ČESKÁ ZEMĚDĚLSKÁ UNIVERZITA V PRAZE

Phytoremediation of Selected Radionuclides by Higher Plants

autoreferát doktorské disertační práce

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Faculty of Environmental Sciences

Department of Applied Ecology

Phytoremediation of Selected Radionuclides by Higher Plants

Executive Summary

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General Introduction

Radiation in environment comes from three sources, namely, cosmic, terrestrial and anthropogenic. Life on earth has been continuously exposed to radiation resulting from radionuclides produced by cosmic ray interaction in the atmosphere and from terrestrial natural materials since the dawn of time. But radiation emanating from man-made activities like application of radioactive minerals in industry, nuclear testing and nuclear power generation including occasional disastrous nuclear accidents is rather a recent development. While background radiation has in some ways contributed to the chemical and biological processes on earth, radionuclides and mining and milling of radioactive materials and their waste disposal has become a cause of concern for the health of all living creatures on this planet. There is a growing trend in developing nuclear energy to meet the energy crisis. However, there are inherent problems in nuclear energy as also in the application of nuclear material for military use since radiation emanating from this anthropogenic radionuclide source is difficult to handle both during normal operation time and in case of nuclear accident. Therefore, it is imperative to understand the environmental implications of radionuclides and mining of nuclear deposits with consequent health issues if nuclear energy is to be developed for meeting the impending energy requirements.

Radioactive contamination can enter into the various ecosystems by two pathways: first, the dry way by atmospheric deposition, and the second, the wet way by precipitation (Pöschl, 2006). Therefore, the main concern is to prevent radioactive elements from penetrating into the alimentary systems of the living organisms. One of the promising and environment-friendly techniques for abating the ingression of radioactive elements into the system seems to be phytoremediation, hence the method remains under research investigation to increase its efficiency (Eapen et al., 2007, Soudek et al., 2008).

In this work, an attempt has been made to study the interaction of plants with radiocesium and natural radioactive thorium to evaluate further the scope of phytoremdiation as a technique to address the problem of radiation effects resulting from these elements in the

use of nuclear energy. The area of interest for radiocesium phytoremediation studies is the Temelin nuclear power plant in the Czech Republic, while focus on thorium-plant interaction is to discern the applicability of this phytoremediation technique in areas of radioactive mineral mining.

Radiocesium in the Environment

Radiocesium is a radioactive element which is released by catastrophic accidents in nuclear power plants, nuclear bomb tests, leaching from waste disposal of radionuclides and nuclear weapons. With a half-life of 30 years it remains in the ecosystem between 180 and 300 years (Starr, 2013). The history of radiocesium begins around 1945 when the US and the USSR started atomic projects for the nuclear weapons and their testing, leading this radioactive element to enter into the various environmental ecosystems as a consequence (Garten et al., 2000). Even though nuclear weapons testing is banned, radiocesium is released from nuclear power plants during normal operation and especially in huge amounts during the accidents like Jaslovske Bohunice (1977), Three Mile Island (1979), Chernobyl (1986), Fukushima (2011) and many others (Ashraf et al., 2014). Radiocesium is sometimes used in nuclear medicine for radiation therapy, and also in industry for detection and gauging. Currently, radiocesium is one of the most investigated chemical components on the ground (Zaborska et al., 2014).

Thorium in the Environment

Thorium is a radioactive actinide metal, present in small amounts in the environment. The concentration of thorium is around 6 ppm in the earth's crust. More than 99% of thorium occurs as a radioisotope ^{232}Th with half-life 14 billion years, emitting especially alpha particles. It can be found in higher concentrations in some rocks (e.g. monazite sand), which are considered as a source of thorium (ATSDR, 1999).

In recent years, the thorium mining has attracted enhanced attention due to its potential use in the nuclear power industry. Thorium content is three to four times higher than that of the other natural actinide – uranium, which is why some countries like India prefer this element for nuclear power generation (IAEA, 2005). Such use can enhance the risk for the environment as thorium may be released during mining, processing or by an occasional accident. The hazard lays in radiological and chemical toxicity. Irradiation increases the probability of the occurrence of lung and pancreatic cancer, changes in the genetic material of somatic cells, liver damage and failure of haematogenesis (ATSDR, 1999). Although most studies have been focused on long-term toxicity caused by irradiation, thorium is also substantially toxic element (Al-Jundi et al., 2004). When thorium exposure is sufficiently high, chemical toxicity exceeds the radiological one (Mizukami-Murata et al., 2006).

