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Phytoremediation of Selected Radionuclides by Higher Plants

Ph.D. Dissertation

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Certification

I, Kateřina Mazari (Marková), declare that this dissertation entitled "Phytoremediation of selected radionuclides by higher plants" submitted in partial fulfillment of the requirements for the degree of Ph.D. in the Faculty of Environmental Sciences, Department of Applied Ecology, Czech University of Life Sciences Prague, is wholly my own work unless otherwise referenced to or acknowledged.

Prague, September 2017 Kateřina Mazari (Marková)

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Abstract

Nuclear power plants can accidently release huge amounts of radioactive elements, thus human beings, animals and plants may experience the exposure of radioactivity. Radioactivity (in certain amount) is potential hazard to the health from both external and internal effects. Therefore, the elimination of radionuclides from the contaminated areas is required. Phytoremediation may be a good solution to this issue even though it is a long-term process. The advantages of phytoremediation are environmental-friendly tool using green plants to abate radionuclides from soil or water, relatively low-cost with maintaining the original soil.

The aim of the present work was to investigate the potential of selected higher plants to accumulate cesium and to find the most suitable species for the phytoremediation of radiocesium. Further, the effect of thorium on gene expression was followed with the aim to elucidate the ways how the plants response to the presence of radioactive heavy metals.

Cesium uptake was measured in three water (*Pistia stratiotes*, *Echornia crassipes* and *Elodea canadensis*), two wetlands (*Phragmites australis* and *Phalaris arundinacea*) and two terrestrial plants (*Helianthus annuus* and *Brassica napus*). Plants were cultivated in semi-controlled and garden conditions using various cultivation substrates and types of soil. Cesium content was measured in various plant parts after different exposure times. Beside cesium uptake, also effect of Cs exposure on the plant growth and stomatal conductivity was followed. Changes in gene expression were investigated in tobacco plants exposed to 200 µM thorium for one week using microarrays.

Aquatic plants *Pistia stratiotes* and *Eichhornia crassipes* accumulated highest Cs concentrations among tested plant species. Among the terrestrial species, *Helianthus annuus* showed the best potential for the phytoremediation of radiocesium. The experiment with three distinct types of soil revealed that the highest cesium concentration was observed in plants grown in peat followed by chernozem and clayey type of soils. It indicates that phytoremediation efficiency can be influenced significantly by growth medium (soil type). The results further showed that seasonal changes influenced uptake of cesium in the case of some of tested plants (*Eichhornia crassipes*) Cs presence did not negatively affect stomatal conductivity as well as plant growth most likely.

Microarray experiment revealed candidate genes potentially involved in thorium detoxification and resistance. Zinc-induced facilitator *ZIF2*, plant cadmium resistance *PCR2*, and ABC-transporter *ABCG40* are suggested for further studies. Knock-out and overexpression studies must confirm if they can increase the phytoremediation potential of plants. The transcriptomic study also revealed that thorium in 200 µM concentration induced stress as demonstrated by increased expression of genes involved in JA and SA signalling pathways.

Tobacco plants probably suffered by lack of phosphorus and iron in the presence of thorium as indicated by changes in expression of phosphorus and iron responsive genes.

In the present work, phytoremediation potential of some plants was investigated. *Pistia stratiotes* and *Eichhornia crassipes* are suggested for remediation of radiocesium contaminated water while *Helianthus annuus* for radiocesium contaminated soils. At molecular level, several genes with thorium (and other radionuclides) detoxification potential were discovered.

Abstrakt

Nukleární elektrárny mohou uvolnit velké množství radioaktivních částic při nehodě, přičemž lidé, zvířata a rostliny jsou v takovém případě vystaveni účinkům záření 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 měřren 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 potencionální

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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List of Abbreviations

AAS	Atomic Absorption Spectroscopy
α	alfa particular
AKT1	a highly selective inward-rectifying potassium channel
AMF	arbuscular miccorhizal fungi
ß- beta	emitting particulars
Bq/L	Becquerel/Liter
Bg/m^3	$Becquerel/metr^3$
Cd	cadmium
Cs	cesium
CsCl	cesium chlorid
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
γ	gamma emitting particulars
Gy, mGy	Grey, miliGrey (unit of ionizing radiation dose)
HAK5	High Affinity K^+ Transporters
HMA	gene family encoding metal transport
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
K^+ Channels	potassium channels
KUPs	potassium uptake permeases
MATE	gene family encoding multidrug and toxic compound extrusion
MeV	mega electron volts
$mg.kg^{-1}dw$	milligram/ kilogram of dry weight
µM	micro Mol
mM	milli Mol
MTP	gene family responsible for metal efflux transport
Ni	nickel
p-adj	p-adjusted
PBq	Peta Becquerel ($1x10^{15}$)

Rubisco	Ribulose-1, 5-bisphosphate carboxylase/oxygenase
Sv, mSV	Sievert, miliSievert (unit of dose equivalent)
TF	transfer factor
US	United States
USSR	Union of Soviet Socialist Republics
VICCs	voltage-insensitive cation channels
W	Wilcox's statistical test
Wt	weighting factor
YSL	gene family involved in metal uptake and long-distance transport
ZIP	gene family encoding metal transport
Zn	zinc

1. 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 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 where can be a higher risk of discharging radioelements with the aim to prevent their further penetrating into ecosystems.

1.1 Health Risks of Radiation

Ever since the Hiroshima and Nagasaki bombing in August 1945, various nuclear accidents in recent decades and nuclear weapon testing, the radiation thus emitted is known to have caused numerous health problems, most of them being life threatening. The damage to body by radionuclides can occur externally by dermal route and internally by inhalation and ingestion of contaminated food and water in nuclear polluted environment. It can affect whole body, organs and tissues, cell and DNA, etc. Oral route is even more dangerous as flushing process is time consuming thereby raising the health concern severely. Among the fission related nuclides, radiocesium is the most dangerous because of its large production during fission, intermediate half-life, and chemically reactive and highly soluble properties. Because of the latter, it quickly spreads into the ecosystem affecting water, soil and life. Thus, it is a persistent health hazard at nuclear accident sites and beyond affecting human life in a variety of ways.

1.1.1 Effect of Radiation on the Body

As we know from different sources the impact of radiation on the human body of population or individuals is dependent on several factors like the intensity of exposure, the age and sex at exposure, and the various routes of exposure. Radioisotope inhaled or ingested precariously affects different organs as its residential time in the body can be several days, months or years until it decays or flushed out. Thus, long-term high doses of radiation may cause mortal damage to the body. According to medical research men are more sensitive to radiation effects than women. The absorption dose of 0.2 Gy (Fox, 2014) can lead to a temporal decrease in sperm production and 2 Gy and higher causes irreversible azoospermia, and a dose of 3 or more Gy induces woman's sterility and earlier climacterium (Huser et al., 2010). Radiation's effects on an embryo can be terminal or highly damaging. The experts consider the critical period for an embryo to be in the range of 4-8 weeks of pregnancy. Additionally, exposure to radiation can cause death of embryo, decrease in weight and physical defects of organs. The critical dose of radiation for an embryo is considered 50 mGy (Yang et al., 2017). Doses >0.05 Gy can reduce intelligence and cause mental retardation within a period of 8-15 weeks of the embryonic stage when the central nervous system

develops and matures (Yang et al., 2017). For the whole time of pregnancy, the embryo is sensitive to inducing malignant tumors; thus, pregnant women cannot work in the area of ionizing radiation exceeding 0.05 Gy (Yang et al., 2017).

The other segment of the population is the children where significant health problems have been noted. These are essentially related with foodstuff grown in contaminated soils containing ^{137}Cesium and also ^{90}Strontium as reported from Chernobyl (Starr, 2011).

1.1.2 Effect of Radiation on the Organs and Tissues

Organ and tissue sensitivity towards the occurrence of tumors varies after the exposure to radiation. The highest organ response to radiation was observed in the thyroid gland, mammary glands, active bone marrow and lungs. Furthermore, the lethal risk of malignant tumors is $5.5x10^{-2}$ Sv^{-1} for the population exposed by chronic radiation, for workers the risk remains at $4.1x10^{-2}$ Sv^{-1} and for children three times higher than the adults (ICRP, 2007). The latency time can be long (15-25 years) for all tumors induced by ionizing radiation (time between the exposure and tumor occurrence). The dermatoid tumors need even more time than usual to express themselves in 25-30 years, for it can often be diagnosed in X-rays of workers and uranium mine workers (Pelclova et al., 2014). The IAEA BSS (Basic Safety Standard) has specified the exact radiation limits for workers even though we may find variations among countries' standards.

The European Union has already prepared the amendment of Directive 29/96, also called EU BSS. The International Commission on Radiation Protection (ICRP) has suggested to radically decrease the limit for eye lentil from the current 150 mSv to 20 mSv, based on new results. The Czech Republic government has accepted atomic law no. 263/2016 Collection of Laws which regulates and specifies conditionings of peaceful use of nuclear power and ionic radiation (SONS, 2016). The dose limits (Table 1) preventively help to protect the employees in high risk jobs from high doses of radiation. We can follow trends in exposition of radiation workers from natural and anthropogenic resources (Table 2). The tissue sensitivity is measured by the tissue weighting factor (W_t) to be able to see differences among the various tissues. The higher the weighting factor (W_t), the more the sensitive tissue towards the radiation. Total W_t is calculated by the sum of original tissue weighting factors for the particular tissue (Fox, 2014). The weighting factors (W_t) and the total weighting factors (Total W_t) are shown in Table 3 for the single tissues.

Table 1
Element limits (effective and equivalent doses) for radiation workers within one year (ICRP, 2007).

Dose equivalent limit ***(no stochastic effect)***	***Workers***
For eye lentil	*150 mSv*
For skin	*500 mSv*
For arms and legs	*500 mSv*
Dose effective limit (stochastic effect)	100 mSv within five followed years

Table 2
World Trends in Exposition of Radiation Workers (mSV*) (UNEP, 2016).

Resources	**1970**	**1980**	**1990**	**2000**
Natural Resources				
Air Stewards	-	3.0	3.0	3.0
Coal Mining	-	0.9	0.7	2.4
Other Mining**	-	1.0	2.7	3.0
Miscellaneous	-	6.0	4.8	4.8
Anthropogenic Resources				
Medicine	0.8	0.6	0.3	0.5
Nuclear Industry	4.4.	3.7	1.8	1.0
Other Industry	1.6	1.4	0.5	0.3
Miscellaneous	1.1	0.6	0.2	0.1

* Estimation of annual effective dose/one worker
** Uranium industry is in nuclear industry

Table 3
Tissue Weighting Factors (according to ICPR in Fox, 2014).

Tissue	W_t	Total Wt
Bone marrow, breast, colon, lung, stomach	0.12	0.60
Bladder, esophagus, gonads, liver, thyroid	0.05	0.25
Bone surface, brain, kidneys, salivary, glands, skin	0.01	0.05
Remaining tissues	0.10	0.10

1.1.3 Effect of Radiation on the Cell and DNA

The harmful effects of radiation on a cell and DNA depend on the kind of emission exposure, whether α, ß, γ or neutrons as they vary in density of the emitted particulars (Van den Heuvel, 2014). An α particle may cause a wide range of destruction in DNA due to the higher density of ionization; however, ß particle or γ ray may not have any effect on DNA whatsoever due to a low density of ionization; in fact, they just penetrate through the sequence (Fox, 2014). Nowadays, scientists use a special model, Monte Carlo, to identify single or double strand breaks in DNA-molecules (Van den Heuvel, 2014). El Ghissassi et al. (2009) considers all particle emitters (α, ß, γ particles emitters, X-rays and gamma rays, and Neutron radiation) as carcinogenic to the human population based on data obtained from animal experiments by using the "risk coefficient". Furthermore, the risk of estimation made by ICPR for the occurrence of genetic consequences starts at 1.3×10^{-2} Sv^{-1} for exposed population (including children) and 0.8×10^{-2} Sv^{-1} for workers with radiation.

1.2 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 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).

1.3 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, 2004). When thorium exposure is sufficiently high, chemical toxicity exceeds the radiological one (Mizukami-Murata et al., 2006).

1.4 Properties of Radiocesium

Radiocesium is an anthropogenic element formed by nuclear fission of uranium 235. It has 82 neutrons and 55 protons in its nucleus; furthermore, its physical half-life is known to last approximately 30.5 years with the decay energy 1.176 MeV (Ashraf et al., 2014). Radiocesium emits ß-particles and γ-rays and forms ^{137}Ba as its decay product. Thus, it is considered as one of the most precarious fission products among all the other products which is persistently present in

the environment. Moreover, this radioactive element can be 100% absorbed by the body - mainly in muscles (Fox, 2014). Importantly, chemical analogue of radiocesium is recognized as potassium which is the critical information in the study of uptake radiocesium by plants (Hampton et al., 2004; Qi et al., 2008).

1.5 Radiocesium in the Air

Radiocesium spreads by the dry pathway and contaminates the face of the earth as a fallout from the polluted atmosphere. It is an element released into the atmosphere by the anthropogenic activities like producing electricity from nuclear power plants and by testing nuclear weapons (banned in 1963) (Povinec et al., 2013). The radiocesium fallout depends on the actual local precipitation occurrence (IAEA, 2009). The growing vegetation possibly retains certain amount of radiocesium fallout which gradually transfers into the soil by precipitation. The initial fallout seems to be pivotal for any radioecologic modelling because the direct deposition of radiocesium can cause relatively high activity in food and feed (IAEA, 2009). Therefore, caution is to be observed in regard to radiocesium and other radionuclides for their intrusion into the food chain and environment. It is vital to perceive knowledge about the behavior, transport and fate of radiocesium in the environment particularly its intake by the plants. Many assessment studies contribute to this knowledge of radiocesium especially from nuclear accidents like Chernobyl (1986), Fukushima (2011), etc. Comparative study of atmospheric deposition of radiocesium released from Chernobyl shows a fallout of 85 PBq (UNSCEAR, 2008) and that of Fukushima 13-15 PBq (Chino et al., 2011), whereas the global fallout of radiocesium in the atmosphere was counted at the level 950 PBq (Povinec et al., 2013). In addition, Povinec et al. (2013) used the Lagrangian particle model to determine the dispersion of particles from the Fukushima source. After radioactive cesium discharges into the atmosphere, it briskly reacts with the aerosol particles; consequently, it is carried away for a long distance due to air turbulence (Povinec et al., 2013). Thus, presence of radiocesium in the atmosphere is a cause of concern for health risk of the living creatures.

1.6 Radiocesium in Water

As radiocesium enters the atmosphere it subsequently finds its way into the water bodies either through direct atmosphere-water contact or during polluted precipitation. Global pollution for the ocean by radiocesium was raised to 600 PBq after Fukushima and Chernobyl contributing approximately 15 PBq and 16 PBq respectively (Povinec et al., 2013). The radiocesium contamination in huge aquatic formations rapidly moves and circulates all around, besides collecting in the bottom sediments, benthos, water plants and fish (Wells and Hancock, 2014). The high accumulation and long-time retention of radiocesium in fish were influenced by low pH, lack of potassium and oligotrophic character at an observed Finnish lake and its catchment (Saxén and Ilus, 2008). The radiocesium transportation and bioavailability vary due to size of particles and geological layout in water elements (Dupré de Boulois et al., 2008). Moreover, radiocesium tends to bind with the organic matter where it can last for a long time as Kim et al. (2006) assert in stating that the organic matter may play a key role in adsorbing radiocesium in marine sediment. Fan et al. (2014) emphasize that the river sediments have high affinity towards radiocesium and it can be excerpted from the sediments while the salinity elevates in the water, for instance seawater.

The following formula can be used to calculate the concentration ratio for an activity of object in a liquid with unit Bq/L (Kinoshita et al., 2011) where K_d means the solid liquid distribution coefficient:

$$Kd \text{ value} = \frac{\text{Activity per unit mass solid}}{\text{Activity per unit volume liquid}}$$

Pollution by radionuclides in the ocean can be generally expressed by using a different unit Bq/m3, also used by Buesseler et al. (2011) who claim that the level of radiocesium is still 10,000 times higher than levels before the Fukushima nuclear disaster in Japan's coastal water, 68 x 10^6 Bq $^{137}Cs/\ m^3$. Radiocesium can persist in the catchment of aquatic bodies for a long time; likewise, it can prevail in the soil for some time.

1.7 Radiocesium in the Soil

Radiocesium usually enters the soil element from the atmospheric fallout or from leaf washout later. Soil plays a significant role in the behavior of radiocesium as it becomes a sink for this

radionuclide after the fallout. Furthermore, a soil element is supposed to be an object of consideration in most radioecological modelling. Ashraf et al. (2014) suggest including several of the following parameters into a model of radiocesium behavior in the environment:

1. Transfer from plant root to tissue
2. Animal ingestion assessment of soil and plants
3. Dry deposition assessment from the air to soil
4. Wet deposition assessment from the air to soil
5. Re-suspension from the soil to air
6. Initial fraction deposited on the plant tissue
7. Removal assessment from plant tissue
8. Washout assessment from the top layer of soil into the deeper soil layers

The behavior of radiocesium in soil is determined by several factors. For this, various authors emphasize different factors but the agreement exists about uptake and transport mainly in the following items: a) soil pH, b) content of clay minerals, and c) content of other cations. Apart from that, Zehnder et al. (1995) highlight the importance of soil texture. Forsberg et al. (2000) add to the soil characteristics the importance of physico-chemical radionuclide form, climatic conditions and landuse with practices of landscape management. Staunton et al. (2002) suggest that addition of the organic matter to the clay minerals causes a decrease of radiocesium distribution coefficient and that the nature of organic matter and its amount is important. Giannakopoulou et al. (2007) indicate that the critical factors for sorption of cesium were principally the clay mineral content and size of particle fraction with diverse soils under various pH levels. The work of Mihalík et al. (2014) reveals that the transfer factor for radiocesium was very high from natural peat land and explain that the unique characteristic of peat land determines the behavior of radiocesium such as low redox potential and low pH.

As scientists needed to unify description of a metal movement from the soil to plant for easier comparison among each other, the Transfer Factor (TF) also known as concentration ratio was established and it is described in the following formula (Ehlken and Kirshner, 2002).

$$TFi = \frac{\text{Activity concentration of nuclide } i \text{ per kg dry plants mass}}{\text{Activity concentration of nuclide } i \text{ in dry soil within the root zone}}$$

As we can see from Fig.1., six total acknowledged factors influence the Transfer Factor. Radiocesium freely binds with organic matter making it even more available to plant uptake. There are three other soil phenomena that influence the fate of radionuclide as follows.

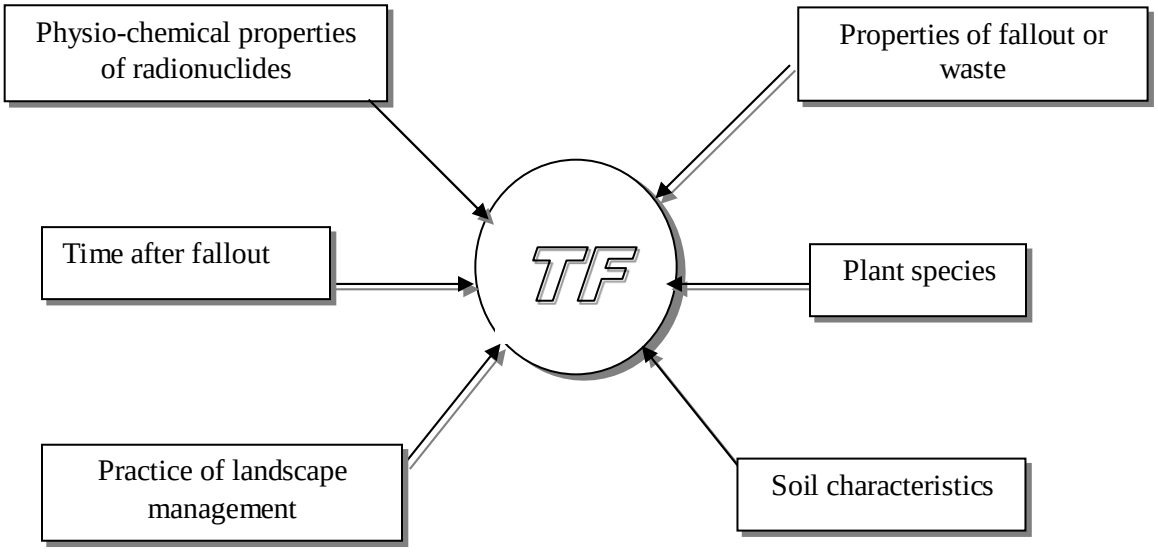


Fig. 1. Factors influencing the uptake of radionuclides by plant roots (IAEA, 2009).

The availability of thorium to plants from soil depends on several factors. These factors were summarized by Hegazy et al. (2013): a) positive correlation was indicated for the concentration of radionuclide in soil and coarse sand volume, b) negative correlation was inferred for the content of organic matter, clay, silt and pH. Guo et al. (2010) revealed that adding phosphate into soil evoked the mineralization of soluble thorium, in consequence, the availability of thorium for plants decreased.

1.7.1 Arbuscular Mycorrhiza

Arbuscular myccorhizal fungi (AMF) passes through the cortical cells of vascular plant root and enclose by intercellular hyphaea (Brundrett, 2008). A hypothesis introduced by Dupré de Boulois et al. (2006) says that AMF may reduce radiocesium uptake into a plant by changing the inclusive transporter expression or action. Furthermore, AMF can play a relevant role in root/shoot radiocesium translocation. Gyuricza et al. (2010) in their *Medicago truncatula* experiment with

influencing AMF and without AMF, reveal that after the examination of radiocesium aggregation the effect of AMF lowered the radiocesium uptake into the plant. This means that AMF acted as a stabilizer in this particular case. Arbuscular mycorrhizal effect was studied also for thorium extraction by *Medicago truncatula* and revaled that the uptake by plant was decreased in the presence of AMF (Roos and Jakobsen 2008). The effect of AMF is not possible to apply on each study in the same way as the plant availability for minerals and radionuclide form should be considered (Ehlken and Kirshner, 2002).

1.7.2 Ectomicorrhiza

Ectomicorrhiza is a symbiotic connection between fungi and mainly trees. Both organisms prosper from this relationship by the enhancement of nutrients in their bodies. Fungi represent a crucial element in the cycling of nutrients and carbon (Vinichuk et al. 2013). Thus, mushrooms have captivated wide attention especially after the Chernobyl accident (1986) for their well-known high accumulation and retention ability of radiocesium in organic soil complex (Parekh et al., 2008). Similarly, Vinichuk et al. (2010) noticed that the activity of cesium in fungi was the highest compared to vascular plants. Moreover, they investigated that ectomycorrhizal fungi had a higher cesium activity than saprotrophic. Bysterzejewska-Piotrowska and Bazala (2008) suggest that two pathways may last in passive transport of cesium in mycelium in *Pleurotus eryngii*. One way is provided via non-specific potassium channel and the second one is provided via extracellular transport. Ashraf et al. (2014) explain that radiocesium uptake from soil to plant can be vigorously influenced by soil properties and plant species, and these are altered by fungi and other microfauna in the rhizosphere.

1.7.3 Microorganisms

Microbes are an important part of the soil. Their function is to decompose organic remains into minerals which are absorbed by plants as their nourishment. Some of the plant families require the soil bacteria for fixing air nitrogen e.g. *Fabacea*e, *Caesalpiniaceae* and *Mimosaceae*. Among cooperative bacteria, *Rhizobium*, *Bradyrhizobium* and *Frankia* can be found with distinct ability to create tubers on the plant root (Štranc et al., 2005). As Djedidi et al., (2014) stated that microorganisms are very useful in stimulating bioremediation by boosting root growth and by raising plant tolerance towards xenobiotics in the soil. With respect to phytoremediation, the

inquiry verified that some of the metals were converted to a more mobile and lesser toxic form after the microbiology treatment (Ashraf et al., 2014). In a study by Wang et al., (2016) found that cesium presence caused higher microbiological activity and diversity in the soil. Djedidi et al., (2014) scrutinized the effect of cesium treatment in the soil with inoculation of two bacteria strains *Azospirilium* and *Bacillus* on five plant species; the best combination was explored in plant species *Brassica rapa* affected by *Azzospirilium* for the efficient cesium uptake. Microbes play important role in environmental fate of radionuclides in both aquatic and terrestrial ecosystems by biological and physiochemical processes (Lloyd and Gadd, 2011).

1.8 Radiocesium and Thorium Uptake by Plants

Once radiocesium penetrates the ecosystems it can be readily taken up by plants. It has a high mobility within plants (Zhu and Smolders, 2000). Radiocesium can be accepted by plants by two pathways: first, via root cells per root pressure, and second, via plant surface mainly through the stomata (Hasegawa et al., 2009). As far as radiocesium reaches the root cells from the soil solution it flows into to the xylem via symplastic way (Hampton et al., 2004). In these circumstances, most of the ions including cesium flow through the symplastic way as well because of the barrier existence in the structure of Casparian band (Ehlken and Kirshner, 2002). Radiocesium transit in the root cell membranes can happen either by K+ Transporters or K+ Channels. K+ Transporters are involved in the case of low concentration of potassium (<0.3 mM) with a little resolution towards radiocesium, whereas K+ Channels are involved in the case of high concentration of potassium with high resolution towards radiocesium (Zhu and Smolders, 2000). The plant uptake of cesium varies from species to species and also within one species due to genetical variation (Hampton et al., 2004)

1.8.1 Terrestrial Species

One huge group of plants which may be good candidates for accumulating radiocesium can be found among terrestrial plants. Some of them were tested in the hydroponic conditions (Moogouei et al., 2011; Soudek et al., 2004, 2006, 2011; Kang et al., 2012; Fu et al., 2016; Lai et al., 2016). Most of them were cultivated in soil (Wu et al., 2009; Soudek et al., 2010; Song et al., 2012; Karunakara et al., 2013; Win et al., 2015; Sugiura et al., 2016) and few of them were examined for

foliar absorption (Zehender et al., 1995; Bystrzejewska-Piotrowska and Urban, 2003; Madoz-Escandez et al., 2004; Fortunati et al., 2004).

Crop plants are investigated principally on the accumulation of radiocesium because of possible harm to the human food chain. It is also important to observe in which part of the plant radiocesium is mainly localized - whether it is root, edible part, leaves or seed. Regarding crop plants, stabilization of radionuclides or heavy metals is more required than accumulation; thus, radiocesium can be stabilized by well-supplied potassium fertilizer in the field (Ashraf et al., 2014). On the other hand, even among crop plants we can find hyper-accumulators (plant with ability to tolerate and accumulate extra amounts of radionuclides or heavy metals, at least 1000 $mg.kg^{-1}$ dw) into shoots (Brooks et al., 1977)).

