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Cationic interactions in caesium uptake by king oyster mushroom (*Pleurotus eryngii*)

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Abstract In order to explain influence of common cations (K^+, Na^+ and Ca^{2+}) on uptake and transport of caesium in macromycetes, a culture of a model mushroom species, king oyster mushroom (*Pleurotus eryngii*) was set up. Fructification in a growing chamber with stabilised temperature (18°C) and humidity (80%) was preceded by mycelial colonization of the sterilized barley seed medium packed into autoclavable plastic containers. Aliquots of test solutions, containing 0.1 mM caesium chloride carrier traced with ^{137}CsCl and the selected ions, were dosed into the interphase between the container wall and the spawn block. This allowed to study influence of the added ions on the uptake of caesium in a way unaffected by the used growing medium, e.g. soil, as it was in the previous studies. The experiments demonstrated that the major amount of radiocaesium was biologically bound and accumulated in the fruitbodies to a higher extent (56–69%) than in the mycelium. Addition of 10 mM Na^+ decreased the transfer factor for caesium (cap/soil) while addition of Ca^{2+} caused an increase of this value. The effect of potassium addition depended on its concentration in the solution. Also the Cs/K ratio in caps was significantly influenced by addition of 10 and 100 mM Na^+. However, the Cs/K ratio in stipes was affected by Ca^{2+}. Discrimination factors, calculated from specific activities (^{137}Cs/^{40}K cap d.w.)/(^{137}Cs/^{40}K stipe d.w.), were also changed after addition of the studied cations. Since the activities of caesium measured in the fruitbodies of single fungal species strongly depend on the content of co-supplied ions, further proofs should be achieved before using mushrooms as bioindicators of the soil caesium contamination.

Key words bioaccumulation • bioremediation • caesium • fungi • mushrooms • mycoextraction • *Pleurotus eryngii* • radionuclides

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Introduction

Fruiting mushrooms are known for their ability to accumulate caesium [8] that may increase the amount of its radioactive isotopes being circulated in ecosystems. However, knowledge on the ability of different species to take up caesium and other radioactive elements from the soil is mostly based on collections and analyses of environmental samples [8, 11]. Variety and instability of conditions in the natural habitats obscure the role of physiological properties of fungi in the uptake of caesium. No physiological models describing uptake and transfer of radiocaesium to the fruitbodies of mushrooms have been established hitherto. Other radionuclides have already been used as tracers in the study of accumulation of heavy metals (e.g. cadmium and mercury) in the fruitbodies of common macromycetes [3].

Several recent reports suggested the use of king oyster mushroom (*Pleurotus eryngii*) as the model species for controlled experiments [2, 10]. The desribed procedures involve spawning and induction of fructification in *ex vitro* conditions. Manjón *et al*. [10] suggested application of caesium solution to the interphase between the walls of culture containers and the spawn block. It is supposed to assure direct contact of test

solutions with the mycelium, without intermediation of the growing substrate. Therefore, this method has been peaked for the study of cationic interactions during the uptake of caesium by mycelium and its transport and distribution in fruitbodies.

Materials and methods

Barley seeds (Plant Breeding and Acclimatization Institute – IHAR, Radzików, Poland) were submerged in water for 24 h. The hydrated seeds were packed into containers (Duchefa, The Netherlands), then covered and sterilized in autoclave at 120°C during 2 h. Each container was inoculated with *ca*. 16 g of the inoculum. The used strain of king oyster mushroom *Pleurotus eryngii* was isolated from a fruitbody found growing on the remains of roots of an *Eryngium campestre* plant found in the locality of Camarma de Esteruelas (3° 22' 45" W, 40° 33' 15" N, 30T 4684489 UTM; Madrid Province, Spain).

The culture protocol described by Manjón *et al*. [10] was followed with some modifications. Mycelium was grown *ca*. 30 days in darkness at 25°C until covering the whole medium and forming a compact spawn block. In order to induce fruiting, the containers were transferred to the growing chamber with temperature 18°C and 80% relative humidity.

All the salts (KCl, NaCl, $CaCl_2$ and CsCl) were analytical grade chemicals (POCh, Gliwice, Poland). The radioactive tracer, *ca*. 100 kBq/ml ^{137}CsCl, was purchased in Polatom (Otwock-Świerk, Poland). The solutions containing stable caesium (0.1 mM), ^{137}Cs tracer, and appropriate salts (10 and 100 mM), as well as controls with caesium only, were pipetted to the containers. 20 ml out of 50 ml was used to moisten the block. Then, it was covered with 15 g sterile peat soil. The remaining 30 ml was split into 3 aliquots (10 ml each) added to the spawn/container interphase every 24 h. The fruitbodies were collected in two series every 7 days. The photoperiod was set to 16 h (8 h night).