Phytoremediation Techniques

Phytoremediation is a set of techniques to extract or alter pollutants from soil or water. Besides, it is associated with soil microorganism, agronomical approach and soil fertilizers (Soudek et al., 2008). They propose six phytoremediation processes as follows.

- Phytostabilisation – plants which are highly tolerant towards the heavy metals or radionuclides used for mechanical stabilization as well as prevention against water and wind erosion.
- Phytoaccumulation – a contaminant is absorbed via plant root on to the shoot and removed by process of phytomining.
- Rhizofiltration – is a suitable method for decontaminating surface water, waste water and groundwater depletion via plant roots.
- Rhizodegradation – organic pollutants start to decay on simpler components after entering plant body or they can decay by the activity of soil bacteria plus secreting root exudates (saccharides or alcohols).
- Phytodegradation – it is a procedure of a contaminant absorption, metamorphism and catabolism within the plant body,
- Phytovolatilisation – it is a transforming and degradation process of volatile substances from soil to the atmosphere by plants and soil

microorganisms (some people consider this procedure controversial, it may be solely transferring pollutants from soil to the atmosphere).

Genetic manipulation

Alkorta et al. (2004) explain that metal pollution is widespread and has become a major environmental issue today. In addition, plant capacity boost to tolerate or accumulate metals through genetic engineering should enlarge the area of phytoremediation usage. Already genetic engineering has achieved some goals. For example, phytochelatin, metallothionein, metal transporter and metal chelator genes were isolated and relocated into plant species. Moreover, transgenic plants were developed to accumulate elements Cd, Pb, Cu, As and Se (Eapen and D'Souza, 2005). In regard to developing desirable traits in plants Wu et al. (2010) suggest focusing on so called crop-accumulators (new designation for crop to cumulate heavy metals). Eapen et al. (2007) point out that plant has natural ability to purify some xenobiotic pollutants; however, it fails to mineralize these compounds as microbes can do. Ruscio and Navari-Izzo (2011) suggest that the driving force for uptake, translocation and accumulation of heavy metals appears to be overexpression of genes encoding trans-membrane transporters as members of ZIP, HMA, MATE, YSL and MTP families. Presently, research takes advantage of genetic engineering to develop transgenic plants for all kinds of purposes including accumulating metals.

Chelate agents

Besides hybridization, the effect of phytoremediation can be enhanced by chelate agents which plants produce naturally when the root reaches the toxic region (Sharma et al., 2015). Organic acid anions such as citrate, maleate, succinate, oxalate, phthalate, salicylate and acetate are generated by plants to minimize the metal toxicity and released by root exudates. Furthermore, citric acid is a well-known chelator which stimulates radionuclide and the heavy metal uptake (Sharma et al., 2015). Investigations of Tahmasbian and Sinegani (2016) focus on the combination of chelate and electrokinetic remediation which may offer new perspective in the field of phyto-extraction. One of the famous synthetically prepared chelator EDTA is used in many phyto-extraction experiments. The chelators have positive impact on metal ions uptake in plants although further addition may cause negative effect on their physiological parameters such as growth or competition, replacing essential elements like cesium and potassium (Sharma et al., 2015). Considering the potential of plants as absorbents of radiation the phytoremediation technique can be used in conditions of both nuclear fallout and mining and milling of radioactive minerals.

Objectives and Benefits of Dissertation

The following studies add to general knowledge about the investigated issue (see above).

The main objective of this study was to find among selected higher plants suitable (with known ability to accumulate radionuclides) bioaccumulators for radiocesium.

It was also aimed to investigate the influence of soil properties on radiocesium uptake by selected higher plants and based on that discern whether growth medium or the plant species played more important role in the radionuclide uptake.

Another objective was to observe seasonal changes in radiocesium uptake by higher plants to enable considering seasonally efficient bioaccumulators in the phyto-remediation model.

In the realm of phytoremediation, we aimed to identified plant genes involved in detoxification process of thorium.

Benefits of Dissertation

The gained knowledge from the experiments will help to select efficient vegetation with aim to prevent and minimalize radionuclides impact on the ecosystem at Temelin nuclear power plant. This information will be possible to apply at different radionuclides sources with respect to site and climatic conditions. Not last, the enhancement of plant threat to detoxify thorium from the various ecosystems may be used in the areas of mill tailings or surroundings of nuclear power plants using thorium as a nuclear fuel.