One of these plants *Brassica juncea* L. was studied by Lai et al. (2016) for high accumulation of cesium and determination of radiological or chemical effect. *Brassica juncea* and *Vicia faba* were also used for experiments to analyze tolerance and enhanced uptake mechanism of cesium into plants; *B. juncea* could accumulate higher concentration of cesium than *V. faba* particularly into the leaves (Fu et al., 2016). Likewise, Djedidi et al. (2016) explored *Brassica napus*, *Brassica juncea* and *Brassica rapa* for radiocesium uptake. Another study conducted by Kubo et al. (2015) with *Fagopyrum esculentum* grain trespassed the standard limits for radioactive materials after the Fukushima wreck (2011); the levels of grain radioactivity however declined back to the limits after supplying the field with potassium fertilizer. Transfer factors for radiocesium and other radionuclides were investigated by Karunakara et al. (2013) by studying an important crop *Oryza sativa* with transfer factor 0.03 which was found low among other plant species. Plant *Vigna mungo* was tested for radiocesium distribution under the different water regimes and a positive correlation relationship was found between the hydraulic conductivity and the ^{137}Cs concentration in leaf and stem (Win et al., 2015). *Amaranthus cruentus* was cultivated on contaminated soils with various levels of Cs, and higher bioaccumulation factor was noticed during elevated CO_2 (Song et al., 2012). Similarly, Wu et al. (2009) used elevated CO_2 level to increase the biomass of *Sorghum vulgare* and to prompt cesium accumulation. Soudek et al. (2011) scrutinized uranium uptake by hydroponically cultivated crop plants. The highest uptake was observed in plants *Zea mays*, *Hordeum vulgare* and *Cannabis sativa* "Benico. Also, *Helianthus annuus* hydroponically cultivated was examined on radiocesium and radiostrontium uptake. The obtained results did not show any difference in uptake of radiocesium and stable cesium (Soudek et al., 2006).

In the case of thorium uptake by plants, there are some studies although in much less extent than for cesium or radiocesium. Shtangeeva et al. (2005) probed uptake of thorium by *Triticum sp*. and found that thorium altered uptake of other macronutrients and the most influenced part was leaf in the exposed plant. Thorium uptake by plants is affected by presence of phosphorus as demonstrate study conducted by Soudek et al. (2013), among other chemical agents the presence of phosphorus was the most significant factor in thorium uptake by *Nicotiana tabacum*. Two plant species (*Triticum aesetivum* and *Secale cereal*) were tested on the thorium accumulation and detected that thorium was absorbed predominantly in the root than upper part (Shtangeeva 2010).

Some of the hyper-accumulators can be found among weed plants, for instance well-studied *Thlaspi caerulescens* for its great ability to tolerate Zn, Cd and sometimes Ni; consequently, this plant can accumulate a high concentration of selected heavy metals into the shoots (Milner and Kochian, 2008). Uptake and translocation of stable cesium were evaluated by Kang et al. (2012) in *Pennisetum purpureum*. They have found that higher concentration of cesium was stored in aboveground biomass; therefore, they suggest *P. purpureum* as a suitable candidate for phytoremediation of radiocesium (TF in range 0.68-1.02 for various levels of Cs concentration). *Vetiveria zizanoides* was exposed to radiocesium and accumulation appeared more in roots than shoots (Singh et al., 2008). Likewise, Eapen et al., 2006 used plant *Calotropis gigantea* to remove radiocesium and radiostrontium from solutions, and accumulation was higher into roots than shoots. Moogouei et al. (2011) worked on the hydroponic solutions of *Calendula alata*, *Amaranthus chlorostachys* and *Chenopodium album* and found that except for *A. chlorostachys* which recorded a value of 4.89 Cs the twoother species showed concentration ratio values above one. As Bystrzejewska-Piotrowska et al. (2005) claim that hyper-accumulator for radiocesium seems to be *Calluna vulgaris*, where accumulation of Cs was much greater into shoots than roots. Cid et al. (2013) developed a mathematical model based on experimental data to predict inclusive process of monovalent inorganic cations by tropical and subtropical fruits. Tagami et al. (2012) examined several herbaceous and woody species after the Fukushima wreck (2011), for instance *Camellia sinensis* showed both isotopes ^{134}Cs and ^{137}Cs within its leaves, even 300 km away from the accident site. Similarly, Sugiura et al. (2016) explored numerous herbaceous and woody plants and observed perennial plant *Houttuynia cordata* and other woody species for radiocesium accumulation ability. In contrast *Amaranthaceae*, *Chenopodiaceae* and *Polygonaceae* did not

accomplish such results to be endorsed as phytoremediators for radiocesium in this experiment. Further investigations are needed on *C. vulgaris* and *P. purpureum* to test for accumulation of radiocesium.

Thorium activity was examined in several medical plants taken up from the contaminated soil (3.41 ppm Th) by Oufni et al. (2011) with mean thorium activity 2.53 $Bg.kg^{-1}$ in root. Simiraly, Xun (2016) tested several native plants for ability to accumulate thorium from uranium mill tailings soils (south China) and found that the highest transfer and phytoremediation factors (0.94 and 22.1 respectively) were determined in plant *Miscanthus floridus*.

Woody plants can be sensitive towards atmospheric deposition especially conifer trees.

For instance, acidic rain (SO_2 released from thermal power plants inhibits enzyme Rubisco in vegetation) caused death to a large area of local spruce forest (*Picea abies*) in North Bohemia in the Czech Republic. However, the present situation has significantly improved due to desulphurisation and *P. abies* being replaced by *Betula pendula* and *Sorbus aucuparius* naturally (Lettl and Hýsek, 1994). The same presumption is valid for radioactivity regarding conifer forest. Whicker (1997) states that the most radiosensitive community from the terrestrial environment is conifer forest as shown by large scale death of pine after Chernobyl wreck (1986). Tagami et al. (2012) analyzed several woody plants for radiocesium concentration in plant tissue after Fukushima wreck (2011). The species included *Prunus* x *yedoensis*, *Dyospyros kaki*, *Rododendron sp.*, *Camellia japonica*, *Fatsia japonica*, *Hydrangea macrophylla* and *Eriobotrya japonica*. Also Sugiura et al. (2016) examined numerous woody species after Fukushima wreck (2011) exposed to radiocesium, for instance *Acer crataegilofolium, Alnus firma, Eurya japonica, Fraxinus sieboldiana, Gamblea innovans, Chengiopanax sciadophylloides, Ilex macropoda, Lithocarpus edulis, Pinus thunbergii, Quercus aliena, Ulmus davidiana var. japonica* and *Viburnum furcatum*. Further investigations were carried out on *A. crataefolium* and *Ch. sciadophylloides* to determine levels of radiocesium accumulation.

Thorium absorbing by trees was also probed in contaminated area. The significant diffirences were analysed in *Nyssa sylvatica* and *Liquidambar styraciflua* (Saritz, 2005).

1.8.2 Water Species

Water plant species having widespread occurrence in water bodies like oceans, rivers and lakes can be involved in the uptake of radiocesium through the water medium (Povinec et al., 2013). Saleh (2012) considers one such plant for high intake of radiocesium like water hyacinth (*Eichhornia crassipes*) which has high productivity of biomass and high ability to accumulate heavy metals. Rezania et al. 2016 indicate that free floating aquatic species like *Eichhornia crassipes*, *Pistia stratiotes*, *Lemna minor*, and *Salvinia spp.* are highly efficient for uptake of radiocesium. Odjegba and Fasidi (2004) tested *P. stratiotes* metal accumulation and potential remediation of eight toxic elements present in industrial wastewater with the highest accumulation of zinc. A newer study about *P. stratiotes* rhizofiltration of cadmium and lead from a solution conducted by Veselý et al., (2011) found that *P. stratiotes* showed enormous ability to remove lead. Another study of cesium uptake by two floating-leaf species aquatic macrophyta *Brasenia schreberi* and *Nymphae odorata*, and two submerged species *Myriophyllum spicatum* and *Utricularia inflata* suggests that foliar pathway was the main access to absorb cesium into to plant body (Pinder III et al., 2006). Different aquatic species were screened for the radiocesium concentration in terrain by Saxén and Ilus (2008). Among the investigated plants namely *Equisetum fluviatile*, *Nuphar lutea*, *Nymphae candida*, *Potamogeton perfoliatus* and *Sparganium gramieum*; the highest radiocesium concentration was measured in *E. fluviatile* 6650 Bq/kg dry weight. Kowata et al. (2014) examined submerged vascular plant *Egeria densa* on the accumulation of radiocesium and found that radiocesium and potassium were mainly stored in the cell wall. In brief, the most promising aquatic plants like *E. crassipes* and *P. stratiotes* can be considered for abating radiocesium which is further supported by the present study.

1.8.3 Wetland Species

Wetland plants besides being used for biological treatment of waste water act as potential accumulators of heavy metals or radionuclides. Laing et al. (2006) surveyed *Phragmites australis* and used its components as a biomonitoring tool with regard to content of heavy metals in tissue. According to Rai (2008) wetland plants are crucial tool for heavy metal removal and suggested *Typha* spp. and *Phragmites* spp. for the abatement of heavy metal pollution. Carvalho et al. (2011) point out that *Typha latiofolia*, *Polygonum spp*. and *P. australis* naturally occurred in sludge dewatering ponds saturated with uranium radionuclides and suggest that wetlands with

these species can be beneficial as a secondary treatment to reduce radioactivity in wet areas. Similarly, Černe et al. (2011) measured activity in plant *P. australis* grown on a former uranium mine and suggest that *P. australis* accumulated uranium mainly in above-ground parts which lead them to propose *P. australis* for phytoremedy purpose. As a hyper-accumulator among wetland species *Salix viminalis* was marked for specifically elements like cadmium, lead and uranium (Schmidt 2003). Interestingly, while many wetland plants can be used for phytoremediation of radionuclides like cesium, Soudek et al. (2004) conducted laboratory analysis of ^{137}Cs uptake by *Phragmites australis* and *Populus simonii* finding no significant results for these species.

Thorium plant investigation indicated that the biggest potential to remove radionuclide from contaminated soil was observed in *Phragmites australis* among other tested plant species (Li et al., 2011).

1.9 Plant Behavior in Relation to Radionuclides

Plants are sessile organisms; therefore, they must develop certain techniques to compromise with abiotic stress caused by heavy metals, etc. One of the options for plants to bypass the metal concentration in soil can be avoidance by way of plant roots growing in different directions where the metal concentration is minimal (Procházka et al., 2003). Another plant strategy to avoid metal toxicity is to form the sensitive tissues without metal content rather than developing resistant proteins (Hall, 2002). If plants could no more use avoidance technique then tolerance mechanisms are involved which include a) removing metals by root exudates, b) metals stored in trichomes, c) metals stored in old leaves, and d) metals stored in decaying tissues (Zehnálek et al., 2005). If metal ions pass through the cell wall, the mechanisms associated with supporting safety environment in plants are complexation and inactivation (metal binds with peptides). The components which participate in these mechanisms are organic acids, free amino acids, gluthathion, phytochelatins, metalothionein, metal chaperones and heat shock proteins (Soudek et al., 2008).

1.10 Effect of Cesium/ Radiocesium on Physiological Parameters of the Plants

Cesium can affect physiological parameters in plants and at high concentration it can be venomous (Adams et al., 2013). The main access for cesium to the root cell from the soil medium is via voltage-insensitive cation channels (VICCs) and K+ uptake permeases (KUPs) at monocation uptake system (Adams et al., 2015). Moreover, High Affinity K+Transporters (HAK5) and K+ uptake (KUP9) are most likely inclusive in the mechanisms of cesium uptake into the plant (Adams et al., 2015). Qi et al., (2008) clone atHAK5 proteine in *Arabidopsis thaliana* and reavel that atHAK5 is involved in the uptake of cesium during K+ starvation and low potassium usage. This finding is in agreement with Hampton et al. (2004), who investigated cesium toxicity in *Arabidopsis* and noticed that the expression of the encoding gene H+/Cs+ symporter atHAK5 increased in plants with K+ shortage. Nieves-Cordones et al. (2014) point out that HAK5 is employed at very low concentrations (<10 μM) of K+, chemical analogue of cesium, while AKT1 channel is activated at intermediate concentrations (1 mM) and non-selective channels at high concentration of potassium (>10 mM). Consequently, these systems are triggered by various mechanisms, for instance phosphorylation of AKT1 or induction of HAK5 transcription. Qi et al. (2008) reveal that the over-expression of atHAK5 elevated cesium tolerance in *Arabidopsis* cultivated at low K+ level. Genetic differences in plant radiocesium uptake are known, and they can be caused by several physiological parameters such as plant demand on potassium, plant growth strategies, growth rate, rooting pattern, degree of mycorrhizal fungi and ion transport systems (Zhu and Smolders, 2000). Some scientists such as Hampton et al. (2004) call for verification of plant genetic responses to the mineral environment prior to their selection for special intention. In the study lead by Adams et al., (2013) jasmonate acid biosynthesis and signaling were probed in plant response to cesium and they observed that cesium inhibits plant growth by initiation of jasmonate pathway and alters potassium uptake mechanisms. Lai et al. (2016) investigated chemical cesium toxicity in *Brassica juncea* and noticed that excessive cesium disturbed the auxin signal transduction pathway and inhibited the indoleacetic acid-induced protein.

Cesium can influence photosynthetic outputs by plants directly or indirectly, for instance by modification of potassium perception mechanisms (Adams et al., 2013). Some photosynthetic outputs were measured in halophyte plant *Codiaeum variegatum*, the cesium concentration caused lower photosynthetic rate, in contrast CO_2 concentration had been increased in mesophyll cells as

well as in stomata (Bystrezejewska-Piotrowska et al., 2004). Some researchers intentionally used elevated CO_2 as an enhancer of phyto-accumulation of cesium, for instance Song et al. (2012) examined *Phytolaca Americana* and *Amaranthus cruentus* grown on soils with various levels of cesium. In a similar manner, Wu et al. (2009) tested *Sorghum vulgare* and *Trifolium pratensis* as hyper-accumulators for cesium during elevated CO_2 level. Another experiment with *Lepidum sativum* conducted by Bystrzejewska-Piotrowska and Urban (2003) found that stomatal conductivity and transpiration ratio were robustly repressed whereas photosynthetic ratio was slightly affected. Atapaththu et al. (2016) measured level of antioxidant enzyme activities and content of peroxide in *Nitella pseudoflabellata* after cesium exposure and their results showed that cesium inhibited plant growth and induced defense mechanism against the oxidative stress. In the case of *Vigna mungo* grown in contaminated soil with radiocesium under water stress the results showed that accumulation and distribution of radiocesium were due to plant growth, water movement and selective absorption (Win et al., 2015). Apparently, cesium/ radiocesium has inhibitory impact on physiological parameters within the plant, it may induce chlorosis in aerial part of plant and restrict plant growth (Adams et al. 2015). There is also hypothetical assumption for decreasing stomatal conductivity due to competitive relationship with K+ except for halophytes see above studies conducted by Bystrzewejska-Piotrowska et al. (2004) although more studies would lead to a clear statement.

1.11 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).

1.11.1 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 accumulation of metals.

1.11.2 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 phytoextraction. One of the famous synthetically prepared chelator EDTA is used in many phytoextraction 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 absorbers of radiation the phytoremediation technique can be used in conditions of both nuclear fallout and mining and milling of radioactive minerals.

2. OBJECTIVES AND HYPOTHESIS

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.

1. Is absorption of stable cesium similar to both annual and perennial plants?
2. If not, do perennial absorb more into any specific organs?
3. Is absorption of stable cesium different from distinct types of soil?
4. Is absorption of stable cesium different due to seasonal changes?
5. Is absorption of stable cesium different among terrestrial and water plant species?
6. Is absorption of stable cesium different from leaf (water solution) and root (from soil)?
7. Does thorium's presence affect plant genes and how?

3. MATERIALS AND METHODS

3.1 Cesium Uptake by Plants

In all, three experiments were conducted to meet the objectives of this work. In the first experiment, four different plant species, namely, *Helianthus annuus* (sunflower) and *Phragmites australis* (reed), *Pistia stratiotes* (water lettuce) and *Elodea canadensis* (water plug) were employed to observe their cesium uptake from sand and water. The second experiment focused on two plant species grown in three different soil types like clay, peat and chernozem, and third experiment targeted the impact of seasonal changes in the cesium uptake by plants. Studies have shown that stable cesium and radiocesium have similar uptake behavior in plants (Soudek et al., 2004) which is why cesium chloride solution was used in the present study to investigate the behavior of radiocesium uptake by plants in the study area.

3.2 Outline Description of Four Different Plant Species

The plant species were selected based on their natural occurrence in the Temelin nuclear power plant area. *Helianthus annuus* L. and *Phragmites australis* L. are easily grown in study area and have good phytoextraction potential (Soudek et al. 2004, 2006). Next non-native species *Pistia stratiotes* was tested because of its high efficiency in the heavy metals uptake as reported by Odjegba and Fasidi (2004) and Veselý et al. (2011). Another non-native species *Elodea canadensis* was included in the present study based on its potential as documented by Pinder III et al., 2006.

Helianthus annuus L. (*Asteraceae*). Known as sunflower in general terms, this terrestrial annual plant is commonly used for such experiments. It has good biomass growth, can reach heights up to 3 m, and is grown as an agriculture crop.

Phragmites australis L. (*Poaceae*). This wetland perennial called as reed, it is able to produce a large amount of biomass and can reach heights ranging from 1 to 4 m (sometimes even 6 m). It can be used for pulp manufacturing and thatching.

Pistia stratiotes (*Aranacea*). This is a non-native plant in the study area. Its temporary occurrence was noticed mostly in backwaters. Generally called as water lettuce, the species is generally used

as algae cleaner and it does not survive winter in the region. This species can therefore be used for cleaning contaminated water without the risk of its further spread.

Elodea canadensis L. (*Hydrocharitеceae*). An aquatic clonal perennial submersed plant, common in Central Europe, *E. canadensis* is a non-native species the abundance of which increases in the secondary distribution area. Called as waterweed, it produces large amounts of biomass and can reach heights from a few cm up to 100 cm in a short time.

3.3 Experiment I - with Four Plant Species Grown in Inert Medium

Helianthus annuus seeds were sterilized for 10 min. in H_2O_2, then placed onto seed plates in greenhouse with semi-controlled conditions at 22° C and 16/ 8 hour light cycles. Three weeks old seedlings were then transplanted into pots (200 mm diameter) with sand and perlite as an inert medium. Seedlings of *Phragmites australis* were purchased as a commercial product and washed with distilled water. These seedlings were then transplanted into pots with sand and perlite. Plants of similar appearance and size were used for the analyses. The experiment was conducted in outdoor conditions in completely randomized block design. The plants were watered using tap water with ¼ Hoagland fertilizer [$Ca(NO_3)_2$ x $4H_2O$ – 1.2492 mM, KNO_3 – 1.2512 mM, KH_2PO_4 – 0.2498 mM, $MgSO_4$ x $7H_2O$ – 0.5103 mM + microelements], 50 ml of solution one time per pot. After 20 days of establishing plants in the garden, 0.5 mM stable Cs [CsCl] was added to the pots at rate 25 ml per pot. The plants were exposed for 8 days. The aquatic species of *Pistia stratiotes* and *Elodea canadensis* were cultivated in a basin with 3 Lit of tap water. At the time of harvesting substrate was washed off the roots with tap water and let them dried (Fig. 2., 3.). Fresh and dry weights, length of roots, length of shoots, number of leaves and their weight were measured (Tables see in Appendix B). The plants were then dried for 24 h at 70° C to a constant weight. Dry biomass was ground using a mill (Fritsch, Palverisette 15, Idar-Oberstein, Germany). Homogenized sample (0.2-0.4g) was transferred in teflon tube and incorporated to HNO_3 + H_2O_2 in the ratio 7:1. After decomposition, sample was subjected to microwave digestion using an Anton Paar device (Anton Paar, Graz, Austria). Leaves, stems and roots were analyzed separately except for *Elodea canadensis* which showed poor root growth.

Fig. 2. Washed root of harvested *Helianthus annuus.*

Fig. 3 Washed root of harvested *Phragmites australis.*

3.3.1 Preparation of Cesium Solution

The solution of CsCl (Sigma Aldrich, Czech Republic) was prepared by measuring on the calibrated scale with a certain amount (0.0421 g/ 0.5L). Then the powder of CsCl was transferred into measuring cup and added 0.5 L of water. The solution was dissolved on a stirrer for few minutes.

3.3.2 Measurement of Cesium Concentration

ICP-MS analysis was employed, it is specified by mass spectrometry with induce bind plasma (ICP MS, 7700x Agilent Technologies Inc., USA) without reacted/ colize gas and with using the internal solution of indium of the concentration 100 μg L^{-1}. For the dose of the sample was used autosampler ASX-500 Series (Agilent Technologies Inc. USA), ultra-low volume application MicroMist (Agilent Technologies Inc. USA) and copper skimmer and nickel conus were employed. The volume of each sample was 50 ml.

3.3.3 Statistical Evaluation

Experimental data were analyzed by statistical method ANCOVA. The dependent variable (content of cesium) was transformed by logarithmization because it did not show normal distribution. The explainatory variables species, plant part followed by categorial variable position in block and covariate dry weight were included in statistical model. Apart from that *Helianthus anuus* was analyzed by ANOVA to see statistical differences in root, leaf and stem. Software R was employed in the analysis. To design figures (boxplot, box and whisker plot and plot) the software R was used as well with the package lattice from library.

3.4 Experiment II - with Two Plant Species Grown in Peat, Clay and Chernozem Soil

Seeds of *Hellianthus annuus* L. were cultivated in a greenhouse with semi-controlled conditions of 22^{o} C temperature and 16/8 day light until the stage of seedling. Then the seedlings were transferred to pots containing different types of soil. The seedlings of *Phragmites australis* were ordered from a garden shop and transferred to pots with different soil types. The fertilizer ¼ Haogland was used for the plants. The pots were organized in a completely random design for both species. After establishing plants to the right conditions, 0.5 mM CsCl was added in the form of an aqueous solution (25 ml each). The exposition time was 20 days to two months old plants. At

the time of harvesting, the shoots and roots of the plants were washed out from substrate and then separated, their fresh weight measured. Plants were dried in the oven at 80º C for 22 hours to a constant weight and homogenized using a mill (Fritsch, Palverisette 15, Idar-Oberstein, Germany). The mineralization of samples (0.25-0.30 g dry mass) was carried out in teflon tubes (Fig. 4, 5). The acids HNO_3: $HClO_4$ were added in the ratio 7:1. The samples were incubated in a microwave oven (Anton Paar, Graz, Austria) for 1 hour. The contents of C and N in soils were measured on the Analyzer Primacs SCN (SCALAR). The method in brief is based on burning of sample at 950-1100 ℃ than adding controlled amount of the oxygen causing nitrogenic compounds to get converted onto NOx. The mixture of gas passes through the scrubbery where the acid aerosols, water and SO_3 are disposable. The stream of helium carries them through the copper reducer where NOx are converted into N_2. The concentration of incurred N_2 was measured by the thermal conductive detector. CO_2 was measured by Infrared detector.

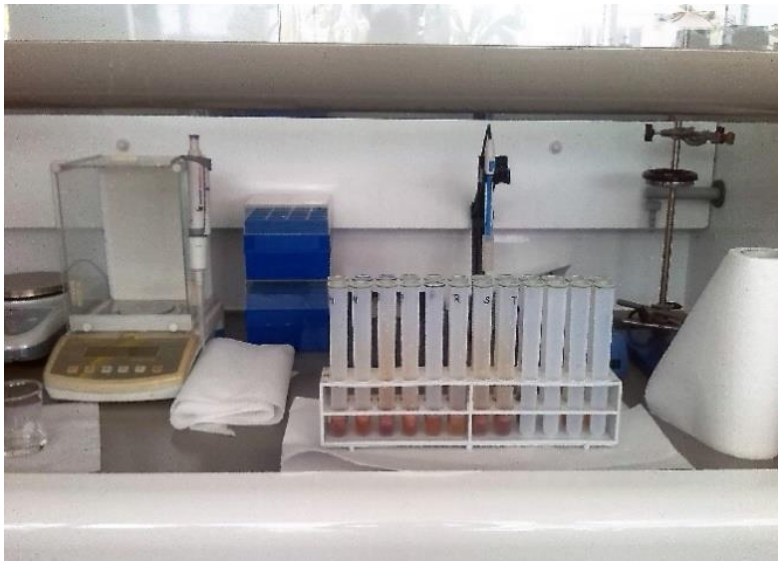


Fig. 4. Samples being digested in teflon tubes.

Fig. 5. Picture of samples prepared for microwave oven.

3.4.1 Preparation of Cesium Solution

Cesium solution for this experiment was prepared the same way as in Experiment I (see Section 3.3.1).

3.4.2 Measurement of Cesium Concentration

Procedure for measuring cesium concentration in this experiment was same as described in Experiment I (see Section 3.3.2).

3.4.3 Measurement of K, Mg, Ca in Soil

Atomic Absorption Spectroscopy was employed. Samples of soil were mineralized and in two replicated samples measured by AAS (Agilent, AAS DUO 55B/240Z) in laminar flame of mixture acetylene with air.

3.4.4 Measurement of pH in KCl Solution – Standard Method by pH Meter

All soil samples were collected from the upper layer (0–15 cm) from localities in Suchdol-Prague, Jilove -Prague, Vomacka-south Bohemia), air-dried, homogenized and sieved through a 2-mm stainless sieve. The soil pH was measured in 1:2.5 soil:1 M KCl suspensions.

3.4.5 Statistical Evaluation

To evaluated experimental data, ANCOVA was used with post-hoc test Tukey*HSD*. As previously the dependent variable (content of cesium) was transformed by logarithmization because of its not normal distribution. The explainatory variables species and soil with continual variable of dry weight as covariate were included in the statistical model.

3.5 Experiment III - with *Phalaris arundinacea* for seasonal changes in cesium uptake

Plants of *Phalaris arudiancea* (Fig. 6) were taken from their natural habitat and washed out their root system with tap water. After adjustment, they were transferred into a pot with sand and perlite. The exposure was repeated each month from April to November and last for eight days. It was not possible to keep plants in outdoor conditions the entire season due to cold temperature in October and November. Plants were relocated in the greenhouse for that time. The plants were watered using tap water with ¼ Hoagland fertilizer [$Ca(NO_3)_2$ x $4H_2O$ – 1.2492 mM, KNO_3 – 1.2512 mM, KH_2PO_4 – 0.2498 mM, $MgSO_4$ x $7H_2O$ – 0.5103 mM + microelements], 50 ml of solution per pot each month.

