# Identification of factors affecting cesium uptake by plants

# Identifikace faktorů ovlivňujících příjem cesia rostlinami

PhD Thesis

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# Identification of factors affecting cesium uptake by plants

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# Thesis

This thesis is submitted in fulfillment of the requirements for the PhD degree at the Czech University of Life Sciences Prague, Faculty of Environmental Sciences.

I declare that I prepared the dissertation with the topic "Identification of factors affecting cesium uptake by plants" independently using the sources cited in the bibliography.

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# Acknowledgement

# Abstract

Humanity's demand for energy is on the rise, paralleled by an increased use of nuclear energy. Although nuclear energy is considered a safe source, it carries the risk of incidents that could immediately threaten human populations, organisms, and ecosystem services with enduring effects, primarily due to the dispersal of highly mobile radionuclides such as cesium (Cs). The work is based on the study of the stable isotope $^{133}$Cs and the radioactive isotope $^{134}$Cs of cesium, utilizing multiple randomized block experiments in climabox, greenhouse and field environments, conducted between 2018 and 2023.

The earliest series of experiments explored the phytoremediation potential of *Ceratophyllum demersum* for cesium removal under various conditions. Exposure to the stable isotope $^{133}$Cs in both the greenhouse and the field, as well as to the radioactive isotope $^{134}$Cs in a climabox, showed significant Cs uptake influenced by concentration and exposure time ($p < 0.001$). Significant differences in Cs uptake were found between the greenhouse and field conditions ($p < 0.001$). Isotopes $^{133}$Cs and $^{134}$Cs were accumulated in a similar manner ($p > 0.05$). The results indicated that increasing the length of exposure reduced the uptake ability, suggesting a limited capacity of the plants for Cs removal. *C. demersum* has shown potential for remediating aquatic habitats, especially in cases of acute events, where a short duration of phytoremediation may be effective.

Following research on *Lactuca sativa*, *Allium cepa*, and *Raphanus sativus* grown in six European soils contaminated with $^{134}$Cs and $^{85}$Sr revealed species-specific aggregated transfer factors ($T_{ag}$). Potassium supply did not significantly affect Cs $T_{ag}$ ($p > 0.05$). Inconclusive results regarding accumulation were obtained for soil pH levels ($p > 0.05$), most of the soil type and vegetable combinations ($p > 0.05$), and clay particle content ($p > 0.05$). Lettuce, onion, and radish showed varying $T_{ag}$ values, with the lowest observed in lettuce despite its growing in the most contaminated soil. This indicates the importance of plant selection in minimizing food contamination from radionuclides and their transfer to the food chain.

Furthermore, the electrokinetic technique coupled with phytoremediation was studied with *Phragmites australis*. The application of an electric field for 8 or 16 days showed a 20-25% reduction in $^{133}$Cs accumulation. Factors such as the initial plant weight significantly impacted uptake ($p < 0.001$), while biomass increment ($p > 0.05$), pH ($p > 0.05$), and electrode decomposition products did not. These findings offer novel insights into the impact of the electric field on Cs uptake by hydroponically grown plants. Moreover, the additional experiments substantiated that potassium supplementation to the aquatic environment significantly reduced Cs accumulation ($p < 0.001$); however, it did not play a significant role in mitigating cesium uptake in terrestrial environments ($p > 0.05$). This suggests a potentially higher sensitivity of aquatic species to such measures.

The main recommendations derived from this work can be summarized across several key areas:

1) The necessity of conducting long-term research encompassing the entire growing season to foster a deeper understanding of the dynamics of cesium uptake throughout the year.

2) Investigation into the effect of root exudates and the soil microbiome on the absorption of cesium by plants under real conditions.

3) Execution of mechanistic studies on cesium absorption by aquatic plants, considering their different physiological adaptations in contrast to terrestrial plants.

4) Monitoring the impact of cesium intake on the physiological processes in plants, including photosynthesis, protein gene expression, and the formation of enzymatic and non-enzymatic antioxidants.

5) Examination of the effects of low, long-term doses of radiation on the primary production of plants.

# Abstrakt

Poptávka lidstva po energii je na vzestupu, paralelně se zvýšeným využíváním jaderné energie. Ačkoli je jaderná energie považována za bezpečný zdroj, nese s sebou riziko nehod, které by mohly bezprostředně ohrozit lidskou populaci, organismy a ekosystémové služby s trvalými dopady, především v důsledku šíření vysoce mobilních radionuklidů, jako je cesium (Cs). Práce je založena na studiu stabilního izotopu $^{133}$Cs a radioaktivního izotopu $^{134}$Cs cesia s využitím randomizovaných blokových experimentů v klimaboxu, skleníku a za polních podmínek, v letech 2018 až 2023.

První série experimentů posuzovala fytoremediační potenciál *Ceratophyllum demersum* pro odstraňování cesia z vodního prostředí za různých podmínek. Expozice stabilnímu izotopu $^{133}$Cs ve skleníku i terénu, stejně jako radioaktivnímu izotopu $^{134}$Cs v klimaboxu, ukázala významný příjem Cs ovlivněný koncentrací a dobou expozice ($p < 0{,}001$). Byly zjištěny signifikantní rozdíly v příjmu Cs mezi skleníkovými a terénními experimenty ($p < 0{,}001$). Izotopy $^{133}$Cs a $^{134}$Cs byly akumulovány podobným způsobem ($p > 0{,}05$). Výsledky ukázaly, že prodlužování délky expozice snižuje schopnost příjmu, což naznačuje omezenou kapacitu rostlin pro odstraňování Cs. *C. demersum* prokázala potenciál pro sanaci vodních stanovišť, zejména v případech akutních událostí, s krátkou dobou trvání fytoremediace.

Výzkum na *Lactuca sativa*, *Allium cepa* a *Raphanus sativus* pěstovaných v šesti evropských půdách kontaminovaných $^{134}$Cs a $^{85}$Sr odhalil druhově specifické agregované transferové koeficienty ($T_{ag}$). Přísun draslíku významně neovlivnil Cs $T_{ag}$ ($p > 0{,}05$). Neprůkazné výsledky týkající se akumulace byly získány pro pH půdy ($p > 0{,}05$), většinu kombinací půdního typu a zeleniny ($p > 0{,}05$) a obsah jílových částic ($p > 0{,}05$). Salát, cibule a ředkvička vykazovaly různé hodnoty $T_{ag}$, přičemž nejnižší byly pozorovány u salátu, přestože rostl v nejvíce kontaminované půdě. To ukazuje na důležitost výběru rostlin pro minimalizaci kontaminace potravin a následného přenosu radionuklidů do potravního řetězce.

Dále byla u *Phragmites australis* sledována možnost využití kombinace elektrokinetického procesu s fytoremediací. Aplikace elektrického pole po dobu 8 nebo 16 dnů ukázala 20-25% snížení akumulace $^{133}$Cs. Faktory jako počáteční hmotnost rostliny významně ovlivnily příjem Cs ($p < 0,001$), zatímco přírůstek biomasy ($p > 0,05$), pH ($p > 0,05$) a produkty rozkladu elektrod nikoli. Tato zjištění nabízejí nový pohled na vliv elektrického pole na příjem Cs hydroponicky pěstovanými rostlinami. Kromě toho další experimenty prokázaly, že suplementace draslíku do vodního prostředí významně snížila akumulaci Cs ($p < 0,001$); přidání K nehrálo však významnou roli při zmírňování absorpce cesia v suchozemském prostředí ($p > 0,05$). To naznačuje potenciálně vyšší citlivost vodních druhů na tato opatření.

Hlavní doporučení vyplývající z této práce lze shrnout do několika oblastí:

1) Nutnost provádění dlouhodobých výzkumů, které by pokrývaly celou vegetační sezónu, s cílem umožnit hlubší porozumění dynamice příjmu cesia v průběhu roku.

2) Studium vlivu kořenových exsudátů a půdního mikrobiomu na absorpci cesia rostlinami v reálných podmínkách.

3) Provádění mechanistických studií absorpce cesia vodními rostlinami, vzhledem k jejich rozdílným fyziologickým adaptacím při srovnání s rostlinami terestrickými.

4) Sledování vlivu příjmu Cs rostlinami na fyziologické procesy, jako jsou fotosyntéza, genová exprese proteinů a tvorba enzymatických a neenzymatických antioxidantů.

5) Studium vlivu nízkých, dlouhodobých dávek záření na primární produkci rostlin.

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# 1 General introduction

In nature, cesium is exclusively represented by the stable isotope [133]Cs (De Laeter et al., 2003). Despite its prevalence, there remains a notable absence of data on the acute and chronic toxicity of [133]Cs, particularly due to its impact on organisms (Melnikov and Zanoni, 2010). In contrast, the remaining 39 artificial isotopes are inherently radioactive (Dayah, 2017). Notably, some of these isotopes emit low-energy β particles, such as [135]Cs, possessing a half-life of 2.3 million years (Singh et al., 2008), while others, like ^{137}Cs emit γ particles with a half-life of 30.08 years (Browne and Tuli, 2007). A substantial proportion of these radioisotopes is produced in nuclear reactors by neutron absorption and fission reactions. Significant fission products include [134,135,137]Cs, which have a high cumulative fission yield of approximately $6 - 8\%$ (Sonzogni, 2002; Sonzogni, 2004; Browne and Tuli, 2007; Singh et al., 2008; Iwamoto, 2011). Among these, ^{137}Cs stands out as one of the most prevalent, with a yield of approximately 6% (Kocherov et al., 1997). Due to the increased energy demands of civilization and the concomitant utilization of nuclear power plants, the United States presently grapples with the disposal of over 88,000 tons of spent nuclear fuel (GAO, 2021). Similarly, Europe faced an estimated 227,000 tons of nuclear waste in 2019 (Besnard et al., 2019). The management of nuclear waste involves storage in special spent fuel pools or in casks, but these solutions are regarded as short-term and carry a higher risk of leakage compared to the option of permanent underground storage (Johnson et al., 2017). A report by the UK Health and Safety Executive in 2009 highlighted a concerning trend, revealing over 1,750 leaks, malfunctions, or other incidents at nuclear power stations in the British Isles between 2001 and 2008 (ONR, 2009). While these incidents may not represent the most precarious events in nuclear energy, addressing such issues is imperative. In contrast, accidents, as significant negative occurrences, have profound implications for the environment and public health. Noteworthy examples include the disasters at the British Windscale/Sellafield power plant in 1957 (Loutit et al., 1960), the American Three Mile Island in 1979 (Kasl et al., 1981), the Chernobyl accident in 1986 (Izrael et al., 1996), and, more recently, the major disaster in Fukushima, Japan, in 2011 (Mori and Takahashi, 2012).

During accidents, the primary focus is on isotopes such as $^{134,137}$Cs and $^{131}$I, given their significant contribution to population exposure (Métivier, 2002). Anspaugh et al. (1988) conducted an assessment of the health effects of the Chernobyl accident on human population in 1988. They concluded that, for a total of 800 million people exposed, the overall increase in cancer risk would be 97%. Moreover, the total number of victims was estimated to 17,400 by the year 2036 (Anspaugh et al., 1988). However, in 2005, the final report of the IAEA conference in Vienna, which evaluated the consequences of the Chernobyl accident, presented a lower estimate of 10,000 victims (IAEA 2005). Subsequently, in 2006, a critical study by Fairlie and Sumner (2006) was published, challenging the conclusions of the Vienna Conference, and proposing an estimate of victims within the range of 30,000 to 60,000.

In the aftermath of this and other accidents, vast areas of terrestrial and aquatic ecosystems have been contaminated. The contamination of any site poses a multigenerational problem for all living organisms (Métivier, 2002). These accidents, along with their direct consequences, have impacted both individual organisms and their populations. Simultaneously, numerous ecosystem services were jeopardized. Due to bioaccumulation, several radionuclides, including $^{134,137}$Cs, permeated into food webs (Wehrden et al., 2012).

Cesium, having capability to substitute for potassium in both animal and plant systems (Collander, 1941; Menzel and Heald, 1955; Relman, 1956), can distribute throughout the plant including flowers and fruits (Zhu and Smolders, 2000). Currently, the characteristic symptoms of cesium metal toxicity include profusion of adventitious roots, abnormal development of plant parts, a reduced dry matter, diminished chlorophylls and carotenoids content, and necrosis in various plant parts or potassium starvation (Cline and Hungate, 1960; Kordan, 1987; Shalygo et al., 1997 ; Hampton et al., 2004). Exposure to radioactive isotopes of Cs leads to a reduction in the number of leaves and new shoots, a decrease in overall growth, or an almost complete cessation of growth (Fan et al., 2014). This exposure results in inhibited germination and development, reduced leaf length/width ratios (Kim et al., 2011), impaired longitudinal leaf growth (Kim et al., 2011; Saakov et al., 1992), and growth abnormalities (Majeed et al., 2018). Furthermore, a decrease in total chlorophyll content in leaves and the amount of carotenoids is observed (Pernis et al., 2020), indicative of acute radiation damage. This damage encompasses genomic

harm, production of free radicals, and reactive oxygen species (Wi et al., 2007; Majeed et al., 2018). Additionally, differential expression of hundreds of genes has been documented, including the down-regulation of metabolic processes that affect chlorophyll metabolism (Volkova et al., 2021) and the formation of proteins related to storage, disease/defense, metabolism, energy, and cell structure (Pernis et al., 2020).

In living organisms, radiocesium toxicity arises from γ-ionizing radiation emitted from external sources such as air, water, and soil, or from its decay products, like $^{137}$Ba. β subatomic particles primarily exert their effects after being incorporated into organisms (UNSCEAR, 2000). Despite decades of research, limited data exists on vegetation responses to radiocesium, as highlighted by Burger and Lichtscheidl (2018). The precise identification of factors influencing Cs uptake is crucial for both *in situ* agricultural/environmental management and engineering strategies of affected areas. Moreover, it is essential for following big scale radiation protection of the population exposed through food webs.

While information on the uptake and accumulation of cesium by the roots of terrestrial higher plants has been abundant over the last 70 years, insight into the uptake of Cs by aquatic macrophytes from the water column and their interactions with other ions are scarce (White and Broadley, 2000; Burger and Lichtscheidl, 2018). Due to the still inadequate amount of data, a study was conducted with the submerged aquatic macrophyte *Ceratophylum demersum* (see Chapter 4).

In contrast, there is a greater abundance of studies detailing the ability of plants to uptake cesium and their transfer coefficients in the terrestrial environment across various soil types. In the context of safeguarding foodstuffs and adhering to hygiene standards aimed at ensuring the radiation safety of the population, the study discussed in Chapter 5 was undertaken. The experimental work occurred in a field environment simulating the early phases following an accident, covering six common types of soil found in the Czech Republic and in the European Union. The impact on commonly cultivated plant species, namely *Lactuca sativa*, *Allium cepa*, and *Raphanus sativus*, was also assessed, considering potassium fertilization as a countermeasure to mitigate the uptake of $^{134}$Cs.

The aforementioned studies primarily focus on the uptake of radionuclides by plant species in aquatic and terrestrial environments. They emphasize the necessity of

understanding the transfer of radionuclides from the growth medium, taking into consideration the ambient conditions and the interplay of multiple factors. This understanding is crucial not only for assessing the environmental state and the potential for radionuclide transfer into the food chain but also for developing effective decontamination strategies. Within the realm of environmental engineering practices aimed at decontamination, the objective is to remediate radionuclides from the environment and mitigate their impact. While decontamination techniques for terrestrial environments are well-established and indispensable (Vovk et al., 1993), the quest for effective methods to decontaminate and protect aquatic habitats is ongoing (Shibata et al., 2016). One of explored practice is the electrokinetic process, which has been tested for pollution reduction in mine waters (Haidari et al., 2023) and applied to the decontamination/desorption of $^{137}$Cs from contaminated soils, utilizing leachate (Korolev, 2009). In the study presented in Chapter 6, we examine the influence of the electrokinetic process on *Phragmites australis* cultivated under hydroponic conditions.

## 1.1  Cesium uptake in terrestrial ecosystems

In the years following the discovery of cesium uptake, researchers primarily focused on the absorption of cesium and radiocesium by consumable plants and the fixation of cesium in the soil. This surge in research stemmed from growing concerns about cesium's harmful effects on plants, particularly regarding ecological disturbances, crop productivity, food safety, and consequent impacts on human health (Wittwer and Teubner, 1959; Okuda et al., 1960; Jung et al., 2015). The uptake of radiocesium by plants is often quantified using the transfer coefficient (TF), a ratio that commonly compares the activity of cesium in the plant's dry matter to that in the soil. Soil's complex nature means that cesium uptake is influenced by numerous factors, including soil composition, pH, temperature, and moisture, as well as the presence of different plant species and ages and the effects of their life processes, along with surrounding environmental factors. These contribute to the overall variability, characterized by countless interactions.

Subsequent studies have shown that in soils with increasing proportions of organic matter, plant species such as *Triticum vulgare* (Monira et al., 2005), *Brassica rapa*, *Brassica juncea*, and *Raphanus sativus* (Aung et al., 2015) exhibit higher uptake of $^{134,137}$Cs. Concurrently, Tegen and Dörr (1996) found in their research on the mobility of $^{137}$Cs in forest soils that fresh organic matter readily binds cesium, which is subsequently released by bacterial activity. In peat and sandy soils with more than 10% organic matter content, there was a noted increase in the transfer of $^{137}$Cs to terrestrial plant species (Van Bergeijk et al., 1992). Correspondingly, Fesenko et al. (2002) observed similar results in various wetland species of peat and bog meadows, such as *Equisetum palustrae*, *Eriophorum vaginatum*, *Scheuchzeria palustris*, and *Sphagnum angustifolium*. In related study, Van Bergeijk et al. (1992) categorized and compared the relative transfer coefficients (TF) of cesium in soils, observing the impact of organic matter content. They found that the effect of organic matter is, on average, a factor of 2 in soils with 5–20% organic content, a factor of 4 in soils with 21–50% organic content, and a factor of 10 in soils containing more than 50% organic matter. Additionally, their study revealed that TF values for cesium were lower in clay or loamy soils compared to sandy soils. Subsequent analyses of extensive arable crop databases indicated that TF for radiocesium appears to be independent of soil radiocesium concentrations (Nisbet and Woodman, 2000). Rather, it is more closely related to the concentration of mobile and exchangeable ions, which significantly influence the plants' ability to uptake cesium (Fesenko et al., 2002). Recent studies have demonstrated that soil organic matter does not significantly contribute to the specific retention of cesium. Instead, the selective retention of cesium in soils is primarily associated with the presence of illite (Cremers et al., 1988; Berns et al., 2018) and other clay minerals (Cornell, 1993). Additionally, loamy soils have been shown to be more effective in fixing cesium radionuclides compared to sandy soils, leading to reduced migration of introduced contaminants in loamy soil types. In sandy loam soils, the availability (exchangeable fraction) of contaminants was found to be lower immediately after application than in sandy soils (Roca and Vallejo, 1995). Regarding the ability of minerals to adsorb cesium, research indicates that zeolite possesses the highest capacity, with an adsorption rate of 200 mg/g. Other minerals such as biotite (Fujita et al., 2013; Mukai et al., 2016), montmorillonite, and muscovite also exhibit high cesium adsorption capacities of around 50 mg/g. In contrast, minerals like feldspar,

hematite, calcite, and carbon exhibit maximum adsorption rates of up to 30 mg/g, while quartz shows the lowest capacity at 13 mg/g (Fujita et al., 2013).

The influence of soil pH on cesium uptake remains a subject of ongoing debate. Van Bergeijk et al. (1992) investigated cesium transfer from soil to plants and found that soil pH had a negligible effect on the cesium TF within a pH range of 3.9 to 8.4. Nisbet and Woodman (2000) arrived at similar conclusions while examining uptake in 28 different soil types. However, contrasting evidence exists from other studies highlighting the impact of pH on cesium uptake. Increased cesium uptake at low pH levels (4.6-5.1) was reported in meadow plant species such as *Trifolium repens*, *Trifolium pratense*, and *Rumex obtusifolius* (Ogura et al., 2014). This trend was also noted in the pH range of 4 to 5, as analyzed from the database of the International Union of Radioecologists (Frissel et al., 2002). Soil pH is influenced by temperature, among other climatic factors, as well as by the activity of soil edaphone (Koarashi et al., 2012). Consequently, temperature is a key factor affecting the uptake of cesium by plants. Initial evidence of this was observed in sheep farm pastures, where data indicated higher exchangeable $^{137}$Cs levels during winter months, leading to increased soil-to-plant transfer in the spring (Rafferty et al., 1994). Kovacheva et al. (2014) documented similar findings. In their study, freezing loamy-sand and sandy-loam soils for a month resulted in a twofold increase in the available fraction of $^{137}$Cs.

The relationships between individual factors and soil types, particularly in relation to their composition, should not be overlooked. This was demonstrated in an experiment by Kovacheva et al. (2014), where, in addition to temperature exposure, soils in some treatments were subjected to drying and varying temperature levels. In fluvisols, drought and rapid temperature decrease led to a reduction in the soluble forms of $^{137}$Cs, whereas in cambisols, the trend was reversed. Moreover, long-term exposure to drought and cold resulted in an increase in the water-soluble fraction of $^{137}$Cs in both soil types. Similar observations have been documented historically; Shalhevet (1973) noted an increase in $^{137}$Cs uptake by *Sorghum vulgare* under drought conditions, and Garten and Paine (1977) reported comparable results for *Sagittaria latifolia*. Ehlken and Kirchner (1996) investigated German pastures with varying soil types and confirmed a negative correlation between $^{137}$Cs uptake and soil moisture. Additionally, Win et al. (2015) observed a similar effect in two varieties of *Vigna mungo* grown in the tested soils. However, contrasting evidence

regarding the effect of humidity on cesium uptake exists. Studies such as those by Prister et al. (2003) and Prorok et al. (2006), which investigated the behavior of ^{137}Cs in the soil-plant system within the Chernobyl exclusion zone, and research by Goncharova (2006) examining the responses of various field crops and environmental factors to ^{137}Cs uptake, provide alternate perspectives.

The uptake of cesium from soil is influenced not only by intraspecific morphological and physiological factors of plants but also by interspecific interactions. This includes the effects arising from biological activities such as the excretion of aliphatic acid exudates (Khan et al., 2023). The role of oxalate anions, derived from oxalic acid exudates, in enhancing the availability of cesium from the rhizosphere by desorbing it from illite minerals has been established (Wendling et al., 2004). Furthermore, increased availability and uptake of cesium have also been observed in the presence of citric and tartaric acids (Chiang et al., 2005). In their 2011 study, Chiang et al. explored the impact of acetic, succinic, oxalic, tartaric, and citric acids on cesium's availability for plants, finding a gradation of effectiveness from the least effective acetic acid to the most effective citric acid.

These factors collectively influence the intake of cesium in plants. However, when considering the uptake by field crops, it is crucial to also consider agronomic practices such as fertilization, particularly the role of potassium, which is a major component of fertilizers.

## 1.2  Role of Potassium in Cesium uptake

Potassium is an essential macronutrient needed in plant cells to activate enzymes, stabilize proteins and nucleotide structure. The concentration required in the cytoplasm for all these functions ranges from 100 to 150 mM (Leigh and Wyn Jones 1984; Marschner 2011). The Cs cation shows almost identical properties to the K cation (Avery 1995). Therefore, $Cs^+$ can inhibit $K^+$ channels in the plasma membrane and compete for binding sites in proteins (White and Broadley 2000). Potassium concentrations are commonly present in the millimolar range in soil solution, as documented by Marschner (2011). In contrast, its concentrations in aquatic environments display considerable variation. For example, rainwater generally

contains about 1 µmol per liter of potassium, while the concentration in Central European lake waters ranges from 10 to 100 µmol per liter. In African lakes, these levels are even more pronounced, ranging from 100 to 10,000 µmol per liter, as reported by Talling (2010). On the other hand, cesium is usually present in much lower concentrations, ranging from the nanomolar to the very low micromolar concentrations (Rai and Kawabata 2020; White and Broadley 2000). Therefore, as noted by Hampton et al. (2004), cesium toxicity is very unlikely to occur in most natural environments.

Collander (1941) was the first to report on plants accumulating $Cs^+$ from a complete nutrient medium, presenting analytical data that showed certain plant species consistently exhibited high contents of $K^+$, $Rb^+$, and $Cs^+$, while others consistently displayed low levels of these elements. This led to the conclusion that $Cs^+$ is absorbed by roots as readily as $Rb^+$ and almost as easily as $K^+$ (Collander, 1941). Epstein and Hagen (1952) observed that $K^+$ and $Cs^+$ competitively influence the absorption of $Rb^+$, suggesting these ions share the same binding sites or reactive centers. Menzel and Heald (1955) identified a discrimination between $Cs^+$ and $K^+$ in nutrient mediums, noting that cesium is absorbed by roots only 0.2 times faster than potassium, on average (Menzel and Heald, 1955). Cline and Hungate (1960) also confirmed average discrimination of $Cs^+$ from a solution containing both $K^+$ and $Cs^+$, with values ranging from 0.05 to 0.8, depending on the concentration of $K^+$ in the substrate. Middleton et al. (1960) reported varying discrimination of $Cs^+$ across different concentrations of potassium and cesium cations in the nutrient solution. A study involving *Hordeum vulgare* roots further confirmed this discrimination in intake, measuring the relative intake of $^{137}Cs$ and $^{42}K$. The interference of other mono- and divalent cations with $Cs^+$ absorption on barley roots was studied, finding that $K^+$, $Rb^+$, and to a lesser extent, $NH_4^+$, inhibit $Cs^+$ uptake, indicating competition for the same binding site (Bange and Overstreet, 1960). However, Zhu and Smolders (2000) noted that the most common cesium uptake discrimination values in the presence of potassium range from 0.1 to 0.8.

Research in the 1950s, recognizing the discrimination of cesium uptake in the presence of potassium, shifted focus to the competition between these two ions in plant uptake from growth mediums and soil. Cline and Hungate (1960) observed a rapid decrease in $^{137}Cs$ content in *Phaseolus vulgaris* dry matter upon the application of potassium, with significant reductions noted at concentrations of 0.25 mM in the

growth medium, followed by a slower decrease up to 20 mM. Interestingly, in leaves, an increase in accumulated $^{137}$Cs was also recorded. The variations observed were as follows: one instance showed an increase in $^{137}$Cs uptake in the range of 0.25 – 2 mM external potassium concentrations, while another indicated an increase between 2 – 20 mM. Complementing these findings, a study by Smolders et al. (1996) on *Triticum aestivum* cv Tonic revealed that potassium fertilization significantly reduced $^{137}$Cs uptake. This was evidenced by a decrease of 123 times in stems and 300 times in roots. Zhu (2001) reported similar findings in hydroponically grown *Triticum aestivum*, noting a hundredfold difference in $^{137}$Cs uptake between the lowest (2 mg/L) and highest (40 mg/L) external potassium concentrations. Wallace et al. (1983) found a decrease in Cs uptake in all parts of *Phaseolus vulgaris* with increasing potassium concentrations. Conversely, Soudek et al. (2004) investigated the effect of varying potassium concentrations on $^{137}$Cs uptake in *Helianthus annuus*, finding that a 1 mM external concentration of K led to a twofold or greater increase in cesium uptake compared to higher potassium levels. In 2006, Soudek et al. expanded this experiment by including $NH_4^+$ in the growth medium, discovering a synergistic effect of external K and $NH_4^+$ on cesium uptake, with the highest $^{137}$Cs accumulation at 10 mM K and 12 mM $NH_4^+$. Smolders et al. (1997) also observed a weak positive correlation between potassium and cesium in hydroponic media for *Spinacia oleracea* cv. Subito, with a more significant reduction of accumulated radionuclides seen for calcium and magnesium.

These results yield conflicting conclusions regarding the effect of added potassium in growth media. In soil environments, the application of potassium fertilizer demonstrates both a reduction and, in some cases, no effect on cesium transfer from soil to plants. Squire and Middleton (1966) studied the uptake of $^{134,137}$Cs by *Lollium perene* in four different soil types with varying concentrations of exchangeable potassium and clay content from 1959 to 1963. They found that potassium fertilization effects varied by soil type: soils with low exchangeable potassium content showed a 20-50% decrease in cesium radionuclide uptake, while those with higher potassium content exhibited little change from pre-fertilization levels. This variability was attributed to the decreased availability of radiocesium to plants and its interaction with clay particle adsorption. Additionally, they noted climatic variability and plant morphological differences as influential factors over the first three years of the study. Similarly, a comprehensive experiment conducted by

Haak (1985) between 1961 and 1982 examined various soil types (loam, clay loam, and silty clay) and agricultural crops (oats, peas, mustard, wheat, barley, and rape) under conditions closely resembling reality. The data analysis from this extended period revealed a consistent decrease in cesium uptake across all soil and crop types, proportional to the amount of fertilizer applied. Likewise, higher $^{137}$Cs TFs in tropical regions for plants like *Cocos nucifera*, *Musa* sp., and *Carica papaya* were reported by Robison et al. (1997), who attributed this to the low content of clay minerals and available potassium in those regions. They observed a significant reduction in TF values following the application of potassium fertilizer. Belli et al. (1995) also conducted a two-year greenhouse experiment with meadow species such as *Phleum pratense*, *Lotus corniculatus*, *Dactylis glomerata*, *Lolium perenne*, *Poa trivialis*, and *Trifolium repens*. Their study, focusing on the impact of different fertilizer compositions, found the lowest $^{137}$Cs uptake with potassium-only fertilizer or a combination of phosphorus and potassium. Furthermore, Guillaume et al. (2012) conducted a study on 11 alpine plant species in pastures and waterlogged meadows, focusing on six different geochemical properties. Their analyses revealed a strong correlation between the TF for $^{137}$Cs and exchangeable potassium in these environments.

The studies presented here provide insights into the effects of potassium fertilization, demonstrating a negative correlation between the amount of external potassium and cesium intake. However, despite a general consensus in the cited publications, it is important to acknowledge studies that did not observe an effect of potassium fertilization on cesium uptake. For instance, Ban-nai and Muramatsu (2002) investigated the TF of *Raphanus sativus* for selected radionuclides across ten different types of Japanese soils. Their evaluation revealed no correlation between soil potassium content and $^{137}$Cs intake in any soil group, even with the inclusion of potassium fertilization. In a similar vein, Malm et al. (1991) carried out a greenhouse study on five common vegetable species — *Cucumis sativus*, *Lycopersicon esculentum*, *Petroselinum crispum*, *Raphanus sativus*, and *Lactuca sativa* — grown in peat and compost highly contaminated after the Chernobyl accident. Their findings indicated no change in $^{137}$Cs accumulation following the application of potassium fertilizer. Additionally, Nisbet and Woodman (2000) conducted an extensive study that included both published and unpublished data from 28 combinations of crops and soils, along with soil characteristics. Despite some studies in their database

showing a negative correlation of $^{137}$Cs intake with increasing exchangeable potassium, this relationship was not universally observed.

As previously mentioned, potassium and cesium compete for the same uptake mechanisms. Bange and Overstreet (1960) established that Cs$^+$ uptake and transport occur through both high and low-affinity potassium channels. Schroeder (1994) categorized high-affinity K$^+$ transporters as either proton pumps or cotransporters, with turnover rates ranging from $10^2$ to $10^4$ ions per second, becoming saturated or suppressed at concentrations higher than 200 μM K$^+$. These transporters exhibit ion translocation rates in the range of $10^6$ to $10^7$ per second, comparable to the fastest diffusion-limited enzyme reaction rates (Heginbotham and Mackinnon, 1993). The KT/KUP/HAK family of proteins, which shows poor discrimination between Rb$^+$ and Cs$^+$ analogues, is considered a crucial group of high-affinity Cs$^+$ transporters in plants (Véry and Sentenac, 2003). Additionally, the Trk/Ktr/HKT family of proteins, while playing a less significant role in high-affinity K$^+$ uptake, is involved in various functions related to K$^+$ or Na$^+$ uptake and adaptation to osmotic or salt stress (Corratgé-Faillie et al., 2010). Significant developments occurred when Spalding et al. (1999) discovered that AKT1, part of the high-affinity system, remains permeable at external K$^+$ concentrations ranging from 10 to 1000 μM. This finding diminished the perceived importance of the low-affinity K$^+$ uptake system outlined by Bange and Overstreet in 1960. Additionally, the voltage-controlled family of Shaker-type proteins has been identified, playing a critical role in the plasma membranes of various plant tissues and organs. The outward-rectifying (ORC) and inward-rectifying (IRC) K$^+$ channels maintain plant cell homeostasis (Gambale and Uozumi, 2006). IRC K$^+$ channels, along with proton pumps, adapt to a range of low-affinity K$^+$ concentrations, influencing membrane potentials and shifting activation potential for efficient K$^+$ uptake. They may act as a backup for K$^+$ uptake under environmental or genetic stress (Schroeder, 1994). Unlike other K$^+$ channels, IRC K$^+$ channels have a unique long-term function, and plant membranes are well-adapted to facilitate K$^+$ uptake under varying conditions. IRCs aid K$^+$ uptake into cells, while ORCs support K$^+$ efflux from root cells. In xylem parenchyma cells, these channels distribute K$^+$ to the xylem for shoot transport (White, 1997; White and Broadley, 2000). Furthermore, calcium permeable channels like HACC and DACC (Hampton et al., 2005), and voltage-dependent Ca$^{2+}$ channels VDCC2 (White, 2000; White et al., 2002) are also important transport mechanisms. While high-affinity and inward-rectifying

transporters mainly uptake monovalent cations such as $K^+$, $Cs^+$, $Rb^+$, $NH_4^+$, $Na^+$, and $Li^+$ (Sacchi et al., 1997; White, 1997), calcium permeable channels facilitate a wider range of monovalent and divalent cations including $K^+$, $Cs^+$, $Rb^+$, $Na^+$, $Li^+$, $Ba^{2+}$, $Sr^{2+}$, $Ca^{2+}$, $Mg^{2+}$, $Co^{2+}$, $Mn^{2+}$ (White, 2000; White et al., 2002). The large number of cations potentially influencing the uptake mechanisms of potassium (K) and cesium (Cs) in substrate or growth media necessitates consideration of the interactions among individual elements.

This section delineates key factors that must be considered when addressing cation interactions within soil matrices. Few studies have addressed the effects of other cations. Some suggest that adding calcium to the growth medium can either increase (Bange and Overstreet, 1960) or decrease cesium uptake (Smolders et al., 1997). Smolders et al. (1997) also noted a decrease in cesium uptake with added magnesium. Bange and Overstreet (1960) observed that adding $Na^+$ reduced the plant roots' ability to uptake cesium. Furthermore, a positive correlation was found for Li with cesium intake (Bange and Overstreet, 1960; White, 1997), and a significant positive correlation for elements like As, Rb, Al, Fe, Ni, and a negative one for Ba (Chu et al., 2015). Other elements, including Cu, Ni, Cd, Sn, Co, were also studied (Bréchignac et al., 2000; Scotti and Carini, 2000; Kamel et al., 2007). It's important to note that ions such as $H^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$, and $Ba^{2+}$ can dissolve or desorb up to 10% of total $^{137}Cs$ from soil (Schulz, 1965), while some divalent cations inhibit plant influx pathways (Rai and Kawabata, 2020). Desorption rates of 20-80% for $^{137}Cs$ were observed for stable isotope Cs, $NH_4^+$, and $K^+$ (Schulz, 1965). Potassium sorption/desorption dynamics in soil are also crucial. Hundal and Pasricha (1998) found that K adsorption capacity in soils was higher at lower temperatures (25°C) compared to higher temperatures (40°C). The lower adsorption of K at higher temperatures was noted in soils with increasing clay content with depth, suggesting significant losses due to percolating water during tropical days (Hundal and Pasricha, 1998). Additionally, Rai and Kawabata (2020) pointed out that the K:$^{134,137}Cs$ ratio is as high as $10^3$ in soil solution, whereas in studies, the K and Cs values are proportionally different or even 1:1. Similarly, Frissel et al. (2002) identified a limit of 19.5 mg/kg of exchangeable potassium in environments contaminated with only $^{134,137}Cs$. Below this limit, Cs uptake is influenced by exchangeable K, but above it, other factors become influential. Excessive exchangeable potassium can cause both dilution of cesium ions and its desorption in soils with high organic matter content

(UNSECAR, 2000). Furthermore, plant species sensitivity to decreased exchangeable K in the soil varies (Guillaume et al., 2012).

These examples illustrate a complex system of interconnected factors. Nisbet and Woodman (2000) summarize that results are often difficult to compare due to non-uniform methodologies, the age of radioactive fallout, and variations in leachable determinations of exchangeable potassium across studies (Nisbet and Woodman, 2000).

## 1.3  Cesium uptake in aquatic ecosystems

Considering that some species of aquatic macrophytes absorb at least some K from the water column, K absorption sites may serve as sites for Cs absorption. Therefore, foliar absorption must be considered a potential pathway for Cs uptake by aquatic macrophytes (Barko and Smart, 1981; Barko, 1982; Barko et al., 1988). As Haynes and Goh (1977) noted, leaf absorption requires entry into the phloem, overcoming the cuticular barrier and transport through epidermal and mesophyll cells' apoplastic pathway or active absorption by leaf cell plasma membranes. Subsequent transport occurs through the symplastic pathway within cells. Monovalent Cs cations use potassium uptake systems, first identified by Bange and Overstreet (1960), for traversal in plants. Hull et al. (1975) identified three main routes for cuticular absorption in terrestrial plants: stomatal, trichome, and direct cuticular. The predominance of each route varies based on factors like plant species, developmental stage, and environmental conditions. Schönherr (1976) found that cuticular membranes of submerged leaves, such as those of *Potamogeton* sp., are three times more permeable to water and lack cuticular waxes, adaptations that enable direct absorption from the water column.