Thorium impact on root transcriptome

Abstract

Thorium is natural actinide metal with potential use in nuclear energetics. Contamination by thorium, originated from mining activities or spills, represents environmental risk due to its radioactivity and chemical toxicity. Promising approach for cleaning of contaminated areas is phytoremediation, which need to be based, however, on detail understanding of the thorium effects on plants. In this study, we investigated transcriptomic response of tobacco roots exposed to 50 µM thorium for one week. Thorium application resulted in up-regulation of 152 and down-regulation of 100 genes (p-value < 0.01, fold change ≥ 2). The stimulated genes were involved in components of jasmonic acid and salicylic acid signalling pathways and various abiotic (e.g. oxidative stress) and biotic stress (e.g. pathogens, wounding) responsive genes. Further, up-regulation of phosphate starvation genes and down-regulation of genes involved in phytic acid biosynthesis indicated that thorium disturbed phosphate uptake or signalling. Also expression of iron responsive genes was influenced. Negative regulation of several aquaporins indicated disturbance of water homeostasis. Genes potentially involved in thorium transport could be zinc-induced facilitator *ZIF2*, plant cadmium resistance *PCR2*, and ABC transporter *ABCG40*. This study provides the first insight at the processes in plants exposed to thorium.

Authorship: Mazari, K., Landa, P., Přerostová, S., Müller, K., Vaňková, R., Soudek, P. and Vaněk, T.

Keywords: Microarray, Thorium, Gene expression, Toxicity, *Nicotiana tabacum*

1. **H$_a$**: Thorium influences transcriptome in tobacco root.

1. **Q**: Which group of genes are affected by thorium presence?

Comparison of the Uptake 133Cs in Sunflowers and Reeds from Three Different Soil Types (Peat, Chernozem and Clay)

Abstract

Radionuclides enter into the many ecosystems, although their presence in organisms is not required. Due to radiation the quality of life of various organisms decrease. Radionuclides belonging to the anthropogenic group are mainly coming from the used nuclear fuel as 90Sr, 131I, 137Cs. This last one (137Cs) has a nonradioactive isotope occurring in a small amount in nature. This nonradioactive isotope 133Cs was used in our experiment because there is no difference in uptake of stable and radioactive elements by plants. Anthropogenic radionuclides and their occurrence could be expected near atomic plants, during normal operations in a small amount and during an accident in a significant amount.

In our study, we compared uptake of 133Cs by plants cultivated in three different soil types: peat, humus and clay. The selection of plant species was based according to those found in the area around the nuclear power plant in Temelín – *Helianthus annuus* L. and *Phragmites australis*. These plants were cultivated in greenhouses in pots with regular irrigation. The solution of ¼ Hoagland was used as a fertilizer. Plants were exposed to Cs, added in the form of CsCl, for 20 days. All samples were analyzed with ICP-MS. The pH and the content of organic carbon were measured in the

soil. Our results show higher accumulation of 133Cs in *Heliantus annuus* L. The highest accumulation was observed in plants cultivated in peat soil.

Authorship: Markova, K., Berchova, K.

Keywords: phytoremediation, stable isotope, earth, annual plant, perennial plant

2. **H$_a$**: The cesium uptake by plants is driven by the characteristic of growth medium.

2. **Q**: What the main factors of growth medium which affect the cesium uptake by plants?

3. **Q**: What plays more important role in the cesium uptake by plants, growth medium or plant species?

Differences in uptake of ^{133}Cs among sunflower and reed, water lettuce and water plug

Abstract

Phytoremediation consists of a set of methods using plants to remove hazardous elements from soil or water. The aim of this experiment was to compare differences in of ^{133}Cs uptake among *Helianthus annuus*, *Phragmites australis*, *Elodea canadensis* and *Pistia stratiotes*. In the study, radioactive caesium was replaced by stable Cs, as there is no difference in uptake of these two forms. The plants at seedling stage were cultivated under constant garden conditions, and the experimental design was completely randomized. To the pots (containing sand and perlite for terrestrial plants and water for water plants) was added 0.5 mM CsCl in solution. The exposure to ^{133}Cs was for 8 d. Measurements were made using inductively coupled plasma mass spectrometry. The greatest accumulation of ^{133}Cs was observed in *P. stratiotes* and the least in *P. australis.*

Authorship: Markova, K., Berchova, K.