Few plants were selected for stomatal conductivity measurement by device AP4 Porosimeter (Delta T-Device, UK) before immediate harvest. The AP4 Leaf Porosimeter measures diffusion conductance by comparing the precise rate of humidification within a small cuvette (chamber) to readings obtained with a calibration plate. The plate has 6 diffusion conductance settings whose values have been accurately determined by Finite Element Analysis. The plants were exposed to 0.5 mM stable Cs [CsCl] which was added to the pots at rate 25 ml per pot for eight days. The plants were then dried for 22 hours at 80 ^{o}C to a constant weight. The plant dry weight was measured on caliber scaled gauge. Additionally, *Eichhornia crassipes* (Fig.7.) was employed in the experiment for four months (Aug. to Nov.) and *Brassica napus* was employed for four months (April to July).

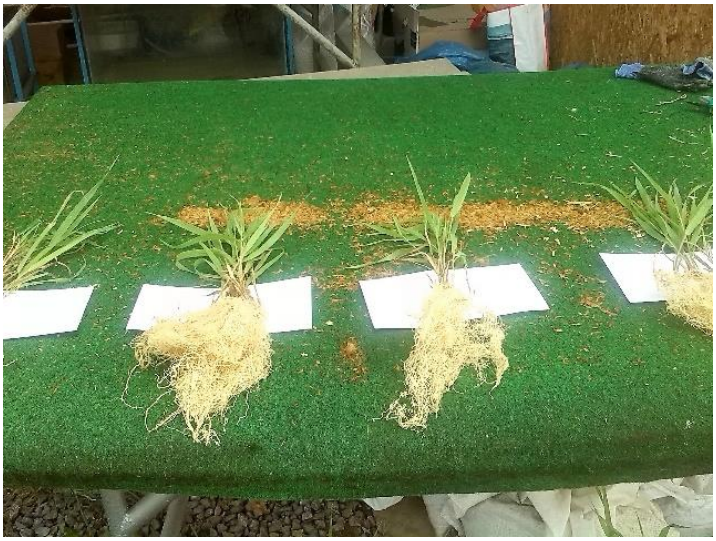


Fig. 6. Washed roots of harvested *Phalaris arudinacea*.

Fig. 7. Photo of *Eichhornia crassipes* (water hyacinth) grown in the botanical garden.

3.5.1 Preparing Cesium Solution and Measuring its Concentration

Procedure for preparing cesium solution and measuring its concentration in plants in this experiment was same as described in Experiment I and II (see Sections 3.3.1, 3.3.2).

3.5.2 Statistical Evaluation

The multiple regression was employed in data evaluation. Similarly, the dependent variable was transformed by logarithmization due to its not normal distribution. The explainatory variables as species its dry weight and month were included in the statistical model and the evaluation for stomatal conductivity was done separately. The values of exposed plants and control plants were compared by using Wilcox's test.

3.6 Thorium Plant Uptake

3.6.1 Experiment IV - thorium impact on tobacco root transcriptome

In a separate experiment, run for a week, impact of thorium on tobacco root transcriptome was investigated. The objective was to understand the potential of tobacco plant as a tool of phytoremediation of thorium contamination resulting from mining activity or spilling.

3.6.2 Plant material and cultivation conditions

Tobacco seeds were sown in perlite and cultivated for two months. All seedlings were watered by Hoagland medium (Hoagland, 1920) every three days. The hydroponic medium with pH was 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. Eight-week old plants were placed for one week into Araponics boxes (Araponics SA, Belgium) supplemented with 2 L of Hoagland hydroponic medium to acclimatize to the hydroponic conditions. The plants were kept at 20 ºC under 16 h light period, humidity about 60%, average irradiation 72 µmol m^{-2} s^{-1} (Soudek et al., 2013).

3.6.3 Treatment of Plants with Thorium

Tobacco plants (*Nicotiana tabacum* L.) grown in hydroponics were transferred into the Hoagland hydroponic medium containing 0.2 mM $Th(NO_3)_4$ (Lachema n.p., Brno, Czech Republic). Plants were exposed to $Th(NO_3)_4$ for seven days. The Th concentration and exposure time were selected according to previous toxicity tests (Soudek et al. 2013). During sampling for gene expression studies, roots and shoots were separated. The roots were then rinsed in demineralized water and

dried on filtrated paper. The fresh weight of the samples was determined before freezing in liquid nitrogen and storing at -80 ºC until RNA isolation.

3.6.4 Microarray Analysis

The RNA was isolated from the roots of *N. tabaccum* plants that were treated with $Th(NO_3)_4$ control, and untreated ones using the Plant RNA Isolation Mini Kit (Agilent Technologies, CA, USA). RNA was then labeled using LowInput QuickAmp Labeling Kit (Agilent Technologies) by Cyanine 3 and Cyanine 5 using a dye swap design to avoid dye-based bias. Labeled cRNA was purified by RNeasy Plant Mini Kit (Qiagen, Germany), fragmented and hybridized on the Nicotiana (V4) Gene Expression Microarray (Agilent Technologies) according to the manufacturer's instructions. After a 17-h hybridization at 65°C, slides were washed in GE Wash Buffers (Agilent Technologies), acetonitrile and Stabilization and Drying Solution (Agilent Technologies). Microarrays were scanned using a GenePix 4000B scanner controlled by GenePix Pro Microarray Analysis Software (Molecular Devices, CA, USA). Experiments were repeated three times with root cRNA prepared independently from individual plants. The data acquired from the scanner were processed in R scripting environment using software package LIMMA according to Smyth and Speed (2003), Smyth (2004), and Smyth et al. (2005). The LOESS normalization method was used to balance the mean fluorescence intensities between the green and red channels in the frame of single arrays, and the Aquantile method was used to normalize signals among arrays. The background intensity was not subtracted from the overall spot intensities. The statistical analyses were performed without spots with zero weights. The false discovery rate (FDR) method was used for statistical evaluation. Genes showing $\geq$ 2-fold change in gene expression (p-value < 0.01) were selected. Tobacco chip was reannotated using recently sequenced *N. tabacum* data (Sierro et al., 2013). Probes sequences were blasted against *N. tabacum* TN90 mRNA database (solgenomics.net) and annotation was assigned to those with resulting E-value < 10^{-6}. Functional classification of up- and down-regulated transcripts was done by Classification Super-Viewer as employed by Provart and Zhu, 2003.

Sample Preparing Procedure

The following procedure was adopted for preparing the sample.

A. Sample Preparation

- Preparing tissue or seed homogenate in appropriate volume of Extraction
- Solution/ß-ME mixture
- Centrifuging up to 600 µL of homogenate through the mini prefiltration column for 3 min. at 16 000 x g
- Adding an equal volume of isopropanol to the filtrate and mixing until homogenous, incubation for 5 min.
- Applying mini column (blue) and centrifuging for 30 sec. at 16 000 x g, discarding flow-through and replacing RNA-loaded column in same collection tube
- Adding 500 µL of prepared Wash Solution to the mini isolation column and centrifuging for 30 sec. at 16 000 x g, discarding flow-through and replace isolation column in the same collection tube
- Adding 500 µL Wash Solution and centrifuging for 2 min. at 16 000 x g
- Eluting purified RNA by adding 10-15 µL of nuclease-free water, waiting 1 min. and centrifuging for 1 min. at 16 000 x g
- Measuring each sample for RNA concentration on the device NanoDrop ND-1000 UV-VIS Spectrophotometer version 3.8.1 (Thermo Fisher Scientific Inc., MA, USA)

B. Labelling of sample

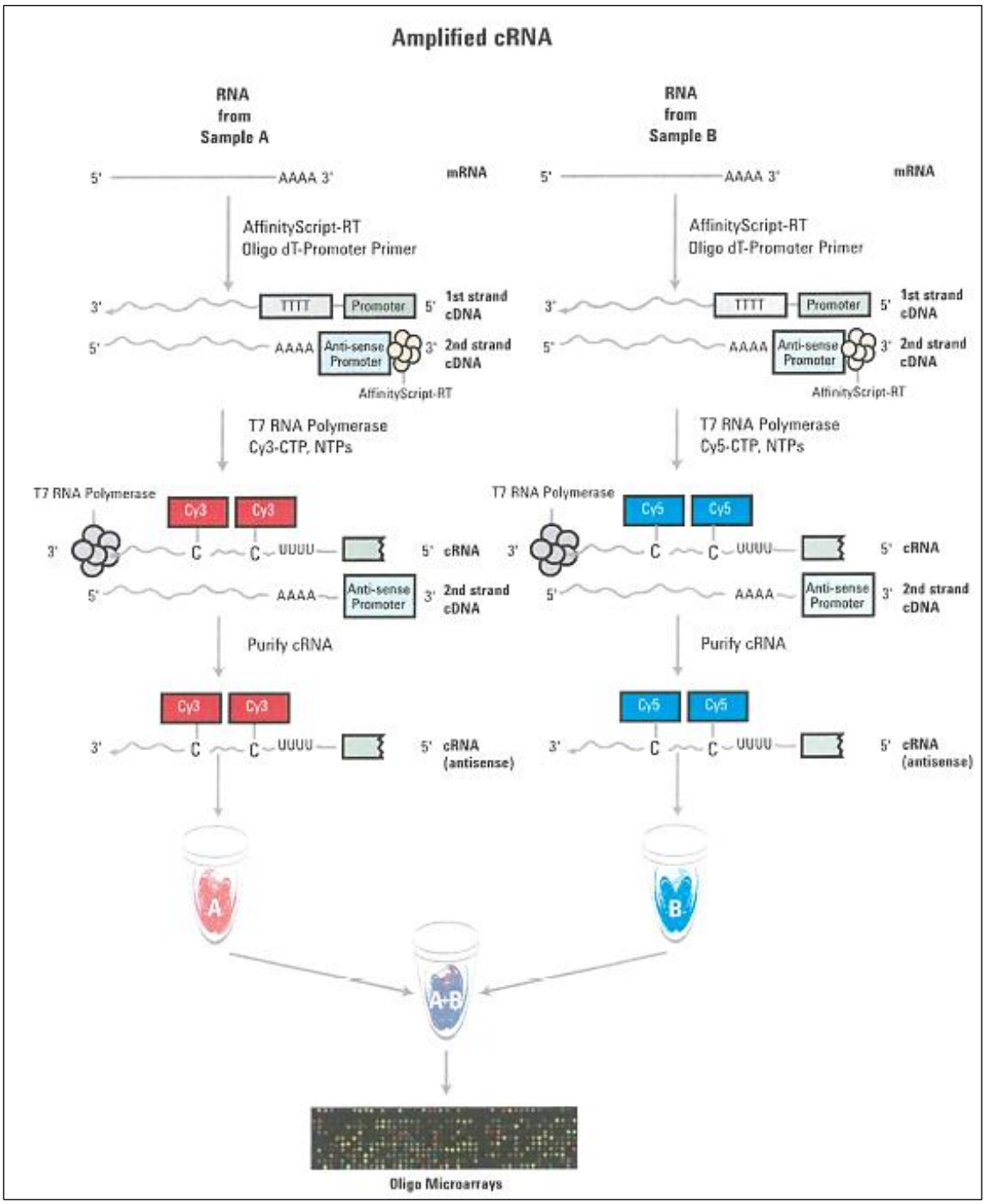


Fig. 8. Schematic of amplified cRNA procedure, Generation of cRNA for a two-color microarray generation is shown. (Adopted from Agilent Technologies Protocol, version 6.6, 2012).

C. Hybridization of sample (steps)

- Preparing 10x Gene Expression Blocking Agent according to Agilent Technologies Protocol
- For each microarray adding Cyanine 3-labeled or Cyanine 5-labeled, 10x Gene Expression Blocking Agent, Nuclease-free water and 25x Fragmentation Buffer to a 1.5 mL nuclease-free microfuge tube
- Mixing well on vortex
- Incubating at 60 ^{o}C for exactly 30 min. to fragment RNA
- Immediate cooling on ice for 1 min.
- Adding 2x Hi-RPM Hybridization Buffer to stop the fragmentation reaction
- Mixing well by pipetting (up and down), no vortex
- Spin for 1 min. at room temperature at 13 000 x g in microcentrifuge to drive the sample off the walls and lid
- Placing sample on ice and loading onto the array as soon as possible
- Placing sample into hybridization chamber: loading a clean gasket slide into the Agilent SureHyb chamber base with the label facing up and aligned with the rectangular section of the chamber base
- Dispensing the volume of hybridization sample onto the gasket well in a "drag and dispense" manner
- Griping the slide on either end and slowly putting the slide "active side" down, verifying that the sandwich-pair is properly aligned
- Placing the SureHyb chamber cover onto the sandwiched slides and sliding the clamps assembly onto both pieces
- Firmly hand-tightening the clamp onto chamber
- Vertically rotating the assembled chamber to wet the gasket and assess the mobility of bubbles
- Placing assembled slide chamber in rotisserie in hybridization oven set to 65^{o}C
- Hybridization takes place at 65^{o}C for 17 hours
- Optional step: Microarray wash

D. Scanning and Feature Extraction

3.6.5 Quantitative Real-Time PCR Analysis

The transcription levels of selected genes obtained from microarrays were verified by the quantitative real-time PCR (RT-qPCR). RNA was treated with Ambion DNA-free kit (Thermo Fisher Scientific Inc., MA, USA) to eliminate the traces of genomic DNA. Complementary DNA (cDNA) was prepared from the total RNA by the M-MLV Reverse Transcriptase (RNase H Minus, Point Mutant, Promega, WI, USA). The protocol for the first strand cDNA synthesis with oligo dT primers was used and the Protector RNase Inhibitor (Roche Applied Science, Mannheim, Germany) was added. 2.5 μL of 20x diluted cDNA was mixed with the LightCycler 480 DNA SYBR Green I Master (Roche Applied Science, Mannheim, Germany) and 500 nM of respective primers to final volume 10 μL. The qPCR cycle included initiation (95°C, 10 s), annealing (60°C, 10 s) and elongation (72°C, 10 s) steps and the reaction was performed by the Light Cycler 480 (Roche Applied Science, Mannheim, Germany). Relative content of RNA in sample was calculated accordingto Hellemans et al. (2007). *RPS4A* and *GTPbEFTu* genes were used for normalization. qPCR efficiency estimated for each primer pair from the calibration curve was used for the calculation. The list of primers is in the Table 4.

Table 4

Primers used for qPCR.

Locus (*A. thaliana* homologue)	Gene	Forward primer	Reverse primer
AT5G58420	RPS4A	ACAATTCGCTACCCTGATCCTC	GTCCCCTGTTTCTACCACCA
AT5G60390	GTPbEFTu	TACTGTCCCTGTTGGTCGTG	TTCAGTTGTCAGACCAGTAGGC
AT2G16060	HG1	ATGAAAAAGAATGCCGGAGA	AGCATGAGGCTTGAGTTTGG
AT1G15520	PDR12	CGTACTGAGATGCCACGAGA	CATAAGCCCATGAAGGGAAA
AT4G00430	PMIP1	GCATGATTTTTGCCCTTGTT	CAGCACCACAGATAGCTCCA
AT3G53420	PMIP2A	GGCATGATTTTCGTCCTTGT	CATAATTGCACGAACCAACG
AT5G05270	ChFIfp	CTCATTTGCAGCAGTGGAAG	TGCGAGCCTTTGATTTCTTT
AT5G10170	mIPS3	CAAAAGGGCAATGGATGAGT	ATTGGAGCAGCCAAAAGAGA
AT4G39730	LH2	TGTTTGACTGCGCCAATCTG	TGTTGGCTGCATCGTTTGTG
AT3G22830	A6B	TAGAGCAAGAAGGGACCAACAC	AACCCAACCTCTCTGCCAAG
AT3G26300	CYP71B34	GTAATGGCGAAAGCACAAGC	TTGGAGGGTGTAATCGGAGTG
AT4G37330	CYP81D4	TTTTGGGACCCCTGTTGAAC	AGCTCCCGAAAATGGTTTGC

4. RESULTS

4.1 Cesium Plant Uptake

4.2 Experiment I – with with four plant species grown in inert medium

4.2.1 *Helianthus annuus* (sunflower)

Plant *Helianthus annuus* showed the ability of cesium uptake after eight days of exposure especially in root average value 40.58± 13.35 mg.kg^{-1}, following by leaf 24.30± 5.91 mg.kg^{1} (Table 9) and stem 12.86± 3.05 mg.kg^{-1}. From obtained results, the cesium concencration rapidly declines in order root>leaf>stem (Fig. 9.). Most of plant species act n similar way except hyperaccumulators. Statistical evaluation showed significant difference in entire data of *H. annuus* p-value (0.0069) and post hoc test (Tukey*HSD*) displays which plant parts are significantly different from each other.

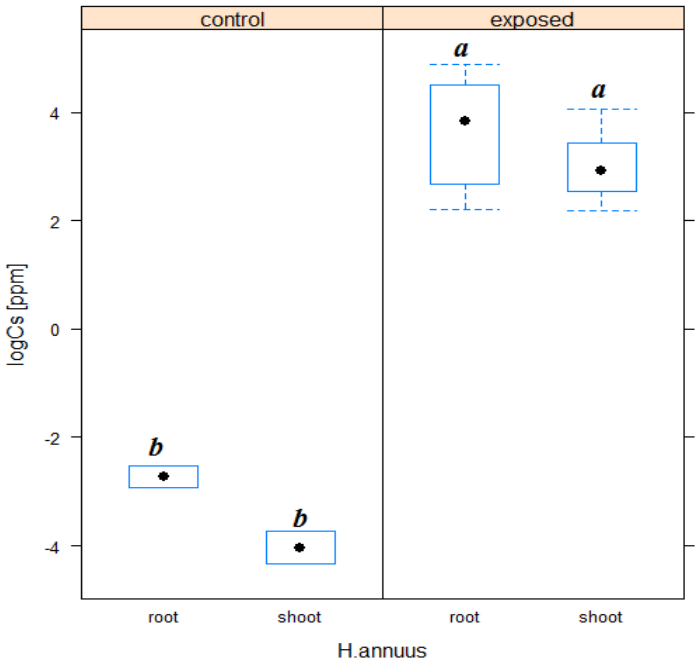


Fig. 9. Concentration of ^{133}Cs in the different plant parts of *H. annuus*. Data were transformed by logarithmization. The letter above boxes shows the statisticaly significant results using post hoc test ($p < 0.05$), central dot shows central value (median), box upper and lower quartiles and whiskers interquartille range. n=8.

The test approved the difference between root and stem p-value (0.005) for α=0.05, no significant difference between root and leaf p-value (0.1999) for α=0.05 and neither any significant difference between stem and leaf p-avalue (0.1998) for α=0.05. Statistical significant difference also exists among exposed and control plants (Fig. 9.) p-value (0.005) for α=0.05.

4.2.2 *Phragmites australis* (reed)

Second plant used in this experiment was *Phragmites australis*. The results also show the higher uptake in root than shoot (Fig. 10.) as in the previous case p-value (0.0087) for α=0.05. The cesium concentration was measured in root and shoot from ten repeating with average value 21.27± 3.05 mg.kg^{-1} and 7.69± 2.26 mg.kg^{1} respectively (Table 9). Likewise, the significant difference exists among exposed and control plants p-value (there is result verging on significance among sunflower and reed cesium concentration in roots, p-value (0.0506).

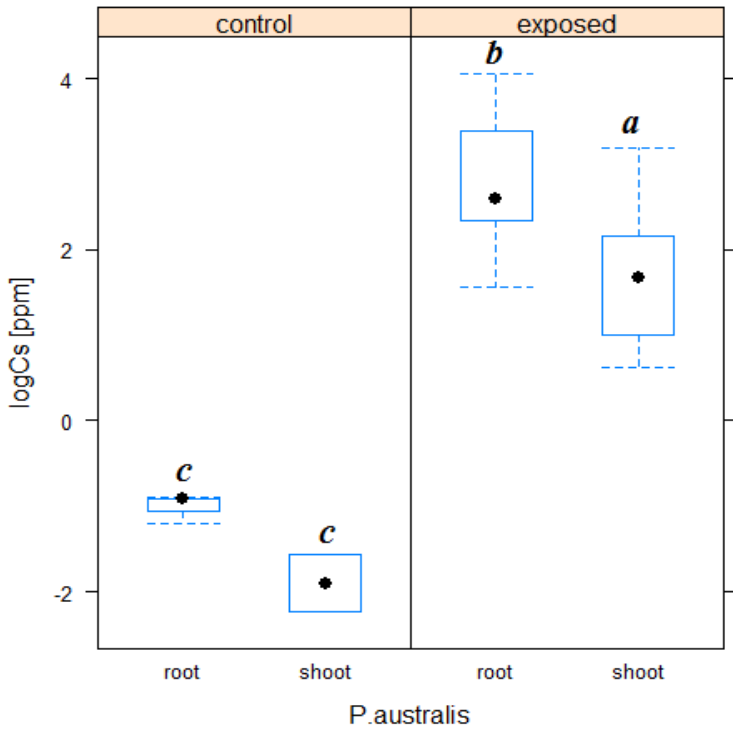


Fig. 10. Concentration ^{133}Cs in different plant parts of *P. australis*. Data were transformed by logarithmization. The letter above boxes shows the statisticaly significant results using post hoc test ($p < 0.05$), central dot shows central value (median), box upper and lower quartiles and whiskers interquartille range. n=10.

4.2.3 *Pistia stratiotes* (water lettuce)

Third plant, *Pistia stratiotes* known for high ability to absorb some of the heavy metals, was exposed to cesium for eight days. From acquired results, *Pistia stratiotes* formed high ability to absorb cesium into root (Fig. 11.) and similarly as previous cases, the concentration was higher in root 388.10± 78.91 $mg.kg^{-1}$ than shoot 34.57± 2.57 $mg.kg^{-1}$ (Table 9). Statistical evaluation confirmed significant difference between root and shoot p-value (6.318e-06).

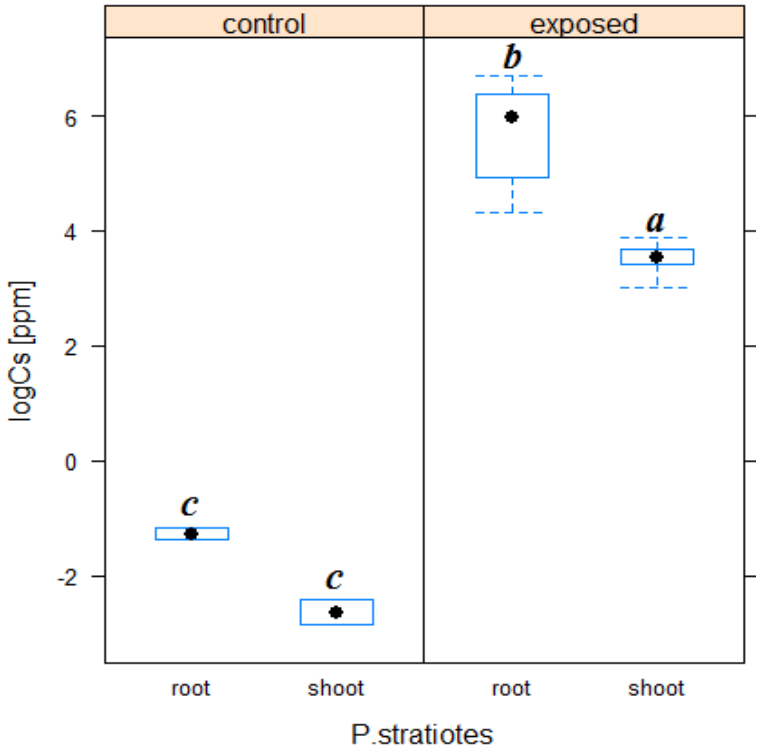


Fig. 11. Concentration of ^{133}Cs in the different parts of *P. stratiotes*. Data were transformed by logarithmization. The letter above boxes shows the statisticaly significant results using post hoc test ($p < 0.05$), central dot shows central value (median), box upper and lower quartiles and whiskers interquartille range. n=11.

4.2.4 *Elodea canadensis* (water plug)

Fourth plant, to investigate ability taking up cesium, was *Elodea canadensis*. In this particular case, the entire plant was measured due to less biomass available. Therefore, statistical evaluation was not neccesary. The entire plant contented 47.36 ± 2.57 mg.kg^{-1}Cs dw (Table 9). The comparison of results among *P. stratiotes* and *E. canadensis* showed much greater ability in cesium uptake in behalf of water lettuce (Fig. 12.), p-value (0.0004, α=0.05).

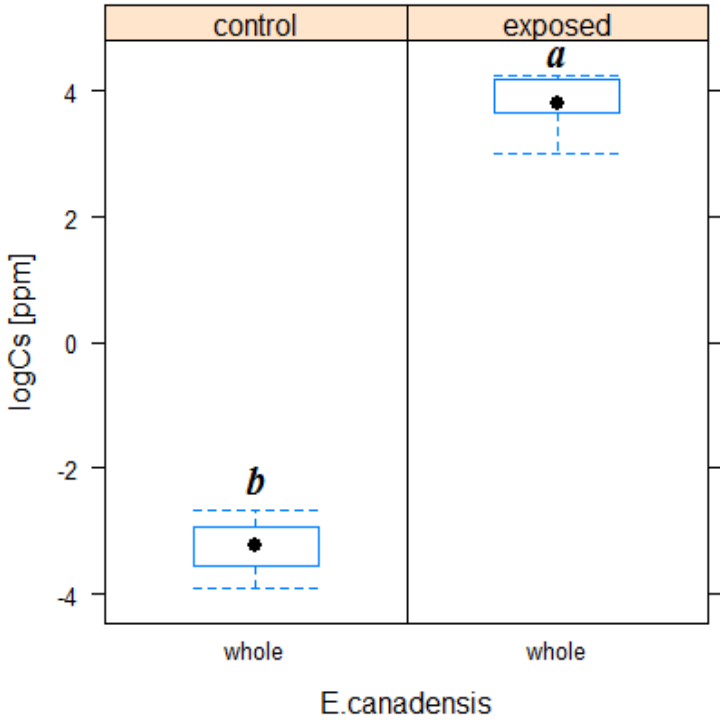


Fig. 12. Concentration of ^{133}Cs in exposed/ controlled plants of *E. canadensis*. Data were transformed by logarithmization. The letter above boxes shows the statisticaly significant results using post hoc test ($p < 0.05$), central dot shows central value (median), box upper and lower quartiles and whiskers interquartille range. n=10.

In summary, the statistical linear model including four plant species classified as most important the factor of plant species p-value=1.719e-13, α=0.05 followed by the factor of plant part p-value=1.035e-08, α=0.05 and their interaction p-value=0.0024, α=0.05, no significance was given to the factor of position in a block p-value=0.5610, α=0.05 and biomass p-value=0.7221, α=0.05 (Fig. 13.).

