Soil to plant transfer of ^{238}U, ^{226}Ra and ^{232}Th on a uranium mining-impacted soil from southeastern China, *J. Environ. Radioact.*, **82**, 223-236.

Eapen S., Suseelan K.N., Tivarekar S., Kotwal S.A. and Mitra R. (2003), Potential for rhizofiltration of uranium using hairy root cultures of *Brassica juncea* and *Chenopodium amaranticolor*, *Environ. Research*, **91**(2), 127–133.

Guo P., Jia X., Duan T., Xu J. and Chen H. (2010), Influence of plant activity and phosphates on thorium bioavailability in soils from Baotou area, Inner Mongolia, *J. Environ. Radioact.*, **101**, 767-772.

IAEA (1985), Thorium-based nuclear fuel: Current status and perspectives. *Proceedings of a technical committee meeting on Utilization of thorium-based nuclear fuel: Current status and perspectives. International atomic energy agency (Vienna, Austria, 2-4 December 1985)*, **IAEA-TECDOC-412**.

http://large.stanford.edu/courses/2012/ph241/bordia1/docs/0412.pdf, on line: 24.5.2017

Knox A.S., Kaplan D.I. and Hinton T.G. (2008), Elevated uptake of Th and U by netted chain fern (Woodwardia areolata), *J. Radioanal. Nucl. Chem.*, **277**(1), 169-173.

Linsalata P., Penna Franca E., Sachett I., Castro M.B., Lobao N., Ballad R., Lei W., Ford H., Morse R.S. and Eisenbud M. (1987), Radium, thorium, and the light rare earth elements in soils and vegetables grown in an area of high natural radioactivity, *DOE Symp., Ser.* **59**, 131-146.

Mihucz V.G., Varga Z., Tatár E., Virág I., van Grieken R., Koleszár Z. and Záray G. (2008), Redistribution of uranium and thorium by soil/plant interaction in a recultivated mining area, *Microchem. J.*, **90**, 44-49.

NIRS (Nuclear Information and Resource Service) (2008), Thorium-based nuclear power: An alternative? *Nuclear Monitor*, **667**, 5-9.

http://www.nirs.org/mononline/nm667.pdf, on line: 24.5.2017

Oufni L., Taj S., Manaut B. and Eddouks M. (2011), Transfer of uranium and thorium from soil to different parts of medicinal plants using SSNTD, *J. Radioanal. Nucl. Chem.*, **287**, 403-410.

Pulhani V.A., Dafauti S., Hegde A.G., Sharma R.M. and Mishra U.C. (2005), Uptake and distribution of natural radioactivity in wheat plants from soil, *J. Environ. Radioact.*, **79**, 331–346.

Shishkunova L.V., Grashchenko S.M. and Strukov V.N. (1989), Entry of uranium, thorium, and radium isotopes into plants from soils and fertilizers, *Sov. Radiochem.*, **30**(3), 362-367.

Shtangeeva, I. and Ayrault, S. (2003), Phytoextraction of thorium from soil and water media. *Int. J. Environ. Pollut.* **154**, 19–35.

Shtangeeva I. and Ayrault S. (2004), Phytoextraction of thorium from soil and water media, *Water Air Soil Pollut.* **154**, 19–35.

Shtangeeva I., Ayrault S. and Jainc J. (2005), Thorium uptake by wheat at different stages of plant growth, *J. Environ. Radioact.*, **81**, 283–293.

Soudek P., Petrová Š., Benešová D., Dvořáková M. and Vaněk T. (2011a), Uranium uptake by hydroponically cultivated crop plants, *J. Environ. Radioact.*, **102**(6), 598-604.

Soudek P., Petrová Š., Benešová D. and Vaněk T. (2011b), Uranium uptake and stress responses of in vitro cultivated hairy root culture of *Armoracia rusticana*, *Agrochimica*, **LV** (1), 15-28.

Soudek P., Kufner D., Petrová Š., Mihaljevič M. and Vaněk T. (2013), Composition of hydroponic medium affects thorium uptake by tobacco plants, *Chemosphere*, **92**, 1090-1098.

Strasser R. J., Srivastava A. and Michael M. T. (2000), The fluorescence transient as a tool to characterize and screen photosynthetic samples. In *Probing photosynthesis: mechanisms, regulation and adaption* (Yunus M., Pathre U and Mohanty P., eds), Taylor & Francis, London and New York, 445-483. ISBN 9780748408214

Zararsiz A., Kirmaz R. and Arikan P. (1997), Field study on thorium uptake by plants within and around of a thorium ore deposit, *J. Radioanal. Nucl. Chem.*, **222**(1-2), 257-262.