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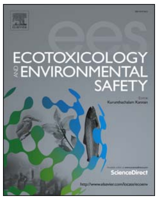

Stable cesium (^{133}Cs) uptake by *Calla palustris* from different substrates

Federica Rinaldi, Dana Komínková*, Kateřina Berchová, Jeremy Daguenet, Emilie Pecharová

Czech University of Life Sciences Prague, Faculty of Environmental Sciences, Kamýcká 129, 165 21 Prague 6, Czech Republic

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ABSTRACT

The uptake of stable cesium (^{133}Cs) by *Calla palustris* was evaluated from four different substrates: water, soil, keramzit (a clay granule) and water with the addition of a potassium compound, after an eight days exposure to a solution of 0.5 mM cesium chloride. Stable cesium was used because it is commonly supposed that its uptake by plants is the same of that of radiocesium (^{137}Cs). The plants were differentiated in their parts (roots, healthy leaves, dead leaves and flowers) and analyzed with ICP-MS. The lowest average concentration of absorbed Cs was found in plants exposed in soil (0.7 mg/kg, S.D. = 96.8), while the highest in plants exposed in water (147 mg/kg, S.D. = 51.7). During the experiment the water planted plants removed 31.6% of provided Cs while those planted in soil removed only 0.06%. The addition of potassium to water was tested because of the competition effect that arises between these two elements: this effect was confirmed with the result that the average uptake in the presence of potassium was lower (41 mg/kg in exposed plants, S.D. = 76.1). The uptake was also lower in the solid-based substrates (soil and keramzit), because of the known tendency of Cs to bind with soil particles, thus becoming less available to plants. There was no evidence that the different parts of the plant showed different uptake effectiveness, or that the health of the plant (evaluated with a qualitative method) had any effect on the uptake of Cs.

1. Introduction

Although atmospheric-based nuclear testing was banned in 1962 and nearly 30 years have passed since the accident and discharge of radioactive material at Chernobyl, increased concentration of radionuclides fallout in the soil, remains as an adverse effect of these events. In particular, it is still a matter of concern for public health in 2016, and represents a worldwide challenge for remediation with implications in medicine, agriculture, botany and many other branches of environmental sciences. Soil contamination from radionuclides fallout is widespread throughout much of Europe, Russia and Japan from Chernobyl and Fukushima Dai-Ichi accidents (Beresford et al., 2016; Cosma et al., 2016; Matsunaka et al., 2016; Pumpanen et al., 2016; Saniewski and Zalewska, 2016; Snow and Snyder, 2016; Yasunari et al., 2011). ^{137}Cs, which is the longest-living radionuclide fallout, has low mobility in soil (Lasat et al., 1998), it binds strongly to clay minerals (Lasat and Kochian, 1997) and it contaminates a wide area with low concentrations. Because of these peculiarities, decontamination from radiocesium with conventional engineering techniques is a difficult and expensive process: contaminated sites would need to be treated with soil disposal, or other chemical and physical treatments that are impractical, inefficient and alter – possibly irreversibly – the properties typically found in agricultural soil. The high biological availability of cesium, caused by its similarities with potassium, represents both a danger for human health, and a possible opportunity for the exploitation of plants in the decontamination of soil through the process of phytoremediation. The current understanding is that some plant species are capable to uptake stable and radioactive cesium that also behave similarly (Soudek et al., 2004), through the same channels used for potassium uptake, and then accumulate it in roots or aboveground parts, with no toxic effect on plant tissue (White and Broadley, 2000).

Published laboratory-scale studies and field trials documenting the success of phytoremediation for stabilization and removal of Cs are abundant in the literature, with the earliest studies dating back to 1941 (White and Broadley, 2000), however to date there are no actual applications which have been implemented. The majority of cesium uptake experiments have been conducted in labs, with pot sized soil samples or lysimeters, because of the obvious difficulty of working with radionuclides in an open field. This difficulty is often overcome with the use of stable cesium (^{133}Cs) in place of radiocesium (^{135}Cs, ^{137}Cs). A perfect analogy between stable and radioactive cesium has often been assume and investigated: Soudek et al. (2004) used both active and non-active cesium to contaminate poplar (*Populus simonii*), reed (*Phragmites australis*) and sunflower (*Helianthus annus*), finding that none of these plants discriminates among ^{133}Cs and ^{137}Cs. The same result was confirmed in Soudek et al. (2006), where another test on

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* Corresponding author.
E-mail address: kominkovad@fzp.czu.cz (D. Komínková).