The studies on cesium uptake by aquatic plants are less extensive than those for terrestrial plants. The first significant study in this area was conducted by Ahamer et al. (1989), who sampled 26 different lake, pond, wetland, and river sites. Their research focused more on the influence of site parameters on the TF of cesium, rather than on plant uptake per se. A notable advancement in this field occurred in 1986 when Kelly and Pinder (1996) investigated three macrophytes in the cooling

tank of a decommissioned nuclear reactor. *Brasenia schreberi*, *Nymphaea odorata*, and *Nymphoides cordata* exhibited rapid increases in ^{137}Cs concentrations in plant matter within hours and days of immersion. By the experiment's end, these plants reached the same ^{137}Cs levels as those growing naturally in the tank. Expanding on this line of research, Pinder et al. in 2006 conducted an experiment to determine the rate and likelihood of leaf absorption of cesium by aquatic macrophytes from the water column. After contaminating a water body with [133]Cs, they observed a swift increase in [133]Cs concentrations in all species. Notably, cesium uptake was seen in two floating species (*Brasenia schreberi* and *Nymphaea odorata*) and two submerged species (*Myriophyllum spicatum* and *Utricularia inflata*). However, the authors concluded that root uptake played a more significant role than foliar absorption. In their long-term observation of cesium behavior in two Finnish ponds and surrounding areas, Saxén and Ilus (2008) cataloged ^{137}Cs activities across various aquatic plants. They noted a decreasing trend in ^{137}Cs activities, from the highest values in *Equisetum fluviatile* to the lowest in *Sparganium gramineum*. This study, however, did not delve into other influencing factors. During a twelve-year study on the Greek river Pinios, Sawidis et al. (2011) investigated six species of macrophytes using stored ^{137}Cs post-Chernobyl accident. They found that *Ceratophyllum demersum* leaves had a higher absorption capacity for cesium compared to other species, followed by *Myriophyllum spicatum*, *Paspalum pasalodes* Scribner, *Cladophora glomerata*, *Cyperus longus*, and *Potamogeton nodosus*, with the latter showing limited cesium absorption capacity. Two years after the Fukushima nuclear power plant accident, Sasaki et al. (2016) surveyed ^{137}Cs in aquatic plants and algae in the vicinity. They recorded concentrations between $10^3$ and $10^4$ Bq/kg, with *Potamogeton crispus* showing the highest accumulation rate of $2.69 \times 10^4$ Bq/kg. *Trapa bispinosa* and *Nymphaea tetragona* exhibited nearly 50% lower values, and in *Potamogeton distinctus*, the accumulation was lower by up to 80%. At this time, Kowata et al. (2014) identified *Egeria densa* as a species efficiently absorbing cesium into its roots and stems near Fukushima as well. Furthering these studies, Nagakawa et al. (2019) investigated cesium transfer in 11 species of emergent, submerged, and floating-leaved macrophytes. While potassium levels remained consistent across species, cesium levels corresponded more to the life form of the plant, with submerged and floating-leaved species accumulating more ^{137}Cs than emergent species.

Cesium transfers and factors affecting its uptake have significant implications for the phytoremediation capabilities of plants. However, the literature focusing on the use of aquatic plants for this purpose is relatively scarce. One of the few species studied is *Myriophyllum spicatum*, as identified by Saleh et al. in 2020. This plant was chosen based on its stable uptake of $^{137}$Cs under varying light conditions and its response to different phytoremediation parameters. For instance, at pH 8, the plant showed a 6% change in cesium intake compared to more acidic hydroponic solutions. Notably, plant weights of three grams were more effective in cesium accumulation than both lighter and heavier plants. Regarding external concentrations of $^{137}$Cs, a decrease in efficiency was observed in the 50 mg/L group compared to other concentrations within the $20 - 150$ mg/L range. *Eichhornia crassipes*, another species identified for phytoremediation, was studied by Saleh in 2012. The research highlighted an ideal pH of 4.5 for optimal cesium uptake. Moreover, plants exposed to light were found to accumulate 30% more cesium compared to those in shade or darkness. In 2017, Rinaldi et al. explored the phytoremediation capabilities of *Calla palustris*. They experimented with different growth media, including expanded clay, sand with perlite, water, and water with added potassium. The plants accumulated more cesium from water than from expanded clay or sand with perlite substrates. Hydroponically grown plants showed a 32% removal rate of cesium, while the values for the sand with perlite mixture did not surpass those of the control. Additionally, adding 114 mg/L potassium to the hydroponic medium decreased cesium accumulation by 74% in green leaves and 83% in roots. Kondo's 1982 study on *Lemna gibba* provided insights into the plant's uptake of potassium and cesium. The research demonstrated that, even with 7.8 mg/L of potassium, cesium was absorbed at a similar rate. However, at a cesium concentration of 5.9 mg/L, potassium uptake was almost entirely inhibited. This finding is particularly relevant in the context of the Fukushima nuclear power plant, where, as Kowata et al. (2014) noted, local potassium concentrations were insufficient to affect accumulation of $^{137}$Cs. Further research by Komínková et al. in 2018 on *Calla palustris* investigated the impact of potassium ($39.1 - 781.9$ mg/L) on cesium accumulation. The results indicated a reduction in cesium uptake by 44-74% across different treatment groups, with the highest accumulation observed in potassium-starved positive control plants.

Considering the anatomical and physiological variations in Cs uptake and transfer in plants, environmental conditions influencing uptake are crucial. Pinder et al. (2006)

observed in experimental water reservoir contamination that dissolved [133]Cs concentrations decreased rapidly within the first 20 days and more gradually over the next 120 days. This initial reduction was primarily due to horizontal and vertical mixing of the water surface. Dissolved Cs in freshwater environments settles on lithogenic particles and sediment surfaces, impeding biological assimilation and subsequent transfers, as shown by Evans et al. (1983) and Comans et al. (1989).

Suspended particles can increase apparent radionuclide concentrations, leading to overestimation of absorption rates and concentration ratios (Sansone et al., 2002). Salo and Saxon (1974) suggested that humic substances in water bodies bind to ^{137}Cs, while in sediments, ^{137}Cs may associate with clay minerals and be displaced by other cations (Lomenick and Tamura, 1965). Binding sites in sediments include iron and manganese oxides or decaying organic matter, but only a small portion (2%) of the total ^{137}Cs is bound by humic and fulvic acids (Brisbin et al., 1974; Evans et al., 1983). Penttilä et al. (1993) showed that humic substances only react with ^{137}Cs under specific laboratory conditions and do not significantly reduce its bioavailability in natural environments.

Cs exhibits strong selective sorption by phyllosilicate components in soil, sediments, and suspended particles (Francis and Brinkley, 1976). It initiates ion exchange with hydrated cations on silicates, following a selective trend of the Hofmeister series (Leberman, 1991; Zachara et al., 2002). Cs is also sorbed selectively to weathered edges of micas and their weathering products (Zachara et al., 2002), demonstrating slowly reversible sorption (Comans et al., 1989). Wang et al. (2017) confirmed that ^{137}Cs sorbed on sediment is displaced by ions in the order $NH_4^+ > Mn^{2+} > K^+ > Mg^{2+} \approx Ca^{2+} > Na^+ > Sr^{2+}$. They found that ^{137}Cs is less mobile in oxic soft water (Kd: $2.0 \times 10^3$ mL/g) and more mobile in anoxic soft water (Kd: $0.2 \times 10^3$ mL/g), with no significant difference in Kd values under oxic and anoxic conditions in hard water. The desorption experiment also confirmed that ^{137}Cs is more mobile in soft water than in hard water.

Temperature significantly influences adsorption capacity and the nature of the sorbate in solution (Salleh et al., 2011; Yagub et al., 2014). An increase in adsorption with rising temperature suggests an endothermic process, while a decrease indicates an exothermic process. Sansone et al. (2002) found that periphyton concentrations of [133]Cs or ^{137}Cs resulted partly from the attachment of suspended

mineral particles, with a significant portion of Cs bound to inorganic particles, contributing to Cs accumulation in periphyton.

These studies collectively represent a small yet significant portion of the research exploring the uptake of cesium in aquatic plants, including the effects of potassium additives and their interactions with the environment. While limited, this research is vital for comprehending the mechanisms of cesium uptake in aquatic species and their application in phytoremediation strategies.

## 1.4  Electrokinetics and Cesium Uptake

Contrasting with phytoremediation, the most common decontamination method in terrestrial habitats involves mechanical removal of the upper soil layer, including vegetation and root mat, which can achieve an efficiency of up to 100% (Vovk et al., 1993). In aquatic environments, the removal of the upper sediment layer was explored by Nishikiori and Suzuki in 2017, specifically in a 170-meter section of the Kami-Oguni River. However, they noted that this method, removing 15-35 cm of sediment, may not be optimal for river decontamination due to the spatial variability of $^{137}$Cs in sediment. The urgency for effective aquatic decontamination techniques was emphasized by Shibata et al. (2016). They tested a new type of sorbent, hydroxyapatite, which reduced radionuclide concentrations in freshwater by 68% over a 13-week trial. Other experimental methods have shown varying degrees of effectiveness. For instance, autoclave leaching of soil at 160°C resulted in 99% Cs removal (Nikolaevsky et al., 2013), while soil washing left 72.6% of Cs in the extract (Saito et al., 2014). Ion exchange using 1 mol of ammonium acetate could replace $^{137}$Cs with $K^+$ or $NH_4^+$ ions (Stepina and Popov, 2011), and magnetic separation had a 20% efficiency (Yoshida et al., 2014). Electrokinetic washing, used for decontaminating concrete in nuclear power plants, achieved up to 76.3% efficiency (Kim et al., 2009).

Electrokinetics, the study of electrically charged fluids dynamics (Castellanos, 1998), involves applying direct current through a soil mass or fluid between two electrodes (Roehl et al., 2005). In environmental engineering, it's seen as an innovative in situ method for decontaminating soils polluted by metals, anions, radionuclides, polar

organic compounds, and other toxic substances (Acar and Alshawabkeh, 1993; Bi et al., 2011; Cameselle et al., 2019; Reddy and Cameselle, 2009; Roehl et al., 2005). This method has also been applied in urban wastewater treatment (Elazzouzi et al., 2017; Murugananthan et al., 2004; Qian et al., 2018), mine waters (Haidary et al., 2023; Nariyan et al., 2018), groundwater (Amarine et al., 2020), and in some cases, in hydroponic systems (Cifuentes-Torres et al., 2021) and coupled with phytoremediation (Haidary et al., 2023). Electrokinetics has been tested for removing $^{137}$Cs from soil, but its main drawback was the use of various chemical leaching agents (Kim et al., 2003; Kim et al., 2013; Prozorov et al., 2000). In subsequent years, tests combined electrokinetics with phytoremediation in soil environments, targeting heavy metals (Aboughalma et al., 2007; Bi et al., 2011; Mao et al., 2016). Haidary et al. in 2023 presented the first results of decontaminating heavy metals from mine waters using electrokinetics paired with phytoremediation. However, as of this date, no studies have been conducted on the use of electrokinetics coupled with phytoremediation for the treatment of $^{137}$Cs in aquatic environments.

# 2 Aims

The objectives of this work and the accompanying research publications are to:

(i) bring a better understanding of $Cs^+$ uptake from the water column; (ii) identify specific factors affecting $Cs^+$ uptake in the aquatic environment; (iii) describe soil $Cs^+$ uptake and discuss effects induced by potassium fertilization; (iv) evaluate the effect on $Cs^+$ uptake by plants grown hydroponically under the action of an electric field; and v) assess the results for their potential application in phytoremediation and radiation protection in foodstuff.

# 3 Methods

This work is based on multiple experiments conducted between 2018 and 2023. Greenhouse experiments were carried out in the experimental greenhouses of the Czech University of Life Sciences Prague, while field experiments took place in ponds near Lomnice nad Lužnicí, Czech Republic. Experiments involving the radioactive isotope $^{134}$Cs were conducted at the National Radiation Protection Institute (NRPI) in Prague for aquatic plant research, and in Hradec Králové for the investigation of agricultural plant species.
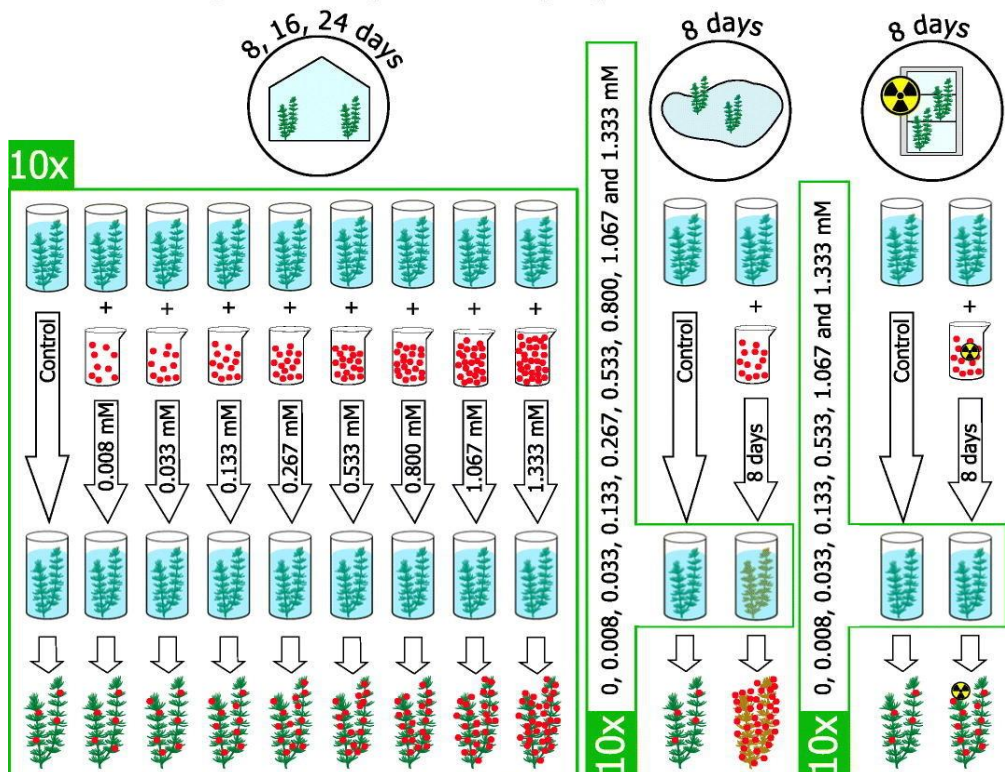
Methodological procedures are described in detail in the relevant chapters of the provided case studies.

# 4 Case study I: Uptake of [133]Cs and [134]Cs by *Ceratophyllum demersum* L. under field and greenhouse conditions

Graphical abstract

## Abstract

The phytoremediation abilities of Hornwort (*Ceratophyllum demersum* L.) were tested under greenhouse and field conditions. Plants were exposed for 8, 16, and 24 days (greenhouse with stable isotope $^{133}$Cs), 8 days (field with $^{133}$Cs), and 8 days (climabox with radioactive isotope $^{134}$Cs). The plants were exposed to different concentration of stable Cs provided as CsCl (0.008, 0.033, 0.133, 0.267, 0.533, 0.800, 1.067, and 1.333 mM) and different activities of $^{134}$Cs (4.46, 4.46, 4.74, 4.64, 2.23 and 2.26 kBq). The results of the experiment revealed a significant effect (p < 0.001) of exposure time on Cs uptake. The results showed highest average $^{133}$Cs removal rates of 11%, 17% and 19% for 8, 16, and 24 days, respectively, in the greenhouse, 10% for the $^{134}$Cs experiment, and 27% for the field experiment with $^{133}$Cs. The results indicated that increasing the length of exposure lowered the uptake ability, hence indicating that the plant has limited capacity for Cs removal. The accumulated amount of Cs by plants is significantly dependent (p < 0.001) on the concentration of treatment and complies to a sigmoid curve. Comparison of experiments revealed the greenhouse experiment with $^{133}$Cs and the experiment with $^{134}$Cs did not differ significantly in their removal rate. However, the field experiment was significantly different from the previous two (p < 0.001), providing a higher removal rate. *C. demersum* was also able to resist phytotoxic effects of Cs in the greenhouse experiment for 16 days without significant effects (p > 0.05) on health. Even after 24 days of exposure, the plant resisted up to 0.267 mM treatment concentration with no significant tissue lesion (p > 0.05). These results indicate that *C. demersum* has potential for remediating aquatic habitats, especially in the case of acute events, where a short duration of phytoremediation may take place.

## Introduction

The radioactive isotopes of cesium (Cs) $^{134}$Cs and $^{137}$Cs are common fission products (Kocherov et al. 1997; Browne et Tuli 2007; Singh et al. 2008). Their routine and accidental releases from nuclear facilities pose severe threats to aquatic ecosystems (Métivier 2002; Wehrden et al. 2012). Subsequent disturbances in environmental relations, disturbance in seasonal synchronism of ecologically-linked groups of organisms, a shift in internal relationships between consumers and producers, and changes in the biological pressure of parasites and predators have become some of the most serious threats to ecosystems (Tikhomirov et Shcheglov 1994). At the same

time, due to bioaccumulation, some of the radionuclides (including [134,137]Cs) penetrate into food webs (Wehrden et al. 2012). This is because Cs is capable of substituting potassium (K) in plant systems (Zhu and Smolders, 2000; Broadley et al., 2001; Hampton et al., 2005; Burger et Lichtscheidl 2018). In the case of plants, it can be relocated to leaves, flowers and fruits (Zhu et Smolders 2000). The abundance of adventitious roots, abnormal development of plant parts, reduction of dry matter, reduction of chlorophylls and carotenoids, and necrosis of various plant parts or K starvation are considered as characteristic of Cs toxicity (Shalygo et al. 1997; Hampton et al., 2004; Burger et Lichtscheidl 2018). However, radioactive isotopes of Cs represent greater health risks than [133]Cs due to the adverse effects of β and γ radiation (Williams et al. 2004). Radioactive effects on plants, ranging from small changes in populations, decreases in shoot growth rate, morphological alterations, degradation of seed quality and quantity, microsatellite mutations, to increased chromosomal aberrations, are summarized by Real et al. (2004). These adverse effects on individual organisms, as well as whole ecosystems, make Cs uptake and transfer in nature an important topic for continuous study.

Research into the intake of Cs from the soil or hydroponic media by terrestrial plants began in the early 1940s. In contrast, in the case of aquatic macrophytes, research began at the end of the 1980s. Smart et Barko (1988) proposed that some types of macrophytes absorb at least a portion of K from the water column. Since K absorption sites may function similarly for Cs, leaves must be considered a potential pathway for Cs uptake by aquatic macrophytes (Smart et Barko 1988), whereas foliage is the primary pathway for K uptake by other species (Madsen et Cedergreen 2002). Several studies have been carried out since the mid-1990s, describing both the behaviour of Cs in experimentally contaminated water bodies and its accumulation by aquatic macrophytes. The results suggest that aquatic macrophytes are capable of rapidly increasing Cs concentrations in plant matter (Kelly et Pinder 1996, Pinder et al 2006). Therefore, it can be assumed that immediately after contamination the average concentration values of dissolved Cs in the first metre of a water column will be rapidly reduced within the first 20 days. In the next 120 days, the concentrations decline more slowly (Pinder et al. 2006). However, all studied aquatic plant species have shown a large and rapid increase in Cs in plant biomass almost immediately after contamination of the water body (Kelly et Pinder 1996, Pinder et al. 2006). It should also be noted that in aquatic plants there are higher

transfer factors consistently found for both stable and radioactive Cs compared with terrestrial plants, even those grown hydroponically (Burger et Lichtscheidl 2018). These findings were supported by a laboratory experiment with water hyacinth (*Eichhornia crassipes*), where a rapid increase in $^{137}$Cs in biomass occurred within 10 hours of contamination (Saleh 2012). The study also considered the effect of light and pH on $^{137}$Cs accumulation. The highest removal rate was achieved when exposed to sunlight, while plants growing in laboratory light or shade had much lower accumulation values. Plants retained the same ability to accumulate Cs in the pH range 4.9-10.9 (Saleh 2012). Saleh (2012) observed the highest uptake of $^{137}$Cs from solution (almost 80%) in a group exposed to sunlight.

Other studies focused on monitoring $^{137}$Cs after the Chernobyl nuclear accident. One of these studies was conducted on the River Thessalia in central Greece, where Sawidis et al. (2011) found that the leaves of *Ceratophyllum demersum* have a high absorption capacity for $^{137}$Cs (54.5 Bq/kg in 1998 and 6.5 Bq/kg in 2010) compared to leaves of other species, for example *Myriophyllum spicatum* (45.7 Bq/kg in 1998 and 10.6 Bq/kg in 2010). *Potamogeton nodosus* had only limited absorption by leaves (3.3 Bq/kg in 1998 and 2.1 Bq/kg in 2010). In the years following the Fukushima accident, a few studies were conducted to monitor the status of freshwater ecosystems and the uptake of radionuclides by aquatic macrophytes. Sasaki et al. (2016) monitored concentrations of $^{137}$Cs in water reservoirs and aquatic plants growing in them at 4 localities no more than 7 km from the Fukushima nuclear power station. The highest concentrations of $^{137}$Cs for water reservoirs were in the range of 0.50-2.98 Bq/L on the perimeter of the power station. The concentration of $^{137}$Cs in aquatic macrophytes and algae was 103-104 Bq/kg, while in *Potamogeton crispus* it was 2.69 × 104 Bq/kg, which was the highest of all studied plant species. *Nymphaea tetragona* and *Potamogeton distinctus* contained the lowest concentrations of 103 Bq/kg (Sasaki et al. 2016). Kowata et al. (2014) measured $^{134,137}$Cs in Brazilian waterweed (*Egeria densa*) and water sediments of rivers, ponds, and drainage canals in Fukushima prefecture. Measured mean values of $^{137}$Cs were higher than 800 Bq/kg in sediment, while in plants it was lower than 75 Bq/kg. At one of the sites the values varied significantly from others with 78 Bq/kg in the sediment and 15 Bq/kg in plants, which could be due to the large amount of plant litter (Kowata et al. 2014). Subsequent laboratory tests with *Egeria densa* also confirmed foliar uptake and, after the addition of 0.2 mM CsCl,

concentrations in shoots were 2800 mg/kg and in the roots 3500 mg/kg (Kowata et al. 2014). Table 1 summarizes the activity of radiocesium in different species of macrophyta.

**Table 1:** Activity of radiocesium measured in different macrophytes collected in a field

| Study | Aquatic plant species | Max. Concentration | Locality |
|---|---|---|---|
| Kelly et Pinder 1996 | *Nymphaea odorata* | 8.5 kBq/kg | NPP cooling pond |
| | *Nymphoides cordata* | 19 kBq/kg | |
| Pinder et al. 2006 | *Myriophyllum spicatum* | 6.6 kBq/kg | NPP cooling pond |
| | *Brasenia schreberi* | 26 kBq/kg | |
| | *Utricularia inflata* | 29 kBq/kg | |
| Sasaki et al. 2016 | *Potamogeton crispus* | 26.9 kBq/kg | Fukushima prefecture |
| | *Trapa bispinosa* | 14.8 kBq/kg | |
| Kowata et al. 2014 | *Egeria densa* | 75 Bq/kg | Fukushima prefecture |
| Sawidis et al. 2011 | *Cyperus longus* | 62.3 Bq/kg | River Thessalia in central Greece |
| | *Paspalum pasalodes* | 75.1 Bq/kg | |
| | *Potamogeton nodosus* | 33.4 Bq/kg | |
| | *Ceratophyllum demersum* | 101.3 Bq/kg | |

Saleh (2012) and Kowata et al. (2014) also mention the use of aquatic macrophytes studied by them for the purpose of water habitat remediation. Phytoremediation is an *in situ* passive method utilizing solar energy, serving to mitigate the impact of contamination from different pollutants, including [134,137]Cs. These methods are safe and cheap, and they are able to remove, transfer or immobilize pollutants from contaminated habitats (Salt et al. 1998). Although aquatic plants are used for phytoremediation of heavy metals at this point, there are not many species suitable for phytoremediation of Cs (Sharma et al. 2015). In a survey of 183 strains of algae and 5 aquatic plants species, Fukuda et al. (2014) found that an appropriate aquatic plant for phytoremediation of ^{137}Cs is *Lemna aoukikusa*, as well as 3 species of algae (*Chloroidium saccharophilum, Batrachospermum virgato-decaisneanum*, and an unidentified eustigmatophycean strain). In addition, the authors of a recent review state that there is a very little information about Cs intake from aquatic ecosystems (Burger et Lichtscheidl 2018).

*Ceratophylum demersum* L. could be a very effective plant for phytoremediation of Cs from water bodies of aquatic habitats as a completely submerged macrophyte with a cosmopolitan distribution (Les 1989). It commonly grows in ponds, lakes, flooded ditches, slow flowing streams and rivers, as well as reed swamps. It is an indicator of slightly acidic to basic conditions (Hejný et Slavík 1988) and can also be an indicator of nitrate-rich waters (Denny 1980). It never occurs under strongly acidic conditions and it is salt tolerant. It does not form roots but absorbs nutrients directly from the water column. In the case of growth at sediment level, it forms rhizoids as a way of anchoring (Godfrey 1981). The plant may produce thick mats and the maximum net production was estimated by Forsberg (1960) on Lake Ösbysjön as 5.7 g of dry matter/m$^2$/day. Denny (1972) found average increments of 9.5 mg of dry matter/plant/day on mud and 4.5 mg of dry matter/plant/day on sandy sediments. . Considering its cosmopolitan distribution and the insufficient knowledge about remediation of Cs in aquatic habitats, *C. demersum* could be a good addition to the list of plants capable of Cs remediation from aquatic environments.

## Methods

This study was conducted as 5 separated experiments. In the first 3 greenhouse experiments, plants were exposed to $^{133}$Cs for 8, 16, and 24 days. The next experiment was conducted in The National Radiation Protection Institute (NRPI) with exposure to $^{134}$Cs for 8 days. In the last experiment, plants were tested in field conditions at Rod pond, where they were exposed to $^{133}$Cs for 8 days. The plant material was taken from Rod and Fišmistr ponds near Lomnice nad Lužnicí (Czech Republic) The ponds were selected as typical macrophyte localities in the vicinity of Temelín nuclear power station.

A stable isotope $^{133}$Cs was used in the field and green house experiments because it has the same chemical behavioural properties as $^{134}$Cs (Rühm et al. 1999) and $^{137}$Cs (Tsukada et al. 2002, Tsukada et Hasegawa 2003). Using a stable isotope is supported by other authors. Tsukada et al. (2002) suggested $^{133}$Cs as an analogue to $^{137}$Cs in agricultural fields. Soudek et al. (2004) draw a similar conclusion when, in hydroponic experiments with sunflower (*Heliantus annus* L.), they did not find any significant differences between accumulation of $^{133}$Cs and $^{137}$Cs; furthermore, accumulation kinetics was found quantitatively similar. Identical chemical behaviour,

uptake and distribution in plant tissue of different species has been reported by many more authors (Tsukada et Hasegawa 2003, Kondo et al. 2015, Shinano et al. 2014, Yoshida et al. 2000).

*Greenhouse experiments*

Plant material was transported from collection sites to the experimental greenhouse, where the plants were transfer to four clean 40 L aquariums with tap water to acclimatize for 24 h. Next day each plant was examined for damage and individually cleaned. All impurities, unhealthy parts and invertebrates were removed. During this process each plant's health condition was rated on a scale of 1-10, where 10 was the healthiest. All plants rated below 7 were excluded from the experiment. Already clean plants were kept in 40 L aquaria for 6 days.

For the experiment, 2 L acid-washed cylindrical glass flasks were used. The treatment concentration of CsCl were: 0.008, 0.033, 0.133, 0.267, 0.533, 0.800, 1.067, and 1.333 mM. A wide molarity range of contamination solutions was based on the findings of White and Broadley (2000), where phytotoxic effects on some plant species began to appear at an external concentration of 0.253 mM . $Cs^+$ ions (atomic weight 133) were provided by 99.9 % CsCl in crystalline form diluted in deionized water. Every trial with the plants exposed to CsCl also contained control plants, without contaminant. Flasks with pipetted solutions were filled with stagnant tap water to a volume of 1.5 L. Each water-filled flask had 40 g of plants added to ensure a sufficient amount for analysis. Each treatment group (plus the control group) contained 10 flasks with plants. All flasks were randomly distributed in clean glass boxes. The experiment was inspected every second day, and stagnant tap water was added whenever the water volume dropped below the 1.5 L mark. During each inspection, the flasks inside the glass boxes were randomized to eliminate any potential effects of microclimatic conditions.

At the end of the experiment, each glass flask was poured through a plastic sieve and thoroughly flushed for 10 seconds under a stream of tap water. The plants were then placed on filter paper, carefully dried, weighed, and the health condition was determined and registered. Plants were oven dried to a constant weight at 40 °C.

*[134]Cs experiment*

The experimental cylindrical glass flasks and plants were prepared in the laboratories of the Czech University of Life Sciences Prague (CULS). Each glass flask was cleaned, acid washed, and appropriately marked with a number and the 1.5 L mark. The plants were cleaned, divided into 40 g samples, and sealed in plastic ziplock bags with water immediately before being transported to The National Radiation Protection Institute (NRPI).

Contamination solutions containing [134]Cs (certificate 1035-SE-40341-17) were prepared in the NRPI Department of Radiochemistry, where small amounts of [134]Cs were added to already predetermined solutions with [133]Cs. Exposure concentrations resulted in 0.008, 0.033, 0.133, 0.267, 0.533, 0.800, 1.067, and 1.333 mM of CsCl. Concentrations of 0.267 and 0.800 mM were omitted due to climabox capacity. There were 15 samples in each treatment group. The glass flasks were filled with tap water to a volume of 1.2 L. Subsequently the contaminant solution was pipetted, and water was added to make a final volume of 1.5 L. At the start of the experiment, activities were determined to 4.46, 4.46, 4.74, 4.64, 2.23, and 2.26 kBq. Glass flasks with plants were placed in climaboxes. The exposure time was 8 days at 16 hours light, 8 hours dark photoperiod and 22 °C.

After 8 days, the plants were removed from the glass flasks with tweezers and transferred to a 10 L bucket with clean tap water, and were immersed for 1.5 minutes. The bucket with water was freshly filled and used on each plant. Every plant sample was dried with paper filter sheets. These plants with paper sheets were moved to a single aluminium foil container and marked. Oven drying was carried out at constant weight at 60 °C.

*Field experiment*

Sealing containers and treatment solutions were prepared in the laboratory. Each 2 L sealing watertight cylindrical glass flask was acid washed, appropriately marked with a number and the 1.5 L volume. [133]Cs^{+} ions were provided by 99.9 % CsCl in crystalline form diluted in deionized water. Final solution concentrations resulted in 0.008, 0.033, 0.133, 0.267, 0.533, 0.800, 1.067 and 1.333 mM (below, also called external concentration). At the site of a field experiment at Rod pond near Lomnice nad Lužnicí (South Bohemia, Czech Republic), the cylindrical flasks were rinsed with

tap water. Contamination solutions were pipetted into each flask and then filled with stagnant tap water to a volume of 1.5 L. The glass flasks were sealed and the content mixed. Every flask was filled with 40 g of pre-prepared plants free of residues of other plants, benthic organisms, and dead parts. Plant health condition was recorded for every sample. Each treatment group, as well as a control group, contained 10 glass flasks with plants. The glass containers were closed and immersed (Fig. 1.) in Rod pond for 8 days. Every 24 h the sealed container groups were raised above the water level and opened one by one to prevent accidental cross contamination. Ventilation of one group lasted 5 minutes to ensure sufficient gas exchange.



**Figure 1:** *Field experiment construction and arrangement. The glass flasks were randomly hung up on floating aluminium square anchored to the bottom of the pond.*

Harvesting of the samples proceeded from control to the highest concentration. For each sample, the water from the cylinder was poured through a sieve into a prepared waste barrel. The plants in the sieve were rinsed with tap water. Health condition was recorded as well as plant weight. After recording the parameters, the plants were placed in marked paper bags and then transported to the laboratory for oven drying to constant weight at 40 °C.

*Laboratory samples processing and analyses*

All samples from the greenhouse and field experiments were ground up by hand using a medical friction dish with a pestle. Dried samples were microwave digested (Microwave PRO by Anton Paar GmbH) with $HNO_3$:$HClO_4$ (7:1); a detailed description is in the methodology provided by Rinaldi et al. (2017). After microwave digestion each sample was poured into polypropylene centrifuge tubes and adjusted to 45 mL with deionized Milli-Q water. Samples were analyzed with mass spectrometry ICP MS 7700x (Agilent Technologies Inc., USA).

Plant samples exposed to $^{134}$Cs were measured in the NRPI Department of Spectrometry. After drying the plants were weighed, ground, and measured in ointment box M60. The geometry of M60 boxes were not adequately met due to lack of sample sizes. The detector used was HPGe (high purity germanium detector) with a relative efficiency of 28.4%, model GEM-25185-S (ORTEC, Advanced Measurement Technology, USA). Measurement time of samples was 2000-3000 seconds. Dimensional correction and self-absorption were calculated by Efficiency transfer and coincidence summing corrections for environmental gamma-ray spectrometry (EFFTRAN). Average correction and estimate of uncertainty were made for the 13 samples selected. This correction was applied to all samples. Correction to true coincidence was performed in LabSys and stated in RPT. The activities of $^{134}$Cs were determined as the arithmetic mean of 605 and 796 keV. The resulting uncertainty was estimated based on the uncertainties of the individual corrected lines and rounded to 10%.

*Statistical analyses*

The data obtained from the sample analysis was processed in an MS Office Excel spreadsheet. RStudio (v 1.0.153) was used for hypothesis testing and chart processing. All analysed data did not met assumption of normality, thus statistical analysis were calculated by Kruskal-Wallis tests, which are non-parametric equivalents of one-way ANOVA. A series of tests were made for comparison of significance between time exposure and $Cs^+$ intake by plants. A series of Dunn's tests were used for calculating significant differences between groups or whole experimental design. Lastly, Wilcoxon signed-rank tests were used to calculate significant differences in plant health before and after experiments.

## Results

### *Control samples*

Values of $Cs^+$ determined by mass spectrometry in control samples were in the range 0.079-0.919 mg/kg. According to Yamagata et al. (1959), Cs concentrations in 50 plant species growing in non-urbanized areas were in a value range of 0.002-1 mg/kg . Another 24 wild growing plant species were examined by Horovitz et al. (1974). They found the concentrations of $Cs^+$ in plants in range 0.01-2.5 mg/kg. Similar concentrations were reported in fresh weight of vegetables 0.2-3.3 mg/kg and fresh weight of fruits < 0.1-2.9 mg/kg (Pais et Jones 1997). Cook et al. (2007) did similar research in the USA, where values of [133]Cs in 124 wild growing plants species were between 0-0.365 mg/kg. By comparing values from our samples with those available in the literature, it can be concluded that the concentrations present in the control samples are in common wild plants. The data obtained indicates the natural background levels of $Cs^+$ in the South Bohemia region.

### *Exposure time significance*

The hypothesis of dependence of experiment exposure time on accumulated amount of Cs was tested. Kruskal-Wallis test found significant (chi-squared = 23.349, df = 2, p < 0.001) dependence of the exposure time on Cs accumulation by plants. Kruskal–Wallis test proved the existence of exposure time's positive impact on Cs intake. In other words, the concentration of $Cs^+$ is significantly dependent on exposure time, which corresponds with the findings made by Scotti in 1996. The same effect was found in greenhouse experiment with ^{137}Cs and rice (Myttenaere et al. 1969) and in many others with different plants, e.g. *Brasenia schreberi*, *Nymphaea odorata*, *Nymphoides cordata*, and *Pennisetum purpureum* (Kelly et Pinder 1996; Pinder et al. 2006; Kang et al. 2012). Dunn's test showed results in more detail by multiple pairwise comparisons of sample groups (Fig. 2). Significant evidence was found (p < 0.001) that Cs intake differs between 8 and 16 days and also between 8 and 24 days. However, exposure time had no significant impact (p > 0.05) on accumulation of Cs between the 16- and 24-day experiments. Similar results between exposure times were found by Scotti (1996), where the only difference was weak but still significant results between the longest exposure times.
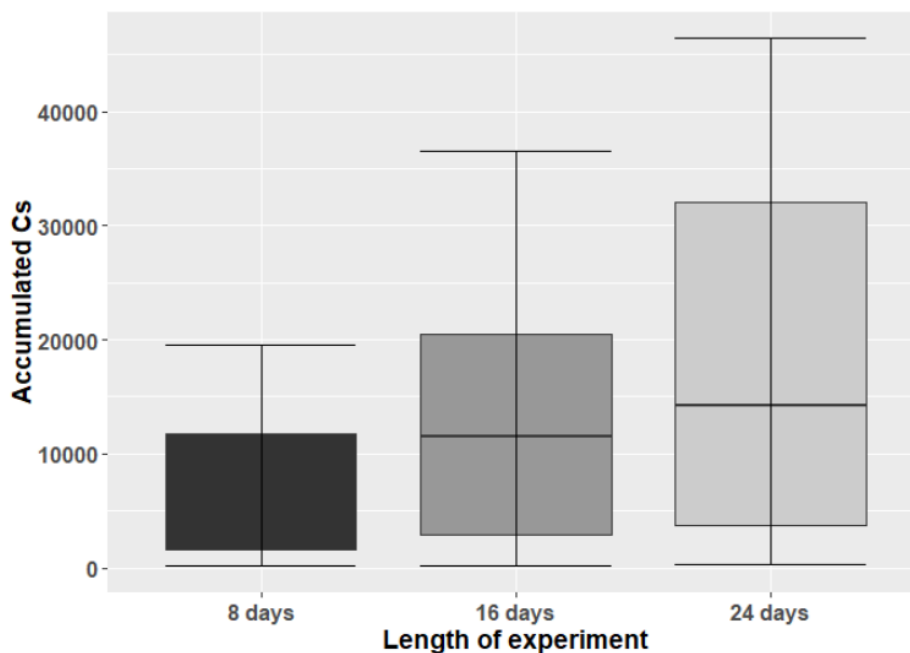
**Figure 2:** *Accumulated amount dependant on exposure time of Cs$^+$ [mg/kg].*

*Intake of Cs$^+$*

Accumulated Cs$^+$ values in the greenhouse experiment grew not only with increasing exposure time but also with increasing external concentration of solution in all groups. The highest external concentration did not reach the maximum uptake limit of plants. While the mean accumulated Cs$^+$ values in the treatment groups were 78, 298, 1022, 1456, 3008, 4383, 4743 and 5756 mg/kg dry matter in an 8-day experiment, they rose to 139, 494, 1936, 3477, 6987, 8043, 10156 and 12175 mg/kg after 16 days. After 24 days, the mean accumulated values further increased to 180, 706, 2403, 4684, 8553, 12441, 15718 and 15806 mg/kg dry matter (Tab. 2). A difference in accumulation was observed in the field experiment, where, in 8 days, the plants in each group were able to uptake on average 143, 523, 1598, 2897, 5817, 7277, 9430, 11697 mg/kg (Tab. 3). Compared to the 8-day greenhouse experiment, the values are much higher. A possible comparison would only be after 16 days of experiment in a greenhouse, where the values are very similar.