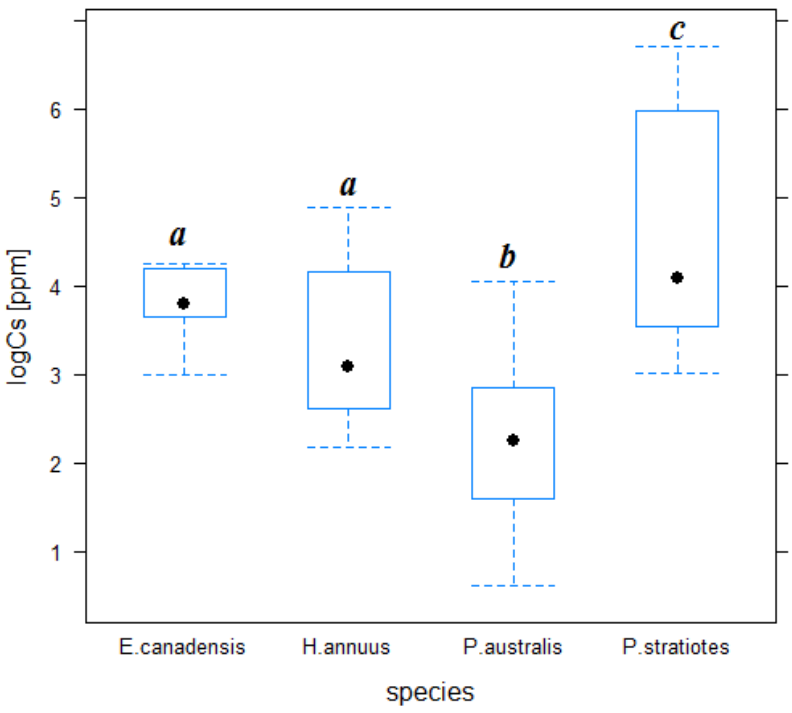


Fig. 13. Concentration of ^{133}Cs in four tested species. The letter above boxes shows the statisticaly significant results using post hoc test ($p < 0.05$), central dot shows central value (median), box upper and lower quartiles and whiskers interquartille range.

Table 5

Mean ± SEM concentration of ^{133}Cs in different plant parts.

Species	Roots [ẋ ± SEM]	Leaves [ẋ ± SEM]
Helianthus annuus L.	40.58 ± 13.35	24.30 ± 5.91
*Phragmites australis**	21.27 ± 5.54	7.69 ± 2.26
Pistia stratiotes	388.10 ± 78.91	34.57 ± 2.57
Elodea canadensis	47.36 ± 5.11**	–

*****Above and below-ground parts of *P. australis* were analysed.
** *E. canadensis* was analysed only as a whole plant.

4.2.5 Morphological Parameters

Along with cesium content in plants, the morphological measurement was taken to verify the correlation of absorbed cesium and biomass. For the phytoremediation purpose, it is necessary to take into account the factor of biomass yield as a criterion for suitable vegetation selection otherwise even hyperaccumulative plants with low biomass yield do not serve the desired purpose of effectively removing the radionuclides from the soil or water. Nevertheless, in the statistical linear model, it is evident that biomass factor did not play any role in the cesium uptake by plants in this experiment p-value=0.7221, α=0.05 (Fig. 14-17.). The details of plant size and weight are given in Tables 4-7 (see Appendix B).

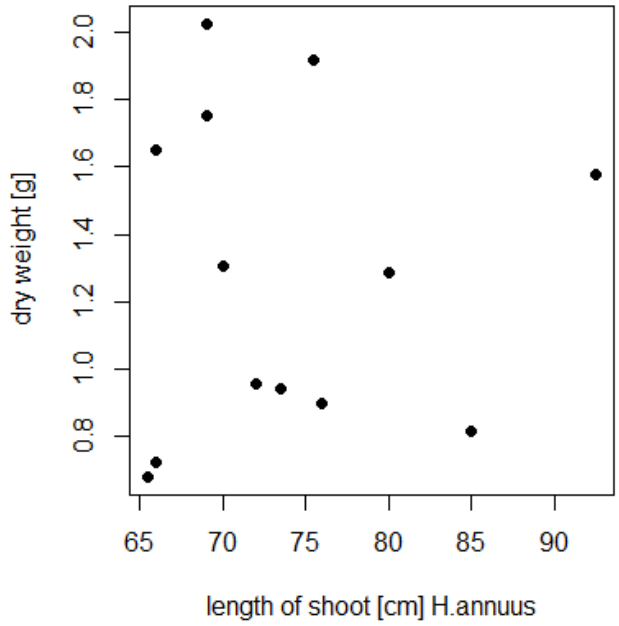


Fig. 14. Frequency of length and dry weight of *H. annuus*. Dot shows the length (cm) with the dry weight of biomass (g).

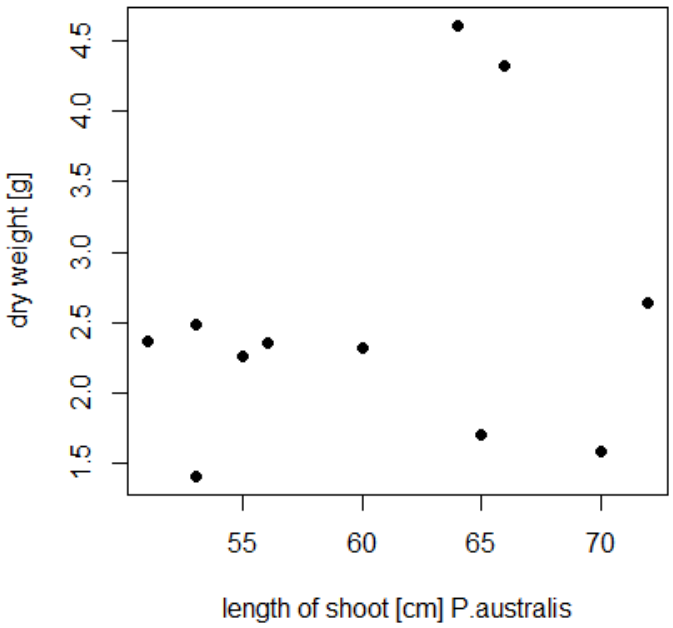


Fig. 15. Frequency of length and dry weight of *P. australis*. Dot shows the length (cm) with the dry weight of biomass (g).

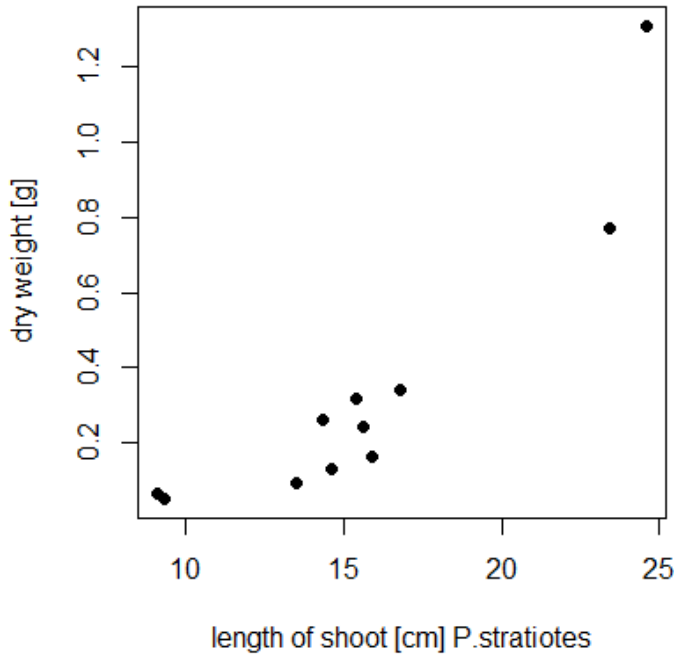


Fig. 16. Frequency of length and dry weight of *P. stratiotes*. Dot shows the length (cm) with the dry weight of biomass (g).

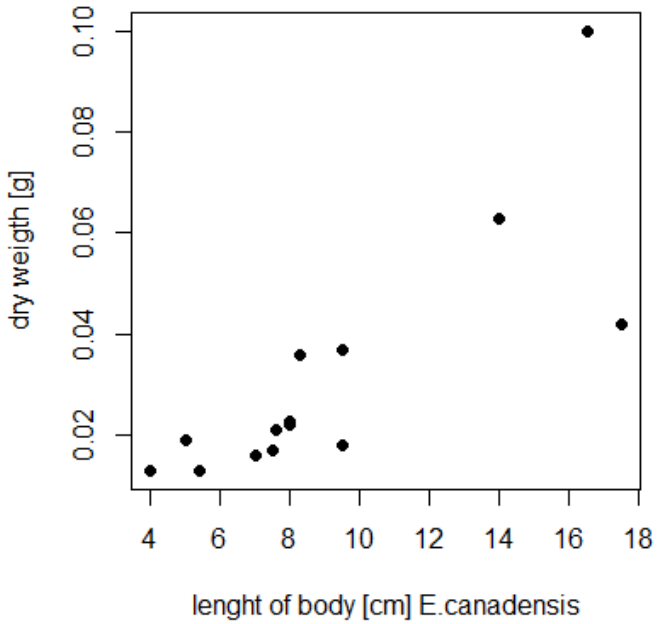


Fig. 17. Frequency of length and dry weight of *E. canadensis*. Dot shows the length (cm) with the dry weight of biomass (g).

4.3 Experiment II - with two plant species grown in peat, clay and chernozem soil

The second experiment was targeted on the cesium uptake by two plant species *Helianthus annuus* and *Phragmites australis* grown on different soil types like peat, chernozem and clay soil. In addition, the cesium concentration contrast between roots and shoots in species was compared to.

4.3.1 *Helianthus annuus* and *Phragmites australis* grown in peat

H. annuus and *P. australis* roots were compared to determine which species has higher ability for cesium uptake from peat soil. This type of soil is characterized by high content of organic matter and low pH, both enhancing metal uptake (Table 9). The results showed that the higher cesium uptake was exhibited by species *H. annuus* rather than *P. australis* (Fig. 18.). Statistical method using Wilcox's test (nonparametric t-test) showed significant difference among plant species p-value=0.0059, α=0.05. Suprisingly, there was no significant difference found in individual root and shoot concentrations (Fig. 18., 19.) $p\text{-value}_{(sunflower)}$= 0.328, α=0.05, $p\text{-value}_{(reed)}$= 0.208, α=0.05 as was found in sand, clay soils and chernozem by *H. annuus* (Fig. 23.-25.).

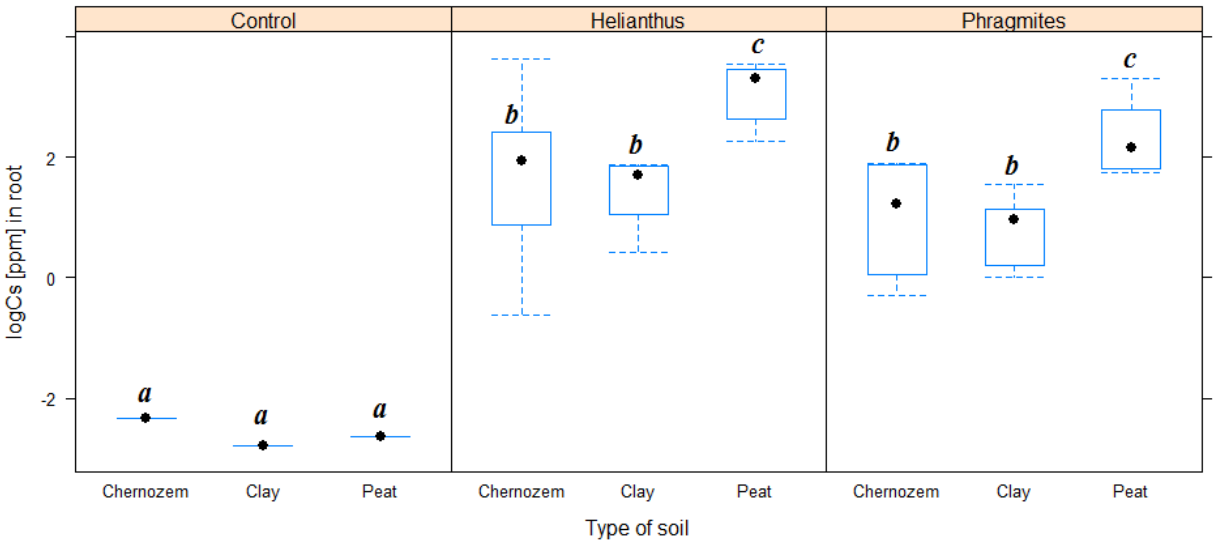


Fig. 18. Differences in ^{133}Cs concentration in *H. annuus* and in *P. australis* root grown on the three distinct types of soil. The letter above boxes shows the statisticaly significant results using post hoc test ($p < 0.05$), central dot shows central value (median), box upper and lower quartiles and whiskers interquartille range.

4.3.2 *Helianthus annuus* and *Phragmites australis* grown in chernozem

Also, in the experiment with chernozem, *H. annuus* and *P. australis* roots were measured for the cesium concentration and determine which species has higher ability to take up cesium. This particular chernozem was well saturated by pottasium which may reduce cesium uptake by plants (Table 6). In the case of chernozem, the results did not indicate significant difference among plant species (Fig. 18.) p-value=0.1471, α=0.05. In additon, the cesium concentration in root and shoot was detected. According to statistical evaluation, there was no important difference in the cesium concentration in root and in shoot in *P. australis* p-value=0.818, α=0.05 (Fig. 25.), although collected data from *H. annuus* signal the meaningful difference in the cesium concetration of root and shoot p-value=0.023, α=0.05 (Fig. 22.).

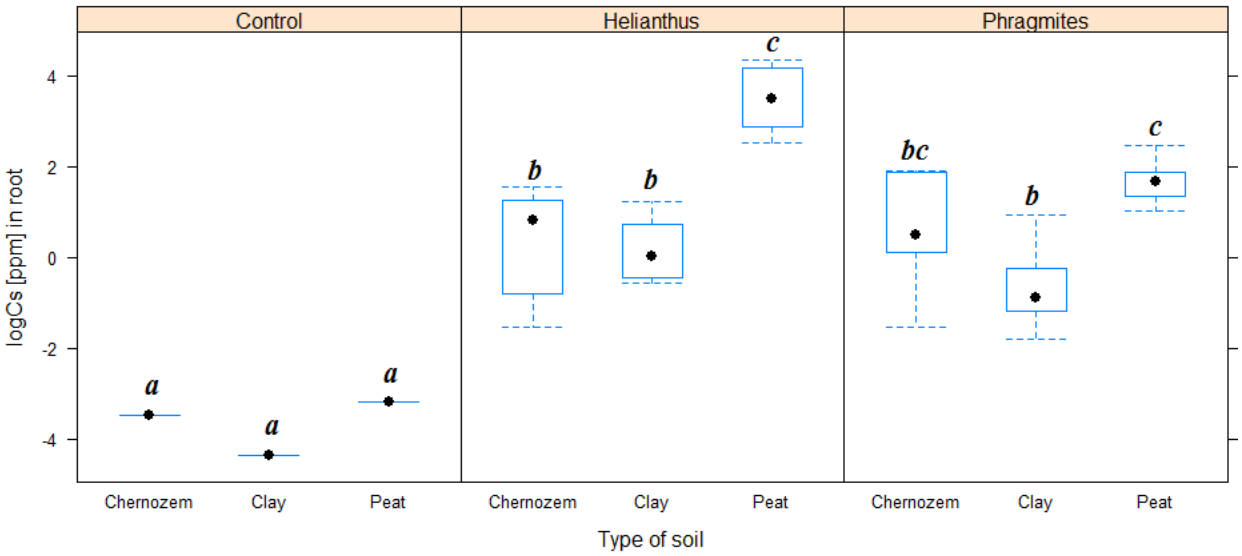


Fig. 19. Differences in ^{133}Cs concentration in *H. annuus and in P. australis the* shoot grown on three distinct types of soil. The values of control plants are below zero due to data transformation. The letter above boxes shows the statisticaly significant results using post hoc test ($p < 0.05$), central dot shows central value (median), box upper and lower quartiles and whiskers interquartille range.

Table 6

Soil pH and other elements in selected types of soil (g. kg^{-1})

Soil type	**pH (KCl)**	**C**	**N**	**K**	**Ca**	**Mg**
Peat	4.4	48.6	5.70	3.17	3.57	5.79
Chernozem	6.9	30.0	1.95	7.92	11.41	6.09
Clay	6.3	15.0	2.70	4.77	2.88	4.66

4.3.3 *Helianthus annuus* and *Phragmites australis* grown in clay soil

Regarding experiment with clay soil as a growth medium, *H. annuus* and *P. australis* roots were examined and compared to find out which species was more successful in the cesium uptake. In clay soil, cesium is usually tightly bound to the clay minerals which prevents its transfer into the plants. Data showed that there was significant difference among plants with *H. annuus* having p-value=0.034, α=0.05 (Fig. 20); moreover, the measured concentration also varies in roots and shoots for both plants (Fig. 23, 26), $p\text{-value}_{(sunflower)}$=0.015 and $p\text{-value}_{(reed)}$=0.011.

In the entire model of soil data (Fig. 27.), the soil factor was more important (p-value=2.26e-05, α=0.05) than factor plant species (p-value=0.00422, α=0.05) that means that the cesium uptake by selected species is highly affected by properties of growth medium followed by plant species and their genetical equipment. Logically, cesium first reacts with medium and afterwards with plant.

4.4 Experiment III - with *Phalaris arundinacea* (reed canary grass), *Eichhornia crassipes* (water hyacinth) and *Brassica napus* for changes in various months in cesium uptake

4.4.1 *Phalaris arundinacea*

The third experiment was conducted to search trend in the cesium uptake by *Phalaris arundinacea* during period April till November with the aim to distinguish variation in the cesium uptake in each month (Fig. 20.). The plant *P. arundinacea* exhibited sufficient ability to absorb cesium (mean 31.46 $mg.kg^{-1}$). The entire statistical model is significantly different p-value= 8.567x10-4, α=0.05.

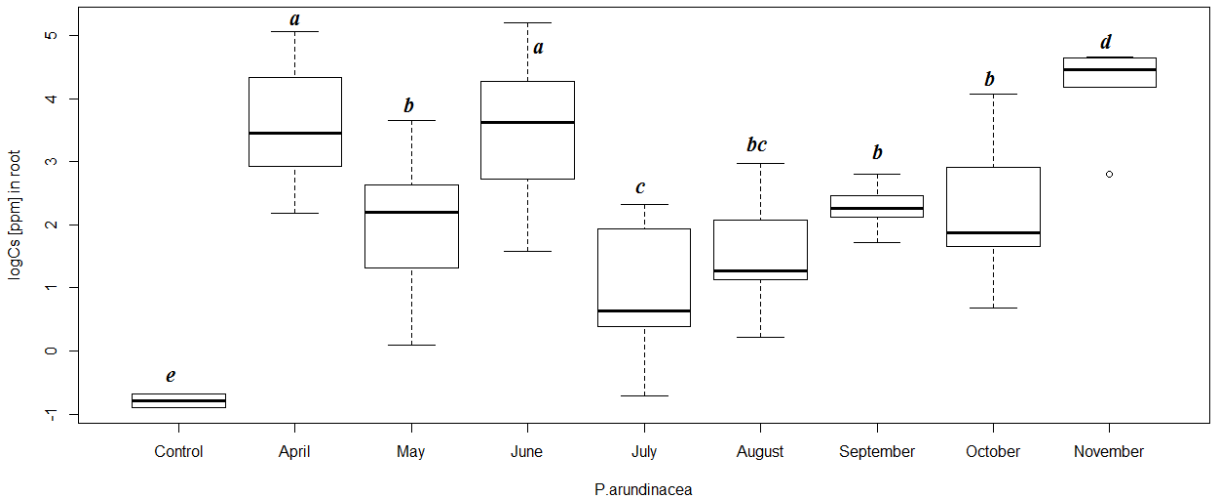


Fig. 20. Monthly changes in ^{133}Cs uptake by *P. arundinucea* in the root. Data were transformed by logarithmization. The letter above boxes shows the statisticaly significant results using post hoc test ($p < 0.05$), central line shows central value (median), box upper and lower quartiles, whiskers interquartille range and solitaire dot outlayer.

Therefore, the changes in cesium uptake by plant exist during a particular period, however; this report cannot be considered as a general statement because in the month of July, plants received extra amount of sun radiation which caused tissue damaged and for obtaining more relevant results,

the experiment requires couple of repetitions. Nevertheless, in the month of May, the cesium uptake by plant showed meaningful decline with compare to months of April and June, similarly August although it started to elevate again in months September, October and especially November.

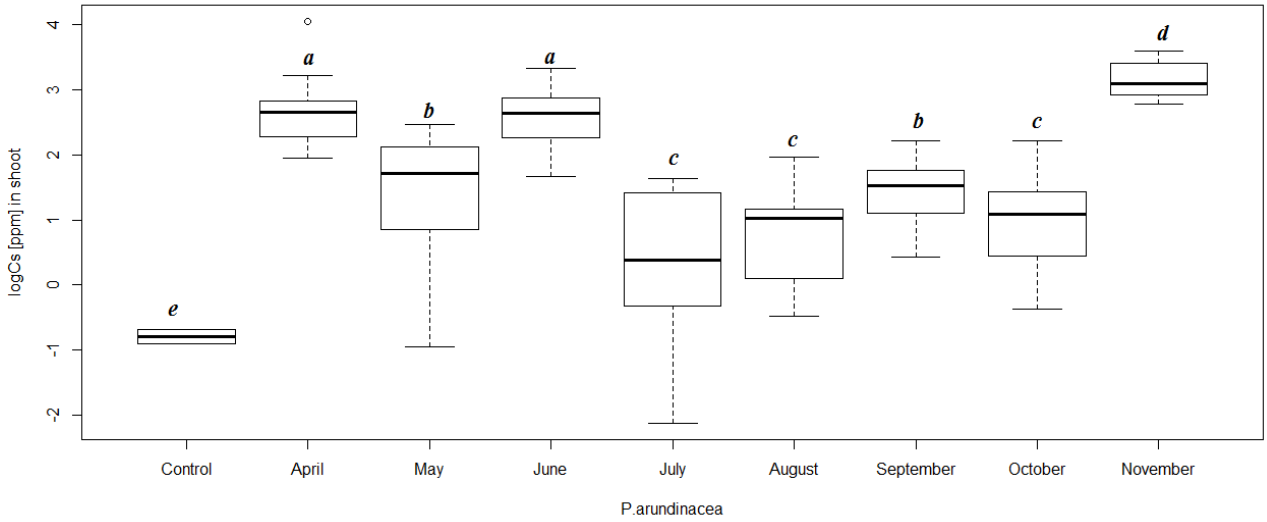


Fig. 21. Monthly changes in ^{133}Cs uptake by *P. arundinacea* in the shoot. Data were transformed by logarithmization. The letter above boxes shows the statisticaly significant results using post hoc test ($p < 0.05$), central line shows central value (median), box upper and lower quartiles, whiskers interquartille range and solitaire dot outlayer.

4.4.2 *Eichhornia crassipes*

The plant *E. crassipes* performed good ability to accumulate cesium (mean 62.92 mg. kg^{-1}). From the obtained results, the exposed plants and controlled plants were significantly different 6.57e-09 for α=0.05. In the months of August and September, there was no statistical significance observed p-value (0.950) for α=0.05. Also, among months of September and October any statistical importance was found p-value (0.119) for α=0.05. However, in the month of November, the elevation of cesium uptake was detected in the plant from the probed months. The month of November was significantly different from August and September p-values (0.4687e-03 and 0.2124e-02, α=0.05) respectively although any disparity from October was recieved p-value (0.323) for α=0.05. The similar pattern was followed by cesium uptake in the shoot of *E. crassipes*. The change was noticed among months of October and November p-value (0.7127e-03) for α=0.05 which indicated significant contrast.

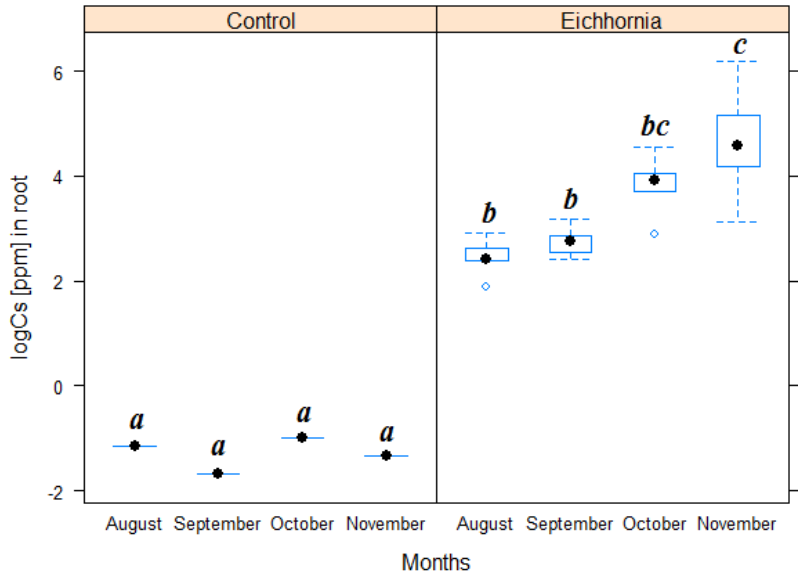


Fig. 22. Monthly changes in ^{133}Cs uptake by *E. crassipes* in the root. Data were transformed by logarithmization. The letter above boxes shows the statisticaly significant results using post hoc test ($p < 0.05$), central line shows central value (median), box upper and lower quartiles, whiskers interquartille range and solitaire dot outlayer.

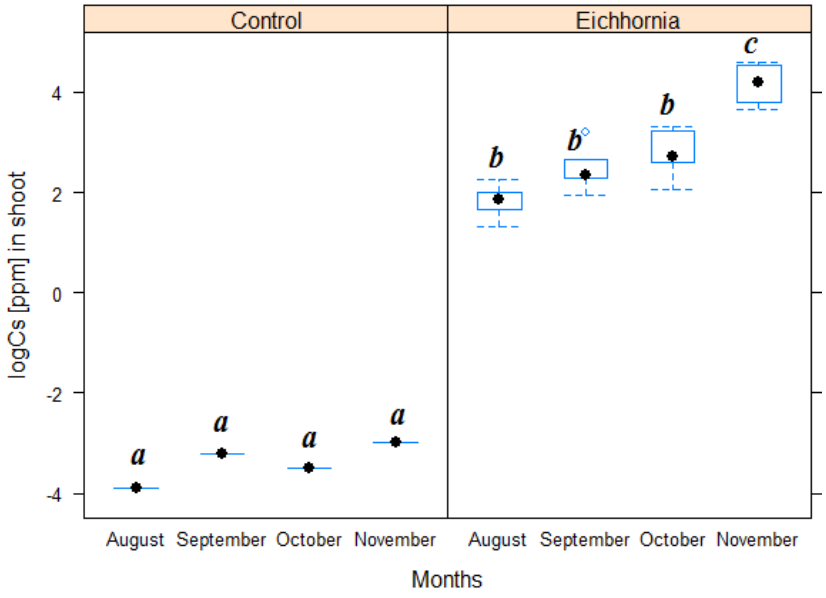


Fig. 23. Monthly changes in ^{133}Cs uptake by *E. crassipes* in the shoot. Data were transformed by logaritmization. The letter above boxes shows the statisticaly significant results using post hoc test ($p < 0.05$), central line shows central value (median), box upper and lower quartiles, whiskers interquartille range and solitaire dot outlayer.