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sunflower showed no difference in the uptake of three different cesium forms. Tsukada et al. (2002) confirmed that plants show the same behavior with the two isotopes, which differs a little from the potassium uptake mechanism, indicating that the chemical similarity between Cs and K, alone, is not enough to predict the fate of Cs based on that of Cook et al. (2009) used ^{133}Cs in the evaluation of the feasibility of phytoremediation of a field near the Idaho National Laboratory, assuming that it was a good indicator for the behavior of ^{137}Cs. Uchida and Tagami (2007) stated that ^{137}Cs from nuclear fallout is more mobile than native ^{133}Cs, even though the concentrations of the two isotopes appear to be strongly correlated: this means that, although part of ^{133}Cs is strongly bound to soil minerals and a replacement with ^{137}Cs is unlikely, 40 years after nuclear fallout the two isotopes found a balance in the bioavailable fraction of soil. Tsukada and Nakamura (1999) tried to correlate, in a potato-soil system, the transfer factor of ^{133}Cs with the same parameter for ^{137}Cs. Results showed that the transfer factor for ^{137}Cs was always bigger than for ^{133}Cs, with a positive linear correlation. This means that radiocesium is more mobile than stable cesium, thus more available to plants. Hence it can be assumed that if phytoremediation can remove ^{133}Cs, its efficiency for ^{137}Cs will be even bigger.

The uptake of radiocesium by plants is a complex process that depends on the peculiarities of the specific soil-plant system, other than the environmental conditions, and other additional factors. Due to the broad variability of boundary conditions, and the complexity of the cause-effect relationships between the system and the external conditions, virtually every research study and result – that can be traced in literature – is the expression of a different intersection of conditions. The effect of a potassium addition in the substrate (Soudek et al., 2006), the effect of amendments to desorb Cs from soil minerals (Lasat and Kochian, 1997), and the correlations among taxa and cesium uptake (Broadley and Willey, 1997) are only some of the factors that have been discussed in previous research studies. However, the main underling processes is the manner in how plant uptake of cesium have been understood and explained. This knowledge allows one to select suitable crops to avoid the entrance of Cs in the food chain, to select suitable plants for phytoremediation, and to implement effective models that can predict the exposition and the fate of the contaminant in various soil/plant systems (Zhu and Smolders, 2000).