Rinaldi et al. (2017) provided data on Cs accumulation by *Calla palustris*. For 8-day hydroponic exposure with external contaminant concentrations of 0.008 mM, the

mean values of accumulated Cs were 196 mg/kg in green leaves and 139 mg/kg in roots (Rinaldi et al. 2017). That is much more than *C. demersum,* where the mean values are 78 mg/kg. However, the experiment conducted by Rinaldi et al. (2017) started at the beginning of the growing season, while the experiment in this study began at the end. Seasonal variability could also be a reason for higher Cs intake values (Komínková et al. 2018).

**Table 2:** Concentration [mg/kg] of Cs+ in 1 kg of plant dry matter. SE = standard error of mean.

| Concentration of Cs⁺ - greenhouse | | | | | |
|---|---|---|---|---|---|
| Exposition | Contamination | Minimum | Maximum | Mean | SE |
| 8 days | 0.008 mM | 63 | 105 | 78 | 4 |
| | 0.033 mM | 236 | 361 | 298 | 11 |
| | 0.133 mM | 708 | 1181 | 1022 | 47 |
| | 0.267 mM | 1226 | 1992 | 1456 | 66 |
| | 0.533 mM | 2344 | 3934 | 3008 | 133 |
| | 0.800 mM | 3038 | 5175 | 4383 | 205 |
| | 1.067 mM | 3807 | 6248 | 4743 | 241 |
| | 1.333 mM | 4856 | 6399 | 5756 | 158 |
| 16 days | 0.008 mM | 127 | 160 | 139 | 3 |
| | 0.033 mM | 443 | 551 | 494 | 10 |
| | 0.133 mM | 1480 | 2176 | 1936 | 57 |
| | 0.267 mM | 3170 | 3804 | 3477 | 60 |
| | 0.533 mM | 5450 | 8527 | 6987 | 287 |
| | 0.800 mM | 6425 | 9672 | 8043 | 299 |
| | 1.067 mM | 7231 | 13972 | 10156 | 520 |
| | 1.333 mM | 9020 | 17496 | 12175 | 978 |
| 24 days | 0.008 mM | 166 | 198 | 180 | 3 |
| | 0.033 mM | 657 | 732 | 706 | 7 |
| | 0.133 mM | 1992 | 2608 | 2403 | 57 |
| | 0.267 mM | 4205 | 5111 | 4684 | 83 |
| | 0.533 mM | 7522 | 8995 | 8553 | 126 |
| | 0.800 mM | 10796 | 13796 | 12441 | 238 |
| | 1.067 mM | 13734 | 17025 | 15718 | 314 |
| | 1.333 mM | 13691 | 17604 | 15806 | 304 |

When comparing accumulated contaminant in plants from Moogouei et al. (2011) or Borghei et al. (2011) it is obvious those plants were more effective, even though *C. demersum* can be harvested almost whole from the water column. *Calendula alata*, *Chenopodium album*, and *Amaranthus chlorostachys* accumulated more than 1000, 900, and 2000 mg/kg of Cs$^+$ in their shoots, respectively (Moogouei et al. 2011). A comparison can be made with respect to the exposure time of 15 days and the concentration of 0.044 mM with the 16 day greenhouse experiment in this study of and the external concentration of 0.033 mM.

**Table 3:** Concentration [mg/kg] of Cs+ in 1 kg of plant dry matter. SE = standard error of mean.

| Concentration of Cs$^+$ - field | | | | |
|---|---|---|---|---|
| Contamination | Minimum | Maximum | Mean | SE |
| 0.008 mM | 128 | 163 | 143 | 3 |
| 0.033 mM | 467 | 575 | 523 | 11 |
| 0.133 mM | 1211 | 1863 | 1598 | 57 |
| 0.267 mM | 2643 | 3134 | 2897 | 44 |
| 0.533 mM | 5236 | 6795 | 5817 | 153 |
| 0.800 mM | 6163 | 8223 | 7277 | 207 |
| 1.067 mM | 6354 | 11117 | 9430 | 410 |
| 1.333 mM | 8131 | 13662 | 11697 | 537 |

*Removal rate*

The results (Fig. 3) showed different Cs removal rates from solution for different time exposures in the greenhouse experiment. A minimum of 5.90% was found in the 8-day trial with an external contaminant concentration of 1.333 mM. A maximum of 11.03% was found in external concentrations of 0.008 mM. The 8-day trial as a whole resulted in 8.00% average removal rate with the median at 7.59%. The 16-day experiment showed the same external concentrations with a minimum of 11.02% and maximum of 17.25%. As a whole, average removal rate was 14.07% with the median at 14.22%. The highest values were found in 24-day exposure. In this case the minimum remained at the same external concentration of 1.333 mM with a removal rate of 14.93%. On the other hand, the maximum shifted to 0.533 mM with 18.97%. Removal rate values from different groups (0.008, 0.033, 0.133, and 0.800 mM) remained above 18%. The average removal rate was 17.75% with the median at 17.76% for the 24-day trial.

For the field experiment, the average removal rate across all treatment groups was 19.37%. Compared with the literature on terrestrial plant species, for example the work of Borghei et al. (2011) on *Calendula alata*, these values are relatively low. With 15 exposure days and treatment corresponding to 0.005 mM, this plant was able to take in 46.84% of Cs from CsCl solution (Borghei et al. 2011). On the other hand, *C. demersum* removal rate was only 17.25% when exposed to 0.008 mM. Another comparison can be made with the 0.044 mM group, where *C. alata* was able to achieve a 52.06% removal rate (Borghei et al. 2011); meanwhile, the *C. demersum* group treated with 0.033 mM only achieved 16.15%. In another study with *Amaranthus chlorostachys*, *Chenopodium album*, and *Calendula alata*, the removal rate after 15 days of 0.044 mM treated groups was 41%, 52%, and 52% respectively (Moogouei et al. 2011). These are extremely high numbers because *C. demersum* had a removal rate of 14.88% in 0.800 mM group.
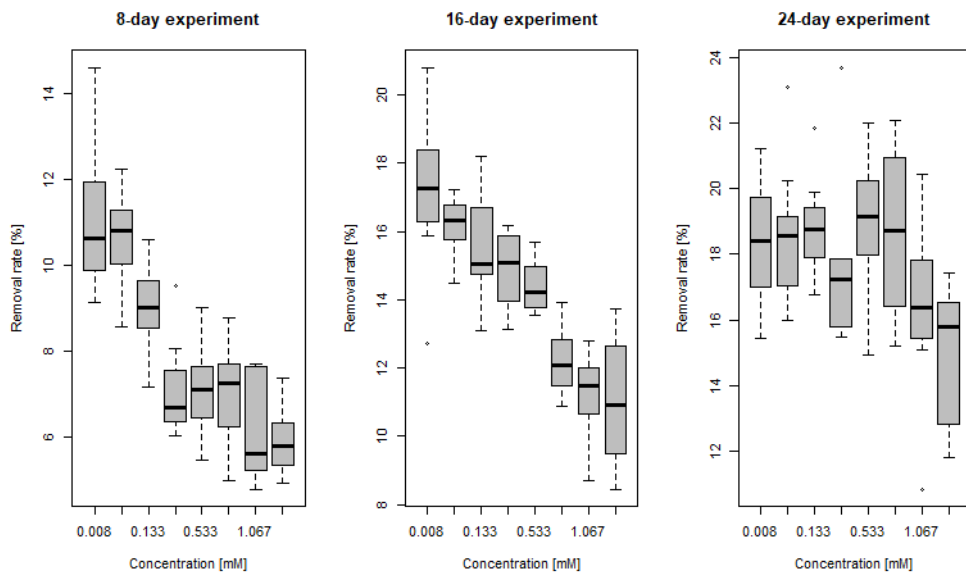


**Figure 3:** *Removal rates per 1 kg of plant dry matter in greenhouse experiment. Removal rates were calculated as accumulated concentration [mg/kg] of Cs$^+$ by plants exposed to different concentrations of Cs$^+$ in solution.*

The results of the average removal rates of the $^{134}$Cs isotope experiment were almost identical to the removal rates in the 8-day $^{133}$Cs experiment. The activities of external concentrations of 0.008, 0.033, 0.133, 0.533, 1.067, and 1.333 mM were, at

the harvest of the experiments, 4.42, 4.42, 4.71, 4.61, 2.21, and 2.24 kBq respectively. Accumulated activities for sample groups with the average activities were 447, 461, 376, 301, 150, and 163 Bq respectively (Fig. 4). In addition, the same groups of plants also resulted in an average activity of 1 kg of dry weight 161, 192, 147, 122, 59, and 65 kBq/kg. These are much higher values than those reported from field studies with aquatic plants. For example, experiments carried out on aquatic plants growing in the reservoir system used to cool a former nuclear power station in South Carolina showed much lower values, namely 6.6 kBq/kg *Myriophyllum spicatum*, 26 kBq/kg *Brasenia schreberi*, and 29 kBq/kg *Utricularia inflata* (Pinder et al. 2006). Similar values were recorded in the experiment of plant transplantation from the clean environment into the environment of the above-mentioned tank system. *Nymphoides cordata* 35 days after immersion in a contaminated water tank showed the highest values of the tested plants, namely 19 kBq/kg (Kelly et Pinder 1996). Higher values were not found even in freshwater habitats within 7 km from Fukushima nuclear power station. In the aquatic plant species *Potamogeton crispus* and *Trapa bispinosa*, average values of 26.9 kBq/kg and 14.8 kBq/kg were recorded (Sasaki et al. 2016).
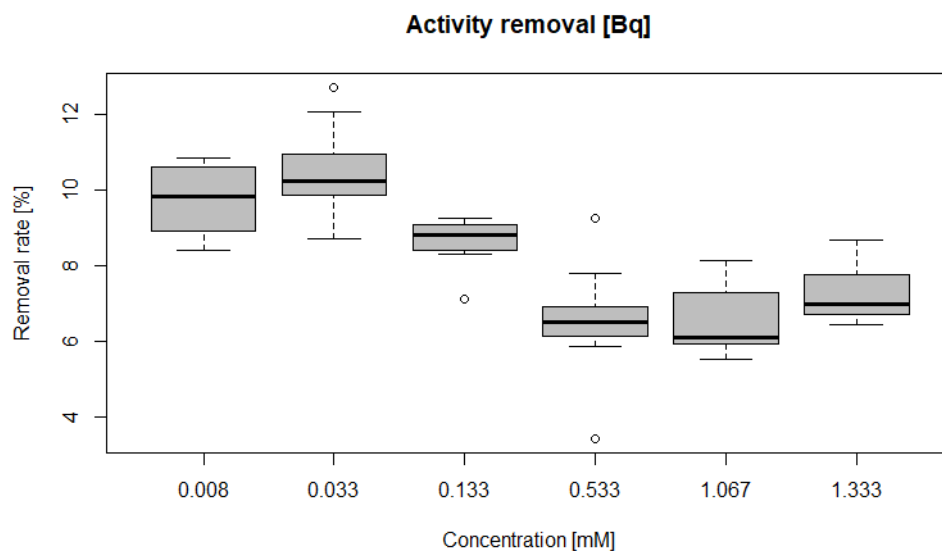


**Figure 4:** *Removal rates of activity [Bq]. Removal rates were calculated as accumulated activity divided by total activity of contamination solution.*

For a concentration of 0.033 mM, the average removal rate was 10.12% (447 Bq). The minimum 6.52% (301 Bq) was found in the group with 0.533 mM. The 1.333 mM group had an average removal rate of 7.28% (163 Bq), which is 1.38% more than the [133]Cs experiment.

The greatest difference in the removal rates compared to the controlled condition experiments was observed in the field experiment (Tab. 4). The maximum recorded average removal rate was 27.29% for the group exposed to 0.008 mM and a minimum average of 14.21% for 1.333 mM. The average for the field experiment as a whole was 19.37% with a median of 17.89%.

**Table 4:** Removal rates per 1 kg of plant dry matter in field experiment. SE = standard error of mean. Removal rates were calculated as accumulated concentration [mg/kg] of Cs+ by plants divided by concentration of Cs+ in solution.

| Field removal rates for 8 days in 2017 | | | | |
|---|---|---|---|---|
| Contamination | Minimum | Maximum | Mean | SE |
| 0.008 mM | 23.67% | 33.13% | 27.29% | 0.0083 |
| 0.033 mM | 23.47% | 26.90% | 25.59% | 0.0036 |
| 0.133 mM | 14.85% | 25.31% | 20.53% | 0.0086 |
| 0.267 mM | 16.92% | 19.91% | 18.64% | 0.0027 |
| 0.533 mM | 16.15% | 18.60% | 17.35% | 0.0022 |
| 0.800 mM | 13.99% | 17.57% | 15.98% | 0.0046 |
| 1.067 mM | 10.76% | 16.99% | 15.02% | 0.0058 |
| 1.333 mM | 7.07% | 17.64% | 14.21% | 0.0096 |

Removal rates were also compared between the greenhouse, [134]Cs, and field experiments (Fig. 5). Analysis was performed without the 0.267 mM and 0.800 mM concentration because they were not designed for the [134]Cs experiment. The Kruskal-Wallis test results suggest that there is a significant dependence ($p < 0.001$) on the type of experiment. The [134]Cs removal rates were not significantly different ($p > 0.1$, Dunn's test) from the [133]Cs greenhouse experiment. On the other hand, removal rates differ significantly ($p < 0.001$, Dunn's test) between field and greenhouse, but also field and radioactive experiments. Such different results are probably due to stress in plants where transport to the laboratory is required. Thus, the field experiment carried out directly *in situ*, in natural conditions to which the plants ware adapted, minimised the stress for the plants relative to their longer manipulation.

Fried et al. (1961) suggested that radiocesium intake is limited in a certain phase by metabolic processes, which was confirmed by Baker (1981) and Shaw et Bell (1989). Comparison of the [133]Cs and [134]Cs removal suggests limitation if we compare the removal rates between isotopes [133]Cs and [134]Cs. However, the values do not differ significantly between the greenhouse and [134]Cs experiments. The only difference in the results is in the minimum removal rate of groups, which are at 0.533 mM instead of 1.333 mM. Limitation of Cs intake mentioned in the literature should probably be more apparent in longer periods of exposure This claim is supported by conclusions made by Kelly et Pinder (1996); they found a decrease in Cs^{+} accumulation in *Nymphaea odorata* and other plants after 20 days of exposure. The rete of decreasing accumulation was dependent on plant species and exposure time (Pinder et al. 2006).
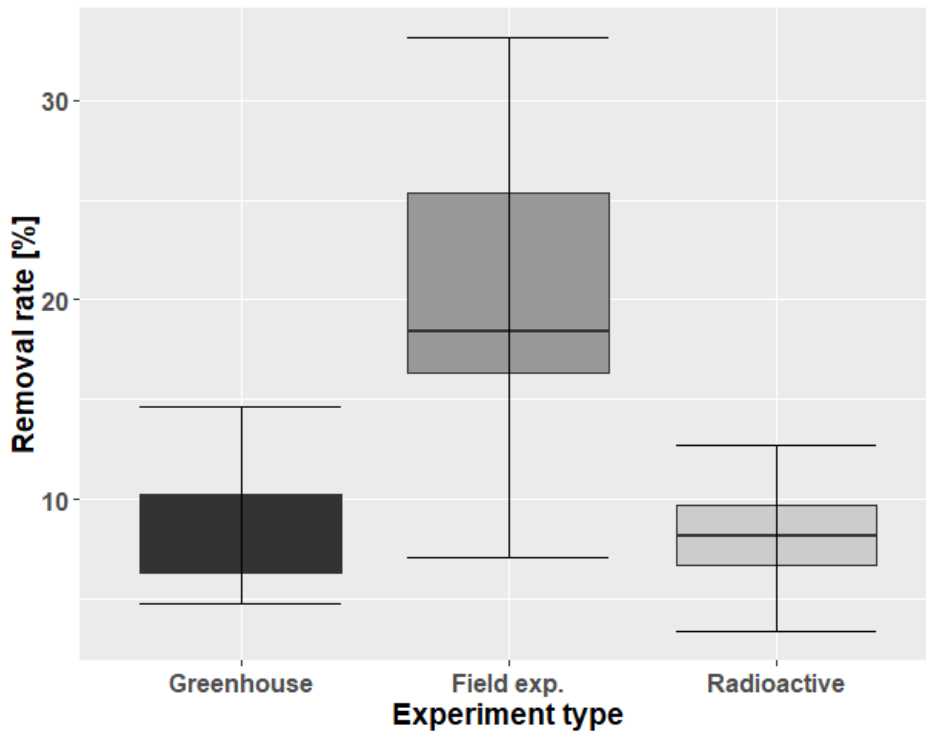


**Figure 5:** *In the greenhouse and field experiments, removal rates were taken as accumulation of Cs^{+} by plants from solution, whereas in the [134]Cs experiment removal rates were taken as the amount of activity accumulated by plants.*

*Health condition*

Health conditions were examined by Wilcoxon signed-rank test for each 8, 16, and 24 day exposure time (Tab. 5). An exposure time of 8 days did not affect plants health at all. On the other hand, in the experiment with 16 days of exposure there were a few changes observed in plants health conditions; however these changes were mostly recorded as positive. These positive assessments could be caused by human error while determining health status. For some plant species the phytotoxic effects of $Cs^+$ begin to appear at 0.2 mM external concentrations (recalculated to 0.253 mM CsCl) (White et Broadley 2000; Borghei et al. 2011), but these manifestations are critically dependent on concentrations of other ions (White et Broadley 2000). While evaluating health conditions it was quite clear that 8 and 16 days of exposure was not enough for the phytotoxic effects of $Cs^+$ to manifest, though values in half of the CsCl treated flasks were above 0.253 mM. More specifically, 0.267, 0.533, 0.800, 1.067, and 1.333 mM were higher values. At a concentration of 1.333 mM CsCl the limit of phytotoxic effects for some plant species was exceeded 5 times. These findings are also supported by Wilcoxon signed-rank test, which did not prove significant differences between health conditions before and after experiments ($p > 0.05$) across all of them.

The experiment with an exposure of 24 days gave very strong evidence of significant deterioration of health condition (Wilcoxon, $p < 0.001$). Detailed analysis of health condition in the 24-day experiment found no observable phytotoxic effects of external concentration up to 0.133 mM (Wilcoxon $p > 0.05$). Observable effects were found in the group 0.267 mM, but still not significant (Wilcoxon, $p > 0.05$). Each group with a higher external concentration than 0.267 mM showed significantly deteriorated health (Wilcoxon, $p < 0.001$).

The [134]Cs experiment had similar results to the 8-day greenhouse trial. Testing all groups together resulted in no changes in plant health (Wilcoxon, $p > 0.05$). The same results were found when groups were tested separately. A small but insignificant ($p > 0.05$) difference was observed at 1.333 mM, where slightly greater biomass loss from plants occurred. However, in assessing their health status, the plants were judged to be healthy as there was no necrosis or chlorosis in addition to newly formed detritus from leaves. Due to the length of the experiment, possible

synergic effects of β and γ radiation with phytotoxic effects of Cs$^+$ were not observed.

In contrast, different results were observed in the 8-day field experiment from both the greenhouse and $^{134}$Cs trials. Wilcox's pairwise analysis of plant health tested as a whole did not show a change in health status ($p > 0.05$). In a more thorough analysis, it was found that 0.267 mM concentration of contaminant solution did not affect plant health, even with removal rates 11.6% higher than in the greenhouse experiment. This could be due to the higher resistance of C. demersum to phytotoxic effects of Cs. This corresponds to the conclusions of the greenhouse experiment, where significant health changes were not observed at 0.267 mM, even after 24 days. Above a concentration of 0.533 mM there was a significant ($p < 0.001$) degradation of plant health. Compared to the greenhouse experiment, the deterioration in condition occurred after 24 days of exposure. It is very likely that this is due to the accumulated amount and rate of accumulation, which was approximately 3 times faster in the field experiment. Strong plant necrosis was observed at a concentration of 1.333 mM and 3 out of 10 samples were considered dead. In groups 0.533 mM and higher, plant health levels deteriorated significantly ($p < 0.001$).

**Table 5:** Influence of external concentrations on plant health. The most significant changes were found in the greenhouse experiment lasting 24 days and the field experiment lasting 8 days.

| Change in plant health | | | | | |
|---|---|---|---|---|---|
| Contamination | Greenhouse 8 | Greenhouse 16 | Greenhouse 24 | Field | $^{134}$Cs |
| 0.008 mM | None | None | None | None | None |
| 0.033 mM | None | None | None | None | None |
| 0.133 mM | None | None | None | None | None |
| 0.267 mM | None | None | Slight $p > 0.05$ | Slight $p > 0.05$ | N/A |
| 0.533 mM | None | None | Significant $p < 0.001$ | Significant $p < 0.001$ | None |
| 0.800 mM | None | None | Significant $p < 0.001$ | Significant $p < 0.001$ | N/A |
| 1.067 mM | None | None | Significant $p < 0.001$ | Significant $p < 0.001$ | None |
| 1.333 mM | None | None | Significant $p < 0.001$ | Significant $p < 0.001$ | None |

Health condition has been assessed and analyzed in many experiments. One of them is Rinaldi et al. (2017), which found health condition to be a significant factor in $Cs^+$ accumulation at a treatment concentration of 0.008 mM in study with *Calla palustris*. After exposing *Arabidopsis thaliana* to 0.016 mM of CsCl treatment, Isaure et al. (2006) found a strong influence of K deficiency on the phytotoxic effect of Cs. The number of leaves was reduced by half and very distinctive chlorosis was observed (Isaure et al. 2006). Kang et al. (2012) also observed 3-57% decrease in chlorophyll content in leaves after 2, 4, and 7 weeks in treatment concentrations 0.016 mM and higher. In this study, no such finding was made in experiments with exposure time of 8, 16, or 24 days, but only to 0.133 mM concentration; some signs of chlorosis were observed above 0.133 mM. In the 1.333 mM group a lack of chlorophyll was apparent. Furthermore, Borghei et al. (2011) state that the majority of *Calendula alata* plants remained healthy, even after 15 days of 0.044 mM CsCl treatment. That is consistent with the findings of this study, where plant health conditions did not change in the 0.133 mM group and were lower across all experiment lengths and designs. After exposing plants to 0.048 mM treatment, concentration growth inhibiting effects of Cs were observed in *Pennisetum purpureum* (var. Merkeron). Two weeks of exposure resulted in 39.3% inhibition effect and four weeks resulted in 47.3% (Kang et al. 2012).

Inhibiting effects were not observed with *C. demersum*; however, detailed evaluation of these effects were not part of this study and claims regarding this topic should be supported by further research. Lastly, it is also appropriate to consider inaccuracies in ratings of health conditions due to human factors. Although maximum attention was dedicated to assessing plant health conditions, it is possible that some samples were overestimated or underestimated by 1 point out of 10. Further experiments will be needed for more detailed information on the effects of stable and radioactive isotope on aquatic plants.

*Factors influencing the results*

One of the factors influencing the uptake of $K^+$ (therefore $Cs^+$) by roots in terrestrial plants is pH (Marschner 2011). Considering the study conducted by Saleh (2012) on *Eichhornia crassipes*, pH should not play a significant role in intake, where pH in the range 4.9-10.9 did not have a notable effect on Cs accumulation from solution.

Furthermore, in both studies conducted by Moogouei et al. (2011) and Borghei et al. (2011), young plants were used in which the metabolism is higher and fast cellular division occurs; consequently these plants had a higher demand for water and nutrients, including K, resulting in higher Cs intake. It should also be noted that the experiments in this study took place in the high season with large biomass, when plant growth slows down considerably.

Kelly et Pinder (1996) and Pinder et al. (2006) determined absorption of $^{133}$Cs by plant leaves from an experimentally contaminated reservoir. Some of the floating plant species (e.g. *Brasenia schreberi*, *Nymphaea odorata*, and completely submerged species *Utricularia inflata* and *Myriophyllum spicatum*) accumulated significant amounts of $^{133}$Cs through their leaves, depending on exposure time. From the available data, and due to many factors resulting from the previously mentioned experiments, it is not possible to compare them with this study. One of the factors is length of exposure, which was more than 55 days. Sediment and periphyton can have a significant influence on the biological availability of $Cs^+$ (Sansone et al. 2002; Zachara et al. 2002). Results of accumulation can be overrated due to $Cs^+$ adsorption to a plant's surface, as suggested by Sansone et al. (2002). In this study, sediment or periphyton was not present in flasks.

Yet another factor can be water temperature. Kelly et Pinder (1996) did their experiment from July to September; temperatures in South Carolina are much lower in September than July or August. A similar time frame was chosen for the experiment done by Pinder et al. (2006) in South Carolina, starting on 1st August and with an exposure time of 120 days. In this study, *C. demersum* was in an experimental greenhouse with a stable temperature or in a climabox. The water surface temperatures in the field part of the experiment were around 22 °C during the day.

Lastly, there is a possible significant influence of algae on $Cs^+$ distribution in the water column (Sasaki et al. 2016). Tap water was used in the experiment with C. demersum, thus stable mineral composition was preserved and, in addition, no algae was detected while harvesting experiments.

## Conclusion

While information on the uptake and accumulation of Cs by roots of terrestrial macrophytes has been abundant during the last 70 years of research, there is still very little information about Cs intake by aquatic macrophytes from the water column. The results of this study bring a new potential candidate, namely *Ceratophyllum demersum* L. as a plant suitable for phytoremediation of aquatic habitats.

In greenhouse experiments, statistical analyses have shown that, with increasing exposure time, the amount of Cs accumulated by plants increases. For 8-day exposure, the average Cs removal from solution ranged from 5.90 to 11.03%. For 16-day and 24-day, these ranges were higher, namely 11.02–17.25% and 14.93–18.97%, respectively. In addition, it was found that even after 16 days of exposure, the plants showed no deterioration in health in the highest concentration of 1.333 mM CsCl in solution. This occurred only in plants exposed for 24 days at concentrations higher than 0.267 mM.

Removal rates for the [134]Cs radioactive isotope did not differ significantly from the Cs stable greenhouse experiment. On the other hand, a statistically significant difference was found in the field experiment, where removal rates at 8 days of exposure were 14.21–27.29%. This also suggests that the conclusions of the greenhouse experiments are transferable to real-world applications, even with higher efficiency of non-stressed plants in natural conditions.

The results of this study may not just lead to a new species suitable for phytoremediation of aquatic habitats. Adding information on Cs intake can also lead to the emergence of new and more accurate models of radionuclide transfer in the food chain, leading to better protection of food resources, and therefore of the population. More accurate models of radionuclide behaviour in aquatic environments and their impact on ecosystem services in the event of contamination of large areas may also arise, ensuring better knowledge and a future for new generations.

## 4.1 Following research to Case study I

Annual differences in field Cs accumulation

Methods

The field experiment adopted the methodology of Case Study I – Field Experiment. For the purposes of the trial, 2L watertight cylindrical glass flasks were selected. These flasks were divided into four treatment groups, with each group comprising 10 samples. The treatment concentrations were set at 0, 0.133, 0.267, and 0.533 mM of CsCl, with a final volume of 1.5L. The same experimental site was consistently used for both years (2017 and 2018): Rod Pond, located near Lomnice nad Lužnicí in South Bohemia, Czech Republic. Experiments were carried out in July and August, spaced at most a few weeks apart. The mass of the *Ceratophyllum demersum* plant in each sample was kept constant at 40g for 2017 and 2018.

Results and Discussion

The removal rates in two separate field experiments conducted in August 2017 and July 2018 were studied. This study investigated whether the concentration of the exposure solutions negatively impacted plant health and whether the results from the 2017 experiment did not significantly differ from those in 2018. Removal rates were normalized to per 1 g of dry matter due to slight variability in sample weight changes in the glass flasks between the years.

Concentrations of cesium from blank samples indicated negligible contamination of the working environment, at 0.02–0.03 mg/kg, which is an order of magnitude lower than that found in the control group. Control group cesium concentrations were 0.11–0.32 mg/kg, consistent with commonly found natural concentrations (Cook et al., 2007). Moreover, results from the control group provided background values of $Cs^+$ concentrations in the Rod pond, in alignment with previous results.

Furthermore, this study tested whether the concentration of contamination solutions resulted in significant $Cs^+$ accumulation compared to the control group. In both years of the experiment, the Kruskal-Wallis test revealed very strong evidence ($p < 0.001$) of the effect of concentration contamination solutions on accumulation.

These findings align with those in the literature (Kelly and Pinder, 1996; Pinder et al., 2006; Rinadli et al., 2017) as well as with previous results.
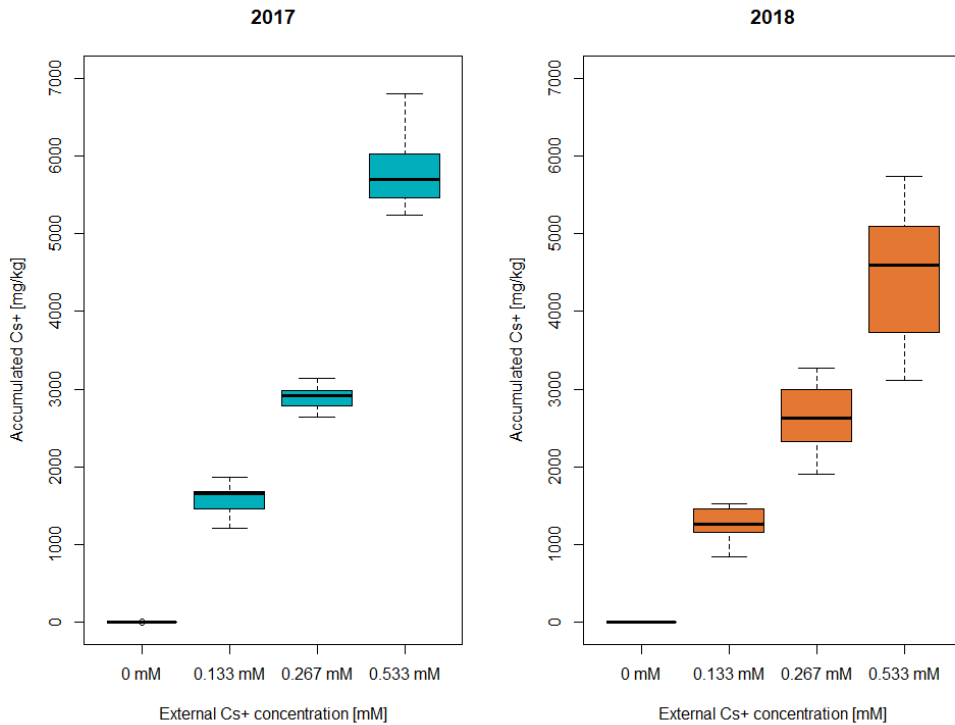


**Figure 1:** *Differences in accumulated Cs+ concentrations [mg/kg] between the years 2017 and 2018.*

Removal rates (as shown in Fig. 1) indicate that in 2018, the accumulation efficiency decreased among individual averages of treated groups. This reduction in efficiency was found to be significant in groups contaminated with 0.133, and 0.533 mM by the Kruskal-Wallis test ($p < 0.001$), with the exception of 0.267 mM where no significant difference was observed. Overall, the difference between 2017 and 2018 was significant, pointing to variances in accumulation in individual years due to the biological dynamics of individuals (Kenzo et al., 2020; Salt et al., 2004) and to the variability of environmental properties, which directly influence physiological dynamics (Zalewska, 2012).

## Comparative analysis of cesium accumulation in greenhouse vs. field conditions and preliminary results on the effect of potassium

### Methods

The experiment comparing field and greenhouse uptake, with the involvement of potassium supplementation under greenhouse conditions, was conducted in 2019. The experiment utilized 2-liter cylindrical glass flasks, which were divided into 12 groups and subsequently subdivided. All treatment groups subjected to a contamination process utilized 99.99% pure CsCl. In the first four treatment groups, each flask was filled with a contamination solution and stagnant tap water, achieving a total volume of 1.5 L and resulting in external $Cs^+$ concentrations of 0, 0.133, 0.267, and 0.533 mM. The next four treatment groups used watertight 2-liter cylindrical glass flasks with identical external concentrations of cesium (0, 0.133, 0.267, and 0.533 mM), filled with stagnant tap water and achieving a volume of 1.5 L. These four groups were subjected to a field experiment at Rod Pond, located near Lomnice nad Lužnicí in South Bohemia, Czech Republic. The remaining four treatment groups received a mixture of a contamination solution, a potassium additive, and stagnant tap water to also reach a volume of 1.5 L. The potassium additive was prepared following the method described by Komínková et al. (2018), involving the use of $K_2SO_4$. The resulting external concentrations of cesium were identical to those of the other groups (0, 0.133, 0.267, and 0.533 mM), while the potassium concentration for all groups was maintained at 39.1 mg/L. The mass of *Ceratophyllum demersum* plant material added to each flask was 20 g. Each group comprised 10 replicates. The preparation and execution of the experiment adhered to the methodology outlined in Case Study I.

### Results and Discussion

In 2019, the experiment was replicated in both field and greenhouse environments, employing stable cesium and incorporating additional potassium in the greenhouse setting. Contamination of the work environment during sample analysis was detected using blank samples. As observed in previous studies, it remained at a low level, ranging from 0.02 to 0.03 mg/kg. Control samples from greenhouse experiments ranged from 0.50 to 0.76 mg/kg, whereas those from field experiments

varied between 0.32 and 0.59 mg/kg, thereby aligning with natural background values (Cook et al., 2007).

The difference between the greenhouse experiment and the experiment conducted in the natural environment was evidenced by significant variations in accumulation at a concentration of 0.533 mM ($p < 0.001$). In contrast, the significant difference was not confirmed at concentrations of 0.133 and 0.267 mM ($p > 0.05$). The disparate results observed among the tested groups can likely be attributed to the enhanced control over environmental variables in greenhouse experiments, as well as to the stressors encountered by the plants during collection and transport to the greenhouse, which, in turn, may influence metabolic processes and subsequent accumulation. However, no deterioration in plant health was observed in any of the tested groups.

To compare the outcomes with those of previous years, it is imperative to consider methodological modifications. Despite the methodology in 2019 remaining consistent, the quantity of plant material required for each sample was reduced by half. This adjustment was necessitated by the limited availability of healthy plants at the time of collection. Ignoring the variance in biomass weights of the samples would lead to the erroneous conclusion that there was a marked improvement in the efficiency of cesium removal from the solution in the field environment in 2019 compared to the years 2017 and 2018. Whereas in 2017, plant concentrations were recorded at 1652, 2909, and 5697 mg/kg across different groups, the 2019 figures significantly surpassed these, doubling or more. However, the discrepancy in values between 2019 and the preceding years can be logically attributed to differences in plant biomass. At identical concentrations across all field experiments conducted in 2017, 2018, and 2019, the doubled weight in the earlier years resulted in a seemingly reduced uptake. This phenomenon can be explained by the interaction of biomass with the contamination solution in 2017 and 2018, where a greater quantity of plant biomass intensified the competitive demand for resources.