The samples of fruitbodies were dried at 105°C for 24 h, homogenized and analyzed using a gamma spectrometer with HPGe detector (Canberra Packard). Activity of the culture medium before and after fructification is denominated as A and B, respectively. Data treatment was carried out with MS Excel 2000 software.

Transfer factor (TF) for ^{137}Cs and ^{137}Cs/^{40}K discrimination factor (DF) are defined as:

$$\text{TF} = \frac{A\left(^{137}\text{Cs}\right)_{up}}{A\left(^{137}\text{Cs}\right)_{low}} \quad (1)$$

$$\text{DF} = \frac{\dfrac{A\left(^{137}\text{Cs}\right)_{up}}{A\left(^{40}\text{K}\right)_{up}}}{\dfrac{A\left(^{137}\text{Cs}\right)_{low}}{A\left(^{40}\text{K}\right)_{low}}} \quad (2)$$

where A are specific activities of ^{137}Cs or ^{40}K measured in the upper and the lower parts of the system (see descriptions).

Results and discussion

Previous studies on uptake and transport of caesium-137 and other radionuclides in fruitbodies of *Pleurotus eryngii* were carried out using a soil-rich system [2], which did not allow to exclude complexing properties of the soil making the physiological interpretation difficult. Herein, application of a method based on supply of ^{137}Cs-traced caesium and a supplementary cation to the interphase at the myceliar block, based on the protocol described by Manjón *et al*. [10], is described for the first time.

The obtained results showed that caesium was accumulated mainly in the caps, and to a lesser extent in the stipes (Figs. 1 and 2), which is in agreement with the radiocaesium distribution described by Heinrich [6] who compared relative caesium fates as follows:

sporophores > fruitbodies > stipes.

The experiments proved that in the culture of *Pleurotus eryngii*, grown under controlled conditions, the major part of radiocaesium is accumulated more in the fruitbodies than in the mycelium. The decrease of ^{137}Cs activity in the culture medium was a result of production of fruitbodies (Table 1). Only 31–44% of primary activity of radiocaesium was found in the medium after

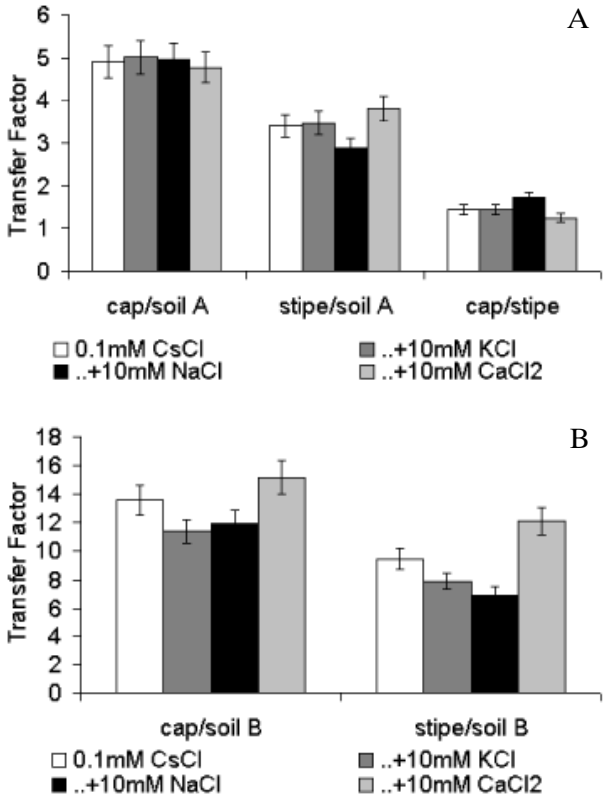

Fig. 1. Transfer factor (TF) for caesium at 10 mM K^+, Na^+ and Ca^{2+} for (A) medium A and (B) medium B.

Table 1. Activities of ^{137}Cs in the growing medium in the 1st and 2nd stage of the experiment and percent share of medium and crop

	Supplement concentration [mM]	Activity [Bq·g^{-1} d.w.]	Medium [%]	Crop [%]
Medium A	none	19.81	100	–
Medium B	none	7.16	36	64
	KCl, 10	8.71	44	56
	KCl, 100	8.79	44	56
	NaCl, 10	8.21	41	59
	NaCl, 100	6.70	34	66
	$CaCl_2$, 10	6.23	31	69
	$CaCl_2$, 100	8.20	41	59

the collection of fruitbodies. It shows that *ca*. 56–69% of radiocaesium was accumulated in the biomass of the fruitbodies. Caesium remaining in the spawn block is supposed to be bound by mycelium. It is expected that this part of fungus has a great ability to bind caesium, especially because of massive share of mycelium in the total weight of the mushroom [12].