Radionuclides transfer in plants is indeed determined not only by the element's bioavailability, but also by plant-specific factors, that intervene both on a molecular and ecological scale (Willey, 2014). Collander (cited in Broadley et al. (1999)) with his comparative ecophysiological studies on Cs uptake by plants, noted different shoot Cs concentrations among 12 taxa. The most likely explanation for these variations is the different cation selectivity between taxa. It was suggested that this is the reason why plants belonging to the *Chenopodiaceae* family can accumulate more Cs in their shoots. Broadley et al. (1999) screened 14 different studies, looking for the taxonomic variation of the variable “concentration of Cs in plant shoots”. Results showed that the higher relative shoot Cs concentration is found in the *Caryophyllidae* superorder, which also includes *Chenopodiaceae* and *Amaranthaceae*, which is a consistent result with their broad niche regarding cation requirements. Tang and Willey (2003) confirmed that radiocesium accumulators are predominantly found among *Chenopodiaceae* and *Amaranthaceae*, however studying ^{134}Cs uptake from four species from *Asteraceae* (among which includes garden lettuce and cornflower), they found out that these plants perform very well on two typical Chinese soils. Another study on a plant from *Asteraceae* was conducted by Singh et al. (2009), which showed a good efficiency in the removal of three different ^{137}Cs concentrations spiked in distilled water by Siam weed (*Chromolaena odorata*). The highest remediation efficiency was 89% after 15 days for a Cs concentration of $1{\cdot}10^3$ kBq/L. Lasat and Kochian (1997) performed a screening on hydroponically grown plants that were high biomass producers and accumulated Cs in their shoots. The aim was to study the feasibility of phytoremediation of radiocesium in a soil which was contaminated 10 cm deep with 15 Bq/g of Cs. Out of ten plants tested, the highest shoots' concentration was found in cabbage (*Brassica oleracea*) and tepary bean (*Phaseolus acutifolius*), with bioaccumulation ratios ranging from 38 to 165. Lasat et al. (1998) tested Indian mustard (*Brassica juncea*), redroot pigweed (*Amaranthus retroflexus*) and tepary bean for the uptake of ^{137}Cs from a naturally contaminated soil. *A. retroflexus* showed the highest shoot concentration of Cs and the greatest production of shoot biomass, confirming that it has a good potential for phytoremediation. In an extensive screening focused on the assessment of a feasible option to phytoremediate a soil in the vicinity of Chernobyl, Dushenkov et al. (1999) found that *Amaranthus retroflexus* had the best performance in terms of both biomass production and Cs accumulation over a sample of eleven plant species exposed on the same soil, among which a wide variety of Amaranth species. Plants that show higher concentrations in their roots than in their shoots are in fact the tolerant ones, since they restrict the upward movement of metals. It is the case of giant milky weed (*Calotropis gigantea*), that removed Cs effectively from a solution of distilled water and ^{137}Cs (Eapen et al., 2006), retaining more Cs in roots than in shoots. The cause of this behavior is probably the fact that roots are the primary uptake mechanism, and thus there might be stringent restrictions at xylem loading, transport, and sequestration of radionuclides towards the shoots. Grasses have also been widely used for phytoremediation experiments, mainly for their high and sometimes huge production of biomass. Vetiver (*Chrysopogon zizanioides*), a fast growing grass with a massive root system, and the ability to grow in adverse climatic conditions, can rapidly colonize soil, and is already used in engineering for stabilization of eroded soil and phytofiltration. It removed 38% of an initial $5{\cdot}10^3$ kBq/L concentration of Cs in 96 h, which became 61% after 169 h (Singh et al., 2008). Switchgrass has been studied by Entry and Watrud (1998) as a perennial fast growing native North American grass and has produced the highest biomass yields of any grass tested. For phytoremediation purposes it could be harvested every three weeks or less, to completely exploit the uptake ability of the plant. In the study it was calculated, using mass balance, that 97% of Cs was removed from the medium after five months with a monthly harvest. Napier grass (*Pennisetum purpureum*) produces the highest shoots mass among herbs (Kang et al., 2012). Its ability to depurate a hydroponic solution spiked with different (up to 3000 μM) concentrations of stable Cs was studied by Kang et al. (2012). A reduction in height of the plant was noted, which positively correlated with the concentration of Cs. However, treated plants also had more tillers than control plants, which could account for the reduction of height. The plant showed a tendency to accumulate more Cs in roots than in shoots for relatively low Cs concentration, and vice versa for relatively high concentrations. Growth strategies of plants can, too, influence Cs uptake. A study on six species from the family *Gramineae*, with growth strategies spanning from “competitive” to “stress tolerator” *sensu* Grime, showed that the species with strong competitive tendencies produced the greatest increase in dry weight, coherently with their strategy, that optimizes the uptake of nutrients through high growth rate; in contrast, plants that are stress tolerant showed less growth. These differences in growth directly result in a significant variation in stable Cs uptake, which is likely to be correlated to the different strategies of nutrient uptake (Willey and Martin, 1997). A number of Cs uptake studies used terrestrial plants, while small attention was given to aquatic or wetland species.

The aim of this research was to investigate whether, to which extent and in which external conditions, *Calla palustris* is capable of accumulating stable cesium and be used for phytoremediation in cases where this is pollution of aquatic and wetland ecosystems. This research aimed to give answer, through a greenhouse experiment, to the following points:

- Whether the substrate in which *Calla palustris* is exposed plays any

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