The greenhouse experiment also revealed differences in cesium uptake between groups contaminated only with cesium and those with cesium plus added potassium. The results showed a 92% decrease in cesium accumulation in all groups treated with potassium. There was a significant decrease ($p < 0.001$) in the accumulation of cesium, indicating higher bioavailability of potassium for free-

floating plant species (as shown in Fig. 1). The concentration of potassium additive used, 39.1 mg/L, thus exceeds the upper limit of potassium concentration present in the water bodies of Central Europe by tenfold (Talling, 2010). A significantly reduced uptake of cesium could indicate that *Ceratophyllum demersum*, as well as possibly other aquatic submerged plant species, are sensitive to changes in potassium levels in the water column, due to their adaptive physiological measures in nutrient uptake. A similar conclusion was reached by Guillaume et al. (2012), who observed varying sensitivities to available potassium among terrestrial grassland plant species. Komínková et al. (2018) applied the same concentrations of cesium and added potassium in their experiment with *Calla palustris*. With comparable parameters to this study, the potassium additive achieved a reduction in cesium accumulation by 44%. However, it is important to note that *Calla palustris* absorbs cesium and potassium through the roots, unlike *Ceratophyllum demersum*. A similar experiment with comparable parameters, in which *Calla palustris* plants were grown hydroponically, was conducted by Rinaldi et al. (2017). With an application of 114 mg/L, the cesium accumulation was reduced by 74% in the above-ground parts and by 83% in the underground parts. These results indicate a negative correlation between available potassium and the amount of cesium accumulated in aquatic plant species, with the dependency in submerged species shifted towards lower concentrations, in line with the adaptation mechanism of plants for nutrient uptake.
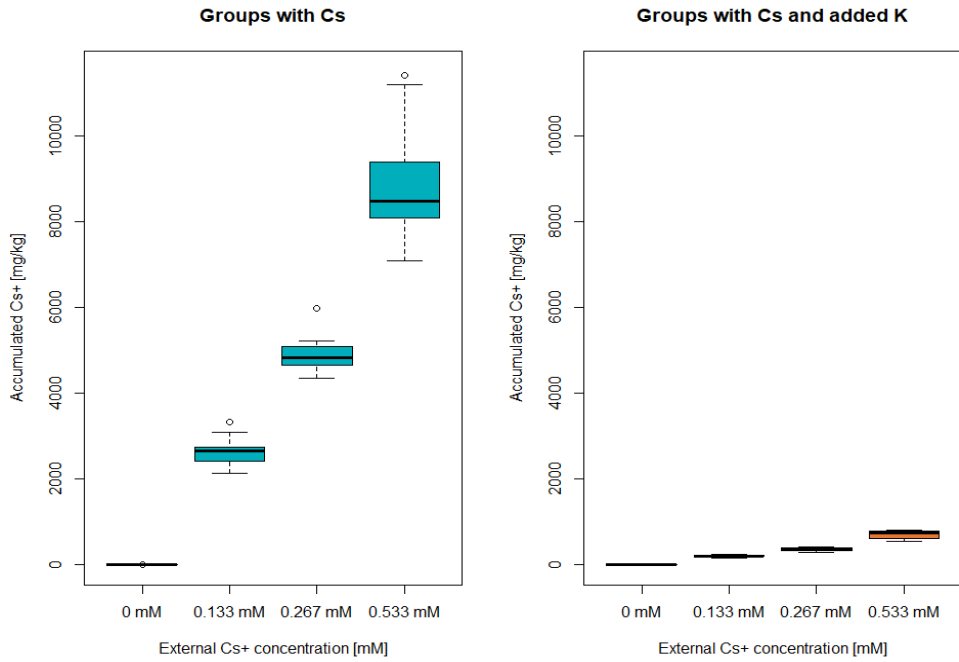
**Figure 1:** *Differences in Cs+ accumulation in experiments conducted without a potassium additive (left) compared to those with a potassium additive (right).*

# 5 Case study II: Aggregated transfer factors of fresh Cs and Sr pollution to various vegetables from six common European soils – mesocosm experiment

Komínková, D., Vacula, J., Záhorová, V., Rulík, P., Škrkal, J., Pecharová, E., Borecký, Z., Šestáková, K., Stádníková, D., Finkous, P., Finkous, M., Hofmanová, K., Doskočilová, V., Pilátová, H. (2023). Aggregated transfer factors of fresh Cs and Sr pollution to various vegetables from six common European soils–mesocosm experiment. Journal of Environmental Management, 345, 118900. (IF 8.7)

**Graphical abstract**

*Case study II: Aggregated transfer factors of fresh Cs and Sr pollution to various vegetables from six common European soils – mesocosm experiment (Komínková et al., 2023)*
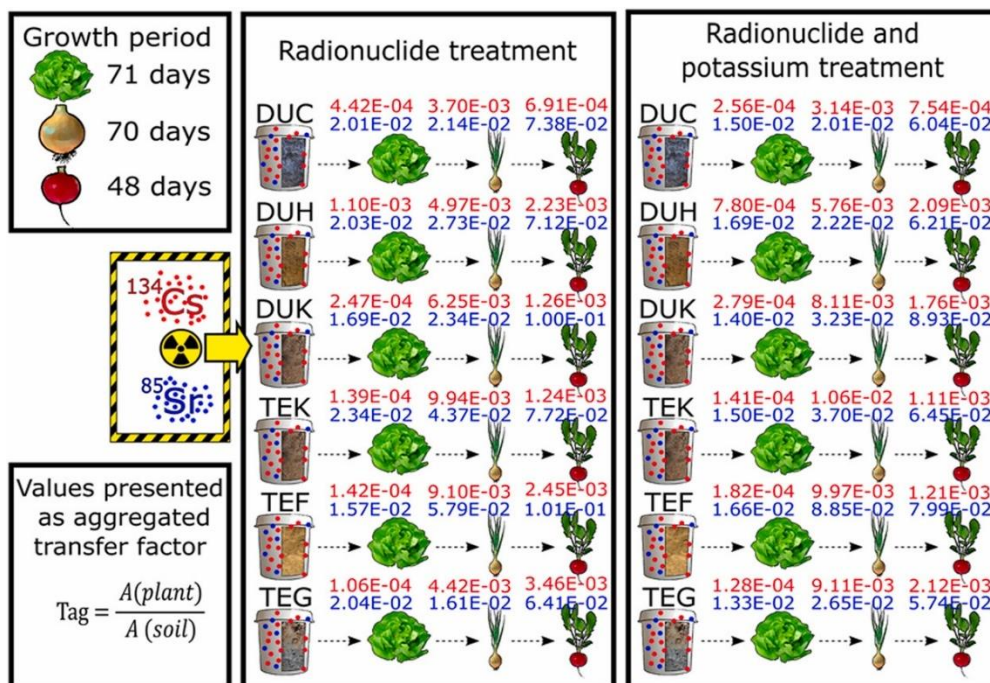
## Abstract

Aggregated transfer factors ($T_{ag}$) were identified for three common vegetables grown in six common European soils freshly contaminated by [134]Cs and [85]Sr. The experiment was carried out as a mesocosm experiment in pots with an average soil weight of 15.8 kg per pot. The vegetables were grown one after the other during one vegetation season, in the order lettuce, onion, and radish (the order usually applied in private gardens and small farms). Despite the fact that lettuce was grown in the most contaminated soil, it had the lowest Tag (in $m^2$/kg) of both radionuclides (3.6E-4 for Cs, 2.0E-2 for Sr), while onion had 6.4E-3 for Cs and 3.2E-2 for Sr and radish had 1.9E-3 for Cs and 8.1E-2 for Sr. Potassium supply did not show any statistically significant effect on Cs $T_{ag}$; there was a significant impact of K on the decrease in Sr $T_{ag}$. The experiments indicated that $T_{ag}$ is more affected by plant species than by soil type; therefore, selection of plants with a lower capacity to uptake radionuclides may be an important measure to reduce food contamination and thus minimize the committed effective dose.

## Introduction

In the case of a nuclear accident and the release of the most common fission products, as caesium (Cs) and strontium (Sr) to the environment, the transfer factor soil to plant is usually one of the first tools used to assess the transfer of radionuclides from the contaminated environment to biota (Veresoglou et al., 1995); this is especially true for agriculture production and the consequent evaluation of the possible risk to human diet and possible radionuclide intake by the human body from the consumed food. The knowledge about ^{137}Cs and ^{90}Sr behavior is important as they are long-lived radionuclides (with half-lives of 30 years for ^{137}Cs and 29 years for ^{90}Sr), once entering the environment in bigger quantity from anthropogenic activities, they cause radiological risk for generations and affecting the whole environment. Their chemical properties, which are similar to those of essential elements such as K (in case of Cs) and Ca (in case of Sr), make them major artificial contributors to the enhanced overall radiological dose that the human population worldwide is exposed to (Wang et al., 2000). The soil to plant transfer factor identifies the uptake of radionuclides from soil to plant biomass, hence identify how much of the radionuclide may enter the trophic chain from the abiotic environment and may indicate the treat to human population related to

contaminated food consumption (IAEA, 1994). The dependence of the transfer factor (TF) on a number of variables is frequently studied, e.g.:

1) contamination ageing – how does TF change as time passes from the release to the environment (al Attar et al., 2016; Wakabayashi et al., 2020);

2) soil type and soil properties – how do soil type and properties affect TF (Abu Khadra et al., 2009; Arapis et al., 1997; Djingova et al., 2005; Schuller et al., 2018; van Bergeijk et al., 1992);

3) plants species and genotype – what is the role of plant species on the transfer of radionuclides from soil (Guillaume et al., 2012; Melnitchouck and Hodson, 2004; Ramadan et al., 2021; Uchida and Tagami, 2018). According to Kilchevsky and Khotylova (1997) in (Melnitchouck and Hodson, 2004), intervarietal differences of $^{137}$Cs and $^{90}$Sr transfer factors are reported to be a factor of 1–3, while interspecies differences can be up to a factor of 30;

4) impact of fertilizers – how is TF affected after application of different fertilizers, mainly potassium (K) for Cs (Komínková et al., 2018; Soliman et al., 2019; Uematsu et al., 2018; Wakabayashi et al., 2020) and calcium (Ca) for Sr uptake (Lapointe et al., 2020);

5) impact of environmental factors – how different environmental factors, such as soil moisture (Nikitin, 2021) and length of day light (Noda et al., 2016), affect TF;

6) soil ploughing (He and Walling, 1997; Li et al., 2019; Massas et al., 2010) can lead to changes in soil properties or lead to the redistribution of radionuclides in the root zone and subsequently affect radionuclide accumulation by crops.

A combination of all the above-mentioned factors can lead to high variability of TF (Melnitchouck and Hodson, 2004). Despite the large number of studies conducted around the world during the last five decades, and the number of papers and reports summarizing the state of the art in the particular period (Anspaugh & Balonov, 2005; Kostiainen et al., 2002; Burger and Lichtscheidl, 2018, 2019; IAEA, 1994, 2003, 2010), there is still limited understanding of the mutual effect of all the factors affecting the transfer of radionuclides from soil to plant. Hence, generic values of TF provided by IAEA (2010) carry quite a high level of inaccuracy (Frissel et al., 2002), and, for safe assessment of the radionuclide transfer to the food chain, site-specific values are necessary (Ban-Nai and Muramatsu, 2002). With an

increasing database of site-specific TF values, together with assessments of local conditions (soil type and properties, plant species, climate, concentration of K and Ca in soil, humidity, temperature, etc.), there is an increasing understanding and source of data for TF predictions and modelling their changes over time. Some level of uncertainty or even confusion is also brought to the values of transfer factors by the way they are calculated; the majority of published transfer factors ((Bq/kg)/(Bq/kg)) are calculated as the ratio of mass activity concentration in dry the weight of the plant (Bq/kg) to mass activity in dry soil (Bq/kg) (Aba et al., 2021; Al-Oudat et al., 2021; Guillén et al., 2022; Ishii et al., 2015; IAEA, 2010). Utilization of this TF is applicable in a situation when the radionuclide's pollution is equally distributed in the soil (Nakai et al., 2014), this is assumed for arable land after a number of years since the accident. The majority of the generic TF published by the IAEA (2010) and globally accepted reflect transfer factors of radionuclides long after the contamination but do not reflect fresh contamination or uneven distribution of the radionuclides in soil as would be the case of contaminated agriculture soil after a nuclear accident. The other approach is to use the aggregated transfer factor ($T_{ag}$) calculated as mass activity in dry weight of plants (Bq/kg) divided by the surface activity of the soil expressed in Bq/m$^2$, which better reflects the situation of fresh contamination and is more useful in the event of an emergency release (Choi et al., 2009; Godyaeva et al., 2018; Koivurova et al., 2015; Kostiainen et al., 2002). Frissel et al. (2002) stated that soil surface area is sometimes a better unit to use. A number of studies examined the presence of Cs and Sr in soil (Anspaugh & Balonov, 2005; He and Walling, 1997), and indicated that vertical movements of radionuclides in soil are very slow (Kamei-Ishikawa et al., 2008) and therefore these elements are mostly located in its upper layer (IAEA, 2010; Matsuda et al., 2015). Application of different approaches to the determination of transfer factors can lead to confusion and incorrect conclusions when comparing different transfer factors. The main aim of this paper is to identify aggregated transfer factors soil to plant for six different soils collected in extended emergency zones of two nuclear power plants (NPP) in the Czech Republic and three common types of vegetables grown in conditions close to the real situation. The aim of the paper is also to identify transfer factors for fresh pollution when the contamination is not yet evenly distributed in the soil, which simulates conditions close to nature in the event of a nuclear accident. The main research questions are:

*Case study II: Aggregated transfer factors of fresh Cs and Sr pollution to various vegetables from six common European soils – mesocosm experiment (Komínková et al., 2023)*

1) How does soil affect $T_{ag}$?

2) Does $T_{ag}$ respond differently to K supply with respect to soil type?

3) What are the differences in $T_{ag}$ according to different vegetables? Is there a species that is capable of uptaking more radionuclide than other vegetable species?

4) Are there significant differences between $T_{ag}$ for Sr and Cs? Do the studied plants prefer to uptake one radionuclide above the other?

The paper is based on the results of a mesocosm pot experiment conducted with soil collected in emergency zones of Temelín and Dukovany NPPs, which took place in the experimental area of the National Radiation Protection Institute (SÚRO) in the city of Hradec Králové during the vegetation season of 2020.

## Material and Methods

*Experimental design*

The main goal of the experiment was to simulate the transfer of a dissolved fraction of radionuclides to the plants during the first vegetation season after contamination, hence to imitate the situation shortly after a nuclear accident when arable land is contaminated by wet or dry deposition prior to the crop starting to grow (prior vegetation season). In our experiments, the radionuclides were used in a soluble form; in the real case, part of the radionuclide activity in the fallout may be bound in an insoluble form, so the transfer coefficients given here are an upper estimate of the real $T_{ag}$. The experiment was conducted in the form of a mesocosm pot experiment, with 25 L pots containing approximately 15.8 kg of semi-fresh soil collected in extended emergency zones of two NPPs in the Czech Republic (Temelín and Dukovany) from agriculture fields. In each area, the 3 most common types of soil were collected (16 pots per soil) during autumn 2019, transported to the experimental area, and left over the winter to settle under large tents. During the vegetation season, the tents also maintained similar microclimatic conditions and minimized the direct impact of wind, dry and wet deposition, and solar radiation on the experimental plants. The experimental pots were rotated weekly during the vegetation season to maintain the same microclimatic conditions for all the pots.

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*Soil properties and soil contamination*

The soil types collected in the emergency zones of NPPs are the most common soil types not only in the emergency areas of NPPs but also cover 79% of the arable land in the Czech Republic and 54% in the EU. Basic soil properties and coordinates of the cultivated soil sampling sites are provided in S1. Prior to contamination of the soil, soil lumps were broken up. The pots were divided into 2 treatments (8 pots per each treatment and soil type): 1) radionuclide treatment – $^{134}$Cs and $^{85}$Sr were added to the pots; 2) radionuclide and K treatment – $^{134}$Cs, $^{85}$Sr, and K were added to the pots. To minimize the amount of hazardous waste after the experiment, short life radionuclides were used for the experiments: $^{134}$Cs with a half-life of 2.06 years and $^{85}$Sr with a half-life of 65 days. Universal radioactive solutions manufactured by the Czech Metrology Institute were used for the preparation of soil contamination solutions (5 g solutions with a specific activity of 100 kBq/g contain a stable carrier of CsCl ($^{134}$Cs standard) or SrCl$_2$ ($^{85}$Sr standard) at a concentration of 20 mg/L and HCl at a concentration of 3 g/L. The radionuclide solution was diluted with tap water and equally distributed onto the soil surface using a kettle equipped with a sprinkler. The soil area in the pot was 531 cm$^2$, and the depth of the soil layer was about 25 cm. The average radionuclide activity in each pot at the start of the experiment was 5.89 ± 0.18 kBq for $^{85}$Sr and 8.09 ± 0.24 kBq for $^{134}$Cs; hence, the surface activity for $^{85}$Sr and $^{134}$Cs was 111 kBq/m$^2$ and 152 kBq/m$^2$, respectively. Treatments with K were watered with 100 mL of K$_2$SO$_4$ solution (18.1 g K$^+$/ L). The pots were left for one week after contamination to stabilize, and then sowing took place. To prevent changes of soil parameters after contamination, we selected pots with a relatively large amount of soil (approximately 15.8 kg) and a large surface. The concentration of radiotracers was very low and the buffering capacity of the soil was high; therefore, we did not observe substantial changes in the soil composition caused by the contamination. However, some changes in the composition and fertility of the soils during the time of the experiments are unavoidable and simulate close to real conditions in the field in the event that fertilizers are not applied during vegetation season.

*Grown vegetable – sowing and harvesting*

Three common types of vegetables were planted during the experiment in the following order: lettuce (*Lactuca sativa*), onion (*Allium cepa*), and radish (*Raphanus*

*sativus* L.). Lettuce was sown in May and harvested after 71 days, while onion was sown in July and harvested after 70 days. Radish was sown in early October and harvested after 48 days. To minimize the negative effects of occasionally low temperatures, pots with radish were covered with food foil. Lettuce and radish were harvested in sizes comparable to those harvested for consumption; in the case of the onion, the size of the plants was smaller and more adequate to the size when the onion is harvested as an herb, but not yet as a bulb. Watering was carried out with respect to the condition in individual pots, as the need for watering changed during the year depending on the weather and the pots dried unevenly. Tap water was used for watering. The amount of water used for one watering varied from 100 mL per pot in the spring to 300 mL in the summer. The total amount of water used per pot was 4750 mL for the lettuce and 7000 mL for the onion. Radishes were grown under foil, so the soil did not dry out and watering was not necessary. After harvest, there was a similar procedure for all the vegetables: plants were rinsed in a bucket of tap water, dried with filter paper, weighed, placed into paper bags, and dried in an oven at 50 °C to a constant weight. While the biomass of lettuce and radish was quite large, we could analyse plants separately from every pot (all together 96 samples per species), but the onion biomass was small, so we had to mix together plants from one treatment and one soil, which means we had only 12 onion samples; hence, result evaluation is limited.

*Activity measurement and results interpretation*

The activities of $^{134}$Cs and $^{85}$Sr were measured in dried homogenized plant samples by semiconductor gamma spectrometry (HPGe detectors with a relative efficiency of 50–150%). The measurement time was chosen so that the uncertainty of Cs and Sr determination was up to 10%, but with a maximum of 1 week. The efficiency calibrations were done using gel standards prepared by Czech Metrology Institute. Since the density, elemental composition and size of the vegetable samples in the measuring container (i.e., measurement geometry) differed from the calibration standard geometry, a correction had to be made. The $^{134}$Cs activities were corrected for true coincidences as well. The all corrections were done using EFFTRAN of MEFFTRAN software (Vidmar, 2005; Vidmar et al., 2011; Bruggeman et al., 2014). The method is accredited in the SÚRO laboratory. The aggregated transfer factor was calculated as:

$$T_{ag} = \frac{A\ (\text{plant})}{A\ (\text{soil})}$$

where A(plant) is the activity concentration measured in dry plant biomass (Bq/kg) and A(soil) is the surface activity at the harvest day of the experiment (Bq/m$^2$) (IAEA, 2010). The complete unit $T_{ag}$ (Bq/kg)/(Bq/m$^2$) is further used in the text in the abbreviated form (m$^2$/kg). Soil activity in the following experiment was not corrected for the activity uptake by the plants in the previous experiment because it was negligible. To compare the obtained aggregated transfer factors with general transfer factors provided by IAEA (2010) or other studies using soil activity in (Bq/kg), the results of these studies were recalculated to aggregated transfer factors using the following formula:

$$T_{ag} = \left[\frac{\text{Bq}.kg^{-1}}{Bq.m^{-2}}\right] = \frac{\text{TF}\left[\frac{\text{Bq}.kg^{-1}}{Bq.m^{-1}}\right] \times soil\ area\ [m^2]}{(soil\ density\ [kg.m^{-3}] \times soil\ volume\ [m^3])}$$
$$= \frac{\text{TF}\left[\frac{\text{Bq}.kg^{-1}}{Bq.m^{-1}}\right] \times soil\ area\ [m^2]}{soil\ weight\ [kg]}$$

where the soil volume of 1 m$^2$ to 20 cm (IAEA, 2010) depth is 0.2 m$^3$ and the soil density was taken as the average reduce bulk density for the soils used in our experiments, which is 1190 kg/m$^3$. After substituting into the relationship above, we get the relationship $T_{ag}$ = TF/238.

*Statistical evaluation*

The obtained data were processed in Excel and in Rstudio Version February 1, 5042. The unpaired Welch's *t*-test was used to compare significant differences of means between groups because the size of the data sets and their unequal variance. If necessary, a nonparametric alternative, the unpaired two-sample Wilcoxon test, was used. To compare whether the means of the groups differ from each other, the parametric ANOVA method was performed followed by Tukey HSD post hoc tests. However, the assumptions for their use were often not met, so the non-parametric equivalent of the Kruskal-Wallis rank sum test followed by Dunn's post hoc test was carried out instead. A one-sample *t*-test was used to compare a grouped sample of onions as expected mean values with measured variables for lettuce and radish plants. If the assumptions were not met, the analysis was replaced by its non-

parametric equivalent, a single-sample Wilcoxon sign rank test. Correlation tests were performed using Pearson's product-moment correlation and Spearman's rank correlation, depending on the achieved assumptions. Exploratory analysis of the data set through k-means clustering and tree clustering was also performed using the software STATISTICA. The values of the variables describing soil properties were standardized before analysis to exclude the effect of scale. The values of transfer coefficients were used in their original form.

## Results

### Soil properties

The experiment was conducted with six soils; three from the vicinity of NPP Temelín (TE) and three from the vicinity of NPP Dukovany (DU). The studied soils were cambisol (DUK, TEK), fluvisol (TEF), chernozem (DUC) and haplic luvisol (DUH) belonging to loam types of soil, and gley (TEG) belonging to clay loam soil. The basic properties of soils are provided in S1. A cluster analysis was performed to determine the similarity of the investigated soil properties; the results are shown in Fig. 1. The distances of the horizontal graph lines from the x-axis indicate the degree of similarity – a smaller distance means greater similarity. As can be seen from Fig. 1, although DUC and DUH soils belong to the same soil type, the DUH properties are so different from other soils that DUH forms a separate cluster. The cluster analysis also indicates that even though there are two cambisols (DUK and TEK), their properties are quite different from each other (they are members of different clusters). This analysis of soil property similarity, as well as the differences in radionuclide uptakes between the soils, suggest that the use of generic values of transfer factors based on soil type brings a high level of uncertainty in assessing pollution and its implication for food contamination in a real situation. Therefore, local specific values need to be identified to increase awareness about possible crop contamination in the event of nuclear accidents and readiness to change the type of crops planted on the contaminated area with respect to crop selection with a lower ability to accumulate radionuclides in their biomass. It is especially important in areas where there is a higher risk of soil contamination, such as emergency zones of NPPs.
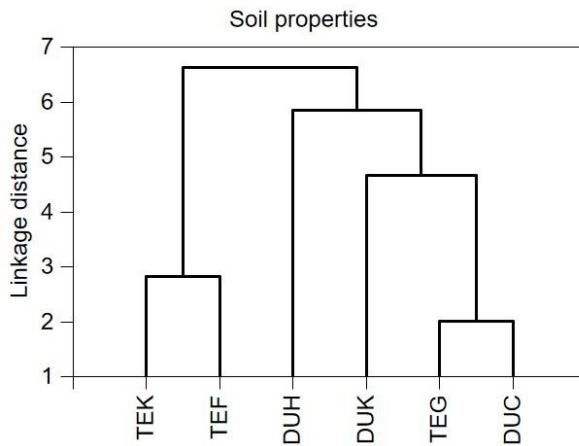
**Fig. 1:** *Results of cluster analysis of soil types.*

*Impact of soils and potassium treatment on radionuclide transfer*

S2 summarizes the aggregated transfer factors of Cs and Sr for all soils and for all three studied vegetables. It can be seen from S2 that plants uptake Sr more easily than Cs and that there are significant differences between the uptake of radionuclides by different plant species. A detailed analysis of the results is presented in the following sections.

*Impact of soils and potassium treatment on radionuclide transfer to lettuce*

Figs. 2 and 3 show aggregated transfer factor of Cs and Sr of lettuce grown in six different soils.

**Fig. 2:** *Aggregated transfer factor $m^2/kg$ of Cs in lettuce grown in six soils without (left) and with (right) potassium treatment.*



**Fig. 3:** *Aggregated transfer factor $m^2/kg$ of Sr in lettuce grown in six soils without (left) and with (right) potassium treatment.*

*Caesium.*

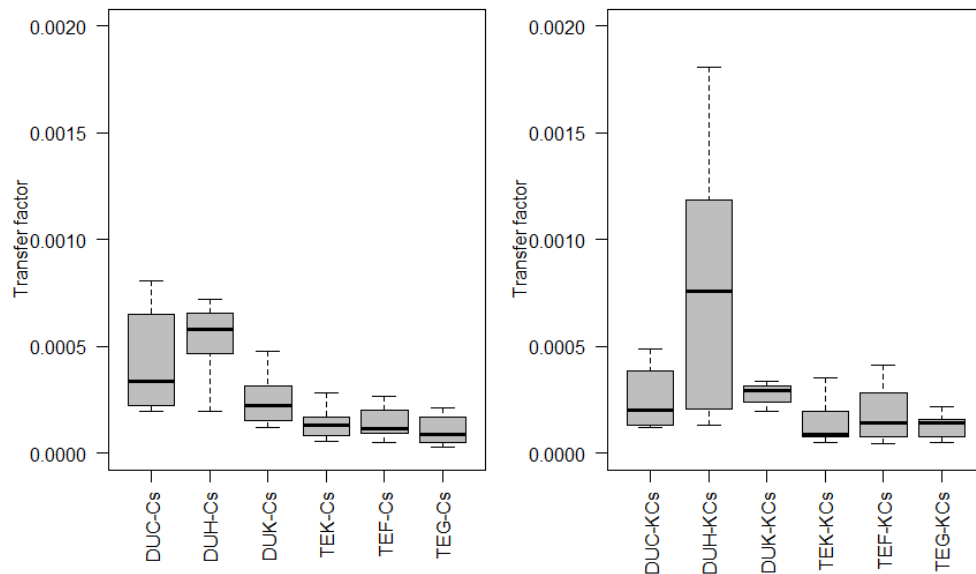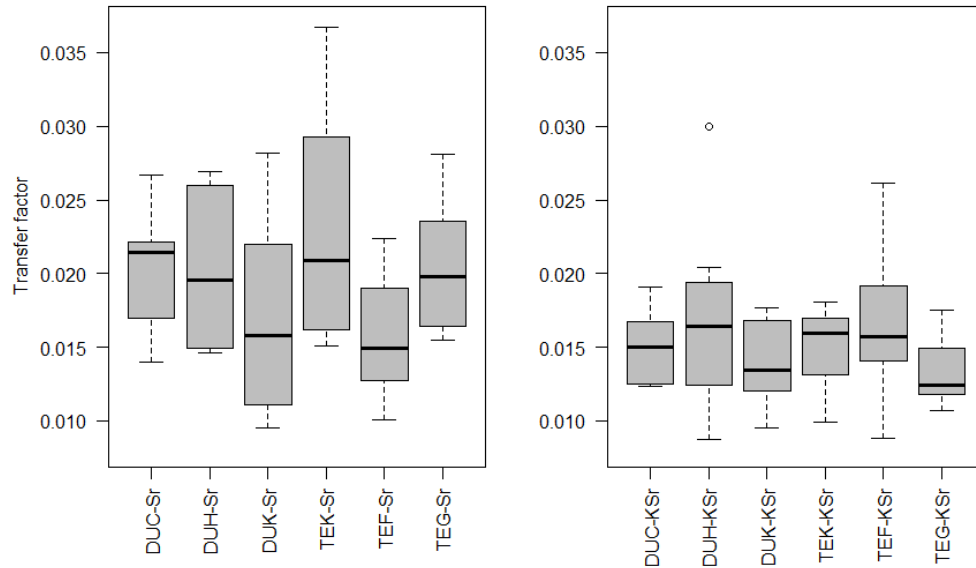A wide range of $T_{ag}$ [134]Cs with the highest arithmetic mean (AM) was found in the soil type DUC (2.0E-04 − 8.1E-4 $m^2$/kg; AM 4.4E-4 $m^2$/kg) and similarly in the DUH (2.0E-4 − 5.1E-3 $m^2$/kg; AM 1.1E-3 $m^2$/kg). The arithmetic means of $T_{ag}$ in the groups of soil types DUK, TEK, TEF, and TEG showed lower values (1.1E-4 − 2.5E-4 $m^2$/kg) (Fig. 2). In statistical tests of the influence of soil types on $T_{ag}$ [134]Cs in lettuce, several cases of statistically significant differences between individual groups of soil types were found (Table 1, S9). Significantly higher $T_{ag}$ were found in lettuce plants growing in soil DUC and DUH in comparison to soil TEK, TEF, and TEG. DUK shows significantly higher $T_{ag}$ only when compared to TEG. The different soil properties, mainly the different proportion of particular particle size and pH, may provide an explanation for the different behaviour. Based on known factors such as clay fraction content, pH, and K, it is not possible to infer the cause of the higher TF in DUC, DUH, or DUK. The differences in these soil parameters compared to the parameters of other soils are not large. The reason may be a combination of conditions. DUC and DUH are distinguished from other soils by a higher amount of calcium and a higher pH value at level 7. However, there is no confirmation in the literature that these conditions would lead to higher TF; in the case of an individual pH value, the opposite is true. A pronounced lower $T_{ag}$ range (1.2E-4 − 4.9E-4 $m^2$/kg; AM 2.6E-4 $m^2$/kg) was found in K-treated DUC compared to K-untreated soil. In contrast, the $T_{ag}$ range of the DUH group was much larger than in all the remaining groups not treated with K, where the AM was 7.8E-4 $m^2$/kg (1.3E-4 − 1.8E-3 $m^2$/kg). The low K concentration in DUH soil could be the reason for the highest transfer factors of Cs to lettuce. For the remaining groups the mean $T_{ag}$ values ranged from 1.3E-4 to 2.8E-4 $m^2$/kg. Despite apparently different $T_{ag}$ values in the K-untreated and K-treated corresponding soils pairs, no significant difference was achieved (S10). This insignificant result can be explained by the large variability of the data in the DUC and DUH groups. For the $T_{ag}$ [134]Cs of all soil types with the K treatment (Table 1, S9), significant differences were maintained in the DUH group if comparing with TEK, TEF, and TEG and the DUK soil type showed significantly higher $T_{ag}$ values than TEK and TEG.

**Table 1:** Statistical significance of $T_{ag}$ differences (p-value) for lettuce and radish grown on different soil types.

Notes: Marking significance of difference 0 - for p > 0.05; 1 - for p < 0.05; 2 - for p < 0.01; 3 - for p < 0.001 ↑ if the value of the variable specified for the row is significantly higher than the value of the variable specified for the column↓ if the value of the variable specified for the row is significantly lower than the value of the variable specified for the column.

| Lettuce | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| without K | DUC | DUH | DUK | TEK | TEF | TEG | | with K | DUC | DUH | DUK | TEK | TEF | TEG |
| DUC | | 0 | 0 | 0 | 0 | 0 | | DUC | | 0 | 0 | 0 | 0 | 0 |
| DUH | 0 | | 0 | 0 | 0 | 0 | | DUH | 0 | | 0 | 0 | 0 | 0 |
| DUK | 0 | 0 | | 0 | 0 | 0 | Sr | DUK | 0 | 0 | | 0 | 0 | 0 |
| TEK | ↓ 2 | ↓ 2 | 0 | | 0 | 0 | | TEK | 0 | ↓ 2 | ↓ 1 | | 0 | 0 |
| TEF | ↓ 1 | ↓ 2 | 0 | 0 | | 0 | | TEF | 0 | ↓ 1 | 0 | 0 | | 0 |
| TEG | ↓ 2 | ↓ 3 | ↓ 1 | 0 | 0 | | | TEG | 0 | ↓ 1 | ↓ 1 | 0 | 0 | |
| Cs | | | | | | | | Cs | | | | | | |

| Radish | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| without K | DUC | DUH | DUK | TEK | TEF | TEG | | with K | DUC | DUH | DUK | TEK | TEF | TEG |
| DUC | | 0 | ↓ 2 | 0 | ↓ 1 | 0 | | DUC | | 0 | ↓ 3 | 0 | ↓ 2 | 0 |
| DUH | ↑ 3 | | ↓ 2 | 0 | ↓ 2 | 0 | | DUH | ↑ 2 | | ↓ 3 | 0 | ↓ 1 | 0 |
| DUK | 0 | 0 | | 0 | 0 | ↓ 3 | Sr | DUK | 0 | 0 | | ↓ 3 | 0 | ↑ 3 |
| TEK | 0 | ↓ 1 | 0 | | 0 | 0 | | TEK | 0 | 0 | 0 | | 0 | 0 |
| TEF | ↑ 3 | 0 | 0 | ↑ 1 | | ↓ 2 | | TEF | 0 | 0 | 0 | 0 | | ↑ 2 |
| TEG | ↑ 2 | 0 | 0 | 0 | 0 | | | TEG | ↑ 2 | 0 | 0 | 0 | 0 | |
| Cs | | | | | | | | Cs | | | | | | |

*Strontium*

The ranges of $T_{ag}$ [85]Sr in lettuces growing in six soils treated with radionuclides only were very similar in all groups, and the values span from 1.1E-2 to 3.7E-2 m$^2$/kg. From statistical tests comparing $T_{ag}$ [85]Sr, no significant difference (Table 1, S9) was found for either group. The DUH group had the largest range of $T_{ag}$ [85]Sr (8.8E-3 – 3.0E-2 m$^2$/kg; AM 1.7E-2 m$^2$/kg) of K-treated soil types followed by soil type TEF (8.8E-3 – 2.6E-2 m$^2$/kg; 1.7E-2 m$^2$/kg). In contrast, TEG showed the smallest range (1.1E-2 – 1.8E-2 m$^2$/kg; AM 1.3E-2). By comparing the $T_{ag}$ of the same pairs of soil types in only [85]Sr treated groups with those treated with [85]Sr and K additive, three

pairs of groups were found to have significant differences (S10). Significantly lower $T_{ag}$ were found in the K-treated DUC, TEK, and TEG groups. However, a decrease in mean $T_{ag}$ [85]Sr values was observed in almost all K-treated groups (i.e. DUC, DUH, DUK, TEK, and TEG) by 17–35%. It has been shown that the differences in $T_{ag}$ in lettuce plants growing in different K-treated soil types are not significant (Table 1, S9). Results of cluster analyses for the aggregated transfer factor of lettuce are provided in the supplements (S3 and S4) and indicate that the characteristics of the transfer factors for individual soil types are very similar. In the case of [85]Sr, TEK and DUH differ the most from the others, both in terms of higher $T_{ag}$ value and higher variability. In the case of [134]Cs, DUH differs the most, again with a higher $T_{ag}$ value and variability, as well as a more pronounced response to the addition of K to the soil. Of the other soils, it most closely resembles DUC.

*Impact of soils and potassium treatment on radionuclide transfer to radish*

Figs. 4 and 5 present the aggregated transfer factors of Cs and Sr for radish grown in six soils with and without K treatment.
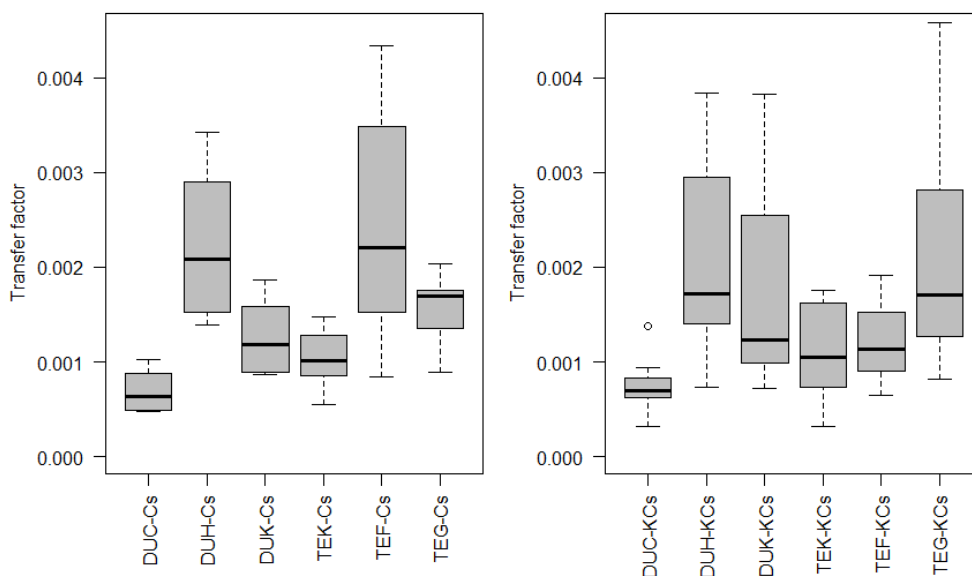


**Fig. 4:** *Aggregated transfer factor $m^2/kg$ of Cs in radish grown in six soils without (left) and with (right) potassium treatment.*

*Case study II: Aggregated transfer factors of fresh Cs and Sr pollution to various vegetables from six common European soils – mesocosm experiment (Komínková et al., 2023)*
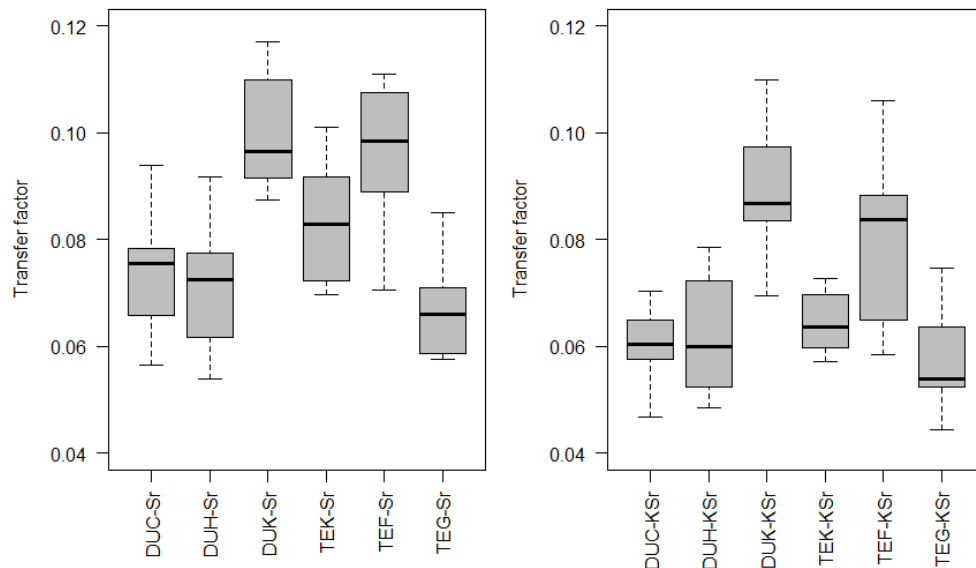


**Fig. 5:** *Aggregated transfer factor $m^2/kg$ of Sr in radish grown in six soils without (left) and with (right) potassium treatment.*

*Caesium.*

The largest range of $T_{ag}$ [134]Cs values in radishes was found in soil type TEF (4.3E-3 − 8.5E-4 $m^2/kg$; AM 2.5E-3 $m^2/kg$), the smallest $T_{ag}$ in DUC (4.7E-4 − 1.0E-3 $m^2/kg$; AM 6.9E-4 $m^2/kg$). The arithmetic means of the $T_{ag}$ values of the remaining soil types ranged from 1.1E-3 to 2.2E-3 $m^2/kg$. Statistical evaluation of radish $T_{ag}$ [134]Cs between soil types revealed significantly lower $T_{ag}$ values in DUC compared to DUH, TEF, and TEG (Table 1, S9). Another group with a significantly lower $T_{ag}$ was the TEK (compared to DUH and TEF). The range of $T_{ag}$ [134]Cs for radishes in the K-treated groups was the highest in the TEG group (8.2E-4 − 4.6E-3 $m^2/kg$; AM 2.1E-3 $m^2/kg$) and the smallest in the DUC group (3.2E-4 − 1.4E-3 (outlying) $m^2/kg$; AM 7.5E-4 $m^2/kg$). The values of the arithmetic mean of the radish $T_{ag}$ for the other groups spread from 1.1E-3 to 2.1E-3 $m^2/kg$. Differences between $T_{ag}$ [134]Cs for radishes in pairs of soil types treated with only [134]Cs and [134]Cs with K additive show that only the TEF group values were significantly lower in K-treated pots, with a decrease to 51%. With the exception of DUH, where only a slight decrease in Tag was also recorded, the Tag of the other K-treated soil groups increased (by 6–40%). Significant

differences in $T_{ag}$ $^{134}$Cs between K-treated soil types were obtained only for soil type DUC. $T_{ag}$ of soil type DUC was significantly lower than for DUH and TEG (Table 1, S9).