Keywords: phytoremediation, cesium, garden experiment, plant, accumulation

3. **H$_a$**: The cesium uptake differs in each tested plant species.

4. **Q**: Is difference in the cesium uptake among tested plants significantly important?

Summary

Nukleární elektrárny mohou uvolnit velké množství radioaktivních částic při nehodě, přičemž lidé, zvířata a rostliny jsou v takovém připadě vystaveni účinkům zářeni z radioaktivity. Radioaktivita (v určitém množství) představuje nebezpečí pro lidské zdraví, jak z interního účinku tak i externího. Proto je nutné zohlednit eliminaci radionuklidů v kontaminovaných oblastech. Fytoremediace se zdá být dobrým řešením tohoto problému, přestože se jedná o dlouhodobý proces. Výhody fytoremediace spočívají v relativně nízkých nákladech, zachování původní zeminy a postupně dochází ke snížení obsahu radionuklidů v půdě nebo vodě pomocí zelených rostlin.

V této práci bylo cílem zjistit potenciál vybraných vyšších rostlin pro akumulaci cesia a najít nejvhodnějsi rostlinnný druh pro fytoremediaci radiocesia. Dále byl zkoumán efekt thoria na genevou expresi s cílem objasnit, jakým způsobem rostliny reagují na přítomnost radioaktivních kovů.

Příjem cesia byl měřen ve třech vodních (*Pistia stratiotes, Eichhornia crassipes* and *Elodea canadensis)* ve dvou mokřadních (*Phragmites australis* and *Phalaris arundinacea*) a ve dvou suchozemských rostlin (*Helianthus annuus* and *Brassica napus)*. Rostliny byly kultivovány v polokontrolovaných a zahradních podmínkách v různých substrátech a typech půd. Obsah cesia byl měřen v rýznych částech rostlin po různé době expozice. Kromě přijmu cesia, byl sledován i vliv cesia na růst

rostlin a stomatální konduktivitu. Změny v genové expresi byly zkoumány u rostlin tabáku vystavených účinkům 200 μM thoria po dobu sedmi dnů za použití mikroerejů.

Vodní rostliny *Pistia stratiotes* a *Eichhornia crassipes* vykázaly nejvyšší příjem cesia z testovaných druhů. Ze suchozemských rostlin se nejlépe osvědčila rostlina *Helianthus annuus*. Experiment se třemi odlišnými typy půd odhalil, že nejvyšší koncentrace cesia byla sledována u rostlin pěstovaných na typu Organozem následně na Černozemi a obdobně na Jílovité zemině. Výsledky z tohoto pokusu nazančují, že účinnost fytoremediace je zásadně ovlivněna růstovým mediem (typ půdy). Další z výsledku týkající se sledování změn v přijmu cesia během sezónního období ukázaly, že určitému trendu podléhají rostliny *P. arundinacea* a *E. crassipes*. Přítomnost cesia neměla negativní vliv na stomatální vodivost exponovaných rostlin, jak by se dalo očekávat ani na růst rostlin s největší pravděpodobností. Experiment s mikroereji odhalil potencionálni genové kandidáty podílející se na detoxifikaci a resistenci vůči thoriu. Zinc-induced facilitor *ZIF2*, plant cadmium resistance *PCR2* a ABC transporter *ABCG40* jsou navrženy pro další výzkum. Knock-out a overexpress studie musí potrvdit, zda uvedené skupiny genů mají schopnost zvýšit fytoremdiační potenciál. Transkriptonická studie též odhalila, že 200 μM koncentrace thoria způsobila stres, jak dokládá zvýšená exprese genů zapojených ve složkách JA a SA signálních drach. Rostliny tabáku se pravděpodobně potýkaly s nedostatkem fosforu a železa v přítomnosti thoria, jak naznačují změny v expresi genů v odpovědi na fosfor a železo.

Fytoremediační potenciál některých rostlin byl studován v této práci. *Pistia stratiotes* a *Eichhornia crassipes* jsou navrženy pro remediace kontaminovaných vod radiocesiem. Kdežto *Helianthus annuus* je doporučena pro půdy kontaminované radiocesiem. Na molekulárni úrovni bylo objeveno několik genů zapojených do detoxifikace thoria (popř. jiných radionuklidů).