Table 1 shows ^{137}Cs activities in the mycelium for different ionic compositions of the interphase solution. Compared to the activity at 0.1 mM Cs^+ (control set), addition of 10 and 100 mM K^+ decreased accumulation of radiocaesium in fruitbodies, which is represented by increase of radiocaesium activity in the medium B, after collection of the fruitbodies (Table 1).

Addition of 10 and 100 mM Na^+ did not cause any remarkable alterations in the content of caesium in the medium (Table 1), however it significantly decreased potassium content in the medium B (Table 2). This led to an increase of $^{137}Cs/^{40}K$ ratio in the medium B. Analysis of the medium before and after mushroom collection (medium A and B) showed that addition of 10 and 100 mM Ca^{2+} did not influence the content of ^{137}Cs (Table 1), and the content of potassium in the medium B (Table 2). Therefore, the $^{137}Cs/^{40}K$ concentration ratio did not change.

Increase of $^{137}Cs/^{40}K$ ratio in the medium due to addition of Na^+ points to the increased uptake of potassium by the fruitbodies in the presence of Na^+, which may lead to an increased discrimination of caesium transport by potassium. Unchanged, after addition of Ca^{2+}, activity of ^{137}Cs and ^{40}K in the medium shows that

Table 2. Activities of ^{40}K in the growing medium in the 1st and 2nd stage of the experiment and percent share of medium and crop

	Supplement concentration [mM]	Activity [Bq·g^{-1} d.w.]	Medium [%]	Crop [%]
Medium A	none	0.17	100	–
Medium B	none	0.10	59	41
	NaCl, 10	0.10	35	65
	NaCl, 100	0.06	24	76
	$CaCl_2$, 10	0.04	53	47
	$CaCl_2$, 100	0.09	59	41

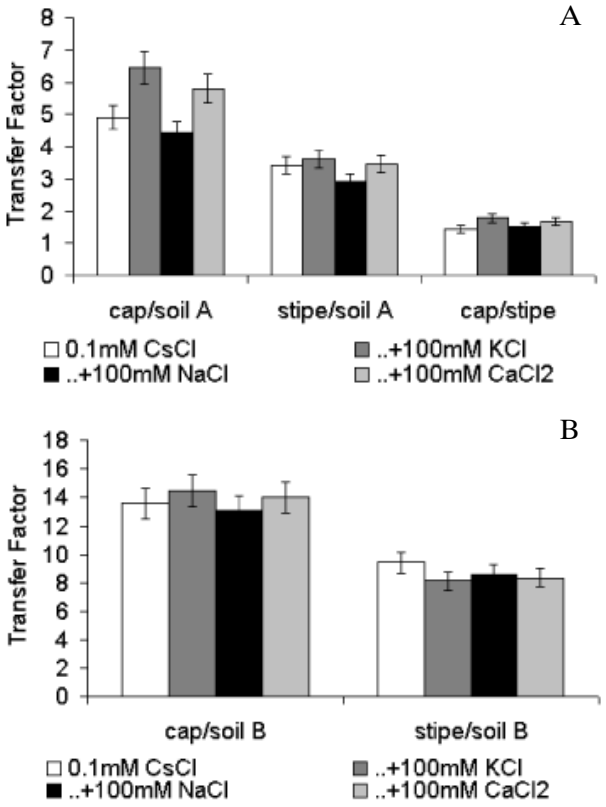

Fig. 2. Transfer factor (TF) for caesium at 100 mM K^+, Na^+ and Ca^{2+} for (A) medium A and (B) medium B.

Ca^{2+} ions do not affect caesium and potassium uptake and transfer to fruitbodies. Analysis of the medium provided relevant information on the influence of K^+ and Na^+ ions on the uptake of caesium and potassium by the whole population of fruitbodies grown in the investigated medium.

Transfer factors for ^{137}Cs, calculated for the medium A and the first crop, showed a decrease in ^{137}Cs transport from the mycelium-coated medium to the stipe at 10 mM NaCl and to the stipe (Fig. 1A,B) and the cap at 100 mM NaCl (Fig. 2A,B). It is also supported by the results on TF from the medium B to the 2nd series of fruitbodies (Fig. 2B). The TFs decreased under influence of Na^+. However, under influence of a bivalent cation Ca^{2+}, TF for ^{137}Cs was increased (Fig. 1A,B). A decrease of TF for ^{137}Cs, as influenced by addition of KCl, was observed only in the 2nd crop (Fig. 2B).