*Strontium.*

The statistical analyses of $T_{ag}$ $^{85}$Sr in radishes growing in various soil types revealed several cases of significant differences (Table 1, S9). Soil types DUC, DUH and TEG showed significantly lower $T_{ag}$ compared to DUK and TEF (Table 1, S9). The range is the smallest for TEG (5.8E-2 − 8.5E-2 m$^2$/kg; AM 6.81E-2 m$^2$/kg) and the largest for TEF (7.1E-2 − 1.1E01 m$^2$/kg; AM 9.4E-2 m$^2$/kg). The average values for the DUC, DUH, DUK, and TEK groups ranged from 7.1E-2 to 1.0E-1 m$^2$/kg. In the K-treated groups, the narrowest range of $T_{ag}$ $^{85}$Sr was found in the TEK group (5.7E-2 − 7.3E-2 m$^2$/kg; AM 6.5E-2 m$^2$/kg), in contrast to the TEF group (5.9E-2 − 1.1E-1 m$^2$/kg; AM 8.0E-2 m$^2$/kg). The average values of the remaining groups ranged from 5.7E-2 to 8.9E-2 m$^2$/kg. When comparing the corresponding groups of soil types treated with $^{85}$Sr and $^{85}$Sr with the addition of K, significantly lower $T_{ag}$ were found for soil types with K treatment in the groups DUC, TEK, and TEG (S10). For all groups, there was a mean decrease of 16%. Statistical differences in radishes $T_{ag}$ $^{85}$Sr between different soils amended with K were observed (Table 1, S9). $T_{ag}$ of radishes growing in soil types DUC, DUH and DUK were significantly lower than DUK and TEF. The change compared to K-non-treated soils occurred in soil type DUK, which showed significantly lower $T_{ag}$ than in soil type TEK. Cluster analyses results (S5 and S6) indicate that radish $T_{ag}$ are very similar in different soils, maybe even more than in the case of lettuce. For $^{85}$Sr, TEF and DUK soils differ from the others by having a higher $T_{ag}$ value. In the case of $^{134}$Cs, TEG appears to be different, mainly due to its variability.

*Impact of soils and potassium treatment on radionuclide transfer to onion*

The biomass of onion obtained during the experiment was very low, so samples collected from pots of similar soil and treatment had to be put together, leading to a limited statistical analysis. Fig. 6 shows the aggregated transfer factors. The results show that $T_{ag}$ of Sr were one to two orders of magnitude higher than those of Cs and the supply of K more affected Sr uptake than Cs uptake. The cluster analysis (S7 and S8) was only performed on the basis of arithmetic means. In the case of both

monitored radionuclides, TEF and TEK soils appear to be the most different, with a higher average value of $T_{ag}$.

*Transfer factor depending on vegetable*

The results (S2) show that variability in the aggregated transfer factor of Sr was lower (1 order of magnitude) than in Cs (2 orders of magnitude). All three vegetables had significantly higher $T_{ag}$ for Sr than Cs, despite the fact that Sr activity in soil was lower than Cs activity (ca. only 73% of Cs at the beginning of the experiment and ca. 26% at the end); on the other hand, Sr has higher mobility in soil and is therefore more easily available to plants. The differences result from the different chemical characteristics of both elements; the alkaline element Sr is, as was already mentioned, an analogue of Ca, while Cs is an analogue of K. The higher TFs for Sr reported e.g. Melnitchouck and Hodson (2004). The ratio of Sr/Cs $T_{ag}$ was in the range of 2.9 up to 192, with the lowest values for onion and the highest for lettuce; this may indicate that each vegetable has a different capability to uptake each radionuclide and has different preferences. Despite the fact that lettuce was grown as the first crop, hence having the highest amounts of radionuclides present in the soil, it had the lowest $T_{ag}$ of both radionuclides in the majority of the soils. The highest aggregated transfer factors of Cs resp. Sr were identified in onion and radish, respectively. Kamei-Ishikawa et al. (2008) indicated that vertical movements of radionuclides in soil are very slow, and therefore these elements are mostly located in its upper layer (IAEA, 2010; Matsuda et al., 2015). Therefore differences among the vegetables should not be attributed to movement of radionuclides to deeper layers as their movements are very limited during the duration of the experiment. The mean $T_{ag}$ values of Cs of all soils were in the order lettuce (3.6E-4 $m^2$/kg), radish (1.9E-3 $m^2$/kg), and onion (6.4E-3 $m^2$/kg) for soil without K supply, and 2.9E-4 $m^2$/kg, 1.5E-3 $m^2$/kg, and 7.8E-3 $m^2$/kg for soil with added K.
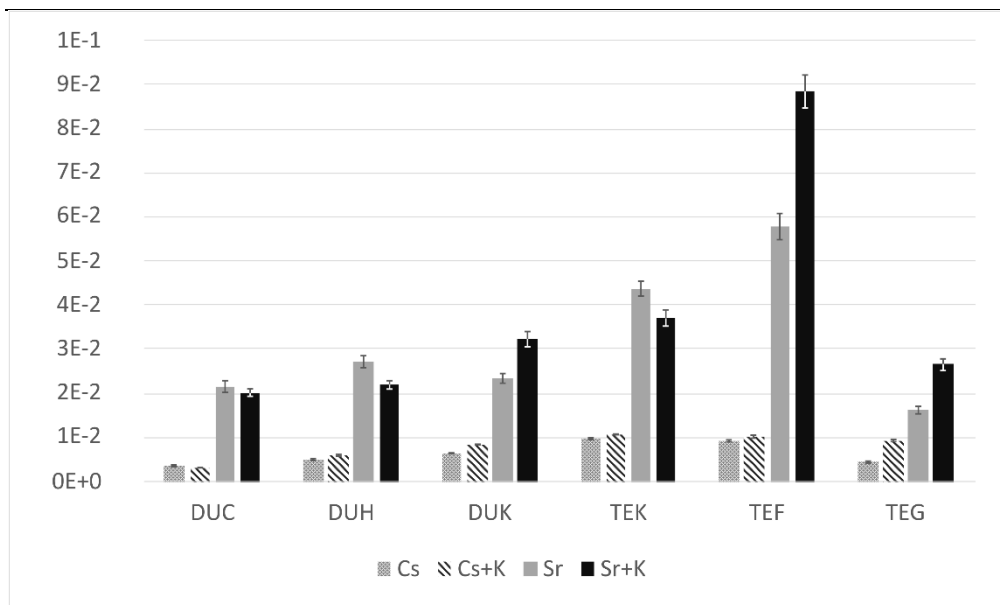
**Fig. 6:** Aggregated transfer factor $m^2/kg$ of Cs and Sr in onion grown in six soils without and with potassium treatment.

In the case of Sr, the mean values were in the order of lettuce (2.0E-2 $m^2/kg$), onion (3.2E-2 $m^2/kg$), and radish (8.1E-2 $m^2/kg$) for soil without K supply, and 1.5E-2 $m^2/kg$, 6.9E-2 $m^2/kg$, and 3.8E-2 $m^2/kg$ for soil with added K. The ratio of Sr $T_{ag}$ for treatments with K to treatment without K was for lettuce in the range of 0.6–1.1, radish in the range of 0.8–0.9, and for onion 0.8 to 1.6; Cs $T_{ag}$ for lettuce was in the range of 0.6–1.3, radish in the range of 0.5–2.4, and onion 0.5 to 1.4. The results of the mesocosms pot experiment show that lettuce took up the lowest concentration of both radionuclides; this is in contradiction to Melnitchouck and Hodson (2004), who observed that lettuce grown in sandy loam soil had the highest uptake of Cs, while radish grown in peat soil had the lowest uptake. They also recorded that plants grown in sandy loam and peat soils had the highest concentration of Sr, regardless of crop species. For both radionuclides applied in two concentrations (1 and 50 mg/kg) to five different soils (silty clay loam, sandy loam from Shuttleworth, sandy loam from Sonning farm, clay loam, and peat soil), the differences in uptake by both plants were significantly different for all soils. The impact of K on Cs uptake has been subject to a large number of studies; while a number of them indicate that Cs uptake is suppressed by K supply (Komínková et al., 2018; Kubo et al., 2017; Rosén and Vinichuk, 2014; Soliman et al., 2019), there are also papers indicating that

the addition of K did not have any clear effect (Ban-Nai and Muramatsu, 2002; Kondo et al., 2015; Tensho et al., 1961), as well as papers indicating that the effect is dependent on the concentration of K (Cline and Hungate, 1960; Soudek et al., 2004). In this study, the impact of K supply on Cs uptake and aggregated transfer factor was not significant in any of the vegetables; a possible explanation could be that the supplied amount (115 mg/kg of soil if we calculate 20 cm layer) was lower than the amount of exchangeable K naturally occurring in the studied soils (118–362 mg/kg). Frissel et al. (2002) reported that K levels respond to threshold mechanism; while below 19.5 mg/kg of exchangeable K there is a strong impact of K on Cs $T_{ag}$, above this concentration other factors determine the TF of Cs. As the natural concentrations of exchangeable K in the soils collected in the emergency zones were one order of magnitude higher than the threshold concentration, increasing the K level in the soil did not bring the required outcome. The non-uniform distribution of K could partially influence the effect, as K was dosed on the surface. However, K is not so significant sorbent like Cs, and its descent freely into deeper layers occurred with watering. This is also confirmed by the influence on Sr. The addition of K decreased transfer into the crops, as can be seen from the above-mentioned ratios of $T_{ag}$ in K treated and untreated soils. It is not conceivable to anticipate that K would take the place of Sr in the transportation pathways into the plant body since K is not analogous to Sr. Sr is mainly captured at the soil surface through ion-exchange and complex formation with organic matter (Berns et al., 2018). According to Sanzharova et al. (2005), various soils contain 64–93% of $^{90}$Sr in exchangeable form. The impact of K on the amount of $^{85}$Sr in soil solution and the resulting decrease in its availability for the plants could be the cause of the lower transfer of $^{85}$Sr from the K untreated soils. A comparison of mean values obtained in this experiment to values obtained from the literature (Aung et al., 2015; Bystrzejewska-Piotrowska and Urban, 2004; Djingova and Kuleff, 2002; Nikitin et al., 2018; Tang and Willey, 2003; Wang et al., 2012) is provided in the supplements (S11). Despite the fact that the obtained transfer factors were within the range of earlier published transfer factors, there is a quite wide range of values for particular vegetables and radionuclide. The ratio of experimental $T_{ag}$ to published data for lettuce was 0.4–38 for Sr and 0.01–8.2 for Cs, for radish 0.04–22 for Sr and 0.3–423 for Cs and in the case of onion 2.1 to 71 for Sr and 0.7 to 397 for Cs. The comparison with published data confirmed the urgent need to identify local transfer factors, especially for areas under higher risk of

contamination as emergency zones of NPPs, as the application of general values may lead to underestimation or overestimation of the agricultural crop contamination in case of nuclear accidents and therefore be misleading in assessing the risk for the human population. The use of general values needs to be supported by soil chemistry and used only in cases where the soil chemistry of particular area is close to the soil chemistry for which the general values were identified.

## Conclusion

The main aim of this paper was to obtain aggregated transfer factor soil to plant for six different soils, collected in extended emergency zones of two NPPs in the Czech Republic and three common types of vegetables (lettuce, onion and radish). The studied soils belong to most common agriculture soils in EU, covering 54% of the arable land. The experimental work reached the following main results.

1) The experiment (testing 6 different soils from 5 subtypes and 2 soil types) did not indicate any statistically significant proof that soil subtype or soil type affect $T_{ag}$.

2) The effect of K treatment on $T_{ag}$ Cs was not observed; the mean $T_{ag}$ Cs ratio with and without K treatment is 1.0. The reason could be that a relatively small amount (115 mg/kg) was added compared to the total exchangeable K already present in the soil (118–362 mg/kg of dry matter). The K supply caused a slight decrease in Sr $T_{ag}$, the mean $T_{ag}$ Sr ratio with and without K treatment is 0.9. Despite there being no statistical evidence of K impact on $T_{ag}$, the decrease as well as the increase in $T_{ag}$ should not be neglected for particular soils due to the high variability of the experimental data.

3) There are differences in the $T_{ag}$ of different vegetables, while lettuce grown first in freshly contaminated soil had the lowest $T_{ag}$ for both radionuclides. Although radish has the highest $T_{ag}$ for Sr and onion for Cs. The radish has a high ability to uptake studied radionuclides from the soil; hence it may cause high radiological risk when present in the human diet after the release of radionuclides to the environment.

4) All the studied vegetables had a higher transfer factor of Sr compared to Cs; while in the case of onion the mean value of Sr/Cs was 4.9, for radish it was

54, and for lettuce it was 86, indicating that the plants had different preferences for uptake of both radionuclides.

In addition to these main conclusions, we can make some other generalizations. For all three vegetables and soil types. We can say that.

1) TEF and TEK reach rather higher values of $T_{ag}$; DUK and DUH reach averages, and DUC and TEG have medium and lower values;

2) in DUC and DUH, the addition of K to the soil lead to a decrease in $T_{ag}$, whereas in the other soils, the response to the addition is different in different cases;

3) the highest concentration of Ca in DUH soil (S1) did not cause a lowering of Sr uptake compared to soils with less Ca;

4) overall, for lettuce and radishes, statistically significant differences in $T_{ag}$ between soils were not observed in most cases; only 19 for Cs and 13 for Sr were significant, in comparison to 88 being insignificant (S9);

5) K treatment had a statistically significant effect on the reduction of $T_{ag}$ in lettuce and radish for Cs in 1 case and for Sr in 6 cases; in comparison to 17 cases the effect was insignificant (S10).

The obtained transfer factors were within the range of transfer factors published in earlier works, but the range of transfer factors for each radionuclide and vegetable species is quite wide (from one up to three orders of magnitude). Therefore, compared to generic values or values obtained from the literature, we believe that site specific transfer factors provide a higher level of accuracy in the process of radiological risk assessment and evaluation of radionuclide transfer to food chains. On the other hand, $T_{ag}$ values were determined for typical crops grown in Europe, and an analysis of basic soil characteristics was performed; therefore, we believe that the transfer coefficients can be used to predict contamination on a wider scale on similar soils in Central Europe and that the study helps to update the state of the art concerning the uptake of radionuclides by common vegetables grown not only for commercial purposes, but also in private gardens.

The contribution of this study is to obtain transfer coefficients that come from an experiment designed to simulate an uptake of fresh pollution during the first

growing season after a nuclear accident and to simulate the conditions on an agricultural land, where the pollution will not be dispersed homogeneously and will be located primarily in the surface layers of the soil. However, the higher variability of the results corresponds to real environmental conditions and points out that the results obtained from studies where the soil is homogeneously contaminated represent an ideal situation, but do not correspond to real environmental conditions.

The results compared with the existing transfer coefficients show a high variability of already existing and commonly used transfer coefficients, where the variability can be caused not only by environmental conditions, type of crop, age of pollution, but also by the design of the experiment.

## Supplementary material

**S1:** Basic soil properties of the soil subtypes used in the experiment

| NPP | | | Temelín | | | Dukovany | | |
|---|---|---|---|---|---|---|---|---|
| Subtype of soil | | | fluvisol | gley | cambisol | cambisol | haplic | chernozem |
| Acronym | | | TEF | TEG | TEK | DUK | DUH | DUC |
| pH KCl | | | 5.9 | 6.5 | 5.4 | 6.0 | 7.1 | 7.0 |
| Cox | | % | 1.82 | 1.67 | 1.83 | 1.1 | 0.88 | 1.38 |
| Humus | | % | 3.14 | 2.88 | 3.15 | 1.89 | 1.52 | 2.38 |
| Ca | | mg/kg dry weight | 2080 | 3160 | 2080 | 1710 | 5860 | 3800 |
| K | | | 233 | 332 | 304 | 362 | 118 | 276 |
| Mg | | | 401 | 294 | 316 | 150 | 178 | 364 |
| P | | | 101 | 98 | 70 | 168 | 25 | 97 |
| Grain size (mm) | <0.001 | % | 11,6 | 22,2 | 13,8 | 16,7 | 19,7 | 22,4 |
| | 0.001 - | | 12,9 | 23,0 | 18,4 | 11,9 | 13,1 | 20,5 |
| | 0.01 - | | 21,6 | 29,8 | 28,5 | 34,8 | 23,9 | 35,6 |
| | 0.05 - | | 16,5 | 9,6 | 14,3 | 24,1 | 31,3 | 10,6 |
| | 0.25 - 2.0 | | 37,4 | 15,3 | 25,0 | 12,5 | 12,0 | 10,8 |
| Reduced vol. | | g/cm³ | 0.99 | 0.98 | 0.99 | 1.2 | 1.16 | 1.04 |
| Coordinates | | | 49.14N, 14.15E | 49.13N, 14.14E | 49.14N, 14.14E | 49.08N, 16.17E | 49.07N, 16.18E | 49.05N, 16.21E |

*Case study II: Aggregated transfer factors of fresh Cs and Sr pollution to various vegetables from six common European soils – mesocosm experiment (Komínková et al., 2023)*

Note: Concentration of P, K, Mg correspond to average value of agricultural soils in the Czech Republic according to Smatanová and Sušil (2018)

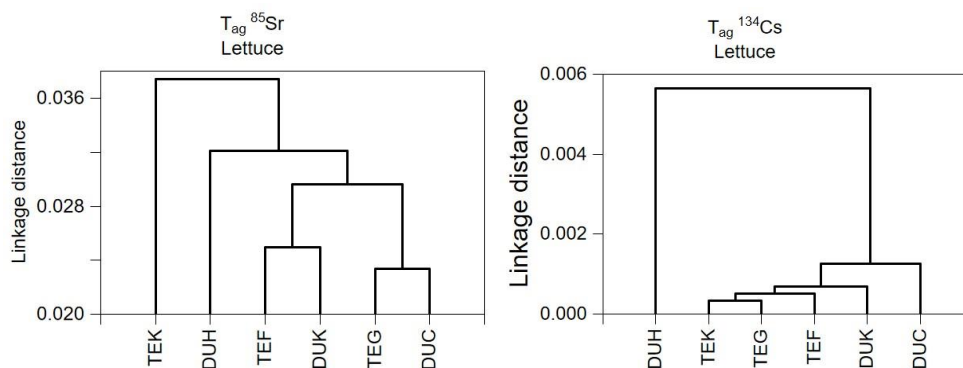**S2:** Aggregated transfer factors of $^{134}$Cs and $^{85}$Sr for lettuce, radish and onion

| | Type of soil | | DUC | DUH | DUK | TEK | TEF | TEG | Mean |
|---|---|---|---|---|---|---|---|---|---|
| **Lettuce** | N. of samples | | 7 | 8 | 8 | 7 | 8 | 7 | 45 |
| | Tag $^{134}$Cs [m$^2$/kg] | Mean | 4.4E-4 | 1.1E-3 | 2.5E-4 | 1.4E-4 | 1.4E-4 | 1.1E-4 | 3.6E-4 |
| | | Min | 2.0E-4 | 2.0E-4 | 1.2E-4 | 5.8E-5 | 5.3E-5 | 2.6E-5 | 2.6E-5 |
| | | Max | 8.1E-4 | 5.1E-3 | 4.8E-4 | 2.8E-4 | 2.7E-4 | 2.1E-4 | 5.1E-3 |
| | | SE | 1.0E-4 | 5.7E-4 | 4.2E-5 | 2.9E-5 | 2.6E-5 | 2.4E-5 | 1.6E-4 |
| | T$_{ag}$ $^{85}$Sr [m$^2$/kg] | Mean | 2.0E-2 | 2.0E-2 | 1.7E-2 | 2.3E-2 | 1.6E-2 | 2.0E-2 | 2.0E-2 |
| | | Min | 1.4E-2 | 1.5E-2 | 9.5E-3 | 1.5E-2 | 1.0E-2 | 1.6E-2 | 9.5E-3 |
| | | Max | 2.7E-2 | 2.7E-2 | 2.8E-2 | 3.7E-2 | 2.2E-2 | 2.8E-2 | 3.7E-2 |
| | | SE | 1.7E-3 | 1.9E-3 | 2.4E-3 | 3.2E-3 | 1.6E-3 | 1.6E-3 | 1.1E-3 |
| | T$_{ag}$ Sr / T$_{ag}$ Cs | | 45 | 18 | 68 | 168 | 111 | 192 | 101 |
| **Lettuce + K** | N. of samples | | 8 | 8 | 8 | 8 | 8 | 8 | 48 |
| | T$_{ag}$ $^{134}$Cs [m$^2$/kg] | Mean | 2.6E-4 | 7.8E-4 | 2.8E-4 | 1.4E-4 | 1.8E-4 | 1.3E-4 | 2.9E-4 |
| | | Min | 1.2E-4 | 1.3E-4 | 2.0E-4 | 5.1E-5 | 4.5E-5 | 4.8E-5 | 4.5E-5 |
| | | Max | 4.9E-4 | 1.8E-3 | 3.4E-4 | 3.5E-4 | 4.2E-4 | 2.2E-4 | 1.8E-3 |
| | | SE | 5.2E-5 | 2.1E-4 | 1.7E-5 | 3.7E-5 | 4.6E-5 | 2.0E-5 | 1.0E-4 |
| | T$_{ag}$ $^{85}$Sr [m$^2$/kg] | Mean | 1.5E-2 | 1.7E-2 | 1.4E-2 | 1.5E-2 | 1.7E-2 | 1.3E-2 | 1.5E-2 |
| | | Min | 1.2E-2 | 8.8E-3 | 9.5E-3 | 9.9E-3 | 8.8E-3 | 1.1E-2 | 8.8E-3 |
| | | Max | 1.9E-2 | 3.0E-2 | 1.8E-2 | 1.8E-2 | 2.6E-2 | 1.8E-2 | 3.0E-2 |
| | | SE | 9.1E-4 | 2.3E-3 | 1.0E-3 | 9.9E-4 | 1.8E-3 | 8.7E-4 | 5.8E-4 |
| | T$_{ag}$ Sr / T$_{ag}$ Cs | | 59 | 22 | 50 | 107 | 91 | 104 | 72 |
| **Radish** | N. of samples | | 8 | 8 | 8 | 7 | 8 | 7 | 46 |
| | T$_{ag}$ $^{134}$Cs [m$^2$/kg] | Mean | 6.9E-4 | 2.2E-3 | 1.3E-3 | 1.2E-3 | 2.5E-3 | 3.5E-3 | 1.9E-3 |
| | | Min | 4.7E-4 | 1.4E-3 | 8.7E-4 | 5.6E-4 | 8.5E-4 | 9.0E-4 | 4.7E-4 |
| | | Max | 1.0E-3 | 3.4E-3 | 1.9E-3 | 2.6E-3 | 4.3E-3 | 1.7E-2 | 1.7E-2 |
| | | SE | 7.9E-5 | 2.8E-4 | 1.4E-4 | 2.2E-4 | 4.4E-4 | 1.9E-3 | 4.1E-4 |
| | T$_{ag}$ $^{85}$Sr [m$^2$/kg] | Mean | 7.4E-2 | 7.1E-2 | 1.0E-1 | 7.7E-2 | 1.0E-1 | 6.4E-2 | 8.1E-2 |
| | | Min | 5.7E-2 | 5.4E-2 | 8.7E-2 | 2.3E-2 | 7.1E-2 | 3.6E-2 | 2.3E-2 |
| | | Max | 9.4E-2 | 9.2E-2 | 1.2E-1 | 1.0E-1 | 1.5E-1 | 8.5E-2 | 1.5E-1 |
| | | SE | 4.0E-3 | 4.2E-3 | 3.9E-3 | 8.6E-3 | 7.6E-3 | 5.0E-3 | 6.3E-3 |
| | T$_{ag}$ Sr / T$_{ag}$ Cs | | 107 | 32 | 80 | 62 | 41 | 19 | 57 |
| **Radish + K** | N. of samples | | 8 | 8 | 8 | 8 | 8 | 8 | 48 |
| | T$_{ag}$ $^{134}$Cs | Mean | 7.5E-4 | 2.1E-3 | 1.8E-3 | 1.1E-3 | 1.2E-3 | 2.1E-3 | 1.5E-3 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | [m²/kg] | Min | 3.2E-4 | 7.4E-4 | 7.2E-4 | 3.2E-4 | 6.5E-4 | 8.2E-4 | 3.2E-4 |
| | | Max | 1.4E-3 | 3.8E-3 | 3.8E-3 | 1.77E- | 1.9E-3 | 4.6E-3 | 4.6E-3 |
| | | SE | 1.1E-4 | 3.7E-4 | 3.9E-4 | 1.9E-4 | 1.5E-4 | 4.4E-4 | 2.3E-4 |
| | $T_{ag}$ $^{85}$Sr [m²/kg] | Mean | 6.0E-2 | 6.2E-2 | 8.9E-2 | 6.5E-2 | 8.0E-2 | 5.7E-2 | 6.9E-2 |
| | | Min | 4.7E-2 | 4.8E-2 | 6.9E-2 | 5.7E-2 | 5.9E-2 | 4.5E-2 | 4.5E-2 |
| | | Max | 7.0E-2 | 7.9E-2 | 1.1E-1 | 7.3E-2 | 1.1E-1 | 7.5E-2 | 1.1E-1 |
| | | SE | 2.5E-3 | 4.0E-3 | 4.3E-3 | 2.0E-3 | 5.6E-3 | 3.3E-3 | 5.2E-3 |
| | $T_{ag}$ Sr / $T_{ag}$ Cs | | 80 | 30 | 51 | 58 | 66 | 27 | 52 |
| Onion | N. of samples | | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| | $T_{ag}$ $^{134}$Cs | Mean | 3.7E-3 | 5.0E-3 | 6.3E-3 | 9.9E-3 | 9.1E-3 | 4.4E-3 | 6.4E-3 |
| | $T_{ag}$ $^{85}$Sr [m²/kg] | Mean | 2.14E- | 2.73E- | 2.34E- | 4.37E- | 5.79E- | 1.61E- | 3.16E- |
| | $T_{ag}$ Sr / $T_{ag}$ Cs | | 5.8 | 5.5 | 3.7 | 4.4 | 6.4 | 3.6 | 4.9 |
| Onion + K | N. of samples | | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| | $T_{ag}$$^{134}$Cs [m²/kg] | Mean | 3.1E-3 | 5.8E-3 | 8.1E-3 | 1.1E-2 | 1.0E-2 | 9.1E-3 | 7.8E-3 |
| | $T_{ag}$ $^{85}$Sr [m²/kg] | Mean | 2.0E-2 | 2.2E-2 | 3.2E-2 | 3.7E-2 | 8.9E-2 | 2.7E-2 | 3.8E-2 |
| | $T_{ag}$ Sr / $T_{ag}$ Cs | | 6.4 | 3.9 | 4.0 | 3.5 | 8.9 | 2.9 | 4.9 |

Note: If the number of samples is less than 8, it indicates that in some group of lettuce or radish, the plants did not grow in several pots or grew too little to provide sufficient biomass for the correct measurement of the activity. Due to the small biomass of *A. cepa*, activity was measured for mixed samples per treatment, hence no mean or SE values are provided. The presented values of onion $T_{ag}$ are informative.

**S3:** Results of cluster analysis of transfer coefficient for lettuce without added potassium (left - $T_{ag}$ $^{85}$Sr, right - $T_{ag}$$^{134}$Cs)
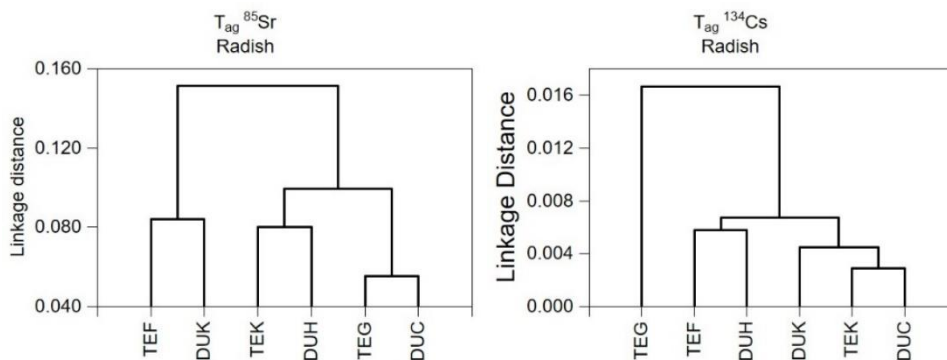
**S4:** Main characteristic of transfer coefficient in individual clusters for lettuce - arithmetic mean (AM), standard error (SE), range without added potassium, and difference in arithmetic means of transfer coefficients between soils without added potassium and with added potassium (Diff AM).

| $T_{ag}$ $^{85}$Sr (m$^2$/kg) | | | | | | |
|---------|--------|--------|--------|--------|--------|--------|
| Soil | TEK | DUH | TEF | DUK | TEG | DUC |
| AM | 1.9E-2 | 1.9E-2 | 1.6E-2 | 1.5E-2 | 1.7E-2 | 1.7E-2 |
| SE | 7.1E-3 | 6.0E-3 | 4.6E-3 | 5.3E-3 | 5.1E-3 | 4.3E-3 |
| Range | 2.7E-2 | 2.1E-2 | 1.7E-2 | 1.9E-2 | 1.7E-2 | 1.4E-2 |
| Diff AM | 8.4E-3 | 3.4E-3 | -8.8E-4 | 3.0E-3 | 7.1E-3 | 5.0E-3 |
| $T_{ag}$ $^{134}$Cs (m$^2$/kg) | | | | | | |
| Soil | DUH | TEK | TEG | TEF | DUK | DUC |
| AM | 9.4E-4 | 1.4E-4 | 1.2E-4 | 1.6E-4 | 2.6E-4 | 3.4E-4 |
| SE | 1.2E-3 | 8.6E-5 | 6.1E-5 | 1.0E-4 | 8.9E-5 | 2.2E-4 |
| Range | 5.0E-3 | 3.0E-4 | 1.9E-4 | 3.7E-4 | 3.6E-4 | 6.9E-4 |
| Diff AM | 3.2E-4 | -2.0E-6 | -2.2E-5 | -4.1E-5 | -3.1E-5 | 1.6E-4 |

Note: The tables contain the characteristics of the transfer coefficient which proved to be the most important for division into clusters during analysis. These are arithmetic mean, standard error, range (indicating the presence of outliers in the set) without added potassium, and difference in arithmetic means of transfer coefficients between soils without added potassium and with added potassium (positive values of this characteristic indicate a reduction in the transfer coefficient after addition of potassium to the soil). The individual soil types are listed in the table in the order that corresponds to their order in the dendrogram.

**S5:** Results of cluster analysis of transfer coefficient for radish without added potassium (left - $T_{ag}$ $^{85}$Sr, right - $T_{ag}$ $^{134}$Cs)
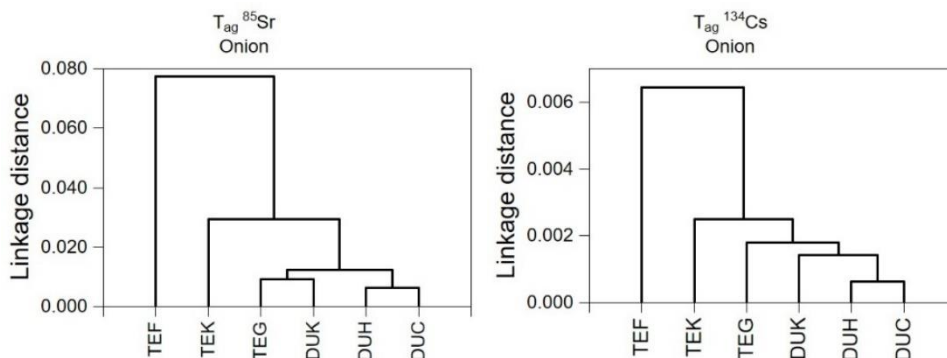
**S6:** Main characteristic of transfer coefficient in individual clusters for radish - arithmetic mean (AM), standard error (SE), range without added potassium, and difference in arithmetic means of transfer coefficients between soils without added potassium and with added potassium (Diff AM).

| $T_{ag}$ $^{85}$Sr (m$^2$/kg) | | | | | | |
|---|---|---|---|---|---|---|
| Soil | TEF | DUK | TEK | DUH | TEG | DUC |
| AM | 9.0E-02 | 9.5E-02 | 7.1E-02 | 6.7E-02 | 6.1E-02 | 6.7E-02 |
| SE | 2.1E-02 | 1.2E-02 | 1.8E-02 | 1.2E-02 | 1.2E-02 | 1.1E-02 |
| Range | 8.6E-02 | 4.7E-02 | 7.8E-02 | 4.3E-02 | 4.9E-02 | 4.7E-02 |
| Diff AM | 2.1E-02 | 1.1E-02 | 1.3E-02 | 9.1E-03 | 6.7E-03 | 1.3E-02 |
| $T_{ag}$ $^{134}$Cs (m$^2$/kg) | | | | | | |
| Soil | TEG | TEF | DUH | DUK | TEK | DUC |
| AM | 2.8E-3 | 1.8E-3 | 2.2E-3 | 1.5E-3 | 1.2E-3 | 7.2E-4 |
| SE | 3.8E-3 | 1.1E-3 | 9.0E-4 | 8.5E-4 | 5.6E-4 | 2.6E-4 |
| Range | 1.6E-2 | 3.7E-3 | 3.1E-3 | 3.1E-3 | 2.2E-3 | 1.1E-3 |
| Diff AM | 1.3E-3 | 1.2E-3 | 1.4E-4 | -5.0E-4 | 1.2E-4 | -6.3E-5 |

Note: The tables contain the characteristics of the transfer coefficient which proved to be the most important for division into clusters during analysis. These are arithmetic mean, standard error, range (indicating the presence of outliers in the set) without added potassium, and difference in arithmetic means of transfer coefficients between soils without added potassium and with added potassium (positive values of this characteristic indicate a reduction in the transfer coefficient after addition of potassium to the soil). The individual soil types are listed in the table in the order that corresponds to their order in the dendrogram.

**S7:** Results of cluster analysis of transfer coefficient for onion without added potassium (left - $T_{ag}$ $^{85}$Sr, right - $T_{ag}$ $^{134}$Cs)

**S8:** Main characteristic of transfer coefficient in individual clusters for onion - arithmetic mean (AM) without added potassium and difference in arithmetic means of transfer coefficients between soils without added potassium and with added potassium (Diff AM).

| $T_{ag}$ $^{85}$Sr (m$^2$/kg) | | | | | | |
|---|---|---|---|---|---|---|
| Soil | TEF | TEK | TEG | DUK | DUH | DUC |
| AM | 7.3E-02 | 4.0E-02 | 2.1E-02 | 2.8E-02 | 2.5E-02 | 2.1E-02 |
| Diff AM | -3.1E-02 | 6.7E-03 | -1.0E-02 | -8.9E-03 | 5.0E-03 | 1.3E-03 |
| $T_{ag}$ $^{134}$Cs (m$^2$/kg) | | | | | | |
| Soil | TEF | TEK | TEG | DUK | DUH | DUC |
| AM | 7.4E-3 | 4.2E-3 | 3.0E-3 | 1.7E-3 | 3.1E-3 | 2.7E-3 |
| Diff AM | -4.9E-3 | 2.7E-3 | -2.5E-3 | 4.7E-4 | 6.3E-5 | 5.3E-4 |

Note: The tables contain the characteristics of the transfer coefficient which proved to be the most important for division into clusters during analysis. These are arithmetic mean and difference in arithmetic means of transfer coefficients between soils without added potassium and with added potassium (positive values of this characteristic indicate a reduction in the transfer coefficient after addition of potassium to the soil). The individual soil types are listed in the table in the order that corresponds to their order in the dendrogram.