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Chapter 7

Curriculum Vitae, Credentials and Publications

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EDUCATION

Doctor of Philosophy, Environmental Science 10/ 2012 – Present
Czech University of Life Sciences, Prague, Czech Republic
Dissertation Title: Phytoremediation of radiocesium by higher plants

Master of Science, Environmental Science 10/ 2006 – 05/ 2009
Czech University of Life Sciences, Prague, Czech Republic
Thesis Title: Leaves conductivity determination and CO_2 uptake in plants cultivated under water stress conditions

Bachelor of Science, Environmental Science 10/ 2003 – 05/ 2006
Czech University of Life Sciences, Prague, Czech Republic
Undergraduate Thesis Title: Remediation of lead contaminated soil

Graduate Courses: Physiological Ecology of Plants, Ecotoxicology, Special Analytical Chemistry, Air-Quality, Land Consolidation and Soil Erosion Control, Ecological Application in Landscape, Environmental Contamination & Remediation, Bio-Monitoring, Land Reclamation, Renewable and Alternative Sources of Energy, Hydrobiology, Human Nutrition & Health.

WORK EXPERIENCE

Laboratory Assistant - Kovohutě Příbram (Leader in lead production and recycling) Příbram, Czech Republic 10/ 2009 – 12/ 2010

- Worked with Atomic Absorption Spectroscopy and Optic Absorption Spectroscopy to analyze the content of extracted metal samples
- Measured pH level and conductivity of water samples
- Carried out pipetting and titration to analyze metal samples

ACADEMIC CREDENTIALS

Graduate Research Assistant, Laboratory of Biotechnology, Department of Experimental Botany, Czech Academy of Science, Prague, Czech Republic

- Performed isolation and hybridization of plant RNA
- Support to establish an experiment and perform mineralization, preparation of mineralization solution and centrifugation of plant samples
- Preparation of plant fertilizer for nourishment of plants
- Co-authored a research paper based on results

Graduate Projects

- Internal grants Agency of Faculty of Environment, 2013, 2014, Accumulation of radionuclides by natural sites I., II.
- Conducted research and collected data and information for the project Minimization of Impacts of Radiated Contamination on the Landscape in the Wrecking Area of Nuclear Power Plant Temelin (VG20122015100), by the Czech Ministry of Interior

TECHNICAL AND SOFT SKILLS

- Windows 10
- Microsoft Office
- Statistical Analysis “R”
- Language Proficiency: English B2, Czech, Turkish, Italian

CERTIFICATIONS

- Certified Indiana Master Naturalist
- Certified OSHA HAZPOWER 40 Hour: Pending
- Certified Associate Environmental Professional: Pending
- Certified ESL (English as Second Language) December, 2016

PROFESSIONAL ACTIVITIES & VOLUNTEERING

- Graduate Research Assistant, Department of Plant Physiology, EGE University, Izmir, Turkey
- Presented a poster at The 14th International Multidisciplinary Scientific Geo-Conferences, Albena, Bulgaria in June 2014

- Presented a paper at The National Conferences of Faculty of Environment, University of Life Sciences, Prague, Czech Republic in November 2012 and 2014.
- Attended workshop on Membrane Separations at The University of Chemistry and Technology, Prague, Czech Republic in June 2016
- Attended a workshop on Statistical Software "R" at University of Economics, Prague, Czech Republic in June 2016
- Active Volunteer with Carmel Clay Parks Recreation. Involved in various indoor, outdoor and adaptive activities for children, teenagers and adults
- Active Volunteer with KIB (Keep Indianapolis Beautiful). Involved in environmental projects.
- Attended training for Monarch Wings Across the Eastern Broadleaf Forest by National Fish and Wildlife Foundation, included training for ArcGis in Fisher Library, April 2017.

PUBLICATIONS

Marková, K. and Berchová, K. 2014. Comparison of the uptake ^{133}Cs in sunflowers and reeds from three different soil types (peat, humus and clay). In *International Multidisciplinary Scientific GeoConference Surveying Geology and Mining Ecology Management, SGEM 17.06.2014, Albena; Bulgaria.* Albena; Bulgaria: International Multidisciplinary Scientific Geoconference, 2014, pages 147-154.

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