4.4.3 *Brassica napus*

In the case of *B. napus* cesium uptake was only detected significantly contrasting in the month of May from controlled plants due to the low cesium concentration. The plant *B. napus* showed very poor cesium tranfering into root (mean 0.30 $mg.kg^{-1}$). There was no significant contrast observed in cesium uptake by *B. napus* in particular month (Fig. 24). The concentration in leaf was under detected limit, therefore the figure was not shown.

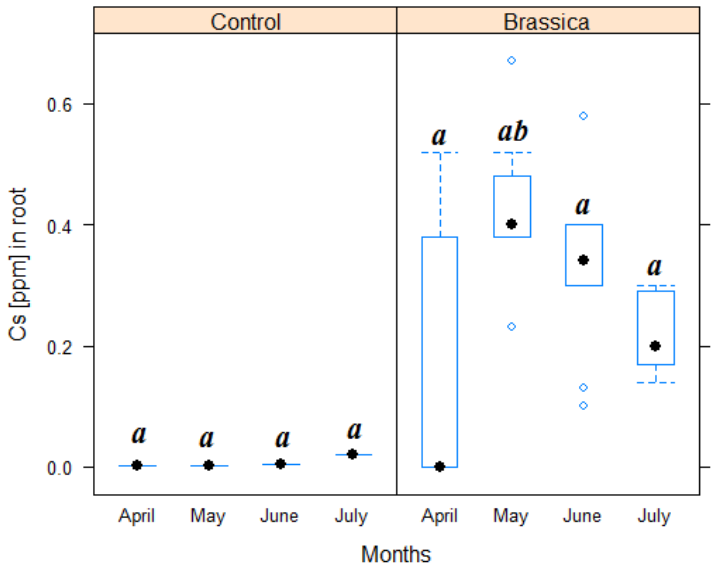


Fig. 24. Monthly changes in ^{133}Cs uptake by *B. napus* in the root. The letter above boxes shows the statisticaly significant results using post hoc test ($p < 0.05$), central line shows central value (median), box upper and lower quartiles, whiskers interquartille range and solitaire dot outlayer.

4.5 Stomatal conductivity

Beside the cesium concentration, stomatal conductivity was measured in few selected plants like *Eichhornia crassipes* and *Phalaris arundinacea* to observe cesium impact on stomata. The reason was that cesium competes with potassium in plant and potassium plays crucial role in opening/closing of stomata. Based on acquired data from this experiment, surprisingly, stomata behaviour seemed to be in correlation with the content of cesium in plant in *P. arundinacea* ($p= 0.0062$) (Fig. 25.) which was completely unexpected and no significant difference among exposed and controlled plant in *E. crassipes* ($p= 0.470$) (Fig. 26.). Following figures (Fig. 27.- 32.) show the stomatal conductivity in particular plant and month.

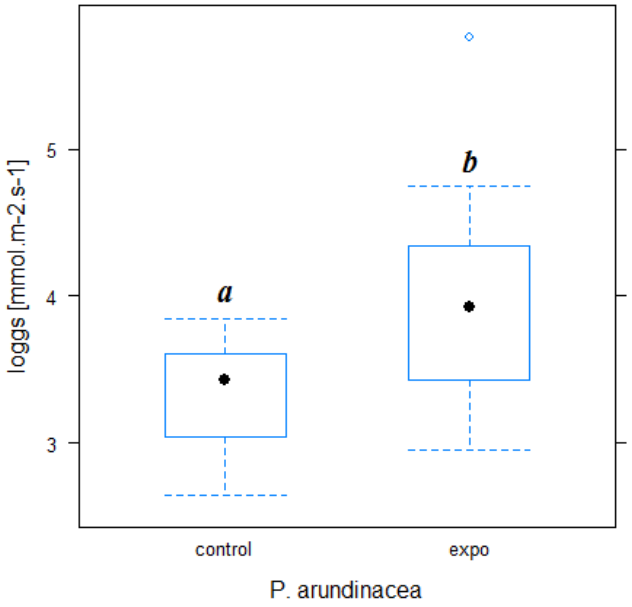


Fig. 25. Total stomatal conductivity measured in controlled / exposed plants of *P. raundinacea*. Data were transformed by logarithmization. The letter above boxes shows the statisticaly significant results using post hoc test ($p < 0.05$), central line shows central value (median), box upper and lower quartiles, whiskers interquartille range and solitaire dot outlayer.

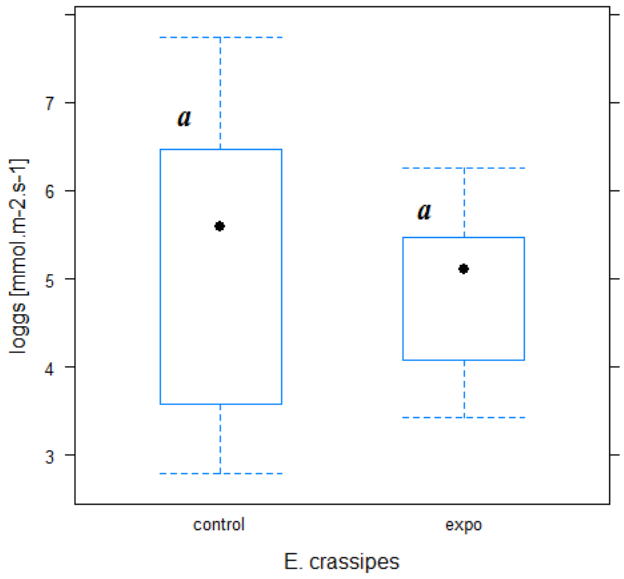


Fig. 26. Total stomatal conductivity measured in controlled / exposed plants of *E. crassipes*. Data were transformed by logarithmization. The letter above boxes shows the statisticaly significant results using post hoc test ($p < 0.05$), central line shows central value (median), box upper and lower quartiles, whiskers interquartille range and solitaire dot outlayer.

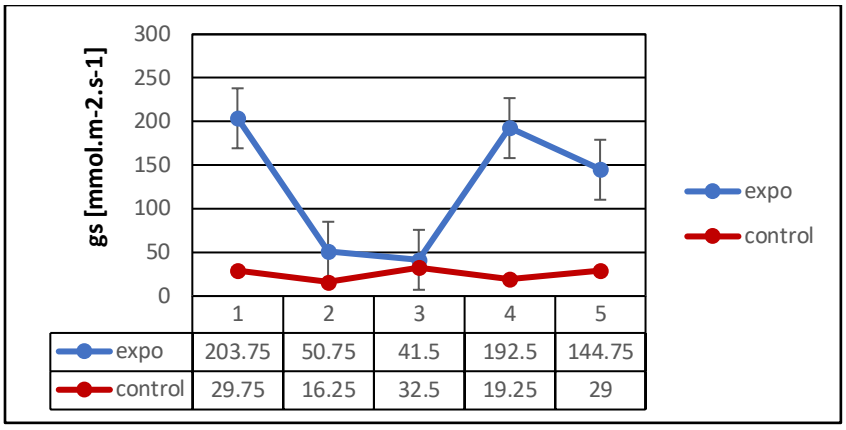


Fig. 27. Different trend in the stomatal conductivity of exposed/ controlled plants *E. crassipes* (August). Bars indicate standard error of mean.

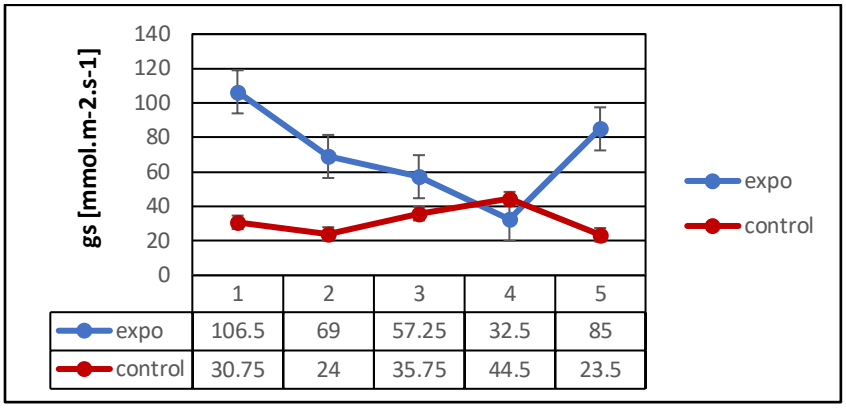


Fig. 28. Different trend in the stomatal conductivity of exposed/ controlled plants *P. arudinacea* (August). Bars indicate standard error of mean.

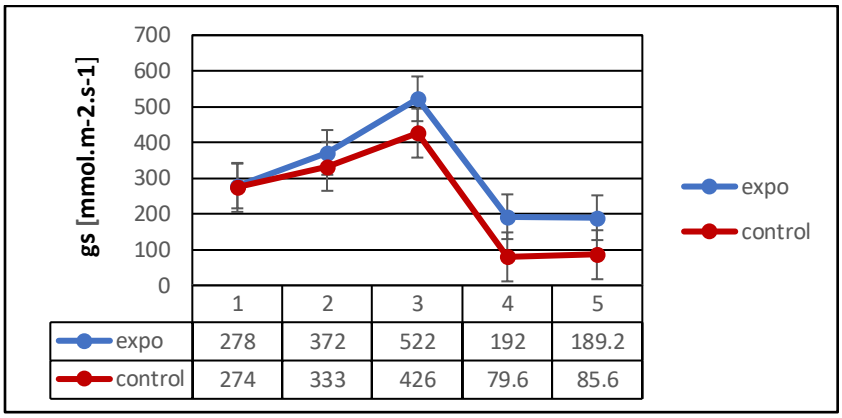
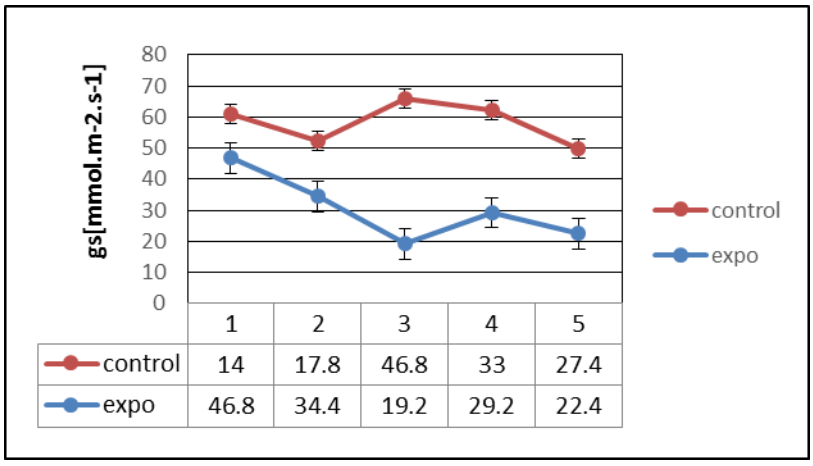
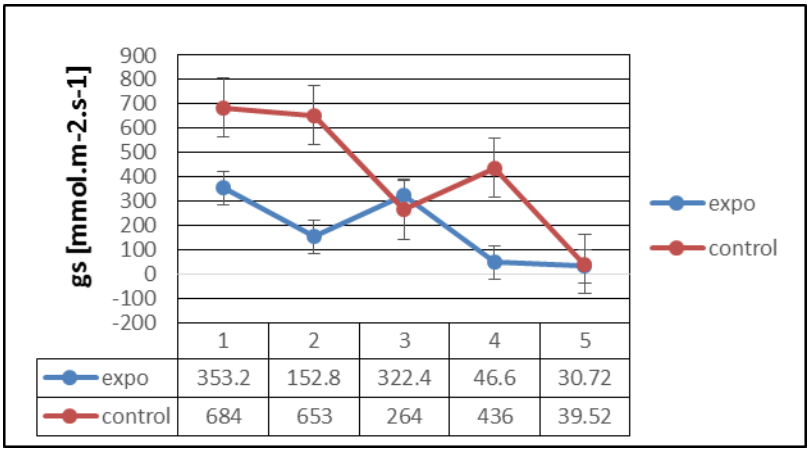


Fig. 29. Similar trend in the stomatal conductivity of exposed/ controlled plants *E. crassipes* (September). Bars indicate standard error of mean.



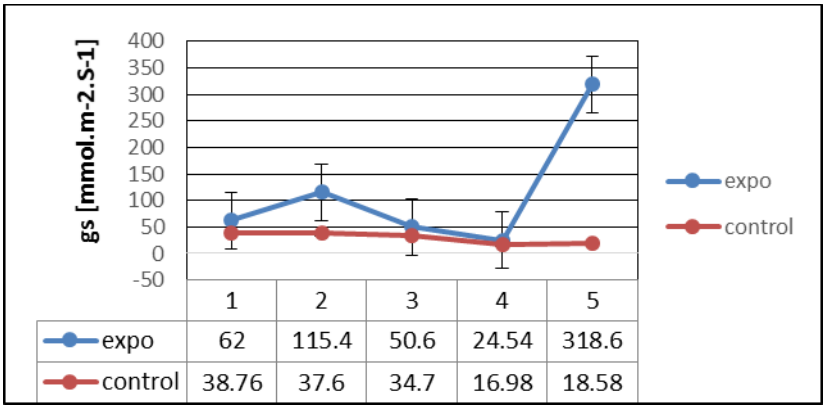
	1	2	3	4	5
control	14	17.8	46.8	33	27.4
expo	46.8	34.4	19.2	29.2	22.4

Fig. 30. Different trend in the stomatal conductivity of exposed/ controlled plants *P. arundinacea* (September). Bars indicate standard error of mean.



	1	2	3	4	5
expo	353.2	152.8	322.4	46.6	30.72
control	684	653	264	436	39.52

Fig. 31. Different trend in the stomatal conductivity of exposed/ controlled plants *E. crassipes* (October). Bars indicate standard error of mean.



	1	2	3	4	5
expo	62	115.4	50.6	24.54	318.6
control	38.76	37.6	34.7	16.98	18.58

Fig. 32. Similar trend in stomatal conductivity of exposed and controlled plants *P. arundinacea* (October). Bars indicate standard error of mean.

4.6 Thorium Plant Uptake

4.7 Experiment IV - thorium impact on tobacco root transcriptome

4.7.1 Transcriptomic response to thorium

Thorium (200 μM) up-regulated 152 and down-regulated 100 genes (p-value < 0.01, fold change ≥ 2) in the tobacco roots after 7-day exposure (see complete list of up- and down-regulated genes in Table 8, Chapter 3). The genes involved in signal transduction represented the relatively most abundant group of up-regulated transcripts (when sorted according to the biological process domain; Fig. 33A). This group comprises genes involved in systemic acquired resistance mediated by salicylic acid (SA) signaling pathway such as two most up-regulated transcripts - *A. thaliana* homologue hemoglobin *AHB1* (*AT2G16060*; also in other cases bellow are always stated *A. thaliana* homologue genes) and alcohol dehydrogenase *ADH1* (*AT1G77120*). Furthermore, other genes involved in SA signaling pathway such as aluminum induced protein with YGL and LRDR motifs *AT4G27450*, sucrose synthase *SUS4* (*AT3G43190*), pyruvate decarboxylase *PDC1* (*AT4G33070*) and LOB domain-containing protein *LBD41* (*AT3G02550*) were up-regulated. Beside SA, also jasmonic acid (JA) mediated signal transduction was initiated by thorium. Genes involved in JA biosynthesis lipoxygenase *LOX5* (*AT3G22400*), two lipase/lipooxygenases *PLAT1* (*AT4G39730*) and *PLAT2* (*AT2G22170*), and cytochrome P450 *CYP94C1* (*AT2G27690*) were up-regulated as well as JA responsive genes methylenetetrahydrofolate reductase family protein *AT5G38710* and allene oxide synthase *AOS* (*AT5G42650*). JA functions in a close cross-talk with another hormone - ethylene. Th up-regulated also ethylene biosynthetic gene ACC oxidase *AtACO2* (*AT1G62380*). These data demonstrate that presence of thorium represented stress for plants, as SA and JA play key roles in the regulation of defense responses to pathogens, wounding and environmental stimuli.

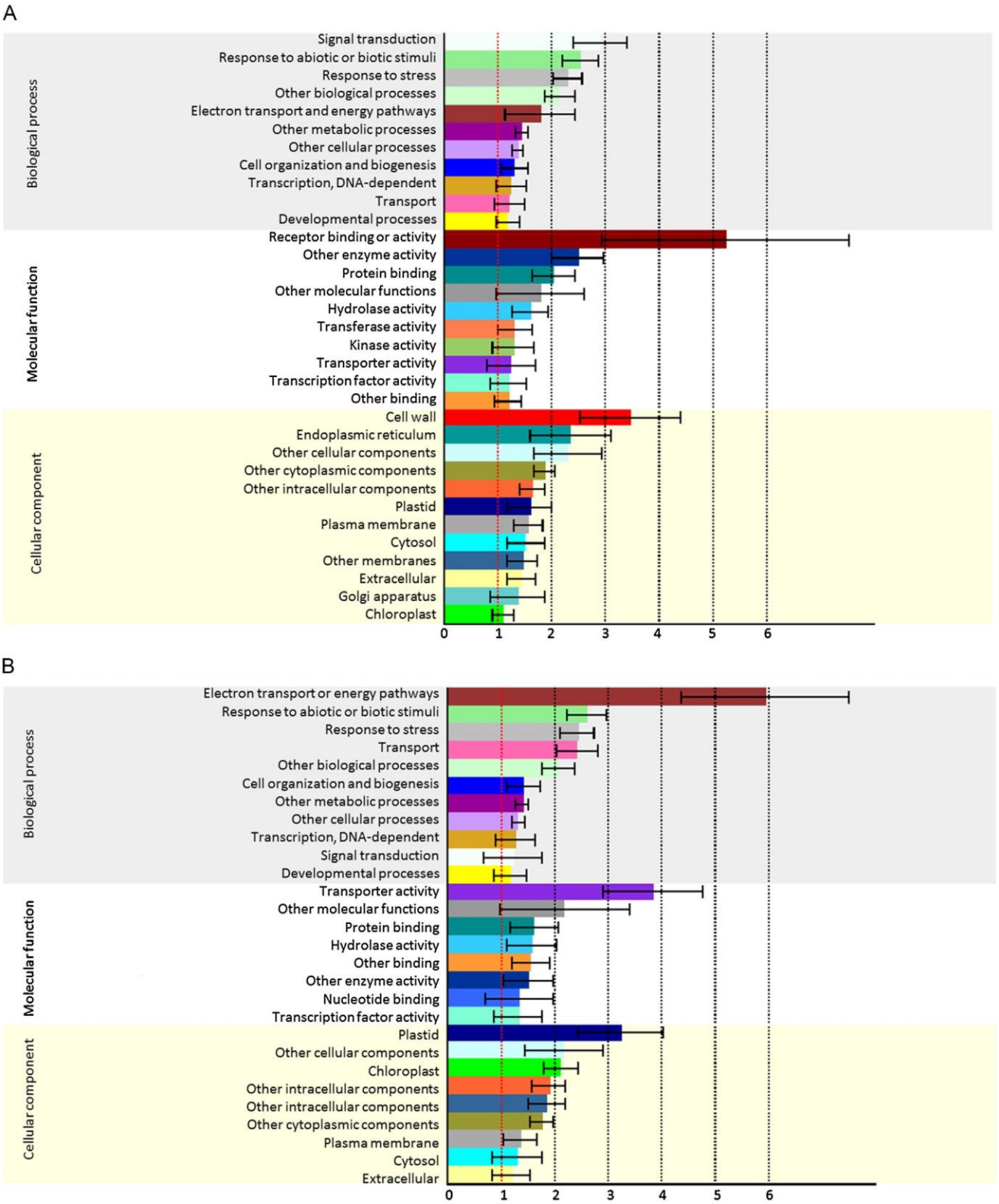


Fig. 33. Proportion of up-regulated genes within the respective categories as calculated with the Classification Super-Viewer (Provart and Zhu, 2003) and expressed as percent (horizontal axis). Only proportions above 1% are shown. Error bars indicate bootstrap standard deviation. (A) genes up-regulated by thorium, (B) genes down-regulated by thorium.

4.7.2 Stress responsive genes induced by thorium

Beside defense and wounding responses also genes up-regulated by salt stress (eukaryotic aspartyl protease family protein *AT1G03220*, alanine:glyoxylate aminotransferase *AT2G13360*, and hydroxy methylglutaryl CoA reductase 1 *HMGR1* [*AT1G76490*]), by oxidative stress (BTB and TAZ domain protein *BT1* [*AT5G63160*], 2-oxoglutarate (2OG) and downy mildew resistant *DMR6* [AT5G24530], plant cysteine oxidase *PCO2* [*AT5G39890*], and dark inducible *DIN10* [*AT5G20250*]), and by cold stress (universal stress protein *AT3G53990*, beta-amylase *BAM3* [*AT4G17090*], and responsive to desiccation *RD22* [*AT5G25610*]) response were stimulated. We recorded up-regulation of several genes associated with response to cadmium (phospholipase D beta *PLDBETA1* [*AT2G42010*], oxidative stress *OXS3* [*AT5G56550*], plant cadmium resistance *PCR2* [*AT1G14870*], and alcohol dehydrogenase *ADH1* [*AT1G77120*]) was observed. The results showed that Th also induced oxidative stress together with response to other abiotic and biotic stimuli in tobacco roots.

4.7.3 Genes potentially involved in thorium detoxification and resistance

No record of elevated expression of genes coding for chelatases or metallothioneins was observed. Surprisingly, heavy metal transport/detoxification superfamily proteins *AT3G24450* and *AT5G03380* as well as natural resistance-associated macrophage protein *NRAMP3* (*AT2G23150*) involved in transport of cadmium ions were down-regulated. Nevertheless, zinc-induced facilitator *ZIF2* (*AT2G48020*) from Major Facilitator Superfamily was up-regulated by Th presence. Further, *PCR2* was another up-regulated zinc transporter. *PCR2* is essential for zinc redistribution and detoxification when zinc is in excess. Additionally, two ABC transporters *ABCA1* (*AT2G41700*) and *ABCG40* (*AT1G15520*) were up-regulated. Taking into account that *ABCG40* is able to detoxify lead, it is possible to assume that this transporter may be involved in thorium detoxification as well. We identified two heat shock proteins *Hsp20* (*AT4G21870*) and *ATHS83* (*AT5G52640*) and heat shock transcription factor *A6B* (*AT3G22830*). Genes potentially included in thorium detoxification process and resistance are showed in Table 7.

Table 7.
Up-regulated genes potentially involved in thorium detoxification and resistance.

Locus (*A. thaliana* homologue)	Annotation	Microarray fold-change log_2
Transport		
AT2G48020	zinc-induced facilitator *2*	3.11
AT1G14870	plant cadmium resistance 2	2.21
AT2G41700	ATP-binding cassette A1	2.07
AT1G15520	ATP-binding cassette G40	1.18
Anti-oxidative protection		
AT5G56550	oxidative stress 3	2.44
Protein reparation		
AT4G21870	HSP20-like chaperones superfamily protein	2.71
AT5G52640	heat shock protein 83	1.89
AT3G22830	heat shock transcription factor A6B	1.84

4.7.4 Thorium effect on uptake of nutrients

Another issue is the interaction of thorium with nutrients. Fe-deficiency induced transcription factor *FIT1* (*AT2G28160)* was down-regulated in plants exposed to thorium. *FIT1* transcription factor regulates expression of *IRT1*, *FRO2*, and *AHA2*, the key genes in iron uptake (García et al., 2010; Colangelo and Guerinot, 2004; Jakoby et al., 2004; Ivanov et al., 2012; Bauer et al., 2007). Changes in expression of these two genes indicate that thorium could suppress iron uptake. It was also observed that changes in transcript abundance of genes occurred in response to phosphorus. Several genes induced by phosphate starvation were up-regulated (glycerophosphodiester phosphodiesterase *GDPD1* [*AT3G02040*], glycerophosphodiester phosphodiesterase *GDPD2* [*AT5G41080*], and responsive to desiccation *RD2* [*AT2G21620*]). Down-regulated were genes (myo-inositol-1-phosphate synthase 3 [*AT5G10170*], granule bound starch synthase *MIPS3* [*AT1G32900*], senescence-associated family protein *AT1G66330*, and B-box domain protein *BBX25* [*AT2G31380*]) involved in phytic acid (myo-inositol hexakisphosphate) biosynthesis. Phytic acid is an important storage form of phosphorus in plants (Alkarawi and Zotz, 2014). These changes in transcription indicate significant decrease of phosphate concentration in the cultivation medium due to reaction of phosphates with thorium or competition of thorium and phosphates in uptake.

4.7.5 Thorium negatively affected energy pathway

Relatively largest group of suppressed transcripts included genes associated with electron transport or energy pathways (when sorted according to the biological process domain, Fig. 33B). The results obtained here are in agreement with other studies focused on the effect of heavy metal on energy pathways (Gill et al., 2015; Yildiz and Terzi, 2016; Dias et al., 2012; Saenen et al., 2014).

4.7.6 Thorium down-regulated transcripts coding for intrinsic proteins

It seems that thorium affects activity of aquaporins similarly as the other mentioned metals. Also, genes involved in response to water deprivation (drought-responsive family protein *AT5G26990*, dehydrin family protein *RAB18* [*AT5G66400*], dehydrin xero 1 *AT3G50980*, proton gradient regulation *PGR5* [*AT2G05620*], beta-amylase *BAM1* [*AT3G23920*], and
ABC-2 type transporter family protein *ABCG22* [*AT5G06530*]) were down-regulated.

4.7.7 Thorium induced transcription o*f* genes involved in callose formation

Genes associated with the cell wall were the most abundant group when sorted according to the cellular component domain (Fig. 33A). Up-regulated genes AGAMOUS-like *AGL15* (*AT5G13790*), ethylene response *ETR1* (*AT1G66340*), and flagellin-sensitive *FLS2* (*AT5G46330*) are involved in defense response by callose deposition in the cell wall.