**S9:** Numbers of significant differences between soils (from Table 3)

| | Lettuce without K | | Radish without K | | Lettuce with K | | Radish with K | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Yes | No | Yes | No | Yes | No | Yes | No | Yes | No |
| Cs | 7 | 8 | 5 | 10 | 5 | 10 | 2 | 13 | 19 | 41 |
| Sr | 0 | 15 | 6 | 9 | 0 | 15 | 7 | 8 | 13 | 47 |

Note: The table provides a comprehensive numerical representation of all statistically significant results obtained for the aggregated transfer coefficient ($T_{ag}$). It highlights the significant (Yes) and nonsignificant (No) differences observed among six different soil types, including five subtypes and two general soil categories, in relation to their treatment effects on lettuce and radish plants. Among the total of 88 cases analyzed, only 19 cases for Cs and 13 cases for Sr exhibited significant differences, indicating that soil types had a limited impact on $T_{ag}$ across the entire experimental dataset. However, the table reveals noteworthy disparities in Sr uptake between lettuce and radish species. Specifically, radish plants displayed varying levels of Sr uptake on different soil types, thereby influencing the $T_{ag}$ outcomes in a significant manner.

**S10:** Statistical significance of $T_{ag}$ differences (p-value) for lettuce and radish grown on a corresponding soil-type pairs without potassium treatment and with potassium treatment

|  | Lettuce | | Radish | |
|---|---|---|---|---|
|  | Cs | Sr | Cs | Sr |
| DUC | 0 | ↑ 1 | 0 | ↑ 1 |
| DUH | 0 | 0 | 0 | 0 |
| DUK | 0 | 0 | 0 | 0 |
| TEK | 0 | ↑ 1 | 0 | ↑ 2 |
| TEF | 0 | 0 | ↑ 1 | 0 |
| TEG | 0 | ↑ 2 | 0 | ↑ 1 |
| Total | 0 | 3 | 1 | 3 |

Notes: Marking the significance of the difference

0 - for p > 0.05    1 - for p < 0.05    2 - for p < 0.01    3 - for p < 0.001

↑   if the value of the variable without treatment K is significantly higher than the value of the variable with treatment K

↓   if the value of the variable without treatment K is significantly lower than the value of the variable with treatment K

**S11:** Comparison of experimental transfer factors and aggregated transfer factors in lettuce, radish, onion, and values obtained from literature (in the case of aggregated transfer factor calculated for average Czech soil weight). Average values of TF provided in the publications were used.

| Lettuce | | | | | | | |
|---|---|---|---|---|---|---|---|
| Soil type | Duration of planting (d) | Cs | | Sr | | Source | Notes |
|  |  | Fv | Fvag | TF | Tag |  |  |
| all soils | 71 | 8,64E-02 | 3,63E-04 | 4,64E+00 | 1,95E-02 | Experiment | Mean values obtained from this experiment |
| clay and peat | 42 | 1,20E-01 | 5,04E-04 | 1,48E+00 | 6,22E-03 | Melnitchouk et al (2004) | 5 different types of soil (lower and upper value) |
| sandy loam |  | 1,91E+01 | 8,03E-02 | 1,46E+01 | 6,13E-02 |  |  |
| paddy soil | 48 | 5,20E-01 | 2,18E-03 | NA | | Tang and Willey (2003) |  |

| | | | | | | Reference | Note |
|---|---|---|---|---|---|---|---|
| red soil | | 6,30E-01 | 2,65E-03 | | | | |
| calcisol | NA | 1,14E-01 | 4,79E-04 | 9,77E-01 | 4,11E-03 | Al-Attar et al (2016) | 1st year after contamination |
| | | 3,00E-02 | 1,26E-04 | 1,03E+00 | 4,34E-03 | | 2nd year after contamination |
| | | 1,30E-02 | 5,46E-05 | 1,03E+00 | 4,34E-03 | | 10th year after contamination |
| sod-podzolic sandy-loam | 75 | 5,10E-02 | 2,14E-04 | NA | | Nikitin et al, (2018) | arable land contaminated after Chernobyl |
| Silty- clay | NA | 1,14E-01 | 4,79E-04 | 9,80E-01 | 4,12E-03 | Al-Oudat (2021) | Harvested in middle of vegetation growth |
| sandy soil | NA | 1,60E-01 | 6,72E-04 | 2,45E+01 | 1,03E-01 | Aba (2021) | only full fruit were harvested |
| all soils | | 6,00E-02 | 2,52E-04 | 7,60E-01 | 3,19E-03 | IAEA, (2010) 1) | Leafy vegetable |
| sand | | 1,20E-01 | 5,04E-04 | 1,70E+00 | 7,14E-03 | | |
| loam | NA | 7,40E-02 | 3,11E-04 | 1,20E+00 | 5,04E-03 | | |
| clay | | 1,80E-02 | 7,56E-05 | 1,50E-01 | 6,30E-04 | | |
| organic | | 2,30E-02 | 9,66E-05 | 2,10E-01 | 8,82E-04 | | |
| all soils | NA | NA | 2.17E-03 | NA | NA | SUJB (online data) | samples collected 1987 in different areas of the Czech Republic 1 year after Chernobyl accident |
| Radish | | | | | | | |
| all soils | 48 | 4,50E-01 | 1,89E-03 | 1,93E+01 | 8,11E-02 | Experiment | Mean values obtained from this experiment |
| clay and peat | 35 | 9,00E-02 | 3,78E-04 | 2,99E+00 | 1,26E-02 | Melnitchouk et al (2004) | 5 different types of soil |

*Case study II: Aggregated transfer factors of fresh Cs and Sr pollution to various vegetables from six common European soils – mesocosm experiment (Komínková et al., 2023)*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| sandy loam | | 1,32E+01 | 5,56E-02 | 9,30E+01 | 3,91E-01 | | (lower and upper value) |
| Yellow earth | 60 | 2,06E+00 | 8,64E-03 | 2,10E+01 | 8,82E-02 | Wang, D et al (2012) | calculated from uptake concentration provided in the paper (5 soil concentration were used 2.5; 5; 10; 20; 40 mg Cs rsp.Sr/kg |
| | | 7,80E-01 | 3,28E-03 | 5,25E+00 | 2,21E-02 | | |
| | | 3,10E-01 | 1,30E-03 | 1,61E+00 | 6,76E-03 | | |
| | | 1,80E-01 | 7,56E-04 | 4,50E-01 | 1,89E-03 | | |
| | | 9,50E-02 | 3,99E-04 | 1,50E-01 | 6,30E-04 | | |
| sandy soil | NA | 2,20E-01 | 9,24E-04 | 1,05E+01 | 4,43E-02 | Aba (2021) | only full fruit were harvested |
| Takanishi soil | NA | 5,90E-02 | 2,48E-04 | NA | | Aung et al. (2015) | 1st year after Fukushima |
| Different type of soil collecetd across Japan | NA | 1,58E-01 | 6,64E-04 | 5,10E-01 | 2,14E-03 | Ban-Nai (2002) | recalculated from activity concentration per wet mass multiplied by the average of DW/FW (17,6); (average, minimum and maximum values) |
| | | 2,50E-02 | 1,05E-04 | 5,60E-02 | 2,35E-04 | | |
| | | 1,13E+00 | 4,73E-03 | 3,17E+00 | 1,33E-02 | | |
| all soils | NA | 4,20E-02 | 1,76E-04 | 7,20E-01 | 3,03E-03 | IAEA 2010 1) | root vegetable |
| sand | | 6,20E-02 | 2,61E-04 | 1,10E+00 | 4,62E-03 | | |
| loam | | 3,00E-02 | 1,26E-04 | 6,10E-01 | 2,56E-03 | | |
| clay | | 2,40E-02 | 1,01E-04 | 4,10E-01 | 1,72E-03 | | |
| organic | | 5,90E-02 | 2,48E-04 | NA | | | |
| all soils | NA | NA | 2.17E-03 | NA | NA | SUJB (online data) | samples collected 1987 in different areas of the Czech Republic 1 |

*Case study II: Aggregated transfer factors of fresh Cs and Sr pollution to various vegetables from six common European soils – mesocosm experiment (Komínková et al., 2023)*

| | | | | | | | year after Chernobyl accident |
|---|---|---|---|---|---|---|---|
| Onion | | | | | | | |
| all soils | 70 | 1,52E+00 | 6,40E-03 | 7,52E+00 | 3,16E-02 | Experiment | Mean values obtained from this experiment |
| | 10 | 2,87E+00 | 1,21E-02 | NA | | Bystrzejewska-Piotrowska (2004) | |
| sandy soil | NA | 1,30E-01 | 5,46E-04 | 4,45E+00 | 1,87E-02 | Aba (2021) | only full fruit were harvested |
| Bulgarian soils | | 4,70E-03 | 1,97E-05 | NA | | Djingova, (2002) | |
| Silty- clay | NA | 2,90E-02 | 1,22E-04 | 1,77E+00 | 7,44E-03 | Al-Oudat (2021) | Harvested in middle of vegetation growth |
| all soils | | 2,10E-02 | 8,82E-05 | 3,60E-01 | 1,51E-03 | IAEA, 2010 1) | non -leafy vegetable |
| sand | | 3,50E-02 | 1,47E-04 | 8,70E-01 | 3,66E-03 | | |
| loam | | 3,30E-02 | 1,39E-04 | 1,40E+00 | 5,88E-03 | | |
| clay | | 9,10E-02 | 3,82E-04 | 1,30E-01 | 5,46E-04 | | |
| organic | | NA | | 2,20E-01 | 9,24E-04 | | |
| all soils | NA | NA | 2.17E-03 | NA | NA | SUJB (online data) | samples collected 1987 in different areas of the Czech Republic 1 year after Chernobyl accident |

Note: The values of aggregated transfer factors $T_{ag}$ were obtained from TF from literature by calculation $T_{ag} = TF/238$ (except for the result from our experiment where the opposite relationship was used, i.e. $TF = T_{ag} \times 238$)

[1] TF relate to the period long after the accident

## 5.1 Following research to Case study II

### Mesocosm experiments

In subsequent years following the Case Study II results, data collection from crops grown in mesocosm experiments at the SÚRO facility in Hradec Králové continued, adhering to the methodological procedures described in Case Study II. After winter frosts subsided, soil profiles were extracted from the experimental containers. Using a soil probe, three profiles were obtained from each container, each comprising seven layers down to a depth of 20 cm. The top five layers were each two centimeters thick, while the bottom two layers were five centimeters thick. Additionally, a simulation of agricultural management practices was applied to the topsoil of all containers, mechanically performed before re-contamination with $^{85}$Sr and $^{134}$Cs. This recontamination ensured the provision of radionuclide amounts necessary according to the methodology, considering their half-life.



**Figure 1:** *A photograph depicting the initial third of the experimental setup, showing barley interspersed with the occasional weed species Chenopodium ficifolium.*

Subsequent procedures were in line with the established methodology in containers initially filled with six prevalent types of European agricultural soils. For the initial planting, barley (*Hordeum vulgare*) was selected (Fig. 1). The entire above-ground portion of the barley was harvested, and the stalks with chaff, and grains were analyzed separately. Following the barley harvest, fodder beet (*Beta vulgaris* var. *rapacea*) was sown. Additionally, the experiment provided an opportunity to measure cesium and strontium uptake in weed species. However, due to their limited number and sporadic presence, these data are not deemed reliable for publication.

## Greenhouse experiments

Due to the observed changes in global climate systems, particularly concerning precipitation activity, a greenhouse experiment was established (Fig. 2). This experiment aimed to ascertain the effects of precipitation regimes and temperature during the growing season on the uptake of $^{134}$Cs and $^{85}$Sr by selected crops.



**Figure 2:** *A photograph captures the completion of three out of four experimental greenhouses at the SÚRO complex in Hradec Králové.*

The experiment utilized the same six soil types as those in Case Study II, including their treatment combinations with and without potassium and calcium additives. The established watering conditions simulated drought (suboptimal soil moisture),

normal conditions (optimal soil moisture), and excess precipitation (waterlogged soil). Building on the methodologies from Case Study II, the initial contamination levels for the greenhouse experiment were set to surface activity values of 152 kBq/m$^2$ for $^{134}$Cs and 111 kBq/m$^2$ for $^{85}$Sr. The greenhouse conditions were meticulously monitored using professional weather stations equipped with indoor and outdoor sensors, along with soil moisture sensors for each soil type and treatment group. The crop cultivation sequence was as follows: radishes (*Raphanus sativus*) were planted first, followed by lettuce (*Lactuca sativa*), and the season concluded with the sowing of fodder beets (*Beta vulgaris* var. *rapacea*). A parallel, smaller-scale experiment was conducted in the experimental greenhouses of the CZU in Prague using stable Cs, where the crops under investigation were beets, radishes, and barley.

## Climabox experiments

Concurrently with outdoor and greenhouse experiments, studies were conducted under the highly controlled conditions of a climabox. Radioactive isotopes $^{134}$Cs and $^{85}$Sr were monitored at the SÚRO complex in Hradec Králové, while stable isotopes $^{133}$Cs and $^{88}$Sr were monitored at the CZU premises in Prague. Unlike the previous two types of experiments, the soils were confined to Temelín gley soil and Dukovany chernozem, both sourced from the outer emergency zones of their respective nuclear power plants. For the climabox experiments, 30 flowerpots with a volume of 1.5 liters each were utilized. Contamination with radioactive isotopes was achieved through uniform pouring to attain an identical area activity of 152 kBq/m$^2$ for $^{134}$Cs and 111 kBq/m$^2$ for $^{85}$Sr, consistent with the other experiments. Similar to the other studied groups, the experimental design involved dividing the pots into two sets: those without additives and those with potassium, calcium, and their combination as additives. In the radioactive isotope experiments, crops of radish (*Raphanus sativus*), lettuce (*Lactuca sativa*), and beetroot (*Beta vulgaris* var. *rapacea*) were sequentially cultivated (Fig. 3).

*Following research to Case study II: Aggregated transfer factors of fresh Cs and Sr pollution to various vegetables from six common European soils – mesocosm experiment (Komínková et al., 2023)*



**Figure 3:** *This image depicts the first crop (radish) harvested from the climabox experiment, involving the use of radioactive isotopes $^{85}$Sr and $^{134}$Cs, conducted at the SÚRO laboratory in Hradec Králové.*

### Future publications

Currently, the data from all the aforementioned experiments have been processed, which includes their statistical evaluation. Work is currently underway on another publication, provisionally titled 'What is More Important for the Uptake of Cesium and Strontium: The Type of Soil or the Type of Vegetable? A Mesocosm Study on Five Common Types of European Soils with Three Common Vegetables.' Furthermore, the forthcoming publication entitled 'Distribution of $^{134}$Cs and $^{85}$Sr in the Above-Ground Parts of *Hordeum vulgare* L. (Barley) Grown on Six Common Soils of the Czech Republic' is in the initial stages of writing.

# 6 Case study III: Electrokinetic effects in hydroponic system contaminated with cesium and its impact on *Phragmites australis* radioprotection

Jaroslav Vacula, Dana Komínková, Lukáš Praus

Adapted from the article under review (2024)

## Graphical abstract
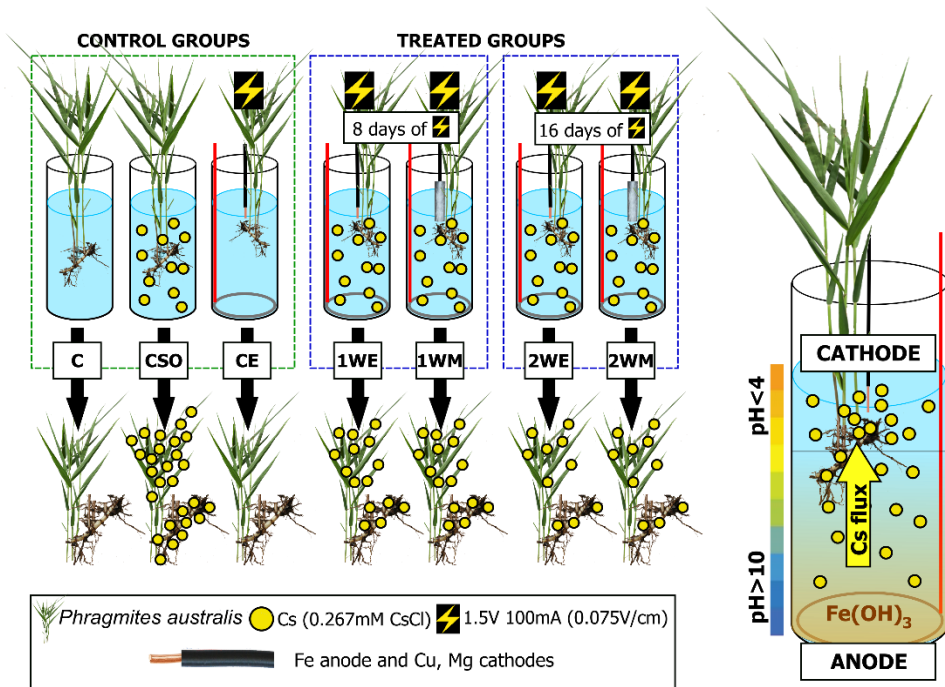
## Abstract

The effects of phytoremediation coupled with electrokinetics were tested on hydroponically grown common reed (*Phragmites australis*) under greenhouse conditions. The plants were exposed to a stable isotope of Cs with an external concentration of 0.267 mM. The experiment followed a randomized block design comprising control groups C, CE (electric field only), CSO (Cs only), and treated groups 1WE, 1WM, 2WE, 2WM (E – copper cathode, M – magnesium cathode), with 5 repetitions within each group. An electric field (source: 100 mA and 1.5 V) with a constant intensity of 0.075 V/cm was applied continuously for 24 hours a day, lasting for 8 days in groups 1WE and 1WM, and for 16 days in groups 2WE and 2WM. The results indicated reductions in accumulated Cs by 25%, 22%, 22%, and 20% in groups 2WE, 2WM, 1WE, and 1WM, respectively. Covariates occurring in the experiment were examined to assess their impact on uptake. Weight was found to be highly significant ($p < 0.001$), as well as the treatment conditions ($p < 0.005$), whereas biomass change and root growth during the experimental period were not significant ($p > 0.05$). Additionally, significant ($p < 0.05$) translocation to the aerial parts from underground parts was observed in all Cs treated groups. The application of an electric field was found to partially mitigate the phytotoxic effects of Cs, while the elemental composition (Mg, K, Mn, Fe, Cu, and Zn) in the reed plants was found to be within normal ranges. The average velocity of a Cs particle in the electrolyte was estimated to be $6.34 \times 10^{-7}$ m/s, resulting in a concentration gradient, with significantly higher ($p < 0.001$) concentrations near the cathode. These findings offer novel insights into the electric field's impact on Cs uptake by hydroponically grown plants, contributing crucial information to pioneering research.

## Introduction

In the field of environmental engineering, in practice, but also in research, electrokinetics is described as an innovative *in situ* method, used for the decontamination of soils polluted by metals, anions, radionuclides, polar organic compounds, hydrophobic and persistent toxic compounds (Acar et al., 1993; Bi et al., 2011; Cameselle et al., 2019; Reddy and Cameselle, 2009; Roehl et al., 2005). Electrokinetics is a study of the dynamics of electrically charged fluids (Castellanos, 1998) encompassing the movement of fluids or particles in electrolytes and ionic liquids. The applied methods are based on the application of direct current (DC)

conducted by the soil mass or liquid between two electrodes (cathode and anode). An electric field passing through the medium can induce desorption of contaminants (Roehl et al., 2005), their transport, capture and removal from the liquid medium (Acar et al., 1993; Castellanos, 1998) under the simultaneous ongoing chemical reactions on the electrodes (Roehl et al., 2005).

Radionuclide decontamination by electrokinetics has been studied with several isotopes, with cesium (Cs) receiving the greatest research attention (Korolev, 2009). Cesium exhibits almost identical properties to the potassium cation (Avery, 1995) and is thus able to substitute potassium in plant and animal systems (Burger et Lichtendail, 2019). The cesium isotope $^{137}$Cs with a half-life of 30.08 years ranks among the most common fission products (Kocherov et al., 1997). Its release into the environment means long-term contamination of the site and a multigenerational problem for communities, populations, and the entire affected terrestrial as well as aquatic ecosystems (Metivier, 2002). In addition to this, the endangerment of many ecosystem services is a cause for concern, compounded by the potential for bioaccumulation within food webs (Wehrden et al., 2012).

Studies of electrokinetics techniques examining decontamination effects of electromagnetic fields on the radionuclides removal and its dynamics were studied for decades. However, some results have shown that decontamination of soil or sediment is found to be more efficient when coupled with a chemical leaching agent (Korolev, 2009). For example, the combination with acetate buffer solution was highly effective in removing radionuclides, including Cs$^+$, from contaminated soil in the anode zone. In this case, electromigration was the main mechanism by which $^{137}$Cs cations were transferred and after period of 10 days the total radioactivity decreased by 25% (Kim et al., 2003). The effects of current and voltage on the extraction dynamics of $^{137}$Cs were investigated by Prozorov et al. (2000). Applied electric fields of 0.10, 0.15 a 0.20 V/cm with a leaching solution of ammonium salt were shown to extract 45%, 85% and 90% of $^{137}$Cs in 120 days, respectively. Encouraging results were presented by Kim et al. (2013) in their experiments with soil flushing with 2M HNO$_3$ coupled with electrokinetic remediation. The highest removal efficiency of 94% was obtained for $^{134}$Cs after 8 days of treatment and 95% efficiency for $^{137}$Cs after 10 days of treatment. Considering the promising results, but at the same time potentially dangerous and even toxic leaching agents for biota, there were also attempts of combining electrokinetic techniques with methods

closer to nature such as phytoremediation. Mao et al. (2016) demonstrated that electromagnetic field increased the metal content in the Indian mustard and spinach tissues. This observation indicated a $40 - 69\%$ higher bioavailability of $Cs^+$ and other studied metals under the action of an electromagnetic field of 1.0 V/cm. A recent contribution towards this end has been made by Haidari et al. (2023). Electrokinetics coupled with phytoremediation in a hydroponic system showed a significant increase in the removal of Fe, Cu, Zn and Pb from landfill leachate. In 90 days of treatment the removal of Fe, Cu, Zn and Pb corresponded to 64, 21, 35 and 28% efficiency, respectively.

In electrokinetic experiments coupled with phytoremediation, the result can be influenced by several factors, and one such factor is the response of plants to an electric field, which depends on the species of the plant (Bi et al., 2011). Other factors are the intensity and orientation of the electric field and the type of electric voltage (DC or AC), which can affect plant growth (Aboughalma et al., 2008; Bi et al., 2011; Mao et al., 2016), as well as the settings and parameters of the used electrodes (Luo et al., 2018). A vertically oriented electric field can contribute to the stimulation of a plant species to a higher uptake of metals in both above-ground and underground parts (Luo et al., 2018). The application of AC electric fields leads to the periodic hyperpolarization and depolarization of plant cells, which promotes the uptake of elements due to the activation of specific ion channels in the tested species. Cho et al. (1999) argues that the same mechanism is applicable for DC currents due to participation of ion channels and pumps in the generation of root electrical potentials. However, it is important to consider that the concentration of heavy metals may increase by their transport by the electric field to the cathode and thereby render the plant incapable of life (Cameselle et al., 2019). Seemingly, weak electric fields show less effect on plant growth and phytoremediation (Chirakkara et al., 2015). Currently, there is insufficient data in the literature to define an electric field strength to produce a positive effect on phytoremediation (Cameselle et al., 2019). However, despite all of the research and applied experiments on electrokinetic treatment of the radionuclide contaminated soils, there is limited information about usage of electrokinetics in solely aquatic habitats.

Fukushima accident yielded critical findings of Cs fate in aquatic habitat as Abukuma River was a subject of extensive research. Notably, from August 2011 to May 2012, evidence indicates that a total of 5.74 TBq of ^{137}Cs and 4.74 TBq of [134]Cs were

discharged (Yamashiki et al., 2014). According to Adhiraga et al. (2015), the predicted discharge until year 2111 was estimated to be 109 Bq per month, totaling 111 TBq for ^{137}Cs and 44 TBq for [134]Cs. Yamashiki's (2014) study showed that cesium transportation was predominantly in particulate form, estimated to range from 85% to 92%. However, the behavior is often dependent on the concentration of carrier or competing nuclides such as $K^+$ (Anderson et al., 1987; Santschi et al., 1990) and $NH_4^+$ (Santschi et al., 1990). All of these factors combined are creating complex interdisciplinary problem of electrokinetic usage in aquatic habitats.

Based on the literature review, there is still a lack of understanding and experimenting with electrokinetic phenomena in wetland habitats. In addition, the effects of electromagnetic fields on hydroponically grown plants are also not well described. The aim of this study is to investigate whether the conditions in a hydroponic system, through which an electromagnetic field passes, will influence the uptake of Cs by *Phragmites australis*. We assume that the electric field will increase the concentration of $Cs^+$ around the cathode, in the proximity of the roots. The secondary objectives will be the evaluation of $Cs^+$ intake on plant health and the determination of the effect of electrodes on the hydroponic system, where the entire experiment was carried out with common, cheap, and easy-to-use materials.

## Material and methods

### *Experimental design*

Plant material of common reed (*Phragmites australis*) was collected in wetland habitats near Třeboň in South Bohemia, Czech Republic. The plants were transported to the experimental greenhouse of the Czech University of Life Sciences Prague (CZU) in 50L plastic containers. After transportation, the removal of all individuals that suffered mechanical damage and the elimination of debris, invertebrates, and algae were executed promptly. The remaining clean and healthy individuals were subsequently transferred to identical 50L containers filled with standing tap water. Leaving the plants in containers for 2 weeks ensured sufficient acclimatization to greenhouse conditions, which were 22°C with air humidity ranging from 45 to 65%. After the acclimatization period, only healthy, thriving reed individuals were included in the experiment.

The experiment took place in acid washed cylindrical glass containers with a volume of 1.8 L. Electrodes were placed in the cylinders. The anode (power source +) formed by a Ø1.2 mm iron wire, twisted into a circle, surrounded the inner perimeter of the bottom wall of the glass container. An insulated wire led from the electrode to the top of the container. The cathode (power source -) was made of copper wire Ø1.2 mm and 35 mm in length, which was fixed 18 mm below the water level, or, alternatively, of magnesium rod 15 mm thick and 100 mm in length. The submerged part of the magnesium cathode was 18 mm below the surface. All electrodes were fixed against the movement during the entire length of the experiment (Fig. 1).
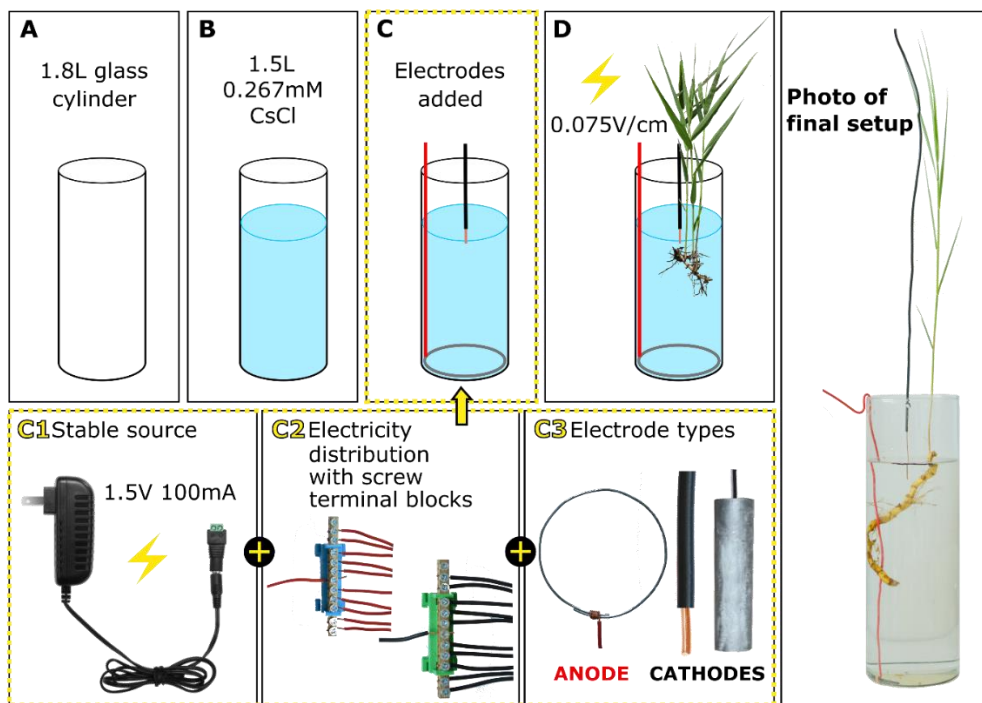


**Fig. 1:** *Graphical method of an experiment with depicted source, electricity distribution parts, electrodes, and photo of a finalized setup*

The deliberate selection of varying dimensions for the cathodes was made with a specific purpose. The thin electrodes in the experiment primarily monitored only the effect of the electric field on the plants, while the larger magnesium electrodes were

chosen to observe any potential precipitation. Seven treatment groups were established as depicted in Table 1.

**Tab 1:** Treatment groups of experimental setup

| Group | Cs concentration | Cs exposure length | Strength of electric field | Electric field exposure | Cathode material | Anode material |
|---|---|---|---|---|---|---|
| C | none | none | none | none | none | none |
| CE | none | none | 0.075 V/cm | day 1-16 | none | none |
| CSO | 0.267 | day 1-16 | none | none | none | none |
| 1WE | 0.267 | day 1-16 | 0.075 V/cm | day 9-16 | Cu | Fe |
| 1WM | 0.267 | day 1-16 | 0.075 V/cm | day 9-16 | Mg | Fe |
| 2WE | 0.267 | day 1-16 | 0.075 V/cm | day 1-16 | Cu | Fe |
| 2WM | 0.267 | day 1-16 | 0.075 V/cm | day 1-16 | Mg | Fe |

Contamination solution was prepared by dissolving ≥99.9% crystalline CsCl with stable isotope [133]Cs (Merck KGaA) in distilled water. The final concentration of CsCl in cylinders was 0.267 mM. The chosen concentration was based on findings reported by White and Broadley (2000) in which they state that at an external concentration of 0.253 mM phytotoxic effects in some plant species begin to manifest.

The main argument for [133]Cs utilization is elimination of potential radiation hazards resulting from handling [134]Cs or [137]Cs. It is cost effective and supported by growing body of literature (Tsukada et Hasegawa, 2003; Soudek et al., 2004; Shinano et al., 2014).

The individual plants were placed in the cylinders, where each plant was pre-weighed, the condition of the roots, their length, the number of leaves, shoots, and the general state of health were recorded. Plants' health was evaluated according to standard botanical visual plant health assessment based on plants' anatomy and physiology (Marschner, 2011). The main monitored indicators were leaf color, morphology, root growth and architecture, monitoring of vascular system and presence of lesions and abnormalities.

A DC source of electric voltage and current provided a stable supply of 100 mA and 1.5 V for all groups growing in the presence of a vertical electric field. The electric field with a stable intensity of 0.075 V/cm acted on the plants 24 hours a day for 16 and 8 days, depending on the treatment.

*Harvest*

At harvest, the health status of the plants, the branching of the roots, the number of fresh and dry leaves were recorded. Subsequently, the plants were divided into underground and above-ground parts, weighed, and dried to a constant weight at 40°C. Water sampling, pH and conductivity measurements were done as supporting data.

*Sample processing and laboratory analysis*

The dried samples were manually ground in a mortar and pestle, homogenized, and digested in triplicate in a closed-vessel microwave system (Microwave PRO by Anton Paar GmbH, Austria) using a mixture consisting of $HNO_3$:$H_2O_2$ (9:1 by volume, both trace analysis grade). After digestion, the samples were poured into 50 ml volumetric flasks and topped up to the mark with deionized MiliQ water. Sample analysis was performed on an ICP-MS 7700x (Agilent Technologies Inc., USA), where the concentration of Cs, K, Mg, Mn, Fe, Cu and Zn was measured. Supplementary data (S1 and S2) on water parameters were provided by an ion chromatography system Metrohm 883 Basic IC Plus (Metrohm AG, Switzerland) while measurement of pH and conductivity was carried out by HACH HQ 30D flexi (Hach Company, USA). Additional elemental analysis of sediment and electrode precipitate was performed by an ICP-OES, Agilent 730 (Agilent Technologies Inc., USA) after the samples had been digested.

*Calculations and statistical analysis*

The translocation factor for Cs between underground and above-ground part of plants was calculated as (Burger et Lichtendail, 2019):

$$\text{TF} = \frac{\text{Concentration in shoots}}{\text{Concentration in roots}} \quad [1] = \frac{\left[\frac{mg}{kg}\right]}{\left[\frac{mg}{kg}\right]} \qquad (1)$$

Plant removal rates were calculated using a standard equation (Moogouei et al., 2011; Urban et Bystrzejewska-Piotrowska 2003):

$$\text{Removal} = \frac{\text{Accumulated Cs by a plant}}{\text{Total Cs in solution}} \cdot 100 \quad [\%] = \frac{mg}{mg} \qquad (2)$$

The change in plant biomass was calculated by following equation:

$$Biomass\ change = Fresh\ weight\ after - Fresh\ weight\ before\quad [g]$$

$$= [g] - [g] \qquad (3)$$

Diffusion coefficient was calculated by Stokes-Einstein equation (Eq. 4) with subsequent calculation of electrical mobility of Cs particle according to Einstein mobility equation (Eq. 5). The last step was drift velocity of charged Cs particle calculation according to equation (Eq. 6).

$$D = \frac{k_B \cdot T}{6 \cdot \pi \cdot \eta \cdot r} \quad \left[\frac{m^2}{s}\right] = \frac{\left[\frac{J}{K}\right] \cdot K}{6 \cdot \pi \cdot \left[\frac{N \cdot s}{m^2}\right] \cdot m} \qquad (4)$$

Where D is diffusion coefficient, $k_B$ Boltzmann constant ($1.380649 \times 10^{-23}$ J/K), T temperature, η dynamic viscosity (0.00089 Pa · s), r radius of spherical particle (0.267 nm) and constant π (Bard et al., 2022).

$$\mu = \frac{q}{k_B \cdot T} \cdot D \quad \left[\frac{m^2}{V \times s}\right] = \frac{[C]}{\left[\frac{J}{K}\right] \cdot [K]} \cdot \left[\frac{m^2}{s}\right] \qquad (5)$$

Where μ is electrical mobility and q electrical charge of a particle ($1.60217663 \times 10^{-19}$ C) (Bard et al., 2022).

$$v_d = \mu \cdot E \quad \left[\frac{m}{s}\right] = \left[\frac{m^2}{V \cdot s}\right] \cdot \left[\frac{V}{m}\right] \qquad (6)$$

Where E is electric field (Bard et al., 2022).

Data processing for the analyses was performed in the Microsoft Excel (v 2212, Microsoft Corporation) and statistical analyses were computed in the R Studio (v 2023.03.1+446, Posit, PBC). If the assumptions for parametric tests were met, ANOVA analysis and the subsequent post-hoc Tukey test were used for the hypothesis testing. The Welch Two Sample t-test was used to test the differences between two groups and the paired t-test in case of testing mean differences between two time sets of an observation. Analysis of covariance (ANCOVA) was

performed to investigate the effects of plants weight, treatment groups, root growth and biomass changes on removal of Cs. If the assumptions of the tests were not met, non-parametric equivalents were selected.

## Results

### Natural background of Cs in P. australis

Before the experiment, natural Cs content in *Phragmites australis* at the collection site was analyzed. Cesium ranged from 0.399 to 0.808 mg/kg in the underground part (roots and rhizomes) and from 0.198 to 0.454 mg/kg in the above-ground part (stem and leaves). Controls were also analyzed: negative control (C) exhibited Cs content ranging from 0.238 to 0.478 mg/kg in the above-ground part and 0.300 to 1.102 mg/kg in the underground part, while positive controls (CE) had Cs values of 0.278 to 0.738 mg/kg in the above-ground part and 0.327 to 1.047 mg/kg in the underground part. Statistical comparison of plants collected at sampling site and two control groups confirmed that no significant difference ($p > 0.05$) in Cs content was found between them. These values are often described as commonly occurring in nature (Cook et al., 2007; Burger et Lichtendail, 2019).

### Cs accumulation in aerial part of plants

In the above-ground portions of the plants, the lowest average content of accumulated Cs was observed in group 2WE, with a mean of 2736 mg/kg, minimum and maximum of 2117 and 3329 mg/kg, respectively. Notably, a significant difference in Cs accumulation ($p < 0.05$) was observed in this group compared to the group that received Cs treatment alone (CSO). Similar results were also obtained for group 1WE, which had an average Cs accumulation of 2825 mg/kg, with minimum and maximum of 2130 and 3496 mg/kg, respectively. The p-values for this group were slightly above the significance level compared to CSO. Likewise, the 2WM group also exhibited p-values above the significance level, with average Cs content of 2830 mg/kg. This group demonstrated the widest range of Cs accumulation among the groups treated with an electric field, with a minimum and maximum of 1870 and 4024 mg/kg, respectively. The average accumulated Cs in the aerial parts of group 1WM was 2890 mg/kg, with a range of 2461–3581 mg/kg. The group treated only with cesium accumulated Cs the most, with an average of 3626 mg/kg,

a minimum of 2674 mg/kg, and a maximum of 4968 mg/kg. The percentage decrease in average accumulated Cs content was also calculated. The use of electric filed resulted in decreases of 25, 22, 22 and 20% in groups 2WE, 2WM, 1WE and 1WM, respectively, when compared to the group treated only with Cs.
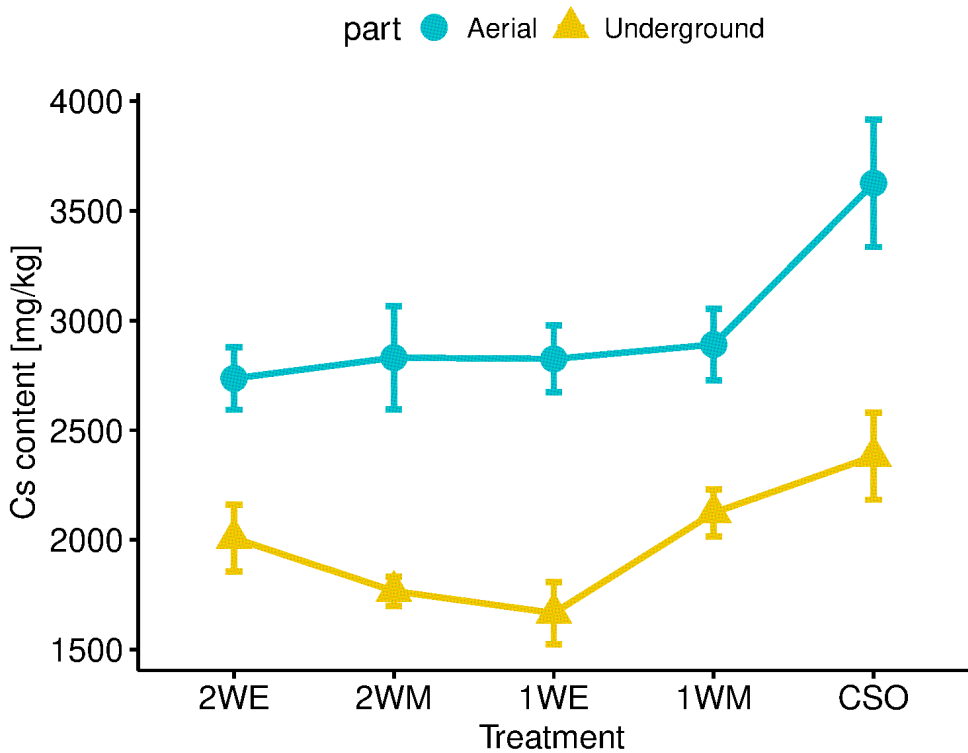


**Fig. 2:** *Cesium accumulation in aerial and underground parts of reed plants (on dry weight basis), where mean (▲ ●) and confidence intervals are plotted (n = 5).*

*Cs accumulation in underground part of plants*

The accumulated amounts of Cs in the underground parts of the plant showed a lower variability than in the above-ground parts. In group 1WE, the lowest average accumulation of Cs was 1666 mg/kg, with a range of 1103–2357 mg/kg. Comparing group 1WE with the CSO, significantly less (p <0.01) Cs was found in 1WE. The 2WM group showed similar contents, with an Cs average of 1766 mg/kg and a narrower range of 1500–2069 mg/kg. Likewise, a significant difference (p <0.05) was found in

the 2WM group when compared to the CSO. The average accumulated content of Cs in the 2WE group was 2007 mg/kg, with a range of 1305–2668 mg/kg. The highest average accumulated Cs in the electric field-treated groups was found in the 1WM group, with 2123 mg/kg and a range of 1711-2562 mg/kg. No significant differences were found in the 2WE and 1WM groups when compared to the cesium-treated only group (Fig. 2). The plants with the highest Cs content were found in the CSO group, with a mean of 2381 mg/kg, with range 1815 to 3350 mg/kg. In all electric field-treated groups (2WE, 2WM, 1WE and 1WM), there was a decrease in the average accumulated values of Cs by 16, 26, 30 and 11%, respectively.

*Translocation of Cs*

The translocation factors (Eq. 1) from the underground to aerial parts of plants for groups 2WM, 2WE, 1WM, 1WE, and CSO corresponded to 1.45, 1.40, 1.23, 1.67, and 1.63, respectively. Significant translocation between plant parts was observed within each group ($p<0.05$). However, when comparing the groups using ANOVA with Tukey's HSD test, no significant differences ($p>0.05$) in translocation were found. The lower accumulated amounts in the underground part of the plants compared to the above-ground parts are also in agreement with the literature, when Soudek et al. (2004) found that reed plants translocated Cs to young shoots, leaf tips and nodes efficiently.

*Covariates influencing uptake*

Overall, the results suggest that the electric field was effective in reducing the accumulated amounts of Cs in both above- and underground parts of the plant. It is worth noting that in every experimental group, the distribution of plant sizes was intentionally varied to cover a broader spectrum of accumulation ranges. This is supported by literature as uptake of Cs was shown to vary across growth stages (Weaver et al., 1981; Soudek et al., 2004; Kondo et al., 2015; Lai et al., 2016) and it is considered as a standard methodological step. However, incorporation of varying plant weights brought high variability in accumulated amounts of Cs. Therefore, an analysis of covariance (ANCOVA) was conducted to examine the effects of covariates, including plant weight, root growth, and plant biomass change, on CS accumulated in the whole plant (dependent variable) in the presence of different treatment groups (independent variables). The model had a good fit to the data ($R^2$

= 0.9211) and revealed a significant (p<0.001) effect of initial plant mass and treatment groups (p<0.005) on the accumulated amounts of Cs. Root growth and biomass change were not found to have significant impact (p>0.05) on uptake. This finding (Tab. 2) helps to account for the wide range of values observed. Since the plants were randomly selected within each group, an initial mass as a predictor also helps to explain the apparent variability in Cs accumulation across underground and above-ground groups.

**Tab 2:** Results for ANCOVA analysis

| Tested parameter | df | Sum Sq. | Mean Sq. | F value | P value |
|---|---|---|---|---|---|
| Treatment groups | 4 | 8.64.E+07 | 2.16.E+07 | 8.59 | **< 0.005** |
| Plant weight | 1 | 2.92.E+08 | 2.92.E+08 | 116.225 | **< 0.001** |
| Biomass change | 1 | 1.06.E+06 | 1.06.E+06 | 0.422 | > 0.05 |
| Root growth | 1 | 1.22.E+04 | 1.22.E+04 | 0.005 | > 0.05 |
| *df* degrees of freedom | | | | | |
| Significant effects (*P*<0.05) are indicated in bold | | | | | |

*Removal rates*

Removal rates were calculated according to Eq. 2. However, the limitation lies in the inclusion of plants of different sizes in the experimental setup and at the same time each treated group as mentioned above. Due to the significant dependence (p<0.001) of accumulated amount of Cs on the plant weight, the removal rates were obtained by predictive modelling based on regression equations of ANCOVA. This approach enabled a more meaningful comparison of groups and provided a clearer understanding of the relationship between the mass of individuals in groups and their removal rates.

Removal rates for 5 g of whole plants dry weight based on predictive modelling are corresponding in each group to 27, 22, 19, 19 and 16% for CSO, 1WM, 1WE, 2WM and 2WE, respectively. To put our results in perspective, in a study with the species *Amaranthus chlorostachys*, *Chenopodium album* and *Calendula alata*, the percentage of Cs removal was 41%, 39% and 65% from the hydroponic solution, respectively. The reported removal rates correspond to 15 days of treatment with an external Cs concentration very similar to this experiment (Moogouei et al., 2011). However, closer comparisons with the literature are difficult, as studies dealing with the removal of Cs include a wide spectrum of mass, concentration, solution volumes

and time horizons, on which the results are significantly dependent (Burger et Lichtendail, 2019).

*Plant biomass changes*

The change in plant biomass was calculated based on Eq. 3. The average weight change of the negative control group was 3.68g and this mass was considered as a baseline for percentage calculations of biomass changes between groups (Fig. 3). The positive controls, treated only with an electric field, showed an average fresh weight gain of 0.94g. Thus, resulting in 74% lower weight gain than plants growing in glass cylinders of group C. The differences in fresh weight of both groups were not significant ($p > 0.05$). However, based on the plant biomass changes, it can be inferred that passing current through the electrolyte of the CE group has an inhibitory effect on plants growth.

On the other hand, significant difference ($p < 0.01$) was found in the average biomass changes of 0.06g in the CSO group compared to group C. This counts for 98% lower biomass production compared to the negative controls and pointing to the obvious phytotoxic effects of Cs occurring at external concentrations above 0.253mM, as described by White and Broadley (2000).

A significant ($p < 0.01$) weight loss of -0.14g (105%) was noted for the 1WM group compared to the negative control. From the observatory records made during the experiment, there is no evidence of serious phytotoxic effects on the plants in this group. The explanation may be the predominance of littering over plant growth in the first week, when only cesium acted on the plant and by the subsequent effect of the electric field with magnesium electrodes.

The remaining groups showed positive changes in the average biomass weight in the range of 0.66 - 1.23g without significant differences to the baseline. However, in groups 2WE, 2WM and 1WE average weight changes of plants recorded were lower by 82, 76 and 67% to the control, respectively. Biomass production was therefore 16, 32 and 23% higher in 2WE, 2WM and 1WE group when comparing them to cesium only treated group, respectively. This result suggests an improved biomass growth in these groups indicating lesser growth inhibition caused by Cs. Therefore, the application of an electric field can partially alleviate the phytotoxic effects of Cs by lowering its accumulation by plants.
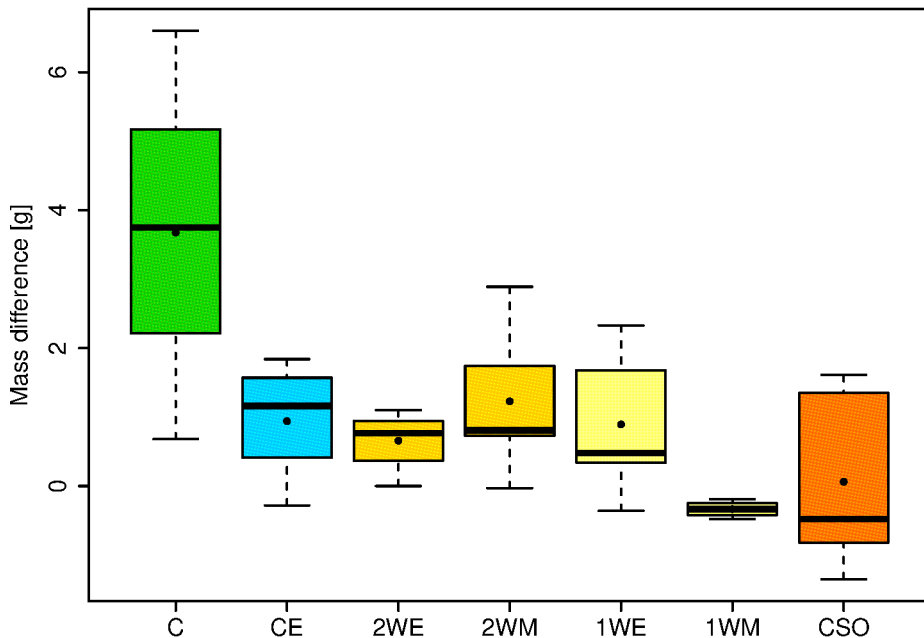
**Fig. 3:** *Plants weight gain (loss) at the end of the experiment*

*Evaluation of leaves and roots growth*

At the end of the experiment, leaf count changes were observed in all groups. In the control plants, the average leaf change (growth – abscission) was zero due to the drying of the plant with the largest number of leaves. However, the health status of this plant at the end of the experiment was stabilized. The plant thrived even after shedding a vast number of leaves in the first week. For all other groups, the average increase in new leaves was in the range of 3.2 - 6.4 leaves per plant. Subsequent paired t-tests found evidence of a significant differences ($p < 0.05$) in the number of leaves before and after the two-week exposure in all pair of groups. However, Kruskal-Wallis test did not find significant leaf count differences ($p > 0.05$) between all treatment groups after experiment.

The root systems of all plant individuals were evaluated depending on their size before and after the end of the experiment. However, results of Wilcoxon signed rank test with continuity correction were not evaluated as significant ($p > 0.05$) for any of the tested pairs.

*Health status of plants*

The C group and the group CE did not show any negative morphological changes or visible signs of nutrient deficiencies or phytotoxicity after the end of the experiment. Mild signs of incipient leaf phytotoxicity were observed in groups 1WM and 1WE. The expression was the formation of tiny brown spots without chlorosis or any other tissue necrosis, where the plants looked healthy. More severe manifestations of phytotoxicity were observed in groups CSO and 2WE, namely formation of tiny brown spots on the leaves, the beginning stages of chlorosis and drying of the leaves. On the other hand, plants in the 2WM group had almost no tiny brown spots, chlorosis, or necrosis on the leaves.

Due to possible ion release from the electrode materials used in the experimental set-up, their potential impact on plant health must be assessed. A variety of studies have reported a wide range of contents of Fe, K, and Zn in reed plant tissue under unpolluted conditions (Bonanno et Giudice., 2010; Rzetala et al., 2011). While observed values for K, Mg and Mn fell within the typical content range found in plant tissues, specifically K (0.7%-2.9% of dry weight), Mg (2000 mg/kg dry weight) and Mn (50 mg/kg dry weight) in a whole plant (Marschner, 2011; Rzetala et al., 2011), the Cu and Zn were elevated from commonly occurring 6 and 20 mg/kg dry weight, respectively. However, experiments reported in the literature, specifically investigating tolerance and accumulation in reed plants, consistently demonstrate that these content ranges are considered tolerable by the plants (Windham et al., 2003; Ye et al., 1997; Ye et al., 2003).

The special attention is paid to Fe, which has increased contents in the underground parts of the plant (Tab. 3). According to Marschner (2011), it is generally agreed that concentrations of around 1100 – 1600 mg/kg of iron in the dry matter of plants can be phytotoxic depending on the plant species and the age of the plant (Batty et Younger, 2003). However, such values are not found in any underground parts of the treated groups. Possible explanation and cause lie in commonly occurring coverage of root and rhizome surface in iron oxyhydroxide plaque (Batty et Younger, 2003; Khan et al., 2016).

**Tab. 3:** Content of (micro)nutrients in aerial and underground parts of reed plants (on dry weight basis). Contents are represented as group means [mg/kg].

| Content [mg/kg] | | | | | | |
|---|---|---|---|---|---|---|
| Group | Mg | K | Mn | Fe | Cu | Zn |
| Aerial | | | | | | |
| C | 2457 | 34841 | 17.6 | 134 | 13.6 | 99.8 |
| CE | 2250 | 41537 | 28.9 | 168 | 30.9 | 168 |
| 2WE | 2381 | 43200 | 30.9 | 537 | 18.8 | 190 |
| 2WM | 2743 | 39438 | 46.5 | 300 | 15.3 | 167 |
| 1WE | 2479 | 28781 | 43.9 | 627 | 14.2 | 360 |
| 1WM | 2680 | 36895 | 41.9 | 223 | 13.2 | 161 |
| Cs | 2240 | 35338 | 41.3 | 233 | 17.4 | 181 |
| Underground | | | | | | |
| C | 1180 | 8497 | 39.8 | 957 | 13.2 | 77.6 |
| CE | 1049 | 3535 | 33.8 | 1323 | 19.5 | 207 |
| 2WE | 1164 | 8087 | 50.3 | 1265 | 14.7 | 190 |
| 2WM | 1058 | 5381 | 126 | 2609 | 27.6 | 420 |
| 1WE | 1057 | 6327 | 82.9 | 980 | 17.9 | 143 |
| 1WM | 1202 | 5823 | 73.5 | 2416 | 19.3 | 505 |
| Cs | 1614 | 7170 | 55.9 | 1653 | 21.0 | 314 |

*Electrode disintegration, precipitate, and Cs interaction*

Observable breakdown of the electrodes was noted on the second day after the start of the experiment. In the first half of an experiment, an average of 1.21g of material was released from the anode in time-dependent linear manner. Then, the mass transfer from anode slowed down as surface area of the electrode, concentration of dissolved oxygen and current density changed. Moreover, surface coverage with oxidation products on the electrode may occurred which can prevent further oxidation of an electrode itself. A total mass release of 2.15g was observed after termination of the 16-day trial. A polynomial regression model (S3) was fitted to ten pairs of time (X) and mass release (Y) data, giving the equation $Y = -0.2023x^2 + 2.8161x + 1.0376$ and $R^2 = 0.998$.

The disintegrated anodes formed a sediment at the bottom of the experimental bottles. All the sediment formed can be derived from anodic dissolution, since the total weight of the sediment in each bottle was equal to the material released. The

homogenized sediment sample was analyzed to quantify Cs adsorption on the sediment particles. The result indicated that only 0.13% of the total cesium in the solution was captured in the sediment. In addition to the sediment, a precipitate was observed on the magnesium electrodes, corresponding, on average, to around 1.0g (S4). It was measured that up to 1.70% of the total Cs in electrolyte was immobilized in the precipitates.

*Electrical mobility of Cs*

The resulting value of diffusion coefficient is $2.17 \times 10^{-9}$ m$^2$/s by calculating the Stokes-Einstein equation with the substitution of the measured variables of this experiment. The obtained electrical mobility is $\mu = 8.45 \times 10^{-8}$. These findings made it possible to estimate the average velocity of a Cs particle in electrolyte while affected by external electric field, drift velocity $v_d = 6.34 \times 10^{-7}$ m/s. The reported diffusion coefficients for Cs in the literature range from $1.77 \times 10^{-9}$ to $2.17 \times 10^{-9}$ m$^2$/s (Yuan-Hui et Gregory, 1974; Sato et al., 1996) and therefore are in agreement with this study.
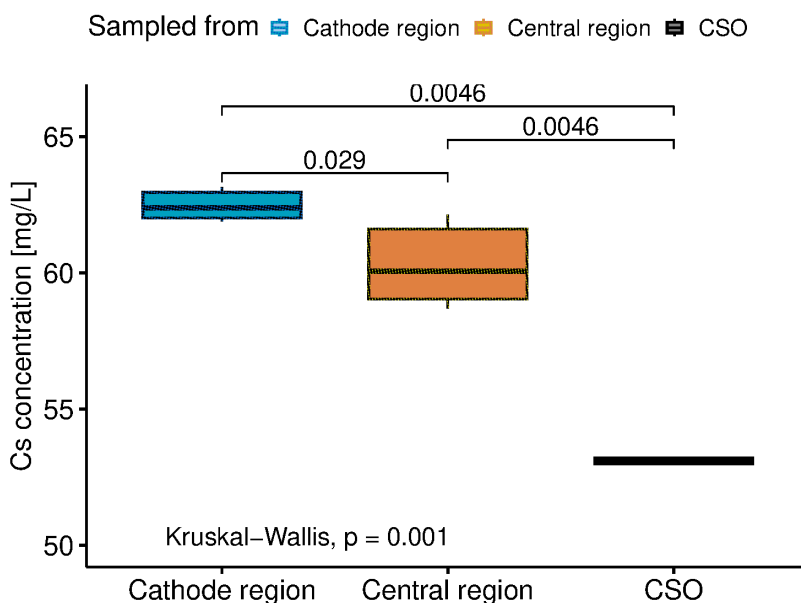


**Fig. 4:** *Spatial differences in Cs concentration when electric current passed through the electrolyte compared to CSO treatment (n=5), with p-values depicted above boxplots.*

The mobility and flux of Cs were tested in experimental cylinders with and without an electric field. Concentration measurements at the cathode and lower central region revealed a significant increase ($p < 0.001$) in Cs concentration after the trial period. In cylinders filled only with Cs solution, the concentration ranged from 52.93 to 53.20 mg/L, while in the central region and cathode region of cylinders treated with an electric field, it ranged from 58.69 to 62.13 mg/L and 61.88 to 63.14 mg/L, respectively (Fig. 4). This suggests that Cs was not adsorbed by the iron residues of the electrodes or formed Fe(II) and Fe(III) complexes, but remained in solution due to the electric field.

*pH changes*

Instant pH changes occurred in the immediate region (10 mm) of the electrodes, where its rate is rather dependent on the response of the measuring device. The pH measurements were conducted within the initial second, with a recorded drop in pH from 6.2 to 2.5 at the cathode. Conversely, the anode exhibited a rise in pH from 10.2 to 11.7. Extended measurements in close proximity to the electrodes were impossible due to electrical interference induced by voltage changes in the surface of the glass electrode, thus not obtaining any readings.

However, pH changes were also recorded in 40 mm distance from the cathode and anode, where in the first second the pH was 4.8 and 9.9, respectively. Subsequent changes over time were observed up to 240 seconds (Fig. 5). After 240 seconds the pH in this area remained stable and unchanged. At the end of the measurement, the cathode region had pH values of 4.8 and the anode region 10.5.
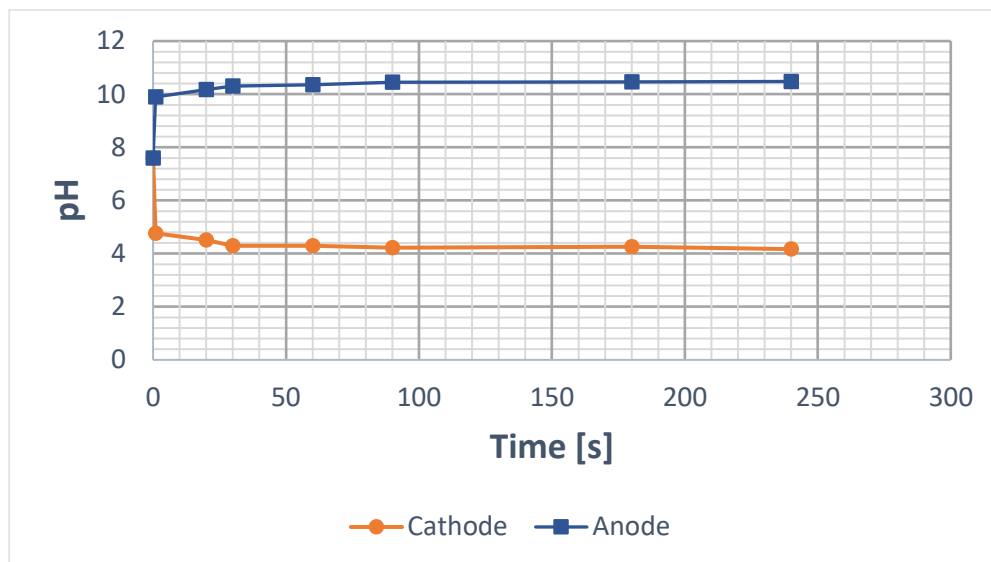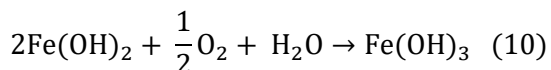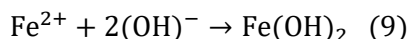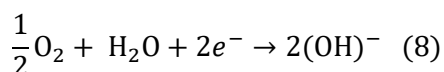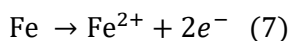
**Fig. 5:** *Measurement of pH near electrodes immersed in CsCl electrolyte solution*

The average pH of the tap water utilized for the experiment was 7.6. Proximity to the iron anode resulted in an alkaline shift in pH due to the accumulation of iron hydroxides as a byproduct of electrode oxidation (Eq. 7).

$$\text{Fe} \rightarrow \text{Fe}^{2+} + 2e^- \quad (7)$$

$$\frac{1}{2}O_2 + H_2O + 2e^- \rightarrow 2(OH)^- \quad (8)$$

$$\text{Fe}^{2+} + 2(OH)^- \rightarrow \text{Fe(OH)}_2 \quad (9)$$

$$2\text{Fe(OH)}_2 + \frac{1}{2}O_2 + H_2O \rightarrow \text{Fe(OH)}_3 \quad (10)$$

In the experimental setup described, this process prevailed over potential acidification caused by the presence of dissolved iron species, specifically Fe(OH)$_2$, within the anode region of the solution. Consequently, the overall pH increased, accompanied by a constant depletion of dissolved oxygen and eventual formation of Fe(OH)$_3$ (Eq. 10). Conversely, at the copper electrode, H$_2$ was generated through the reduction of water molecules (Eq. 11). The acidification observed at the cathode can

be attributed to the predominant presence of $H^+$ ions in its vicinity, resulting from the consumption of $OH^-$ by iron ions (Eq. 8, 9).

$$2H_2O + 2e^- \rightarrow H_2(g) + 2OH^- \quad (11)$$
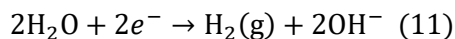
At the conclusion of the experiment, pH measurements were conducted for the entire volume of each group, including C, CE, 2WE, 2WM, 1WE, 1WM, and CSO, yielding pH values of 8.2, 9.8, 10.2, 9.8, 10.2, 10.2, and 7.9, respectively. These findings consistently indicate the presence of dissolved iron species, as evidenced by a significant increase ($p<0.001$) in pH across all experimental groups subjected to the electric field treatment.

The detailed description of pH changes in experimental setup is needed due to its impacts on reed plants and possible Cs uptake. For example, Heredia et al. (2002) reported the maximum uptake of Cs by aquatic free-floating plants at pH 7.5. While a pronounced decrease in uptake occurred in an alkaline environment up to pH 9 and, conversely, a smaller decrease was observed up to pH 6.5 (Heredia et al., 2002). Urban and Bystrzejewska-Piotrowska (2003) reported a different result with *Allium cepa* grown hydroponically, where the highest uptake occurred at pH 4-5. In all measured points across the pH range 6-9, uptake was very similar, but with an overall decreasing trend.

While the uptake of Cs by plants in the soil solution is known to be influenced by a range of chemical, physical, and biological factors, it should be noted that the contents of Cs were found to be inversely related to soil pH, which in turn increases the availability of Cs (Fredriksson et al., 1966; Glabiszewski et al., 1985; Tyler et Olsson, 2001; Burger et Lichtendail, 2019).

In this experiment, the roots of most reed plants were immersed in a depth with corresponding acidic or neutral pH while the system was under the action of an electric field. In terms of ecological valence, reed plants are able to tolerate a wide range of pH from 2.5 to 9.8 (Packer et al., 2017). While the direct role of pH on uptake cannot be determined in this experiment, the results suggest that pH had a negligible effect on uptake, as supported by the calculated ANCOVA. A negligible effect of pH corresponds to the results reported by Van Bergeijk et al. (1992) who reported that no correlation was found between plant Cs uptake and soil pH.

## Conclusion

The aim of the presented study was to investigate an electric field effect on hydroponic system containing 0.267 mM Cs and its impact on uptake of Cs by *Phragmites australis*. The application of an electric field (0.075 V/cm) resulted in a 20 – 25% reduction in Cs uptake by the aerial parts of reed plants, as compared to the Cs control, regardless of cathode material and exposure length to the electric field. For underground parts, a decrease in uptake ranging from 11% – 30% was observed, with the lowest uptake occurring in the treatment involving a copper cathode and one week of exposure to the electric field. With a lower uptake of Cs, related phenomena such as a reduction of phytotoxic manifestations were observed. These plant protective effects occurred despite the significantly increased concentration of Cs ions in the electric field around the roots and rhizomes. Furthermore, the decrease in Cs uptake could not be explained by whole plant biomass growth, root prolongation and branching, changes in electrolyte pH, nor by immobilization of Cs on electrodes or their disintegration products. However, variables influencing uptake were found to be a treatment conditions (i.e., application of electric field with minor disparities between electrode material and one or two weeks of exposition) and initial fresh weight of a plant. The experiment presented herein describes a complex system with multiple variables, each of which warrants further research in order to fully understand its impact.

In conclusion, this study has shed new light on the action of electric field on aquatic emergent plants ability to uptake cesium while grown hydroponically, shaping new directions for the establishment of pioneering remediation strategies. And providing basis for better radioprotective solution in the future.

## Supplementary material

### S1: Conductivity analysis

Conductivity of tap water used for the experiment had a mean value of 339.5 µS/cm. After the addition of the contamination solution, the range of values was 389 ± 3 µS/cm.

After switching on the circuit, in the first 5-10 minutes there was a slight decrease to 378-381 µS/cm in the central region of the applied electric field. It continued to

slowly decrease to 369 µS/cm in 20 minutes and after 165 minutes it reached 341 µS/cm. Visible corrosion of the electrode in the form of turbidity was also noted at this time.

However, increased conductivity due to released zinc $Zn^{2+}$ and later iron ions $Fe^{2+}$ or $Fe^{3+}$ from corroding anode were measurable after 20 seconds. In the region of anode, the conductivity rose from mean value for contaminated solution to 489 – 491 µS/cm.

After the end of the experiment, the final conductivity of all experimental groups C, CE, 2WE, 2WM, 1WE, 1WM and CSO was measured and the average values corresponded to 413, 401, 450, 524, 300, 491 and 392 µS/cm respectively. An increase in conductivity was thus achieved in all groups regardless of electric field treatment except for group 1WE. The explanation for the increased conductivity after the end of the experiment could be the degradation of an electrodes. To confirm this statements, a small additional experiment was set up, where two groups were treated with CsCl and with an electric field and CsCl only, while all parameters stayed identical to presented experimental setup.

The result of the experiment was the finding that, while the conductivity of the cylinders treated with CsCl only did not change over time, the conductivity of the cylinders treated with CsCl and the electric field decreased after 16 days to 261 – 270 µS/cm despite electrode deterioration. Which suggests that the breakdown of the electrodes and the formation of iron hydroxides is not a driving factor of the conductivity increase at the end of the experiment. Another and most likely explanation is the presence of plant litter and its decomposition products or the possible presence of plant exudates (Marschner, 2011).

### S2: Water analysis

According to Haidari et al. (2023) in an experiment with electrokinetics coupled with phytoremediation, significant changes occurred in the anion concentrations of mine water leachate. Therefore, the concentrations of $F^-$, $Cl^-$, $NO_2^-$, $Br^-$, $NO_3^-$, $PO_4^{-2}$ and $SO_4^{-2}$ anions were measured in the water samples taken after the experiment. Tap water served as the baseline for sample comparison.

The analysis of $F^-$, $NO_2^-$, $Br^-$, and $PO_4^{-2}$ ions, across all experimental groups, showed no significant changes to the concentrations found in tap water.

The chlorides measured in the tap water samples and controls ranged from 24.07 – 25.21 mg/L with an average of 24.70 mg/L. The expected higher values of average $Cl^-$ concentration was 36.63 mg/L after CsCl application in the CSO group. Despite the differences in concentrations between the C and CSO groups, no significant differences ($p>0.05$) were found in the amount of $Cl^-$ ions. However, significant differences ($p <0.01$) were found between tap water samples and all electric field-treated groups. The mean concentrations of $Cl^-$ in groups CE, 2WE, 2WM, 1WE and 1WM were 3.92, 1.61, 2.01, 3.30 and 3.61 mg/L. This reduction in concentration corresponds to 84.12 – 93.49% removal from solution. Significant chloride removal rates of 39-70% were also reported by Haidary et al. (2023), during an experiment lasting 90 days. The removal of chlorides can be explained by several mechanisms, including partial precipitation on electrodes, formation of other forms such as solid iron chloride ($FeCl_2$) or chlorine gas ($Cl_2$).

Nitrate ions measured in tap water ranged from 16.46 – 23.32 mg/L with an average value of 17.94 mg/L. A decrease in average values was found in group C with 5.33 mg/L and group CSO with 5.78 mg/L. However, a more pronounced decrease to average concentration of 1.63, 0.48, 0.26, 0.38 and 1.47 mg/L was recorded in all electric field treated groups CE, 2WE, 2WM, 1WE and 1WM. Statistical analysis of $NO_3^-$ differences of all groups revealed significant differences ($p<0.01$) between tap water and all groups. Removal rate in negative control plants and those treated only with Cs was 67.78 – 70.30%. In the groups treated with the electric field, the removal rate was in the range of 90.90 - 98.53%. The $NO_3^-$ removal from the hydroponic system can be partially attributed to the efficient uptake of this compound by reed roots, which utilize it as a major and readily available nutrient (Marschner, 2011). This observation is supported by a substantial body of scientific literature (Cifuentes-Torres et al., 2021). However, the major role of the electric field on the removal of nitrates can be assumed due to the current results and advancements in usage of electrocoagulation and bio-electrocoagulation systems in the removal of nitrates (Elazzouzi et al., 2017; Sharma et Chopra, 2017; Qian et al., 2018). Electrocoagulation or its coupling was shown to be efficient treatment options for urban wastewater (Elazzouzi et al., 2017; Qian et al., 2018) or groundwater (Amarine et al., 2020).

The concentration of sulfate ions measured in tap water ranged from 36.48 to 43.53 mg/L with an average of 43.53 mg/L. In the negative control group C, the average

$SO_4^{-2}$ concentrations were 45.11 mg/L and in the CSO group 56.20 mg/L. However, groups CE, 2WE, 2WM, 1WE and 1WM treated with electric current recorded a decrease of $SO_4^{-2}$ to 2.48, 1.21, 1.57, 3.03, 3.08 mg/L. These values correspond to removal rates in the range of 92.10 – 96.90% when compared to tap water. However, in the negative control and the cesium-treated groups, $SO_4^{-2}$ increased by 15.59 and 44.00%. Application of electric filed was shown to be effective for the removal of sulfides, sulfites and sulfates in recent studies (Murugananthan et al., 2004; Sharma et Chopra, 2017; Nariyan et al., 2018). Murugananthan et al. (2004) reported removal rate of sulfates up to 72% in 10 minutes of electrocoagulation treatment time. Another example of effective sulfates removal is from acidic mine water treated with electrocoagulation. Most efficient experimental setup removed 84% sulfates with pretreatment with calcium oxide to reach pH 12 (Nariyan et al., 2018). On the other hand, the increase in sulfate concentration in the electric field-untreated groups could be explained both by exudates (Marschner, 2011) from reed roots and also by partial decomposition of plant littler in experimental flasks.

**S3:** Disintegration of an iron anode depending on time of electric field action
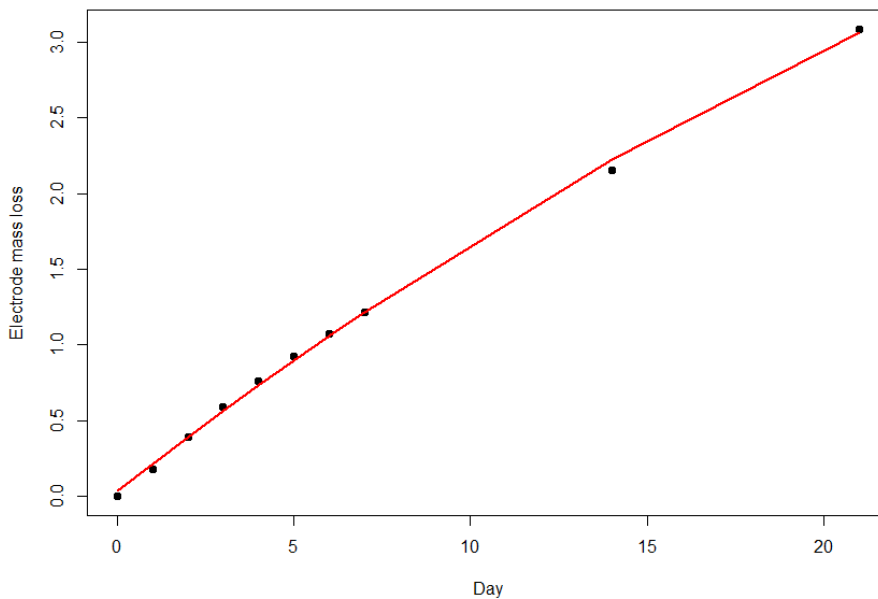
**S4:** Mineral composition of sediment and precipitate

| Sediment | | | Precipitate | | |
|---|---|---|---|---|---|
| Element | [mg/g] | % | Element | [mg/g] | % |
| Fe | 532.05 | 89.77 | Mg | 263.75 | 93.23 |
| Zn | 38.94 | 6.57 | Al | 7.90 | 2.79 |
| Ca | 10.17 | 1.72 | Zn | 3.00 | 1.06 |
| Mg | 4.79 | 0.81 | Fe | 1.79 | 0.63 |
| S | 2.83 | 0.48 | Mn | 1.33 | 0.47 |
| Si | 1.58 | 0.27 | Na | 1.33 | 0.47 |
| Mn | 1.33 | 0.22 | Ca | 0.99 | 0.35 |
| Ni | 0.37 | 0.062 | Cs | 0.90 | 0.32 |
| Cu | 0.20 | 0.033 | K | 0.87 | 0.31 |
| Cd | 0.098 | 0.017 | Cu | 0.45 | 0.16 |
| P | 0.076 | 0.013 | Si | 0.25 | 0.088 |
| Co | 0.070 | 0.012 | As | 0.082 | 0.029 |
| Sr | 0.055 | 9.25E-03 | S | 0.066 | 0.023 |
| B | 0.038 | 6.39E-03 | P | 0.054 | 0.019 |
| Cs | 0.036 | 6.04E-03 | B | 0.037 | 0.013 |
| Al | 0.033 | 5.55E-03 | V | 0.036 | 0.013 |
| V | 0.018 | 3.10E-03 | Tl | 0.013 | 4.47E-03 |
| Ba | 0.011 | 1.91E-03 | Sr | 6.06E-03 | 2.14E-03 |
| Pb | 0.011 | 1.82E-03 | Ni | 5.77E-03 | 2.04E-03 |
| Mo | 3.50E-03 | 5.90E-04 | Co | 5.64E-03 | 1.99E-03 |
| Be | 6.93E-04 | 1.17E-04 | Cd | 2.26E-03 | 7.98E-04 |
| Na | 2.58E-04 | 4.36E-05 | Be | 1.86E-03 | 6.56E-04 |
| Sb | 2.00E-04 | 3.38E-05 | Sb | 1.76E-03 | 6.22E-04 |
| Se | 3.98E-05 | 6.71E-06 | Ti | 2.10E-04 | 7.42E-05 |
| K | 2.17E-05 | 3.66E-06 | Mo | 1.04E-05 | 3.68E-06 |
| As | 1.11E-05 | 1.87E-06 | Cr | 8.09E-06 | 2.86E-06 |
| Tl | 5.26E-06 | 8.87E-07 | Se | 7.53E-06 | 2.66E-06 |
| Li | 4.39E-06 | 7.41E-07 | Li | 3.85E-06 | 1.36E-06 |
| Cr | 1.73E-06 | 2.91E-07 | Ba | 2.97E-07 | 1.05E-07 |
| Ti | 4.27E-07 | 7.21E-08 | Pb | 1.69E-07 | 5.97E-08 |

# 7 General discussion

## 7.1 Natural background levels

Throughout the experimental campaigns, both unpublished and published results of this study have been found to align with the literature-reported values for environmental background levels. Analyses conducted on plants such as *Calla palustris*, *Elodea canadensis*, and *Potamogeton pectinatus* from experiments not yet published consistently confirmed the same range of cesium levels as those reported here for *Ceratophyllum demersum* and *Phragmites australis*. Control samples exhibited cesium concentrations ranging from 0.079 to 0.919 mg/kg, which are considered typical for natural background values. The relatively high content of the stable cesium isotope is attributable to its greater abundance in the Earth's crust compared to other elements such as cadmium, tin, tungsten, silver, and mercury (Norton, 1973). Furthermore, Yamagata et al. (1959) were the first to report on stable cesium concentrations in 50 plant species across Japan, finding that cesium levels commonly ranged between 0.002 and 1 mg/kg. Similarly, Horovitz et al. (1974) examined another 24 plant species and found that $Cs^+$ concentrations could reach higher levels than those reported in the previous studies, with a range from 0.01 to 2.5 mg/kg. Pais and Jones (1997) also reported higher background levels for the fresh weight of vegetables (0.2 - 3.3 mg/kg) and fruits (<0.1 - 2.9 mg/kg). Adding to this body of research, Cook et al. (2007) conducted a comprehensive survey in the USA, observing stable cesium values in 124 plant species ranging from 0 to 0.365 mg/kg.