4.7.8 Validation of the microarray results

The values acquired by microarrays were verified using quantitative real-time PCR. Table 8. shows general agreement of microarray and q-PCR data. Differences in absolute values reflect differences in both methods and their sensitivities (Rajeevan et al., 2001).

Table 8.

Variation of transcription profile by qRT-PCR.

Locus (*A. thaliana* homologue)	Annotation	Microarray fold-change log_2	qRT-PCR fold-change log_2
AT2G16060	hemoglobin 1	3.87	4.01
AT4G39730	Lipase/lipooxygenase, PLAT/LH2 family protein	2.40	3.11
AT3G26300	cytochrome P450, family 71, subfamily B, polypeptide 34	2.34	3.88
AT3G22830	heat shock transcription factor A6B	1.84	2.37
AT4G37330	cytochrome P450, family 81, subfamily D, polypeptide 4	1.71	1.02
AT1G15520	pleiotropic drug resistance 12	1.18	2.25
AT3G53420	plasma membrane intrinsic protein 2A	-4.66	-2.87
AT4G00430	plasma membrane intrinsic protein 1	-2.72	-1.98
AT5G05270	Chalcone-flavanone isomerase family protein	-1.93	-1.87
AT5G10170	myo-inositol-1-phosphate synthase 3	-1.34	-0.19

(conducted by Prerostova, S.)

5. DISCUSSION

In an attempt to observe the ability of higher plants to absorb cesium as a means of phytoremediation, three experiments were conducted on plant species using different growth mediums and seasonality, both known for influencing the process of plant metal uptake. The underlying objective was to find out a suitable bioaccumulator for radiocesium absorption around Temelin nuclear power plant area both during its normal operation and any kind of nuclear accident. Tobacco plants were investigated to understand the mechanism of resistance to thorium exposition and to determine which group of genes are involved in that process. The results obtained from experiments carried out in the present study, evaluate the suitability of the higher plants in reference as remediators of the hazardous contaminants in the environment.

5.1 Experiment I – with Four Plant Species Grown in Inert Medium

In the first experiment, four plant species were examined for their potential usage in the field of phytoremediation of radiocesium, these are: *Helianthus annuus*, *Phragmites australis*, *Pistia stratiotes* and *Elodea canadensis*. Field studies have demonstrated *Helianthus annuus* as a potential bioaccumulator of various radionuclides and heavy metals, like in Fukushima prefecture (Japan) for absorbing radiocesium from the soil (Suzuki et al., 2012) and former uranium site in Thuringia (Germany) for absorbing uranium from water (Kötschau et al., 2013). Based on the data acquired from the present study, *H. annuus* exhibited higher cesium uptake than *P. australis*. Similar results were obtained by Soudek et al., (2004) when they treated *H. annuus*, *P. australis* and *Populus simonii* cultivated in hydroponic medium with radiocesium; with *H. annuus* recording higher uptake than *P. australis* and *P. simonii*. In our case, *H. annuus* showed higher cesium uptake into the roots than shoots, a phenomenon reported by other studies as well (Eapen et al. 2006, Gyuricza et al. 2010, Moogouei et al. 2011, Soudek et al. 2011 and 2013, Karunakara et al. 2013). For instance, Karunakara et al. (2013) analyzed plant *Oryza sativa* in the field conditions and found that ^{137}Cs remained mainly in the root although ^{40}K was present in aboveground part of the plant. That supports the observations made in this study as well. Also, Win et al. (2015) studied radiocesium distribution and accumulation in *Vigna mungo* plant and discovered that the radionuclide was driven by plant growth, water movement and selective absorption; additionally,

potassium moved in easier way than radiocesium in salt-tolerant plants as mentioned previously by Karunakara et al. (2013). Their work (op cit) suggests that even though potassium and cesium are chemical analogues the plant operates with distinguished mechanism towards cesium or the barrier exists within the plant for cesium. There are Casparian bands as mentioned in the Introduction chapter of this dissertation, nevertheless, they should work for all cations equally.

Cesium is mobile within the plant (Zhu and Smolders, 2000) against the lead which is immobile (Kumar et al., 1995) though there is blockage which prevents cesium to transfer further in aerial part in larger amount. Competitive relationship among cesium and potassium may be excluded here up to a point from the reason that potassium would be affected as well as cesium. Hampton et al. (2004) explored potassium/cesium ratio in *Arabidopsis thaliana* plant and acknowledged that potassium and cesium being chemical analogues their behavior in plants is different. The explanation may lay in potassium channels. As documented by Zhu and Smolders (2000) the radiocesium transport may happen via K+ Transporters at low external potassium concentration with little discrimination against cesium while the K+ Channel is dominant at high external potassium concentration but with high discrimination against cesium. Further investigations are needed might be in to focus at reducing cesium discrimination in K+ Channels to allow optimum cesium uptake in the plants.

However, there are cases where contrasting results have been reported (e.g. Kang et al., 2012, Soudek et al., 2006 and Song et al., 2012). Soudek et al. (2010) add that for using phytoextraction potential it is important to characterize three parameters: a) plant species, b) biomass yield and c) amount of contaminate. Morevover, the hyperaccumalative ability was scrutinized in *Calluna vulgaris* as the radiocesium concentration was higher in shoot than root (Bystrzejewska-Piotrowska et al., 2005). Therefore, some promising natural hyperaccumulative plants do not succeed in phytoremedy usage due to a low biomass productivity for instance, *Thlaspi caerulescens* and as mentioned above *Calluna vulgaris*. Another ornamental plant *Callendula oficinalis* was indicated as hyperaccumulator for copper even so the content of chlorophyll and carotenoid pigment suffered from remarkable decay caused by metal stress (Goswami and Das 2016). These results are in contrast with the results of this work because none of the tested plants absorbed more into the shoot than root. The current investigation tends to combine hyperaccumulative threat character with a high production of biomass by genetic engineering.

In such approach, already some plants were advanced to absorb an extra amount of As, Cd, Cu, Pb and Se (Eapen and D'Souza, 2005).

Thus, *P. australis* was tested for cesium absorption but did not performed great ability for such purpose even though it produces large amount of biomass. The transfer ratio root/shoot was also on low level.

Aquatic non-native plant *P. stratiotes* demonstrated reasonably good ability in cesium uptake from water. The species also showed much higher phytoextraction potential than *E. canadensis*. Odjegba and Fasidi (2004) suggested *P. stratiotes* as a suitable eliminator of Zn, Cr, Cu, Cd, Pb, Ag and Hg and added that this plant can possibly be utilized in water environment with low metal concentration. After harvesting contaminated plant may be burnt to ash and packed for safe disposal. Or, the extracted metals could be reused in industry. Like data of this study, Odjegba and Fisidi (2004) also measured higher concentration in roots than shoots in regard to metal testing in *P. stratiotes.* They also found that *P. stratiotes* can be used as a phytoextractor for concentration of Pb. Interestingly, they detected almost twice higher concentrations of Cd, Cu and Zn metal in *P. stratiotes* than Pb. This implies that *P. stratiotes* effectively absorbs heavy metals like Cd, Cu and Zn than other plants and therefore merits as a phytoextractor of high potential. Another study conducted by Vesely et al., (2011) examined heavy metal (Cd and Pb) uptake by *P. stratiotes* and indicated that this plant species has great extraction prospect for Cd. They also observed higher metal concentration in roots than shoots. Similarly, Rezania et al. (2016) evaluated four aquatic species as effective radiocesium removers like *Eichhornia crassipes*, *Pistia stratiotes*, *Lemna minor* and *Salvinia spp*. Their findings are in accordance with results of this work, except *Lemna minor* and *Salvinia spp*. which were not tested in the present experiment. Based on the present investigation with substantiation from other cited works it can be inferred that *P. stratiotes* holds a high potential as phytoextractor of metals and can advantageously be utilized in contaminated water bodies and might be recommended for further investigation for phytoextraction of radionuclides.

In the case of *E. canadensis*, the plant did not display results like *P. stratiotes.* Further, the plant did not develop rich root system during its growth period, obviously rendering it disadvantageous in the field of phytoremediation. Pinder III et al., (2006) reported brisk uptake of radiocesium from water by *E. canadensis* at least in the initial period of time. In the present study, however, the plant did not show any great ability with regard to the metal uptake

Thiébaut et al., (2010) noticed relatively high cadmium concentration in *E. canadensis* grown in a French lake which contrasts with our data. Barring any isolated cases the plant *E. canadensis* does not appear to be a promising phytoextractor of metals, however, further research is needed to raise the conclusive inference.

5.2 Experiment II - with two plant species grown in peat, clay and chernozem soils

The second experiment continued with *H. annuus* and *P. australis* cultivated in three different types of soil to elucidate the role of growth medium and plant species in the cesium uptake of plants. Seedlings of these species raised as plants in three sets of pots containing different soils like peat, chernozem and clay were measured for cesium uptake after exposure time of 20 days. Cesium measurement included all parts of the plant like root, shoot and leaf to understand the absorption distribution of this metal into the plant system. In this case, terrestrial species *H. annuus* recorded higher cesium uptake than wetland species *P. australis* that is in consonance with the results of the first experiment. This finding is also in conformity with the work of Soudek et al., (2004) who report highest radiocesium uptake in growth medium with *H. annuus* (sunflower); *P. australis* (reed) also performed reasonably well in the removal of radiocesium using the growth medium. In contrast, however, Soudek et al., (2006) noted a different behavior where localized highest radiocesium activity was observed in the leaf veins and in the stem of *H. annuus* which made them to conclude that radiocesium was speedily transferred from root to shoot, while in the present experiment the cesium uptake occurred mainly in the root and the leaf. The possible reason could be the characteristic presence of K^+ and NH_4^+ serving as nutrition in the growth medium aiding in rich uptake of cesium in *H. annuus*.

Results obtained from chernozem showed no statistical significance in *H. annuus* and *P. australis* either in their root or shoot which may be due to the presence of saturated potassium and higher pH compared to the peat soil. These factors may cause delay as also inhibit cesium movement from soil to plant (Zhu and Smolders 2000; Hampton et al., 2004; Soudek et al., 2008; Ashraf et al., 2014). From the field study conducted by Kubo et al. (2015) sufficient supplement of potassium inflicted lower radiocesium concentration in *Fagopyrum esculentum* in Fukushima Prefecture. According to Staunton et al., (2002) the most influencing cations to alter cesium uptake from the soil are potassium and ammonium.

H. annuus and *P. australis* cultivated in clay soil showed significant difference in cesium uptake, being more in the case of *H. annuus.* That could be explained by profound production of root exudates which made cesium more accessible to *H. annuus* or *P. australis* unloading cesium back to soil in larger amount than *H. annuus*. Also, Prochazka et al., (2003) and Soudek et al., (2008) contend that plant can discharge organic compounds from root to enhance solubility of a pollutant consequently making it more approachable to the plant for absorption. This can go even further as some authors claim that plant can release xenobiotics back to soil through the root exudates (Zehnder et al., 1995; Zehnálek et al., 2005). For phytoremediation, mature plants are more suitable because young plants only recycle cesium from roots back to the soil (Staunton et al., 2002). The root exudates can play important role in cesium as well as metal/radionuclide uptake. Further investigation needs to be done to substantiate this inference. It is well known that cesium gets bound to clay minerals especially illite and vermiculite as referred to in review article by Staunton et al., (2002). It implies that for this reason the cesium uptake by plants from clay soil was relatively low compared to the peat soil in this experiment. As it was indicated in the previous chapter (Results), the characteristics and the content of other elements in the growth medium is vital in the radionuclides uptake by plants. The selection of suitable plant species for phytoremediation purposes is too germane but in lesser extent and even in the same plant species can be genetic variations. Therefore, it is advisable to scrutinize the genetic treats of plant species prior to their use (Hampton et al., 2004).

5.3 Experiment III – with *Phalaris arundinacea*, *Eichhornia crassipes* and *Brassica napus* for Monthly Changes in Cesium Uptake

The third experiment focused on the seasonal changes in the cesium uptake by *Phalaris arundinacea*. It is because change in season affects the vegetation in terms of plant growth, photosynthesis ratio, and higher accumulation of essential elements, their usage and storage. As for illustration, Thiébaut et al. (2010) in their study on *E. canadensis* did not find any regular pattern in the behavior of cesium uptake. Most likely, this kind of experiment requires more annual repetitions than only one season data collection. In the present study, data from July are irrelevant due to overdose by sun radiation. Decline in the cesium uptake by *P. arundinacea* can be noticed in the months of May and August.

According to Weis and Weis (2004) the factor of seasonal change is far from consistency; hence it is not easy to rely solely on this factor in which many variables outer and inner influence the uptake process.

As Brezinova and Vymazal (2015) revealed that it is hard to determine the peak of seasonal heavy metals uptake by *P. arundinacea* although they agree it could be the early growing season for potent abating heavy metals from constructed wetlands. In the same way, the present results showed higher cesium uptake in April month as well as June and then again reaching winter time. That may indicate that plants of the local origin possibly accumulate heavy metals or radionuclides the most during early growing season. Vymazal and Brezinova (2015) discovered that the seasonal distribution of heavy metals in biomass differs in heavy metals themselves and does not copy the model for nutrients. They also added that it was necessary to consider the factor of biomass into the investigation of metal accumulation. In the case of *Eichhornia crassipes*, the cesium uptake was measured exclusively in the months of August, September, October and November with soaring trend towards the winter season. Saleh (2012) and Rezania et al. (2016) consider *E. crassipes* as efficient eliminator of radiocesium thought without the consideration of the seasonal development. In the study of Zalewska (2012a) on the seasonal radiocesium uptake by *Zostera marina* and it was observed that the concentration hiked from spring to autumn. That is in contrast with the results obtained in the present study, where *E. crassipes* started to elevate the cesium concentration in late summer (September) although the Czech Republic is not a natural habitat for *E. crassipes*. Another study of Zalewska (2012b) examined the variability in season depended on plant biomass and the effect of dilatation in radiocesium bioaccumulation by *Polysiphonia fucoides* and *Cladophora glomerata* in the water column as these plants belong to the lower group of the plants. The present results were independent of the biomass factor. *Brassica napus* was observed for cesium uptake in the moths of April till July. Plant *B. napus* performed the highest cesium absorption in May and lowest in other inspected months. The cesium concentration in the root of *B. napus* was low similar or little higher than the concentration in controlled plants. The cesium concentration in shoot was very low, in fact, hard to detect. In a like manner, Djedidi et al. (2016) measured radiocesium content in the root and the shoot of *Brassica rapa* which performed the limited radionuclide quantity especially in the shoot; consequently, they suggested *B. rapa* as radiocesium plant preventer from accessing the food chain.

Similarly, based on the obtained results, *B. napus* did not tend to absorb the cesium contamination especially not into the aboveground part.

Some of the tested plants exhibited good phytoremedion potential. The greatest ability among all selected species was observed in *P. stratiotes* to uptake cesium. Therefore, it can be recommended to detoxify contaminated water bodies as it has abating abilities for various heavy metals. Plant *E. crassipes* also showed promising results in reducing cesium and similarly it can be suggested for cleaning water bodies, although none of these plants survive winter season in the Czech Republic, thus, the annual harvest is necessary. Another plant, which may be used for abating radiocesium in both ecosystems (terrestrial and aquatic), is *H. annuus*, and in wetland ecosystem, *P. arundinacea* could be eventually employed until more efficient wetland species is verified.

With regards to agricultural fields, it is highly endorsed to maintain higher potassium levels in order to prevent radiocesium transfer from the soil to crops in case a nuclear accident occurs. As *B. napus* was analyzed for cesium uptake, it could be set for fields with the nuclide contamination due to low or zero cesium transfer. To protect water bodies, it is essential to localize organic matter where radiocesium tends to bind and efficient plants can be utilized to reduce the nuclide. In coniferous forest, radiocesium may easily migrate given the fact of lower pH in environment. Organisms growing in forests which are willing to accumulate radiocesium in great amounts are; ectomyccorhizal fungi, linchen and moss. In an event where contamination takes place, the surrounding population needs to be informed about it to avoid exposure (internal and external). Leafed trees can serve as bioindicators to measure toxins in rural as well as urban regions

5.4 Stomatal Conductivity

One of the physiological plant parameters, stomatal conductivity, was carried out on some plants like *Phalaris arundinacea* and *Eichhornia crassipes* to verify the cesium impact on stomata mechanism. Stomata work in plant as a regulator of entering CO_2 molecules and releasing H_2O molecules. In the time of sufficient water level, plant can afford to lose extra H_2O molecules by fully opening stomata, thus more molecules of CO_2 can be absorbed for photosynthesis which consequently leads to increase biomass yield. As, Wu et al. (2009) reported that intensified CO_2 led to higher biomass yield and also the raised concentration of cesium in *Sorghum vulgare* and *Trifolium pretense*.

In the present study plants were not exposed to elevated CO_2, but probably there would not be much change given the fact that the biomass was not the determinant factor. Likewise, Song et al. (2012) investigated the increased level of CO_2 influencing cesium uptake, microbial community and biomass yield in *Amaranthus cruentus* and *Phytolacca americana* and suggested CO_2 as fertilizer for soils contaminated by radionuclides due to enhancing effect in all the three investigated aspects.

Stomatal conductivity is not influenced solely by saturation of water but also by sensitivity towards blue light especially by the presence of ion potassium, the leader of H_2O molecules in plant. On the other hand, in the time of water shortage, Abscisic acid induces outflow of potassium ion from guard cells causing reduction in stomatal conductivity. As reported by Bystrzejewska-Piotrowska and Urban (2003) transpiration rate and stomatal conductivity were heavily repressed by the higher cesium concentration (3 mM) and water uptake was distracted in *Lepidium sativum*. In contrast, the present study noted elevation in stomatal conductivity of the exposed plants compared to the controlled plants in the plant *P. arundinacea*, no difference in *E. crassipes* This deduction is in the agreement with the study of Bystrejewska-Piotrowska et al. (2004) suggesting higher concentration of CsCl caused decline in photosynthesis but enhanced stomatal conductivity in *Codiaeum variegatum*. More experimental data with respect to cesium impact on plant physiology would help in better understanding of this phenomenon.

In summary, the present work suggests that plant species and P. *stratiotes, E. crassipes and H. annuus* hold high potential as phytoextractor of metals and can advantageously be utilized in contaminated areas including water bodies. Furthermore, plant uptake is immensely influenced by the type of the growth medium, being highest in the peat soil from the examined types of soil following by plant speices.

5.5 Experiment IV - Thorium Impact on Tobacco Root Transcriptome

After exposing *Nicotina tabacum* L. to $Th(NO_3)_4$ for seven days several changes were recorded in regard to the plant behavior. The first change observed was that thorium (200 µM) up-regulated 152 and down-regulated 100 genes (p-value < 0.01, fold change ≥ 2) in the tobacco plant roots. The overall impact on gene characteristics suggests that SA and JA immensely help regulate the defense response of abating stress in plants due to thorium contamination. The results also demonstrate that thorium contamination induced oxidative stress in the plant roots.

This has been observed in a variety of cases and with different metal intakes by plants. A study by Mizukami-Murata et al. (2006) reported thorium induced transcription of oxidative stress responsive genes even in the yeast. Oxidative stress was also caused by uranium, as demonstrated by increased transcription of reactive oxygen species-producing enzyme *LOX1* and guaiacol peroxidase in *A. thaliana* roots after 7-day exposure to 100 µM concentration (Vanhoudt et al., 2011). Stress of various sorts can be different with different metals like H_2O_2 level and catalase expression were increased in *Vicia faba* roots cultivated eight days in soil containing radionuclides ^{232}Th, ^{226}Ra and ^{40}K (Haghighat et al., 2014). Horemans et al. (2015) observed that uranium caused oxidative stress different from cadmium. In another illustration, it is reported that Generally, heavy metals induce generation of reactive oxygen species as primary reaction in plants (Yadav et al., 2010) and although there could be differences among various metals, lipid peroxidation and induction of oxidative stress were observed in the plants exposed to lead (Hattab et al., 2016), copper (Goswami and Das., 2016), mercury (Malar et al., 2015), and arsenic (Campos et al., 2015). Plants possess several detoxification mechanisms to cope with heavy metals such as chelation, exudation, binding to metallothioneins and compartmentation to vacuole (Hall, 2002). In the present work, it was noticed that the genes involved in the component of cell wall were enhanced especially those associated with receptor of binding or activity. Therefore, it was assumed that thorium was most likely attached to the cell wall as resistance response. The corresponding findings of Kowata et al. (2014) who intended to probe the exact place in plant where cesium is stocked revealed that cesium was mainly stored in cell wall and apoplastic sector which may be quite interesting because cesium enters plant via symplastic way.

While no elevated expression of genes coding for chelatases was observed, transport/detoxification by superfamily proteins *AT3G24450* and *AT5G03380* and natural resistance-associated macrophage protein *NRAMP3* (*AT2G23150*) in heavy metal ions like cadmium were down-regulated, whereas zinc-induced facilitator *ZIF2* (*AT2G48020*) was up-regulated by Th presence (Mazari, et al., 2017). As is known that *ABCG40* transporter is able to detoxify lead, it is possible that this can help in thorium detoxification as well. *ZIF2* plays an important role in zinc tolerance via root vacuolar sequestration avoiding the accumulation of zinc in shoots (Remy et al., 2014). Heat shock proteins play a crucial role in protein protection (like chaperons) facilitating their recovery during heavy metal stress (Hall, 2002).

ABCG40-overexpressing *Arabidopsis* plants were found to be more resistant to lead and had lower lead content than wild type plants when grown on medium containing lead. Mazari et al. (2017) suggest that *ABCG40* works as a pump to exclude lead ions and/or lead-containing compounds from the cytoplasm (Lee et al., 2005).

One of the interesting aspects is the thorium-nutrient interaction. The study has observed that Fe-deficiency induced transcription factor *FIT1* (*AT2G28160)* was down-regulated in plants exposed to thorium. *FIT1* transcription factor regulates expression of *IRT1*, *FRO2*, and *AHA2*, the key genes in iron uptake (García et al., 2010; Colangelo and Guerinot, 2004; Jakoby et al., 2004; Ivanov et al., 2012; Bauer et al., 2007). Changes in expression of these two genes indicate that thorium could suppress iron uptake. Similar effect was observed in the case of *A. thaliana* exposure to uranium where *FIT1* and other genes involved in iron uptake were down-regulated (Doustaly et al., 2014). Also cadmium down-regulated *FIT1* expression and decreased accumulation of iron in *A. thaliana* roots (Besson-Bard et al., 2009). Phosphates decrease thorium uptake probably via formation of insoluble phosphate salts (Soudek et al., 2013; Wang et al., 2015). Phytic acid is an important storage form of phosphorus in plants (Alkarawi and Zotz, 2014). These changes in transcription indicate significant decrease of phosphate concentration in the cultivation medium due to reaction of phosphates with thorium or competition of thorium and phosphates in tissues. Presence of uranium (in 0.1, 1, 10, 100 µM) in the medium (for 1, 3, and 7 days) did not negatively affect phosphorus content in the *A. thaliana* roots, in contrast to leaves (Vanhoudt et al., 2011). The results of this work are in agreement with other studies focused on the effect of heavy metal on energy pathways. The expression of many water deficit-induced genes is stimulated by abscisic acid (ABA) (Bray, 1997). Heavy metals were reported to decrease ABA concentration (Barcelo et al., 1986). Since this work recorded down-regulation of zeaxanthin epoxidase *ABA1* (*AT5G67030*), which catalyzes the early step of the ABA biosynthesis (Barrero et al., 2005), down-regulation of drought responsive genes could be affected by decreased ABA level.

Formation of callose was induced also by aluminum; moreover, it was even used as an indicator of aluminum sensitivity of plant species and crop genotypes (Basu et al., 2001; Ezaki et al., 2001; Ermolayev et al., 2003). Lead was found as another metal which induced callose formation. However, unequal callose deposition was not able to decrease Pb accumulation in *Lemna minor* (Samardakiewiezc et al., 2012).

In summary, thorium in 200 µM concentration caused stress in plants, as demonstrated by increased expression of genes involved in JA and SA signaling pathways and various abiotic and biotic stress responsive genes.

6. Conclusions

The present study on selected higher plants as phytoremediators for radiocesium uptake leads to the following conclusions. Second part contains thorium conclusion and discovering potential genes involved in its detoxification. All selected plants were analyzed with the intention of their usage in the Temelin area due to presence of nuclear power plant.

- *Pistia stratiotes* (*Aracaae*), an aquatic plant with higher metal concentration as recorded in its roots in this study can be comfortably considered for radiocesium extraction. As an added advantage this plant species does not survive winter season in the Czech Republic, thus there is no risk of overspreading of this plant as a free-floating species as it can be easily harvested. Another aquatic plant species *Eichhornia crassipes* (*Pontederiaceae*) with higher metal intake ability can also be considered as a good phytoaccumulator for cesium radionuclide. In the terrestrial plants, *Helianthus annuus* (*Asteraceae*) appears to be a good phytoextractor for radiocesium.
- From the acquired results, it is obvious that the soil properties and the content of other elements play a crucial role in the radiocesium uptake by higher plants. The highest cesium uptake was observed in plants grown in peat soil. Additionally, landscape management and climatic conditions need to be taken into account in the phytoremediation model.
- In regard to the seasonal changes, *E. crassipes* registered higher cesium concentration than *Phalaris arundinacea* (*Poaceae*) during late autumn(right before winter)in the Czech Republic. Likewise, *P. arundinacea* showed similar trend in cesium uptake in late autumn.
- Stomatal conductivity was measured in some selected plants to validate the cesium impact on stomata as it is known for competiton relationship with potassium. Neverthless, *P. arundinacea* did not show any stomatal disturbance by cesium presence. On contrary, the greater stomatal conductivity was detected in exposed plants than controlled. Stomatal conductivity scoped out among exposed and controlled plants of *E. crassipes* did not vary.
- Potential candidate genes for thorium detoxification are zinc-induced facilitator *ZIF2* and plant cadmium resistance *PCR2*. Both up-regulated genes ensure tolerance to zinc excess and therefore it is suggested that they could facilitate also thorium compartmentation to vacuoles and extrusion.

- Up-regulated ABC-transporter *ABCG40* has been capable to transport lead from cytoplasm and, thus it is proposed to be another candidate for thorium detoxification.
- Antioxidative *OXS3* could mitigate oxidative stress induced by thorium presence and heat shock proteins *Hsp20* and *ATHS83* might be involved in reparation of damaged proteins. Overexpression or knock-down studies should confirm if genes suggested here play role in thorium resistance and detoxification.
- Thorium in 200 μM concentration induced stress as demonstrated by increased transcription of genes involved in JA and SA signaling pathways and various abiotic and biotic responsive genes. Tobacco plants probably suffered by lack of phosphorus and iron in the presence of thorium as indicated by changes in expression of phosphorus and iron responsive genes.
- Negative regulation of membrane intrinsic proteins and drought responsive genes indicated that thorium could negatively influence water balance in plants. Negatively affected were also genes included in energy pathways. Up-regulation of transcripts taking part in callose formation indicated possible defense mechanism against thorium uptake.