## 7.2 Accumulation dynamics

The accumulation of the stable cesium isotope depends on several factors, including the concentration of external cesium and the duration of exposure. Thus, the accumulation of cesium in aquatic submerged free-floating species can be categorized into two main factors affecting uptake: the first being the effect of the external cesium concentration, and the second being the exposure time. The

magnitude of the external concentrations significantly influences the amount of cesium accumulated. In the initial days of exposure, the dynamics of accumulation can be described as follows: at lower concentrations, mimicking a real radiation event, the slow rate of accumulation is attributed to the diffusion of cesium particles and the volume of liquid, i.e., the concentration and availability of the given element. With gradually increasing low concentrations of external cesium on the order of mg/L, the amount of accumulated cesium begins to rise in a largely linear fashion. Upon reaching high concentrations of external cesium in the order of hundreds of mg/L, and depending on the species exposed, the plant reaches the limits of possible intake dictated by internal physiological and biochemical processes, resulting in a plateau in accumulation. This phenomenon provides further evidence of the findings by Fried et al. in 1961, which suggested that cesium uptake is constrained at a certain stage by metabolic processes. This concept was corroborated two decades later by research conducted by Baker in 1981 and Shaw and Bell in 1989.

However, extending the exposure time at the same concentration results in continued accumulation by the plants. In Case Study I, the rate of cesium accumulation in plants was highest during the initial eight days, aligning with observations made by Kelly and Pinder (1996) and Pinder et al. (2006), which noted rapid accumulation in the early stages of exposure to the contaminant. As the exposure duration increased to 16 days, the rate of accumulation continued to rise, albeit at a slower pace compared to the initial days following contamination. For the longest exposure period of 24 days, the rate of cesium accumulation in plant biomass was the slowest. Scotti (1996) observed similar patterns in their study with *Phaseolus vulgaris*, and Kelly and Pinder (1996) reported a decrease in Cs$^+$ accumulation in *Nymphaea odorata* after 20 days of exposure. A decline in accumulation capacity was also observed in other plant species, which varied according to both the plant species and the duration of exposure (Pinder et al., 2006). Thus, the findings from Case Study I suggest that the plant exhibits saturation kinetics in cesium uptake at high external concentrations of the contaminant, as observed over the specified time frame.

In the portion of the case study discussed thus far, the focus has been on the uptake of the stable cesium isotope. It is necessary to distinguish this uptake from that of radioactive isotopes. External $^{133}$Cs concentrations were set at milligrams per liter

(mg/L) for the experiments. In contrast, for radioactive isotopes, approximately three nanograms correspond to an activity of 10 kBq (Rai and Kawabata, 2020). However, even in this scenario, the limitations imposed by metabolic processes still apply (Fried et al., 1961; Baker, 1981; Shaw and Bell, 1989). The main factors limiting the uptake of radionuclides are not only their low concentration but also the plant's resilience against the production of free radicals and reactive oxygen species (Wi et al., 2007; Majeed et al., 2018). Thus, the plant's response depends not only on the species (Ramchander et al., 2015; Roslim and Fiatin, 2015; Saakov et al., 1992; Luckey, 1980; Fokam et al., 2020; Majeed et al., 2018) but also on its variety (Ramchander et al., 2015), age, and consequently, the growth stage of the individual (Kim et al., 2011; Kurimoto et al., 2010).

Furthermore, when comparing the results of cesium accumulation in a greenhouse experiment with $^{133}$Cs and $^{134}$Cs, it was demonstrated that cesium was removed from the solution at the same rate in both cases, as commonly described in the literature (Tsukada and Hasegawa, 2003; Soudek et al., 2004; Shinano et al., 2014). Additionally, one kg of dry weight of *Ceratophyllum demersum* absorbed up to 192 kBq—much higher than any reported value in the literature (Sasaki et al., 2016; Kowata et al., 2014; Nagakawa et al., 2019).

These facts thus offer insights into the complex nature of assessing and remediating radiation pollution. The current body of literature lacks comprehensive studies on the long-term effects of ionizing radiation on aquatic plants, particularly concerning its impact on certain metabolic functions. Simultaneously, the existing gap in radioecological research regarding the effects of ionizing radiation on plants stems from an inadequate distinction between the direct effects of ionizing radiation and the toxic effects of specific elements. These deficiencies highlight the need for further investigation into radionuclides in aquatic environments.

However, as mentioned by Vymazal and Březinová (2015), for the correct assessment of accumulation, it is also necessary to consider the appropriate part of the plant. Soudek et al. (2004) reached a similar conclusion when documenting the accumulation of $^{137}$Cs in various parts of reeds, mainly in young parts such as leaf tips, young shoots, or nodes. These findings align with the processes described by Marschner (2012), wherein after cell division, cell extension is largely facilitated by the accumulation of potassium cations. Due to its properties, the cesium cation

follows potassium to sites of cell extension, as indicated by Bange and Overstreet (1960), White and Broadley (2000), and Zhu (2000). However, these findings underscore the need for long-term field experiments that directly examine or simulate the natural habitats of species. For instance, in the case of the submerged, free-floating species *Ceratophyllum demersum*, rapid growth leads to the formation of dense mats. However, the fitness of a species is influenced by environmental factors such as nutrient availability, which generally predict species variability, composition, and density (Dar et al., 2014; Szabó et al., 2022). The distribution of the macrophyte in the water body is also dependent on other environmental factors, such as light and temperature, as well as the plant's life cycle, growth, and detritus formation—i.e., the uptake and return of contaminants to the environment (Zalewska, 2012; Dar et al., 2014). Sediment properties generally affect growth rates. Forsberg (1960) estimated the maximum net production of *Ceratophyllum demersum* to be 5.7 g dry matter/m²/day at Lake Ösby, based on the increase of floating plants near the surface. Edwards and Owens (1961) reported the net production of these macrophytes in a Cretaceous stream as 4.0 g dry matter/m²/day from June to September, with estimates using the same method. In 1972, Denny experimentally confirmed the difference in the growth rate of *C. demersum* depending on the substrate, with plants growing on mud averaging gains of 9.5 mg dry matter/plant/day and on sand 4.5 mg dry matter/plant/day.

As previously discussed, the impact of environmental factors, such as sediment type, can significantly influence the uptake and, consequently, the remediation capabilities of plant populations. The effectiveness of phytoremediation is contingent upon the biomass amount exposed to the contaminant. This assertion, while intuitive, has been substantiated by research, particularly in the analysis of Case Studies I and III, which examine the factors affecting cesium accumulation. It was observed that plants with a greater initial mass, indicative of higher potassium uptake capacity, nutrient demand, photosynthetic activity, and other metabolic functions, exhibited increased cesium accumulation from the growth medium. Conversely, the change in biomass—measured as the difference in dry matter before and after the experiment's commencement—was not identified as a significant factor influencing uptake over a short duration, specifically up to 16 days. Similarly, the extension of root length also yielded insignificant results in the context of cesium uptake.

However, as stated in Case Study III, plant uptake can be influenced not only by the natural biotic and abiotic factors of the environment but also by those introduced for research purposes. The electric field, an artificially introduced abiotic factor, is seldom explored in current literature for its role in decontaminating aquatic environments when used in conjunction with phytoremediation techniques. The findings from Case Study III demonstrate the impact of the electric field on the plants studied, notably a 20-25% reduction in cesium accumulation. This outcome was verified through a covariate analysis across multiple experimental setups. The treatment group—subjected to an electric current—exhibited a significant decrease in cesium accumulation. Moreover, plants exposed to both cesium and the electric field accumulated higher cesium concentrations in their aerial parts, suggesting enhanced translocation to these areas. Nonetheless, this increase in translocation was not statistically significant, partially aligning with the findings of Soudek et al. (2004), who reported augmented translocation of cesium to above-ground reed plant parts.

## 7.3  Annual variations in accumulation

The difference in accumulation observed between the years 2017 and 2018 was tested for a smaller number of groups in subsequent research related to Case Study I. A significantly different accumulation of cesium by plants was found between the years 2017 and 2018, despite the same external concentrations, plant biomass, and a one-month time period. These results can be explained by environmental characteristics and the metabolic processes associated with them, leading to the dynamics of seasonal changes in plant accumulation (Jackson, 1998; Marschner, 2012; Vymazal & Březinová, 2015). An overview of the dynamics of $^{137}$Cs accumulation throughout the year was provided by Zalewska (2012), who described the processes during different seasons for the submerged vascular sea species *Zostera marina* and the algae *Polysiphonia fucoides*. Zalewska (2012) describes the seasonal dynamics as follows: at the beginning of the growing season, the plant starts to grow and absorbs available cesium from the environment for a short period. As the season transitions from spring to summer, individuals mature, leading to a dilution of cesium content in the biomass, i.e., a decrease in the total accumulated amount per unit of biomass. In peak summer, metabolic processes

and, consequently, uptake by the plant intensify, leading to an increase in cesium content. The onset of autumn brings a slowdown in metabolic processes and a lower intake of metals, resulting in lower accumulated amounts compared to summer, as analyses indicate. At the transition from autumn to winter, the biomass of the individual decreases, and the cesium content in the plant increases. During winter, the biomass dies, and cesium is released back into the environment. However, it should be noted that while some studies, such as the one conducted by Malea and Haritonidis (1999), report a similar relationship for the iron and copper contents in marine algae, many publications report varying dynamics in the uptake of individual metals for different species of higher aquatic and terrestrial plants. Vymazal and Březinová (2015) emphasize that although it can generally be said for heavy metals that concentrations increase during the spring and are later diluted by biomass growth, predicting the details of seasonal variability for individual species and elements is challenging due to unpredictable patterns, which differ from those of plant nutrients. Similar to heavy metals, the kinetics of cesium uptake by plants can also be assumed to be less predictable. This variability depends not only on the plant species but also on numerous metabolic processes. Among these processes is the well-documented autumn storage of nutrients in rhizomes and their mobilization in spring to support growth in *Phragmites australis* (Granéli et al., 1992). There is also a decrease in root and rhizome biomass during the autumn and winter months, including subsequent metabolic losses of elements (Granéli et al., 1992). The discussed translocation and preservation of elements in the rhizomes of reed plants or in the root biomass of higher plants thus indicate differences in the cycle of cesium-contaminated water bodies compared to that of submerged plant species. Other metabolic processes to consider include the remobilization, where nutrients from old leaves are transferred to new leaves, and re-translocation between the xylem and phloem (Marschner, 2012). Additionally, seasonal and annual variations in the management of stored nutrients, depending on the life cycle of the plant, were described by Asaeda et al. (2006), covering the entire life cycle of the plant over several successive vegetation periods.

Seasonal variability in the accumulation of heavy metals is documented in the literature for terrestrial species (Bidar et al., 2009), freshwater emergent species (Vymazal and Březinová, 2015; Kastratović et al., 2013), riparian plants (Kim and Kim, 2018), floating-leaved plants (Duman and Obali, 2008), and submerged aquatic

plants (Duman et al., 2006). Conversely, descriptions of cesium behavior within freshwater aquatic plant species are inadequate and are expected to differ to some extent from those in marine environments, as Zalewska (2012) described. The findings from the follow-up study on Case Study I present one of the initial demonstrations of how environmental alterations can prompt changes in plant development and metabolism. Moreover, even under certain controlled conditions, these modifications can result in a markedly different uptake of cesium. Such findings are deemed essential for risk assessment and environmental management in areas affected by radiation events.

## 7.4  Differences in natural and controlled conditions

The amount of stable cesium accumulated in the greenhouse experiment from Case Study I was lower than those reported in the literature, even under comparable experimental conditions. However, it is important to note that the number of studies with comparable conditions is limited, rendering it challenging to make definitive conclusions based on a small dataset (Saleh et al., 2020; Rinaldi et al., 2017; Kondo, 1982), particularly when methodological approaches vary. In contrast, the field experiment demonstrated a significantly higher accumulation of $Cs^+$, highlighting the discrepancy between controlled and natural environments. This difference underscores the importance of conducting extensive field-based research to enhance our understanding of $Cs^+$ accumulation dynamics in natural settings. Such research is crucial for improving the accuracy of environmental monitoring strategies and remediation efforts following nuclear incidents.

## 7.5  Impact of cesium on health status of plants

In assessing the impact of stable cesium on plant health, no significant deterioration was observed in the health of plants within the 0.267 mM $Cs^+$ group across any exposure durations. Furthermore, no decline in plant health was evident in either group after 8 or 16 days of exposure. Similarly, no deterioration in health status was noted in plants exposed to $^{134}Cs$ for eight days. This suggests the short-term resilience of plants in resisting cesium toxicity, even when exceeding the 0.2 mM threshold outlined in the literature (White and Broadley, 2000), and further

underscores plant resistance to the chronic effects of radiation, with activity values up to 10 kBq, for at least eight days. Conversely, during the 24-day exposure to $^{133}$Cs, groups treated with 0.267 mM and higher concentrations exhibited visible health deterioration, although these signs were not deemed significant. These findings underscore the necessity of understanding the resilience limits of individual plant species to both $^{133}$Cs and radioactive isotopes, as well as a more nuanced understanding of the seasonal distribution of accumulation, due to potential impacts on plant productivity and limitations on phytoremediation capabilities. This includes considerations from the perspective of reintroducing contaminants to the environment as detritus.

The health status of plants in Case Study III was also monitored. Plants exposed solely to cesium at an external concentration of 0.267 mM began showing symptoms of cesium toxicity. However, in plants grown concurrently within an electric field, such symptoms were generally not observed. A significant increase in new leaf growth was detected in all groups, with the exception of the cesium positive control. When considering electrode materials, it is vital to evaluate the impact of their decay products on the adsorption of cesium from the water column or on the plants' ability to absorb cesium. Subsequent analyses and evaluations revealed that iron hydroxides, as the primary products of electrode decay, did not affect the adsorption of cesium. Moreover, there was no observed negative impact of electrode decay products on plant health.

## 7.6  Impact of potassium on cesium uptake

Potassium additives and their effects on cesium accumulation were tested in both aquatic and terrestrial environments. In additional research related to Case Study I, the results from the potassium-supplemented groups revealed a reduction in cesium accumulation compared to their cesium-contaminated counterparts, with a 92% reduction observed across all groups. However, Case Study II showed, across all experiments, that potassium fertilization had no effect on the uptake of cesium. These results highlight the complex nature of evaluating potassium additives in growth media. While some literature on the impact of potassium additives on cesium accumulation in hydroponic media reports a significant reduction in $^{137}$Cs uptake (Zhu, 2001; Smolders et al., 1996) across all plant parts (Wallace et al., 1983),

other studies report an increase in cesium content at higher potassium concentrations (Smolders et al., 1997; Soudek et al., 2004; Soudek et al., 2006) or even identify calcium and magnesium as elements negatively correlated with cesium uptake (Smolders et al., 1997). Similarly, the literature on soil environments presents mixed findings. On one hand, several studies indicate a reduction in $^{137}$Cs accumulation when potassium fertilizer is applied (Haak, 1985; Robison et al., 1997; Belli et al., 1995); on the other hand, there are studies that find little or no effect (Ban-nai et Muramatsu, 2002; Malm et al., 1991; Nisbet and Woodman, 2000).

However, the issue of potassium fertilizer application needs to be examined in the context of soil chemistry and the nuances introduced by biota and environmental factors. While Nisbet and Woodman (2000) did not find a correlation between cesium uptake and exchangeable potassium content across a database of 28 combinations of soil and crop types, Frissel et al. (2002) expanded upon these findings. The authors identified a threshold of 19.5 mg/kg as the critical concentration level affecting the uptake of cesium by crops. Below this value, cesium uptake is influenced by exchangeable potassium, but above it, other factors become more influential. A similar conclusion was reached by Squire and Middleton (1966), who observed that the response of cesium accumulation depends on the amount of exchangeable potassium present in the soil. They noted that soils with a low content of exchangeable potassium exhibit a 20-50% reduction in $^{137}$Cs accumulation following potassium fertilization, whereas in soils with higher potassium levels, no significant change occurs.

In Case Study II, the application of potassium fertilizer did not result in a significant reduction in cesium accumulation. For instance, the average $T_{ag}$ value of *Lactuca sativa* grown on haplic luvisol increased insignificantly after potassium fertilization. This outcome aligns with the findings of Soudek et al. (2004 and 2006), which suggested that increasing the concentration of added potassium could lead to an increased uptake of cesium. Additionally, higher transfer coefficients due to potassium fertilization were observed in *Allium cepa*, where the $T_{ag}$ value decreased only in chernozem soil. Conversely, *Raphanus sativus* grown on fluvisol soil demonstrated a 51% reduction in cesium accumulation. Applying the conclusions of Squire and Middleton (1966), it appears that soils with a low level of exchangeable potassium, when supplemented with potassium fertilizer, experienced an increase in potassium availability, thereby reducing cesium accumulation. However, across the

soil types studied in Case Study II, average soil potassium concentrations ranged from 118 to 362 mg/kg, where Rai and Kawabata (2020) noted that the exchangeable fraction of potassium is typically up to 10%. With the application of potassium fertilizer at a concentration of 115 mg/kg, the soils should, therefore, exceed the threshold values identified by Frissel et al. (2002). When exploring reasons why potassium fertilizer is ineffective in mitigating cesium uptake, it is necessary to consider other influencing factors. These may include the synergistic effect of nutrients (Belli et al., 1995), the roles of calcium, sodium, or magnesium (Bange and Overstreet, 1960; Smolders et al., 1997), other monovalent or divalent cations (Chu et al., 2015; Burger and Lichtscheidl, 2018), the dynamics of potassium sorption and desorption considering environmental factors (Hundal and Pasricha, 1998), or the influence of other elements (Schulz, 1965), alongside other environmental factors or the direct effects emanating from the plants and their biological processes.

In contrast, in aquatic environments, the addition of potassium acts as a mitigation measure against cesium uptake, particularly at high concentrations. This observation is supported by the work of Rinaldi et al. (2017), who found that adding 114 mg/L of potassium to the hydroponic medium decreased cesium accumulation by 74% in the green leaves and 83% in the roots of *Calla palustris*. Similarly, Komínková et al. (2018) observed a reduction in cesium uptake by 44-74% across all groups treated with external potassium concentrations of 39.1, 78.2, 156.4, 390.9, and 781.9 mg/L. However, Kondo (1982) noted that at lower potassium concentrations of 7.8 mg/L, cesium absorption occurred at a similar rate, while at a cesium concentration of 5.9 mg/L, potassium uptake was almost entirely inhibited.

Literature indicates that potassium and cesium compete for the same uptake mechanisms (Bange and Overstreet, 1960; White and Broadley, 2000). Given the interactions with the soil environment, such as adsorption, it is conceivable to apply and shift the conceptual framework provided by Frissel et al. (2002) for soil environments to aquatic settings. The results presented not only suggest a higher sensitivity of aquatic species to naturally lower concentrations of potassium in the water column, as compared to soil concentrations (Talling, 2010; Marschner, 2011; Rai and Kawabata, 2020), but also indicate faster cesium uptake and greater efficacy of potassium additives in reducing cesium uptake in aquatic environments, as highlighted by additional research related to Case Study II. However, to substantiate

these findings, further studies are required to explore the interaction between potassium and cesium, including uptake mechanisms and their sensitivity to cesium concentrations in aquatic species with varying life strategies.

## 7.7  Species specific uptake

While *Lactuca sativa* was grown as the first crop, its transfer coefficients were lower than those observed in subsequent crops, such as *Allium cepa* and *Raphanus sativus*. This observation confirms that the type of cultivated crop significantly affects the transfer coefficient. The variation in uptake among different crop types might be attributed to their distinct requirements for available potassium during cultivation. However, it is noted that *Lactuca sativa* and *Allium cepa* share very similar needs for available potassium in unfertilized soils (Greenwood et al., 1980; Brandenberger et al., 2009). To understand the differences in uptake, it is necessary to consider intraspecific factors, including the specificity and efficiency of membrane transport systems, the architecture and depth of root systems, the varying metabolic needs and processes, the chemistry at the soil-root interface, and the ecosystem comprising plant roots and soil microorganisms.

In the case of potassium transport systems, Lai and Luo (2019) identified differing degrees of involvement in cesium uptake between *Brassica juncea* and *Vicia faba*. They also confirmed varying degrees of inhibition of potassium uptake by cesium in each plant species. Thus, their findings align with the inhibition of potassium uptake by cesium observed in an aquatic species by Kondo (1982). Additionally, Lai and Luo (2019) reported that cesium uptake inhibits the expression of related transporter protein genes in roots. However, this effect was significant only in *Vicia faba*, not in *Brassica juncea*, indicating a variable impact of cesium on gene expression and, consequently, on plant development. These findings may provide insights into the observed developmental retardation in *Allium cepa* in Case Study II. Furthermore, the results suggest a potentially lesser effect of cesium on the expression of root transporter protein genes in *Lactuca sativa* and *Raphanus sativus*.

In addition, the literature also highlights the sensitivity of plant species to exchangeable potassium, as described in relation to cesium uptake by Guillaume et al. (2012). A closer examination of the average potassium uptake in *Allium cepa*,

*Lactuca sativa*, and *Raphanus sativus* reveals that potassium fertilization significantly affects its absorption by plants. Notably, onions planted in the autumn consume nearly twice as much potassium as those planted in the spring. Analyses of optimal and maximum potassium fertilizer rates and their impact on potassium uptake show that *Raphanus sativus* exhibits the lowest potassium consumption (39 kg/ha) even at the highest fertilization rates. Conversely, under the same conditions, *Allium cepa* demonstrates a substantially higher potassium uptake during spring planting (100 kg/ha) and autumn planting (117 kg/ha) (Greenwood et al., 1980). These findings reinforce the earlier conclusion that potassium fertilization had an insignificant effect on potassium utilization in *Lactuca sativa* and *Raphanus sativus*. Thus, a speculative explanation for the cesium uptake and the impact of potassium fertilization in *Allium cepa* is proposed: In the early stages of development, the plant absorbs large quantities of both potassium and cesium, facilitated by cell division and extension (Marschner, 2012), likely due to less discrimination against cesium by uptake mechanisms. In groups fertilized with potassium, the quantity of potassium absorbed increased proportionally, as suggested by Greenwood et al. (1980), but so did the amount of cesium uptake. However, over time, the adverse effects of cesium within the plants began to emerge, leading to a blockade of potassium transport mechanisms and affecting gene expression, which resulted in the retardation of root development.

## 7.8  Soil ecosystem and soil parameters

Regarding the chemistry and ecosystem of the soil-root interface, Khan et al. (2023) provided some explanations for the observed differences in cesium uptake in Case Study II, describing how plant exudates enhance the availability of cesium in the rhizosphere. Neumann et al. (2014) added that the production of plant exudates is strongly influenced by the soil type in which the plants grow. Variations in root exudation, depending on the soil type, can span from one to three orders of magnitude, even without stress factors known to affect exudate production. The authors attribute these differences in exudate production to the interaction between the plant and soil-specific microbiomes. For instance, Schreiter et al. (2014) described soil type-dependent bacterial diversity in *Lactuca sativa* plants during field experiments, leading to the conclusion that soil type significantly

influences the microbial community composition within the same plant species. Berg and Smalla (2009) further noted that the composition of microbial communities is influenced by numerous factors, including root morphology, soil type and structure, plant species, development stage, and the root exudates themselves. Although this information provides insight into potential explanations for the differences in cesium uptake between the species in Case Study II, it does not offer a definitive answer due to the lack of data on microbial communities and exudate production.

Differences among individual soil types (cambisol, fluvisol, chernozem, haplic luvisol, and gley) for specific crops were noted in Case Study II. Simplifying, the soils for lettuce could be visually categorized into two groups based on $T_{ag}$ values—Temelin and Dukovan soils—whereas this categorization differed for radish plants. The radishes could be divided into two groups, with chernozem, haplic luvisol, and gley forming the first group with a lower $T_{ag}$, and cambisol from Temelin and Dukovany with fluvisol comprising the second group with a higher $T_{ag}$. These findings suggest potential differences in the microbial community compositions and types of exudates across different soil types, which could have contributed to variations in plant uptake. This is also indicated by analyzed soil parameters such as clay fraction content and pH, which did not explain the differences in $T_{ag}$.

The analysis of clay fraction content did not reveal statistical differences in cesium uptake between plants grown on loam and clay loam soil types for any cultivated crop. As documented in the literature, soils containing clay minerals are known to possess a high selective retention for cesium (Cremers et al., 1988; Berns et al., 2018; Cornell, 1993). Although a high percentage of clay minerals in the soil typically ensures a high selective retention of cesium, other types of experiments may require consideration of the specific type of clay mineral due to their varied adsorption capacities (Fujita et al., 2013; Mukai et al., 2016). However, owing to the methodology of Case Study II, which involved comparing the soil ratio and the applied amount of [134]Cs contaminant, it can be inferred that the soil's adsorption capacity was not exceeded. Consequently, it is plausible to assume a similar influence of both loam and clay loam soil types on the adsorption of cesium, thus yielding a non-significant difference between them.

Additionally, the effect of pH on cesium uptake by *Lactuca sativa*, *Allium cepa*, and *Raphanus sativus* was not confirmed in soil-grown plants, nor was it observed in hydroponically grown *Phragmites australis* plants in Case Study III. These results align with the findings of Van Bergeijk et al. (1992), who reported a negligible effect of pH on cesium uptake, and Nisbet and Woodman (2000), who observed no effect of pH on uptake in their meta-analysis. In Case Study II, the plants were grown in soil with a pH range of 5.5-7, while in Case Study III, the root system was exposed to a pH range of 6-8. This suggests that plants growing within this neutral pH range do not exhibit a response in terms of cesium uptake. A response to pH, if any, would likely be observed in the pH range of 4-5, where some studies have reported an effect (Frissel et al., 2002; Urban and Bystrzejewska-Piotrowska, 2003; Saleh, 2012; Ogura et al., 2014).

Despite the controlled parameters and the lack of statistically significant findings, the variability in cesium uptake activities by plants in Case Study II exhibited wide ranges. This observation holds true across all experimental conditions for all studied crops (*Lactuca sativa*, *Allium cepa*, and *Raphanus sativus*) and soil types. Such variability aligns more closely with the natural distribution than with laboratory measurements, reflecting the heterogeneous nature of radioactive fallout, which can vary within individual agricultural areas (Korobova and Romanov, 2011; Tsuiki and Maeda, 2012). As with previous findings, this study underscores the importance of further research into the parameters influencing uptake within field environments. Field studies assess results against the backdrop of natural variability, which may not be present under highly controlled conditions. Thus, when applying laboratory-derived knowledge to real-world environments, it is expected to encounter wide margins that are intrinsic to the biota and its environment.

# 8 Conclusions

This dissertation focuses on the study of factors influencing the uptake of cesium by higher plants, targeting and comparing the intake for both aquatic and terrestrial species. It contributes to our understanding of cesium dynamics in plant systems and explores promising strategies for environmental engineering and phytoremediation. The findings highlight the complex interplay of metal-specific, environmental, and plant-specific factors in determining cesium uptake, thereby opening avenues for further research in this critical area. Additionally, this work introduces a new potential candidate for phytoremediation techniques— *Ceratophyllum demersum*—investigates the uptake by three vegetable species in the most common agricultural soils in the EU under conditions of fresh contamination and explores remediation strategies using an electrokinetic process coupled with phytoremediation.

The research delineates a nuanced understanding of the uptake dynamics of stable cesium, noting a roughly equal uptake at lower concentrations and a linear increase until reaching a saturation point at high concentrations. The duration of exposure is crucial, with initial rapid uptake slowing after one week. However, variations exist between aquatic and terrestrial species, attributable to the different physicochemical properties of their growth media, environmental influences, and biological processes. Furthermore, this work critically evaluates the influence of biomass growth, pH levels, soil types, and clay particle content on cesium uptake, revealing a lack of significant impact in short-term experiments. Conversely, the initial biomass of the plants, seasonality, and the introduction of an abiotic factor such as an electric field markedly impacted cesium uptake. Similarly, the addition of potassium was proven to be a significant factor in reducing cesium uptake in aquatic but not terrestrial environments, suggesting a potentially higher sensitivity of aquatic species to such mitigation measures.

Disparities between results obtained from controlled greenhouse experiments and field studies underscore the challenge of replicating natural variability in laboratory settings, emphasizing the need for field research that accounts for environmental complexities. Similarly, species-specific uptake of $^{134}$Cs highlights the critical roles of

environmental factors, plant adaptation mechanisms, microbial ecosystems, the chemistry of the rhizosphere, and the impact of cesium on plant transport mechanisms. Despite the data heterogeneity reflecting the complex reality of environmental contamination, the plant species demonstrated temporary resistance to the phytotoxic effects of cesium, even above the established toxicity threshold.

This work ambitiously seeks to broaden the scope of knowledge regarding cesium uptake, highlighting under-researched factors that could influence the intake process. Consequently, this may lead to observable disparities in transfer coefficients or overall accumulation, as well as the efficacy of phytoremediation. It emphasizes the necessity of conducting field experiments to fully understand the real-world implications of cesium uptake, advocating for a closer examination of plant exudates, microbial interactions, and soil chemistry. Additionally, it calls for further research into the behavior of cesium in aquatic species across different seasons and their specific uptake mechanisms. It advances our understanding of cesium uptake during electric field stimulation of plants and also identifies critical areas for future research into this technique. By doing so, it lays a foundation for the development of more effective environmental management practices aimed at mitigating the movement of radiation contamination through food chains and enhancing the utilization of meaningful phytoremediation efforts.

# 9 Related projects

This dissertation was written as part of contributions to multiple projects, specifically those supported by the Ministry of the Interior of the Czech Republic: project number VI20172020098, titled "Disposal of Radiation-Contaminated Biomass after NPP Accident: Distribution, Logistics of Harvesting, and Exploration in Biogas Technology" (2017-2020); and project number MV VI20192022153, titled "Optimization of Procedures for the Implementation of Crop Production in Areas Affected by a Nuclear Accident" (2019-2022). It also received support from the Internal Grant Agency of the Faculty of Environmental Sciences at the Czech University of Life Sciences, Prague, located at Kamýcká 129, Praha – Suchdol, 165 00, Czech Republic. The project from this agency, named "Vliv elektrického pole a rostlinných antioxidantů na fytoremediaci cesia" (The Influence of Electric Field and Plant Antioxidants on Cesium Phytoremediation), was assigned registration number 20194207 and project number 42220/1312/3162 (2019-2020).

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# 11 Curriculum vitae & List of publications

Jaroslav Vacula

Born: 24. 8. 1988

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## Education

November 2019 – March 2020: Internship

**Francisk Skorina Gomel State University, Belarus**

**Institute of Radiobiology of the National Academy of Sciences of Belarus**

2018 – present: Ph.D. studies

Applied and Landscape Ecology, Faculty of Environmental Sciences

**Czech University of Life Sciences Prague, Czech Republic**

Thesis: *Identification of factors affecting cesium uptake by plants*

2016 – 2018: Master study program

Applied Ecology, Faculty of Environmental Sciences

**Czech University of Life Sciences Prague, Czech Republic**

Thesis: *Effect of exposure time on Cs uptake by Ceratophyllum demersum L.*

2013 – 2016: Bachelor study program

Applied Ecology, Faculty of Environmental Sciences

**Czech University of Life Sciences Prague, Czech Republic**

Thesis: *Cesium uptake by Calla palustris*

## List of publications

**Vacula, J**., Kominková, D., & Praus, L. Electrokinetic Effects in Hydroponic System Contaminated with Cesium and its Impact on Phragmites Australis Radioprotection. Under review – International Biodeterioration & Biodegradation

Kadlecová, M., Vojík, M., **Vacula, J**., & Berchová-Bímová, K. Grab to fill the gap: Key factors Influencing Fallopia japonica germination and seedling establishment in the secondary distribution rang. Under review – Plant Ecology

Çömez, F.Ö., Sochacki, A., **Vacula, J.**, Bárta, J., Zídková, L., Lexa, M. and Komínková, D., 2024. Biochar as a superior material for enhancing the performance and microbial diversity in biofilters treating greywater. Journal of Water Process Engineering, 59, p.105066.

Komínková, D., **Vacula, J.**, Záhorová, V., Rulík, P., Škrkal, J., Pecharová, E., Borecký, Z., Šestáková, K., Stádníková, D., Finkous, P., Finkous, M., Hofmanová Kautská, V., Doskočilová, V., & Pilátová, H. (2023). Aggregated transfer factors of fresh Cs and Sr pollution to various vegetables from six common European soils–mesocosm experiment. Journal of Environmental Management, 345, 118900.

**Vacula, J**., Komínková, D., Pecharová, E., Doksanská, T., & Pechar, L. (2020). Uptake of 133Cs and 134Cs by Ceratophyllum demersum L. under field and greenhouse conditions. Science of The Total Environment, 720, 137292.

## List of utility models

Petreje, M., Hnátková, T., Komínková, D., **Vacula, J**., Çömez, Ö., F., Hladký, R., Sochacki, A. (2023). Vsakovací příkop. 37243.

Petreje, M., Hnátková, T., Komínková, D., **Vacula, J**., Çömez, Ö., F., Hladký, R., Sochacki, A. (2022). Zařízení pro snížení antropogenní zátěže z šedé vody. 36709.

## Grants and projects

| | |
|---|---|
| 2023 – 2028 | Biorefining and circular economy for sustainability (TN02000044) – funded by Technology Agency of the Czech Republic |
| 2022 – 2024 | Grow safely – do not support invasive species – funded by Norway Grants and State Environmental Fund of the Czech republic (3211100006) |
| 2022 – 2024 | Elimination of invasive plant species in practise (3211100010) – funded by Norway Grants and State Environmental Fund of the Czech republic |
| 2018 – 2022 | SWAMP - Responsible water management in built-up areas in relation to the surrounding landscape (CZ.02.1.01/0.0/0.0/16_026/0008403) – funded by Ministry of Education, Youth and Sport of the Czech Republic |
| 2019 – 2022 | Optimization of procedures for realization of crop production in the region affected by a nuclear accident (VI20192022153) – funded by Ministry of the Interior of the Czech Republic |
| 2019 – 2020 | Influence of electric field and plant antioxidants on phytoremediation of cesium (IGA 20194207) |
| 2017 – 2020 | Disposal of radiation-contaminated biomass after NPP accident-distribution, logistic of harvesting, exploring in biogas technology (VI20172020098) - funded by Ministry of the Interior of the Czech Republic |

# 12 Participation in conferences

(i)    Green Deal Biotechnology 2023 (Poznań, Poland)

(ii)   Bioremid 2023 (Muttenz, Switzerland)

(iii)  17th International Conference on Wetland Systems for Water Pollution Control 2022 (Lyon, France)

(iv)   XLIII. Dni radiačnej ochrany 2022 (Stará Lesná, Slovak Republic)

(v)    Ecological and Environmental Engineering 2022 (Poznań, Poland)

(vi)   14. bienální konference CzWA VODA 2021 (Litomyšl, Czech Republic)

(vii)  15th International Conference on Urban Drainage 2021 (Melbourne, Australia)

(viii) Goldschmidt 2020 (Honolulu, USA)

(ix)   Bioremid 2019 (Porto, Portugal)

(x)    RADIOBIOLOGY: CONTEMPORARY ISSUES 2019 (Gomel, Belarus)

(xi)   Kostelecké inspirování 2018 (Kostelec, Czech Republic)