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Appendix A: *Publications related to the thesis*

COMPARISON OF THE UPTAKE ^{133}Cs IN SUNFLOWERS AND REEDS FROM THREE DIFFERENT SOIL TYPES (PEAT, HUMUS AND CLAY)

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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 ^{90}Sr, ^{131}I, ^{137}Cs. This last one (^{137}Cs) has a nonradioactive isotope occurring in a small amount in nature. This nonradioactive isotope ^{133}Cs 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 ^{133}Cs 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 analysed with ICP-MS. The pH and the content of organic carbon were measured in the soil. Our results show higher accumulation of ^{133}Cs in *Helianthus annuus* L. cultivated on all mentioned soil types. The highest accumulation was observed in plants cultivated in peat soil.

KEY WORDS: phytoremediation, stable isotope, earth, annual plant, perennial plant

INTRODUCTION

Radiocesium is spread around the world from nuclear fission as nuclear testing weapons or nuclear accidents such Chernobyl (1986). It can stay in the environment for a long time (30.5 y) for this and not for the last reason it is required to pay special attention to this element. Radiocesium after penetrating into the various ecosystems can easily reach the human food chain via plants and animals. This radionuclide is the γ – emitter, it has genotoxic and carcinogenic effects on the organism. Hence, we should consider radiocesium as a potential hazardous element in soil or water which can serve as a reservoir for Cs.

As some authors claim [3, 14] soil properties directly influence the transfer factor between soil and plants. Among those characteristics belong the following: content of organic matter, pH, content of cations and texture. In the study by Takenaka et al. [13] it was found that the distribution of ^{137}Cs in surface soil correlated with the content of organic carbon and on the basis of their results, a regression model was made by them to predict the movement of ^{137}Cs present in decomposed organic matter.

As presented by Ehlken et Kirchner [2] the current effects of roots and microbial organisms can make different bioavailability from that found in the remaining bulk of the soil. In the three field studies [1] negative correlation was observed between the concentration of ^{137}Cs and soil moisture in pasture vegetation growing on the different soil types.

In some studies radiocesium was used as a pointer of the erosion degree of soil [5].

Mostly, radiocesium remains in the upper 10 cm soil depth which is easily reached by plant roots. It is a general desire to find the appropriate hyperacumulator of Cs to remove it from soil or water in any geographical location. Some promising plants belong to the family *Chenopodiaceae, Amaranthaceae* and *Asteraceae* [7].

To understand how Cs is taken up by roots, it is necessary to see first of all how potassium (essential element) is taken up. Both of them have similar behaviour – both belonging to alkaline metals and Cs is the biological analogue of K. The ions can penetrate into the apoplasm of the root tissue to the endodermis then enter the symplasm until they reach the Casparian bands. K transport through the plasma membranes of the root cell is provided by carriers at the low potassium concentrations, contrary to transport at high potassium concentrations which is supplied by K+ -channels [2], which, in turn, may be influenced by the competition between Cs and K.

Our aim was to compare three types of soil and the ability of sunflowers and reeds to accumulate cesium by testing plants cultivated on them. Sunflowers were chosen because of the possibility of high accumulation of Cs and reeds often occur in the Temelin area.

METHODOLOGY

Seeds of *Helianthus annuus* L. were cultivated in a greenhouse until the stage of seedling. Then the seedlings were transferred to pots with different types of soil. The seedlings of *Phragmites australis* were ordered from a garden shop and transferred to pots with different soil types. The fertilizer ¼ Haogland was used for the plants. The pots were organized in a completely random design for both species. After establishing plants to the right conditions, 0.5 mM CsCl was added in the form of an aqueous solution. The exposition time was 20 days. At the time of harvesting, the shoots and roots of the plants were then separated (washed out from substrate) and their fresh weight measured. Plants were dried in the oven at 80 ^0C for 22 hours and homogenized using a mill. The mineralization of samples (0.25-0.30 g dry mass) was carried out in teflon tubes. The acids HNO_3: $HClO_4$ were added in the ratio 7:1. The samples were incubated in a microwave oven (Anton Paar) for 1 hour. The solution was transferred into the falcon tubes and demi water was added into the final volume of 50 ml.

ICP-MS analysis was employed, it is specified by mass spectrometry with induce bind plasma (ICP MS, 7700x Agilent Technologies Inc., USA) without reacted/ colize gas and with using the internal solution of indium of the concentration 100 µg L^{-1}. For the dose of the sample was used autosampler ASX-500 Series (Agilent Technologies Inc. USA), ultra-low volume application MicroMist (Agilent Technologies Inc. USA) and copper skimmer and nickel conus were employed.

The content of C and N in soils was measured on the Analyzer Primacs SCN (firka SCALAR). The method in brief is based on burning of sample at 950-1100 °C. Than adding of control amount of the oxygen causes that nitrogenic compunds are converted onto NOx. The mixture of gas passes through the scrubbery where the acid aerosols, water and SO_3 are disposaled. The stream of helium carries them through the copper reducer where NOx are converted onto N_2. The concentration of incurred N_2 is measured by the thermal conductive detector. CO_2 is measured by Infrared detector.

The content of K, Ca and Mg by AAS- samples of soil were mineralized and in two replicated samples measured by AAS in laminar flame of mixture acetylen with air.

The measurment of pH in KCl solution – standard method by pH metr.

Data were analysed by statistical software R. For comparing results between sunflowers and reeds a t-test was used but not in the case of peat soil because there was not a full condition of homogeneity of variance, instead of that the nonparametric test, Wilcox´s test was used. In the case of humus – data were transformed by logarithm function to meet a distribution not significantly different from normal, then the t-test was applied to the data do find the differences in Cs uptake. For the whole data was applied multiway ANOVA with consider soil factor in a model and multicomparison test - Tukey*HSD*.

RESULTS

From the following results, it emerges that the highest ability of the accumulation of Cs was observed from sunflowers grown in peat soil. Statistical analysis verified a significant difference between sunflowers and reeds (roots). See Figure 1. with p-value = 0.01265 on the level of α=0.05.

For the humus soil, results show that the mean of sunflowers is higher than the mean of reeds but the difference is not statistically significant (W = 43, p-value = 0.08761). There is just a marginal difference between them according to p-value = 0.08761 on the level of α=0.05 in Figure 2.

The results for clay soil show significant difference between sunflowers and reeds according to p-value = 0.04501 on the level of α=0.05 in Figure 3. The values of the concentration of Cs were the lowest (in the range 1.5-6.5 ppm in clay soil) in tested plants from selected soil types.

The comparison of soils between each other was following: sunflower from peat soil was significantly differenced from sunflowers from both soils humus and clay (p-value= 0.0011203, p-value=0.0010885, respectively, α=0.05). On the contrary there was no difference between sunflowers from humus and clay soil (p-value=0.9922908, α=0.05).

At reeds was significant difference just between peat and clay soil (p-value=0.0211190, α=0.05), reed from humus and peat (p-value=0.1080347, α=0.05), reed from humus and clay (p-value=0.9909250, α=0.05). The properties of soils are mentioned below in Table 1.

Table 1. The pH, content of carbon, nitrogen, potassium, calcium, magnesium

Soil type	pH (KCl)	C (g.kg^{-1})	N (g kg^{-1})	K (g. kg^{-1})	Ca (g. kg^{-1})	Mg (g. kg^{-1})
Peat	4.4	48.6	5.70	3.17	3.57	5.79
Humus	6.9	30.0	1.95	7.92	11.41	6.09
Clay	6.3	15.0	2.70	4.77	2.88	4.66

Fig.1. Difference among plants from peat,
t = 3.0771, p-value = 0.01265, α=0.05.

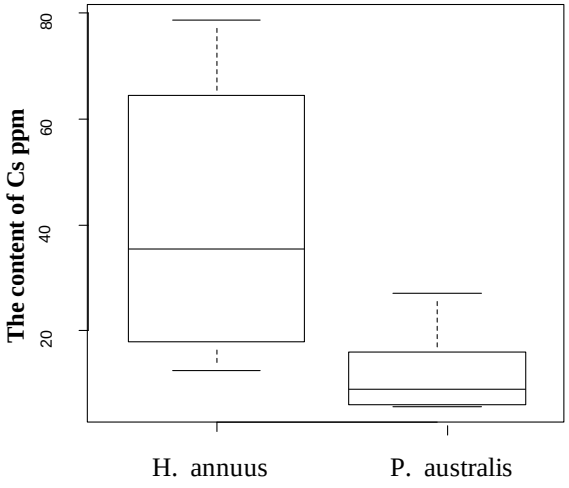


Fig. 2. Difference among plants from humus,
W = 43, p-value = 0.08761, α=0.05

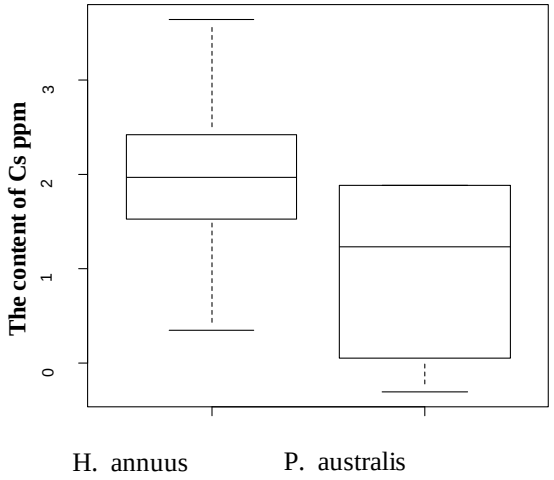
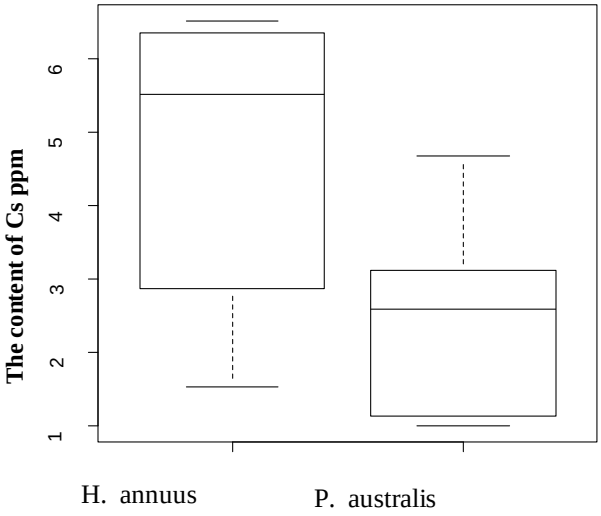


Fig 3. Difference among plants from clay,
t = -3.4064, p-value = 0.04501, α=0.05



DISCUSSION

The cesium was mainly accumulated into the root of plants. From selected plants that root of sunflower had the higher concentration than of reed roots (Figure 1-3). The lower concentration was found in the aboveground parts of plants in each soil type. On the contrary [9] found higher activity of ^{137}Cs in young leaves, stem and nodal segmentation. And also [8] found out difference between sunflower and reed but it was two times higher capacity for reed than sunflower, even for the whole part of plant Cs was distributed. Possible explanation is just in another cultivation method (hydrpony with different level of K and NH4). Same as Soudek. [10] found out in their study that uranium is located mostly in roots of plants, this is with agreement with our results.

Hence, the uranium was taken up the most by *Zea mays* and the lowest by *Arabidopsis thaliana*. Our experiment based on the cultivation of plants (annual and perenial sp.) growing on three different soil types proved that there exists a distinguished difference in values in the uptake of cesium by various species cultivated on the same soil. This is in agreement with As authors Moogue [6] investigated three different species-*Calendula alata*, *Chenopodium album* and *Amaranthus chlorostachys* for potential effective accumulation of cesium. Additionally, they found out differences between selected species in the uptake of cesium.

Our results also showed for humus soil (Figure 2.) that when soil or medium is enough saturated by K than cesium uptake by plants is influenced to lower values, neverless pH. Soudek [11] found in their study with thorium that tabacco increased the uptake by chelating properties, not by pH values which is not in agreement with [14]. As said by author Stouton [12] the cations which contest the most willingly with cesium are potassium and ammonium in soil or medium, which explains why cesium is not accumulated by plants from humus soil easily (Table 1.). The possible explanation could be a high saturation of potassium (7.92 $g.kg^{-1}$) in humus soil which causes a lower uptake of Cs into the plants 2 and also a higher concentration of calcium (11.4 $g.kg^{-1}$) and magnesium (6.09 $g.kg^{-1}$).

The first graph (Figure 1.) shows plants cultivated on peat soil. It is affected by quite low content of other cations in comparison with humus soil. However, it may be caused by the relatively high content of organic carbon in peat soil (Table 1) [12]. Not a last another cause can be affected by deepest migration of cesium in peat soil [4]. The third graph (Figure 3.) shows plants cultivated on clay soil. The uptake of cesium is affected by the lower content of organic carbon [12, 13] more than the other soil types and by the higher content of clay minerals [12].

CONCLUSION

Taken together the obtained results show that the highest concentration of cesium was reached by sunflower (roots) planted on peat soil > sunflower (roots) were found to have a little bit higher concentration of cesium in humus soil than in clay soil > and although it was found that the lowest concentration was in clay soil, there was still a significantly higher concentration of cesium found in sunflower (roots) than in reed (roots) in clay soil. It was unexpected that there was no observed difference between humus and clay soil for both species.

The further aim was to compare the difference between how annual and perennial species take up cesium. It was found that sunflowers were more effective than reeds thus annual species proving more effective than perennial species.

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Differences in ^{133}C uptake by common sunflower, common reed, and two macrophytes (*Elodea canadensis* and *Pistia stratiotes)*

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Highlights

- **The aquatic plant *Pistia stratiotes* had the highest cesium accumulation of all tested species.**
- **The greatest cesium accumulation was measured in the root.**
- **The factor of biomass did not play relevant role in this experiment.**

Abstract

Producing nuclear power comes with the risk of releasing radionuclides into environment. The most precarious and long-lasting anthropogenic radionuclide is radiocesium; therefore, green plants may be useful in eliminating the spread of radiocesium into the food chain. This paper compares differences in ^{133}C uptake for *Helianthus annuus, Phragmites australis,* and two macrophytes (*Elodea canadensis,* and *Pistia stratiotes*) inorder to assess their potential for use in phytoremediation. Building on prior research indicating radioactive cesium can be replaced with stable Cs in uptake experiments, seedlings were cultivated under constant, randomized experimental garden conditions with 8 d ^{133}C exposure created by adding a 0.5 mM CsCl solution to plants in pots containing sand and perlite (sunflower and reed) or water (macrophytes). Plasma mass spectrometry was used for measuring ^{133}C accumulation, with *P. stratiotes* showing the most accumulation with an average value 388±78.91 $mg.kg^{-1}$ in the root. *P. australis* had the least average value, 21.27± 5.54 $mg.kg^{-1}$ in the root. Keywords: phytoremediation; cesium; experimental garden experiment; *Hellianthus annuus*, *Phragmites australis*, *Pistia stratiotes*, *Elodea canadensis*

Introduction

Nuclear fission can produce radioactive substances in small amounts during normal operations and enormous amounts in the case of accidents. Plants are among the first organisms exposed to radioactive emissions but also can be used for removing hazardous waste through a process known as phytoremediation (Soudek et al. 2008). 137Cs is released during anthropogenic activities, including as a result of fallout from the accident at the Chernobyl (Ukraine) nuclear power plant in 1986 (Saxén and Ilus 2008). In that case, radionuclides were spread through dry deposition by wind at great distances across Europe. During the most recent accident at Fukushima (Japan) in 2011, radionuclides were spread into sea water (Saleh 2012). An additional significant radiocesium contribution was generated by testing nuclear weapons in the early 1960s, peaking in 1964. As a result, radionuclides are present today in both terrestrial and aquatic ecosystems. Radioactive cesium persists in the environment for years. ^{137}Cs, for example, has a half-life of 30.5 years, and ^{134}Cs a half-life of 2.5 years.

Therefore, it is necessary to prevent the spreading of anthropogenic radionuclides in the environment. The environmental-friendly method phytoremediation appears to be a reasonable mechanism for preventing the spread of cesium in the environment, even though it is a long-term process. The primary aim of this study was to evaluate the efficiency of several plant species in decreasing radiocesium from both an inert substrate and water, since radiocesium penetrates both terrestrial and acquatic ecosystems. The secondary aim of this study was to determine which part of the plants accumulated the most cesium

Material and Methodology

The plant species were selected on the basis of their natural occurrence in the area around the Temelin (Czech Republic) nuclear power plant. *Helianthus annuus* L. and *Phragmites australis* L. are easily grown in the Czech Republic and have proven suitable for phytoextraction (Soudek et al. 2004, 2006). The species *Pistia stratiotes*, not native to the Czech Republic, was tested because of its high efficiency in abating heavy metals uptake as reported by Odjegba and Fasidi (2004) and Vesely et al. (2011). Another non-native species, *Elodea Canadensis*, was also included in the study based on its potential for phytoremediation (Pinder III et al., 2006).

Plant descriptions

Helianthus annuus L. (*Asteraceae*). Known as sunflower, this terrestrial annual plant is commonly used for phytoremediation experiments. It has good biomass growth, can reach heights up to 3 m, and is grown as an agricultural crop.

Phragmites australis L. (*Poaceae*). This wetland perennial is often found near Temelin. Its common name is reed and it is capable of producing a large amount of biomass and can reach heights ranging from 1 to 4 m (sometimes even 6 m). It is used in pulp manufacturing and thatching.

Pistia stratiotes (*Aranacea*). This is a non-native plant in the Czech Republic. Its is found mostly in backwaters and is generally called water lettuce. The species is generally used as an algae cleaner and it does not survive winter in Central Europe. This species can therefore be used for cleaning contaminated water without spreading contamination.

Elodea canadensis L. (*Hydrocharitеceae*). An aquatic clonal perennial submersed plant common in Central Europe but not native to the Czech Republic, *E. canadensis* increases in abundance in the secondary distribution area. Its common name is waterweed and it produces large amounts of biomass and can reach heights of up to 100 cm in a short time.

Experimental design

Helianthus annuus and *Phragmites australis* were employed in the study so that the difference in 133Cs uptake between plants with different life strategies could be compared. *Elodea canandensis* and *Pistia stratiotes* were included in the study on the basis of a search of the relevant scientific literature indicating their suitability for phytoremediation. In our experiment, radioactive Cs was replaced with stable Cs because it does not emit radioactivity and its uptake by plants occurs in the same manner as that for radioactive Cs (Soudek et al. 2006, Vinichuk et al. 2010). *Helianthus annuus* seeds were sterilized for 10 minutes in H_2O_2 then placed on seed plates in a greenhouse in semi-controlled conditions: 22° C and 16/8 hour light cycles.

Three-week-old seedlings were transplanted into pots (200 mm diameter) with sand and perlite as an inert medium. *Phragmites australis* seedlings were purchased as a commercial product and washed with distilled water. These seedlings were then transplanted into pots with sand and perlite. Plants with similar appearances and sizes were analyzed. The experiment was conducted in outdoor conditions in completely randomized block design. The plants were watered using tap water enriched with ¼ Hoagland fertilizer [$Ca(NO_3)_2$ x $4H_2O$ – 1.2492 mM, KNO_3 – 1.2512 mM, KH_2PO_4 – 0.2498 mM, $MgSO_4$ x $7H_2O$ – 0.5103 mM + microelements], with 50 ml of this solution added once a day to each pot. After 20 days of establishing plants in an outdoor garden, 0.5 mM stable Cs [CsCl] was added to the pots at the rate of 25 ml per pot. Plants were then exposed for 8 days.

The aquatic species *Pistia stratiotes* and *Elodea canadensis* were cultivated in a basin with 3 l. of tap water. Upon harvesting, substrate was washed off the roots with tap water and the plants were left to dry (Fig. 1, 2, 3, 4). Fresh and dry weights, lengths of roots, lengths of shoots, numbers of leaves and their weights were calculated. The plants were then dried for 24 h at 70° C to a constant weight. The resulting dry biomass was ground using a mill (Fritsch, Palverisette 15, Idar-Oberstein, Germany) and each homogenized sample (0.2-0.4g) was transferred into a teflon tube and mixed with HNO_3 + H_2O_2 (7:1 ratio). After decomposition, each sample was subjected to microwave digestion using an Anton Paar device (Anton Paar, Graz, Austria). Leaves, stems, and roots were analyzed separately except for *Elodea canadensis,* which showed poor root growth.

Preparation of cesium solution

A CsCl (Sigma Aldrich, Czech Republic) solution was prepared and measured using a calibrated scale with a certain amount of CsCl (0.0421 g/ 0.5 L). The resulting CsCl powder was transferred into a measuring cup and mixed with 0.5 L of water. The solution was dissolved using a stirrer for few minutes.

Measurement of cesium concentration

ICP-MS analysis was employed, it is specified by mass spectrometry with induce bind plasma (ICP MS, 7700x Agilent Technologies Inc., USA) without reacted/ colize gas and with using the internal solution of indium of the concentration 100 µg L-1. For the dose of the sample was used autosampler ASX-500 Series (Agilent Technologies Inc. USA), ultra-low volume application MicroMist (Agilent Technologies Inc. USA) and copper skimmer and nickel conus were employed. The volume of each sample was 50 ml.

Statistical Evaluation

Analysis of covariance (ANCOVA) was the statistical method employed for evaluating experimental data.

In all statistical analyses, we examined the statistical impact of dry weight and the block of pots as covariates in the model. The results showed there were no significant effect from these covariates. In the model, the independent variables species and plant parts were statistically significant and important. The cesium concentration was primarily dependent on plants species and secondarily on parts of plants. The statistical software R was used to evaluate data.

Results and discussion

Results for all selected species show statistically significant differences among species ($F = 29.18$, $df = 61$, $P < 0.001$) and plant parts ($F = 26.16$, $df = 61$, $P < 0.001$). The highest accumulation of ^{133}Cs was found in *P. stratiotes* (Table 1), followed respectively by *H. annuus*, *E. canadensis*, and *P. australis*. Because *P. stratiotes* is a free floating aquatic plant, cesium uptake from its water environment is generally easier. Thus, the concentration of Cs in this experiment was nearly 100 times greater in *P. stratiotes* than for *E. canadensis* (Table 1). This is in agreement with Odjegba & Fasidi (2004), who claim that *P. stratiotes* can be effective in removing trace metals (Ag, Cd, Cr, Cu, Hg, Ni, Pb, Zn). Cs concentration in *E. canadensis* reached roughly the same level as that of the terrestrial species *H. annuus*. These results correspond with Bělousová and Štukkenberg (1972), who found that the concentration of some elements and their accumulation in some plants or animals can be even higher than source concentrations in the environment. Pinder III et al. (2006) have more recently confirmed these findings, noting the accumulation of ^{137}Cs from water into fish and water macrophytes relates to a lack of K and low pH and that such conditions lead to the long-term retention of ^{137}Cs in those organisms. As mentioned above, radiocesium and stable cesium are absorbed in the same way and in the same amount by plants (Soudek et al. 2006).

Saleh (2012) found that the aquatic species *Eichhornia crassipes* effectively absorbs ^{137}Cs from nuclear waste water, with the ratio (concentration in plant/concentration in solution) of radiocesium uptake is directly dependent on increasing plant biomass. In our experiment, the uptake of Cs depended primarily on plant species. The results for *P. stratiotes* showed the concentration of ^{133}Cs was highest in the roots, with a significant difference between uptake in roots and leaves ($t = 7.69$, $df = 11.771$, $P = 6.318x10\text{-}6$) (Table 1). These results correspond with Zhu (2001), Malek et al. (2002), and Soudek et al. (2011, 2013).

Pinder III et al. (2006) found that *E. canadensis* and *Potamogeton perfoliatus* (a submerged species) showed a rapid uptake of ^{137}Cs (330 and 630 L/ kg/d, respectively). In our experiment, *E. canandensis* exhibited cesium uptake of 47.36 ± 5.11 mg.kg^{-1}.

H. annuus stored ^{133}Cs mainly in the roots, then in the leaves (Fig. 1.), and finally in the stem (as had been expected). Individually, a statistically significant difference was found only between roots and stems ($df = 21, 145$ p-value = 0.026) and no significant differences were found either between roots and leaves ($df = 21$, P 146 = 0.2675369) or between leaves and stems ($df = 21$, $P = 0.450$). This might be explained by the fact that roots and leaves act as a sink for cesium, with the stem acting as a ^{133}Cs transmitter. Malek et al. (2002) verified root uptake of 90Sr as being 200 times greater than foliar absorption in *Brassica oleracea*. Soudek et al. (2011) found the highest uranium uptake in *Zea mays*, *Hordeum vulgare*, and *Cannabis sativa*; moreover, uranium was principally present in the roots of the selected plants. I a similar experiment, Soudek et al. (2013) examined thorium uptake in tobacco plants and found Th to be present mainly in the roots of the plants. Zhu (2001), in a hydroponic experiment, found the highest concentration of Cs in *Triticum aestivum* roots.

However, Soudek et al. (2006) found the distribution of 137Cs in *H. annuus*. to be dependent on its transport together with water and nutrients, with the strongest exposure occurring in young leaves, leaf veins, and nodal segments autoradiogram, that was in contrast with our results showing that *H. annuus* accumulated Cs mostly in the roots.

Table 1.

Mean ± SEM concentration of ^{133}Cs in plant parts [mg. kg^{-1}].

Species	Roots [ẋ ± SEM]	Leaves [ẋ ± SEM]
Helianthus annuus	40.58 ± 13.35	24.30 ± 5.91
Phragmites australis	21.27 ± 5.54	7.69 ± 2.26
Pistia stratiotes	388.10 ± 78.91	34.57 ± 2.57
Elodea canadensis		47.36 ± 5.11*

**E. canandensis* was analyzed as whole plant due to the lack of root biomass productivity

Although Soudek et al. (2006) stated that roots did not show substantial exposure in an autoradiogram, this could have been expected because of he differences in the manner of cultivation. In our experiment, *H. annuus* was cultivated in sand with perlite. On the other hand, these results were in agreement with Soudek et al.'s (2006) claim that *H. annuus* cannot be confirmed as a hyperaccumulator of Cs.

Karunakara et al. (2013) observed 137Cs uptake of, of 40K and 210Pb in the plant *Oryza sativa* near a nuclear power plant. Their results showed that *O. sativa* accumulates 40K in the shoots more than in the roots, with 137Cs and 210Pb absorbed into the roots and with a transfer factor significantly lower in the upper part of the plant. This matches our results for *H. annuus*, *P. australis*, *P. stratiotes,* and *E. canadensis* (Figure 1). All tested species accumulated 133Cs mostly in the roots (Table 1). In contrast, Moogouei et al. (2011) found that *Calendula alata*, *Amaranthus chlorostachys* and *Chenopodium album* had higher concentrations of 133Cs in their shoots than roots, with the highest concentration of 133Cs measured in *A. chlorostachys* shoots. Their experiment was conducted hydroponically, as were the experiments in Soudek et al. (2006).

Zhu (2001) tested 137Cs uptake with hydroponically cultivated *Triticum aestivum* and varying levels of K, observing the highest concentration of 137Cs in roots with the lowest supply of K (2 mg/L). In our experiment, the best bioaccumulator for 133Cs from the tested species was *P. stratiotes* (Table 1). It would be possible to employ this plant for photomediation in the Czech Republic only during the growing season because would not able to survive the winter.

However, this means there is no risk of the plant's uncontrolled spread. *P. stratiotes* could therefore be used as a bioaccumulator of Cs (or other metals) during the growing season in lakes or ponds and then removed from the water source. This process could be repeated until cesium concentration was decreased to acceptable levels.

The lowest concentration of 133Cs was observed in *P. australis*, withaccumulation of 133Cs occuring in the below-ground part of the plant. The difference between that in the above- and below-ground parts was significant ($t = 2.167$, $df = 13.137$, $p\text{-value} = 0.04$). These results are in contrast with those of Soudek et al. (2004) regarding uptake of 137Cs and stable Cs in *H. annuus, P. australis, P. simonii*. Their results showed that reed performed two times more cesium accumulation than sunflower after 32 days of hydroponic cultivation in a laboratory while adding K+ and NH4+. Our experiment was conducted under garden conditions without the addition of any chemical substances other than fertilizer.

Conclusion

The most promising plant extract radiocesium/ cesium isotopes from water from all examined species was the aquatic species *P. stratiotes* followed by terrestrial *H. annuus* and the aquatic species *E. canadensis*. The lowest accumulation was observed in wetland species *P. australis*. Nevertheless, all plant species had the highest cesium concetration in their roots. The authors recommend *P. stratiotes* and *H. annuus* for future investigations because of their potential abilities to reduce radiocesium/cesium.

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Thorium impact on tobacco root transcriptome

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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. A 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 200 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.

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

1. Introduction

Thorium is a radioactive actinide metal, present in small amounts in the environment The concentration of thorium is around 6 ppm in the earth crust. More than 99% of thorium occurs as a radioisotope 232Th 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 [1].The thorium mining has recently attracted enhanced attention due to its potential use in the nuclear industry (power-plants). Thorium content is three up to four times higher than that of the other natural actinide - uranium. This is the reason why some countries, such as India, prefer this element as potential fuel for nuclear power-plants [2]. 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 [1]. Although most studies have been focused on long-term toxicity caused by irradiation, thorium is also substantially toxic element [3]. When Th exposure is sufficiently high, chemical toxicity exceeds the radiological one [4].

Plants can be used for the phytoremediation of areas contaminated by Th. The necessary prerequisite is, however, understanding of Th effect on the plants. Up-to-now, there is a lack of knowledge about this topic. Published studies mostly deal with the accumulation of thorium in the human food [5], medical plants [6], mushrooms [7], fern [8] or crops such as wheat [9]. Nowadays most investigations have been focused on the distribution of thorium in plants with the aim to investigate their ability to bind this metal. Zhou et al. [10] described the localization of thorium on molecular level but they did not determinate chemical structure of the precipitated thorium compound in the cell wall. Selected plants were tested in situ on uranium [11-14] or thorium mill tailings [15] and also on artificially supplied contaminated soils [16] or in liquid media [16-18] in laboratory conditions. Thorium transfer into the shoots was very low and most of it was accumulated in the root system. Presence of phosphate decreased Th as well as uranium uptake, while other modifications of cultivation substrates (e.g. addition of organic acids and polyamines) did not significantly influence Th accumulation in plants [17-20].

Exposition of plants to Th or uranium induced formation of reactive oxygen species (ROS) and subsequent activation of anti-oxidant defense system [21,22]. Recently, the effect of uranium on root transcriptome of Arabidopsis thaliana was studied [23]. Similar study on plants exposed to Th is, according to our knowledge, missing. Only transcriptomic response of Saccharomyces cerevisiae exposed to Th was explored [4]. Therefore we decided to investigate the effect of Th on the gene transcription in tobacco roots with the aim to reveal how this metal affects processes at molecular level in plants.

2. Material and methods

2.1. Plant material and cultivation conditions

Tobacco seeds were sown in Perlite and cultivated for two months. All seedlings were watered by Hoagland medium [24] every three days. The hydroponic medium with pH adjusted to 5.0 contained 4 mM CaCl2, 2 mM K2SO4, 2 mM NH4NO3, 2 mM NaH2PO4, 1.5 mM MgSO4, 4 mM NaNO3, 4 mM NH4Cl, 0.2 mM FeSO4, 138.8 µM H3BO3, 20.8 µM MnSO4, 2.3 µM ZnSO4, 3.3 µM CuSO4 and 0.2 µM Na2MoO4. Eight-week old plants were placed for one week into Araponics boxes (Araponics SA, Belgium) supplemented with 2 L of Hoagland hydroponic medium to acclimate to the hydroponic conditions. The plants were kept at 20 ºC under 16 h light period, humidity about 60%, average irradiation 72 µmol m-2 s-1 [17].

2.2. Plant treatment with thorium

Tobacco plants (N. tabacum L. cv. La Burley 21) grown in hydroponics were transferred into the Hoagland hydroponic medium containing 0.2 mM Th(NO3)4 (Lachema n.p., Brno, Czech Republic). Plants were exposed to Th(NO3)4 for seven days. The Th concentration and exposure time were selected according to previous toxicity tests [17]. During sampling for gene expression studies, roots and shoots were separated. The roots were then rinsed in demineralized water and dried on filtrated paper. The fresh weight of the samples was determined before freezing in liquid nitrogen and storing at -80 ºC until RNA isolation.

2.3. Microarray Analysis

The RNA was isolated from the roots of N. tabaccum plants that were treated with Th(NO3)4 and of control, untreated ones using the Plant RNA Isolation Mini Kit (Agilent Technologies, CA, USA). RNA was then labeled using LowInput QuickAmp Labeling Kit (Agilent Technologies) by Cyanine 3 and Cyanine 5 using a dye swap design to avoid dye-based bias. Labeled cRNA was purified by RNeasy Plant Mini Kit (Qiagen, Germany), fragmented and hybridized on the Nicotiana (V4) Gene Expression Microarray (Agilent Technologies) according to the manufacturer's instructions. After a 17-h hybridization at 65°C, slides were washed in GE Wash Buffers (Agilent Technologies), acetonitrile and Stabilization and Drying Solution (Agilent Technologies). Microarrays were scanned using a GenePix 4000B scanner controlled by GenePix Pro Microarray Analysis Software (Molecular Devices, CA, USA). Experiments were repeated three times with root cRNA prepared independently from individual plants. The data acquired from the scanner were processed in R scripting environment using software package LIMMA according to Smyth and Speed [25], Smyth [26], and Smyth et al. [27]. The LOESS normalization method was used to balance the mean fluorescence intensities between the green and red channels in the frame of single arrays, and the Aquantile method was used to normalize signals among arrays. The background intensity was not subtracted from the overall spot intensities. The statistical analyses were performed without spots with zero weights. The false discovery rate (FDR) method was used for statistical evaluation. Genes showing $\geq$ 2-fold change in gene expression (p-value < 0.01) were selected. Tobacco chip was reannotated using recently sequenced N. tabacum data [28]. Probes sequences were blasted against N. tabacum TN90 mRNA database (solgenomics.net) and annotation was assigned to those with resulting E-value < 10-6. Functional classification of up- and down-regulated transcripts was done by Classification Super-Viewer (http://bar.utoronto.ca/ntools/cgi-bin/ntools_classification_superviewer.cgi) [29].

2.4. Quantitative Real-Time PCR Analysis

The transcription levels of selected genes obtained from microarrays were verified by the quantitative real-time PCR (RT-qPCR). RNA was treated with Ambion DNA-free kit (Thermo Fisher Scientific Inc., MA, USA) to eliminate the traces of genomic DNA. Complementary DNA (cDNA) was prepared from the total RNA by the M-MLV Reverse Transcriptase (RNase H Minus, Point Mutant, Promega, WI, USA). The protocol for the first strand cDNA synthesis with oligo dT primers was used and the Protector RNase Inhibitor (Roche Applied Science, Mannheim, Germany) was added. 2.5 µL of 20x diluted cDNA was mixed with the LightCycler 480 DNA SYBR Green I Master (Roche Applied Science, Mannheim, Germany) and 500 nM of respective primers to final volume 10 µL. The qPCR cycle included initiation (95°C, 10 s), annealing (60°C, 10 s) and elongation (72°C, 10 s) steps and the reaction was performed by the Light Cycler 480 (Roche Applied Science, Mannheim, Germany).

Relative content of RNA in sample was calculated according to Hellemans et al. [30]. RPS4A and GTPbEFTu genes were used for normalization. qPCR efficiency estimated for each primer pair from the calibration curve was used for the calculation. The list of primers is in the Table 1.

3. Results and discussion

Thorium (200 μM) up-regulated 152 and down-regulated 100 genes (p-value < 0.01, fold change ≥ 2) in the tobacco roots after 7-day exposure (complete list of up- and down-regulated genes is in Supplementary table 1).

3.1. Thorium up-regulated transcription of genes involved in signal transduction

The genes involved in signal transduction represented the relatively most abundant group of up-regulated transcripts (when sorted according to the biological process domain; Fig. 1A). This group comprises genes involved in systemic acquired resistance mediated by salicylic acid (SA) signaling pathway such as two most up-regulated transcripts - A. thaliana homologue hemoglobin AHB1 (AT2G16060; also in other cases bellow are always stated A. thaliana homologue genes) and alcohol dehydrogenase ADH1 (AT1G77120). Furthermore, other genes involved in SA signaling pathway such as aluminum induced protein with YGL and LRDR motifs AT4G27450, sucrose synthase SUS4 (AT3G43190), pyruvate decarboxylase PDC1 (AT4G33070) and LOB domain-containing protein LBD41 (AT3G02550) were up-regulated. Beside SA, also jasmonic acid (JA) mediated signal transduction was initiated by thorium. Genes involved in JA biosynthesis lipoxygenase LOX5 (AT3G22400), two lipase/lipooxygenases PLAT1 (AT4G39730) and PLAT2 (AT2G22170), and cytochrome P450 CYP94C1 (AT2G27690) were up-regulated as well as JA responsive genes methylenetetrahydrofolate reductase family protein AT5G38710 and allene oxide synthase AOS (AT5G42650). JA functions in a close cross-talk with another hormone - ethylene. Th up-regulated also ethylene biosynthetic gene ACC oxidase AtACO2 (AT1G62380). These data demonstrate that presence of thorium represented stress for plants, as SA and JA play key roles in the regulation of defense responses to pathogens, wounding and environmental stimuli [31,32].

3.2. Stress responsive genes induced by thorium

Beside defense and wounding responses also genes up-regulated by salt stress (eukaryotic aspartyl protease family protein AT1G03220, alanine:glyoxylate aminotransferase AT2G13360, and hydroxy methylglutaryl CoA reductase 1 HMGR1 [AT1G76490]), by oxidative stress (BTB and TAZ domain protein BT1 [AT5G63160], 2-oxoglutarate (2OG) and downy mildew resistant DMR6 [AT5G24530], plant cysteine oxidase PCO2 [AT5G39890], and dark inducible DIN10 [AT5G20250]), and by cold stress (universal stress protein AT3G53990, beta-amylase BAM3 [AT4G17090], and responsive to desiccation RD22 [AT5G25610]) response were stimulated. Thorium induced transcription of oxidative stress responsive genes was reported also in yeast [4]. Oxidative stress was caused also by uranium, as demonstrated by increased transcription of reactive oxygen species-producing enzyme LOX1 and guaiacol peroxidase in A. thaliana roots after 7-day exposure to 100 μM concentration [33]. H_2O_2 level and catalase expression were increased in Vicia faba roots cultivated eight days in soil containing radionuclides 232Th, 226Ra and 40K [34]. Horemans et al. [35] observed that uranium caused oxidative stress different from cadmium.

However, we recorded up-regulation of several genes associated with response to cadmium (phospholipase D beta PLDBETA1 [AT2G42010], oxidative stress OXS3 [AT5G56550], plant cadmium resistance PCR2 [AT1G14870], and alcohol dehydrogenase ADH1 [AT1G77120]). Generally, heavy metals induce generation of reactive oxygen species as primary reaction in plants [36] and although there could be differences among various metals, lipid peroxidation and induction of oxidative stress were observed in the plants exposed to lead [37], copper [38], mercury [39], and arsenic [40]. Our results showed that also Th induced oxidative stress together with response to other abiotic and biotic stimuli in tobacco roots.

3.3. Genes potentially involved in thorium detoxification and resistance

Plants possess several detoxification mechanisms to cope with heavy metals such as chelation, exudation, binding to metallothioneins and compartmentation to vacuole [41]. However, we did not record elevated expression of genes coding for chelatases or metallothioneins. Surprisingly, heavy metal transport/detoxification superfamily proteins AT3G24450 and AT5G03380 as well as natural resistance-associated macrophage protein NRAMP3 (AT2G23150) involved in transport of cadmium ions [42] were down-regulated. Nevertheless, zinc-induced facilitator ZIF2 (AT2G48020) from Major Facilitator Superfamily was up-regulated by Th presence. ZIF2 plays an important role in zinc tolerance via root vacuolar sequestration avoiding the accumulation of zinc in shoots [43]. Further, PCR2 was another up-regulated zinc transporter. PCR2 is essential for zinc redistribution and detoxification when zinc is in excess [44]. Additionally, two ABC transporters ABCA1 (AT2G41700) and ABCG40 (AT1G15520) were up-regulated. ABCG40-overexpressing Arabidopsis plants were found to be more resistant to lead and had lower lead content than wild type plants when grown on medium containing lead. Authors suggest that ABCG40 works as a pump to exclude lead ions and/or lead-containing compounds from the cytoplasm [45]. Taking into account that ABCG40 is able to detoxify lead, it is possible to assume that this transporter may be involved in thorium detoxification as well. Another up-regulated gene was OXS3, which was reported to enhance tolerance to cadmium, probably via antioxidant protection [46]. Heat shock proteins play a crucial role in protein protection (like chaperons), facilitating their recovery during heavy metal stress [41]. We identified two heat shock proteins Hsp20 (AT4G21870) and ATHS83 (AT5G52640) and heat shock transcription factor A6B (AT3G22830). Up-regulation of HS83 by arsenite (but not by cadmium) was reported by Takahashi et al. [47]. Genes potentially included in thorium detoxification process and resistance are showed in Table 2.

3.4. Thorium effect on uptake of nutrients

Another issue is the interaction of thorium with nutrients. Fe-deficiency induced transcription factor FIT1 (AT2G28160) was down-regulated in plants exposed to thorium. FIT1 transcription factor regulates expression of IRT1, FRO2, and AHA2, the key genes in iron uptake [48-52]. Changes in expression of these two genes indicate that thorium could suppress iron uptake. Similar effect was observed in the case of A. thaliana exposure to uranium where FIT1 and other genes involved in iron uptake were down-regulated [23]. Also cadmium down-regulated FIT1 expression and decreased accumulation of iron in A. thaliana roots [53]. Phosphates decrease thorium uptake probably via formation of insoluble phosphate salts [17,18]. We recorded changes of transcript abundance of genes involved in response to phosphorus. Several genes induced by phosphate starvation were up-regulated (glycerophosphodiester phosphodiesterase GDPD1 [AT3G02040], glycerophosphodiester phosphodiesterase GDPD2 [AT5G41080], and responsive to desiccation RD2 [AT2G21620]).

Down-regulated were genes (myo-inositol-1-phosphate synthase 3 [AT5G10170], granule bound starch synthase MIPS3 [AT1G32900], senescence-associated family protein AT1G66330, and B-box domain protein BBX25 [AT2G31380]) involved in phytic acid (myo-inositol hexakisphosphate) biosynthesis. Phytic acid is an important storage form of phosphorus in plants [54]. These changes in transcription indicate significant decrease of phosphate concentration in the cultivation medium due to reaction of phosphates with thorium or competition of thorium and phosphates in tissues. Presence of uranium (in 0.1, 1, 10, 100 μM) in the medium (for 1, 3, and 7 days) did not negatively affected phosphorus content in the A. thaliana roots, in contrast to leaves [33].

3.5. Thorium negatively affected energy pathways

Relatively largest group of suppressed transcripts included genes associated with electron transport or energy pathways (when sorted according to the biological process domain, Fig. 1B). Genes from this group are involved in glycolytic process (B-BOX domain protein BBX24 [AT1G06040], aspartic proteinase APA1 [AT1G11910], and alpha/beta-Hydrolases superfamily protein AT3G23600) and photosynthetic process (rubisco activase AT2G39730, plastocyanin PETE1 [AT1G76100], pyridine nucleotide-disulphide oxidoreductase family protein AT1G74470, hypersensitive to high light HHL1 [AT1G67700], and transcriptional coactivator/pterin dehydratase AT5G51110). Our results are in agreement with other studies focused on the effect of heavy metal on energy pathways. Chromium stress resulted in reduction of net photosynthetic rate in Brassica napus [55]. Proteomic analyses of B. napus leaves exposed to chromium revealed that chloroplast proteins were most affected [56]. Cadmium in 10 and 50 μM concentrations decreased photochemical efficiency in Lactuca sativa [57] and also uranium negatively affected photosynthetic processes in A. thaliana [58].

3.6. Thorium down-regulated transcripts coding for intrinsic proteins

Genes involved in transporter activity represented the relatively most abundant group of down-regulated transcripts (sorted according to the molecular function domain, Fig. 1B). Four transcripts coding for intrinsic proteins (aquaporins: plasma membrane intrinsic protein PIP2A [AT3G53420], plasma membrane intrinsic protein PIP1;4 [AT4G00430], tonoplast intrinsic protein TIP1;3 [AT4G01470], and tonoplast intrinsic protein DELTA-TIP3 [AT5G47450]) were down-regulated in the presence of thorium. Also uranium in 50 μM concentration decreased transcription of tonoplast intrinsic protein TIP4;1 (AT2G25810) and aquaporin NIP2.1 (AT2G34390) in A. thaliana roots[23]. However, in case of earlier or later response (exposure time in our study was 7 days) the transcriptomic profile could be different. As example can be stated mentioned study with uranium where 1061, 256, 823 transcripts were changed after2, 6, and 30 h exposure, respectively, while 111 transcripts were changed at all three time points [23]. It demonstrates that the expression profile can change substantially during the exposure time. Previously, we recorded down-regulation of intrinsic proteins PIP1;4, PIP2;3, PIP3, and DELTA-TIP in A. thaliana roots exposed to 4 μM zinc for 7 days [59]. Activity of water channels was inhibited also by Hg^{2+} [60] and other metals, such as gold and silver [61,62]. Intrinsic proteins (aquaporins) facilitate water transport through cellular membrane and are important for water homeostasis [63]. It seems that thorium affects activity of aquaporins similarly as the other mentioned metals. Interestingly, also genes involved in response to water deprivation (drought-responsive family protein AT5G26990, dehydrin family protein RAB18 [AT5G66400], dehydrin xero 1 AT3G50980, proton gradient regulation PGR5 [AT2G05620], beta-amylase BAM1 [AT3G23920], and ABC-2 type transporter family protein ABCG22 [AT5G06530]) were down-regulated.

The expression of many water deficit-induced genes is stimulated by abscisic acid (ABA) [64]. Heavy metals were reported to decrease ABA concentration [65]. Since we recorded down-regulation of zeaxanthin epoxidase ABA1 (AT5G67030), which catalyzes the early step of the ABA biosynthesis [66], down-regulation of drought responsive genes could be affected by decreased ABA level.

3.7. Thorium induced transcription of genes involved in callose formation

Genes associated with the cell wall were the most abundant group when sorted according to the cellular component domain (Fig. 1A). Up-regulated genes AGAMOUS-like AGL15 (AT5G13790), ethylene response ETR1 (AT1G66340), and flagellin-sensitive FLS2 (AT5G46330) are involved in defense response by callose deposition in the cell wall. Callose formation was induced also by aluminum; it has been even used as an indicator of aluminum sensitivity of plant species and crop genotypes [67-69]. Lead is another metal which induced callose formation. However, irregular callose deposition was not able to decrease Pb accumulation in Lemna minor [70].

3.8. Validation of the microarray results

The values acquired by microarrays were verified using quantitative real-time PCR. Ten genes representing highly and slightly up- and down-regulated transcripts were selected. Table 3 shows general agreement of microarray and q-PCR data. Differences in absolute values reflect differences in both methods and their sensitivities [71].

4. Conclusions

In summary, thorium in 200 µM concentration caused stress in plants, as demonstrated by increased transcription of genes involved in JA and SA signaling pathways and various abiotic and biotic stress responsive genes. In the presence of thorium, tobacco plants probably suffered from the lack of phosphorus and iron, as indicated by changes in expression of phosphorus and iron responsive genes. Negative regulation of aquaporins and drought responsive genes indicated that thorium could negatively influence water balance in plants. Negatively affected were also genes included in energy pathways. Up-regulation of transcripts taking part in callose formation indicated possible defense mechanism against thorium uptake. Potential candidate genes for thorium detoxification are zinc-induced facilitator ZIF2 and plant cadmium resistance PCR2. Both up-regulated genes ensure tolerance to zinc excess [43,44] and therefore we suggest that they could facilitate also thorium compartmentation to vacuoles and extrusion. Up-regulated ABC-transporter ABCG40 was reported to transport lead from cytoplasm [45] and thus we propose it as another candidate for thorium detoxification. Chromatin-associated factor OXS3 could mitigate oxidative stress induced by thorium presence and heat shock proteins Hsp20 and HS83 might be involved in protection and reparation of proteins. Overexpression or knock-down studies should confirm if the genes pinpointed by transcriptome analysis here really play a role in thorium resistance and detoxification.

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Appendix B: Morphological parameters

Table 9.

Morphological parameters of *Helianthus annuus*

Helianthus											
plant number	**No. of leaves**	**length of stem (cm)**	**length of root (cm)**	**leaf**		**fresh weight(g)**			**dry weight (g)**		
				length (cm)	**width (cm)**	**root**	**stem**	**leaves**	**root**	**stem**	**leaves**
1	12	40	26	9	6	3.585	10.287	6.034	0.544	1.107	0.986
2	14	39	27	7.5	4	1.304	9.626	3.808	0.223	0.675	0.553
3	11	42	27	9.2	5	3.52	11.644	4.819	0.492	1.261	0.702
4	14	47.5	45	8	6	2.648	11.281	4.901	0.383	1.194	0.704
5	10	40	45	7	4	0.647	6.678	2.656	0.137	0.681	0.417
6	9	42	24	6	3.5	0.962	5.716	2.129	0.145	0.579	0.334
7	9	41.5	32	7	4	1.908	6.426	2.593	0.255	0.689	0.403
8	11	47	22	8.5	5	2.668	14.301	5.032	0.365	1.658	0.778
9	9	39	33	7.5	5	1.198	6.175	2.507	0.23	0.728	0.424
10	8	34.5	31	6.5	3.5	1.369	4.052	1.959	0.224	0.459	0.344
K1	12	44.5	31	9.5	6.2	3.952	12.059	6.03	0.548	1.371	0.987
K2	12	38	32	9.5	5.8	2.297	8.777	5.24	0.397	0.93	0.777
K3	13	46	34	6.5	4	1.751	10.305	3.167	0.273	1.013	0.467

Table 10.

Morphological parameters of *Phragmites Australis.*

Phragmites						
plant number	**length of shoot (cm)**	**length of root (cm)**	**fresh weight (g)**		**dry weight (g)**	
			root	**shoot**	**root**	**shoot**
R1	57	13	5.567	2.622	0.971	0.793
R2	35	20	6.034	4.352	0.94	1.318
R3	38	18	6.712	3.834	1.078	1.282
R4	44	22	7.711	6.268	1.749	2.572
R5	43	17	6.164	4.345	1.027	1.297
R6	53	21	11.521	7.089	1.819	2.788
R7	32	19	5.261	3.478	1.109	1.262
R8	45	20	3.494	3.041	0.617	1.085
R9	32	21	5.413	4.874	0.977	1.512
R10	35	18	4.542	3.008	0.545	0.865
R11	52	20	5.749	4.522	1.136	1.5
RK1	53.5	25	6.009	4.524	0.848	1.483
RK2	36	12	2.888	3.45	0.411	1.319
RK3	31	13	5.438	3.802	0.642	1.069

Table 11.

Morphological parameters of *Pistia stratiotes.*

Pistia							
plant number	**length root (cm)**	**leaf**		**fresh weight (g)**		**dry weight (g)**	
		length (cm)	**width (cm)**	**root**	**leaves**	**root**	**leaves**
I	17.9	5.5	3.6	2.027	6.993	0.163	0.609
II	6.5	2.6	2.2	0.125	0.528	0.013	0.05
III	12.1	2.5	2.1	0.347	1.038	0.034	0.095
IV	12.2	4.6	2.4	0.852	3.099	0.072	0.268
V	10.6	3.7	2.7	0.567	2.152	0.06	0.202
VI	18.6	6	4.7	2.613	10.465	0.236	1.072
VII	6.4	2.9	2	0.119	0.328	0.014	0.038
VIII	11.2	2.3	2.1	0.235	0.73	0.024	0.069
IX	11	4.4	3.2	0.735	2.516	0.066	0.252
X	3.8	1.9	1.8	0.045	0.214	0.008	0.023
XI	13.1	2.8	2.4	0.353	1.28	0.035	0.128
XII	12.1	3.5	3	0.663	2.191	0.058	0.186

Table 12.

Morphological parameters of *Elodea canadensis.*

Elodea					
plant number	**length of plant (cm)**	**leaf**		**fresh weight (g)**	**dry weight (g)**
		length (cm)	**width (cm)**		
A	8	1	0.2	0.266	0.023
B	17.5	1	0.3	0.485	0.042
C	16.5	1.5	0.4	1.141	0.1
D	9.5	1.1	0.3	0.208	0.018
E	8	0.9	0.2	0.223	0.022
F	7	1.1	0.2	0.225	0.016
G	4	1.4	0.3	0.137	0.013
H	7.6	1.3	0.3	0.28	0.021
I	8.3	0.8	0.2	0.414	0.036
J	7.5	1.5	0.3	0.128	0.017
K	5.4	0.9	0.2	0.171	0.013
KA	14	1.4	0.4	0.777	0.063
KB	5	1.3	0.3	0.228	0.019
KC	9.5	0.9	0.2	0.348	0.037