Although, a possibility of Cs/K replacement has been assumed until now, apparently, caesium and potassium may occupy different transport pathways in fungi. It is also expressed in the higher efficiency of ^{137}Cs transfer from stipe to cap than in the case of potassium [4]. However, Terada *et al*. [14] observed a decrease in the ratio of ^{137}Cs content mycelium/fruitbody with an increase of K^+ concentration in the medium. There are also figures showing possible replacement of K^+ by Na^+ [13] which may take place in the case of potassium deficit. Our results confirmed earlier data on the prevalence of ^{137}Cs accumulation in caps of mature fruitbodies over accumulation in stipes [4, 15].

Analysis of $^{137}Cs/^{40}K$ concentration ratios in the second crop shows that this ratio, calculated for caps,

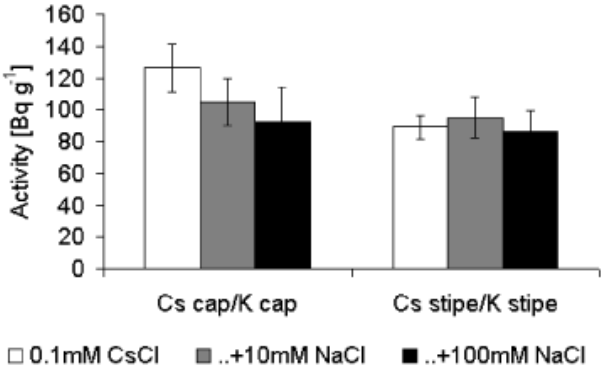

Fig. 3. Ratio of caesium and potassium activity in cap and stipe at 10 mM and 100 mM Na^+ (2nd crop).

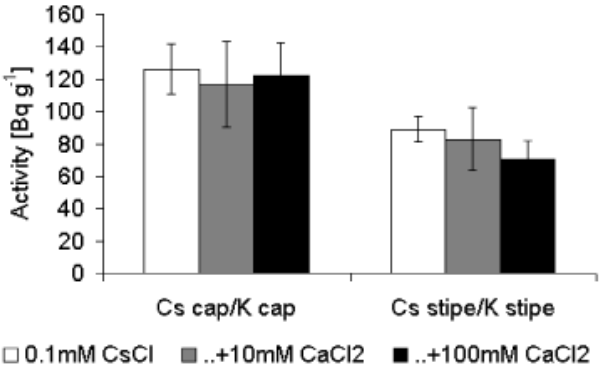

Fig. 5. Ratio of caesium and potassium activity in cap and stipe at 10 mM and 100 mM Ca^{2+} (2nd crop).

decreases as Na^+ concentration increases (Fig. 3), and does not change in stipes. This points to intensification of discrimination of caesium by potassium during the transport from stipe to cap (Fig. 4). Under influence of Na^+, the DFs cap/medium A and stipe/medium B decreased (Fig. 4).

The DFs cap/medium and stipe/medium were decreasing after application of Ca^{2+} what confirms that Ca^{2+} ions increased discrimination of caesium by potassium in the uptake and transport of caesium from the myceliar spawn to the fruitbodies. However, 10 mM Ca^{2+} did not affect discrimination of caesium by potassium in the transport of caesium from the stipe to the cap, and 100 mM Ca^{2+} even decreased that discrimination (Fig. 5). As already mentioned, content of calcium in the fruitbodies of *Pleurotus ostreatus* from a laboratory culture is remarkably low [7], while content of this element in the wild mushrooms is usually high [1].

So far, there has been no data on characteristics of artificial media and changes in their composition due to the growth of mushrooms. The results of this study show a need for analysis of the whole crop and not only for sampling fruitbodies obtained during successive series of fructification.

The unquestioned advantage of the applied method is possibility of supplementing *ex vitro* cultures with test solutions, in such a way, that the liquid has a direct contact with the mycelium. Therefore, uptake of caesium and possible regulation of ionic transport by added substances take place directly in the interphase solution/mycelium, without intermediation of the growing medium. To our best knowledge, such an experimental design has never yet been used within *Pleurotus eryngii* culture, however accumulation of caesium isotopes (^{133}Cs and ^{137}Cs) in the oyster mushroom (*Pleurotus ostreatus*) has been studied previously [9, 14].

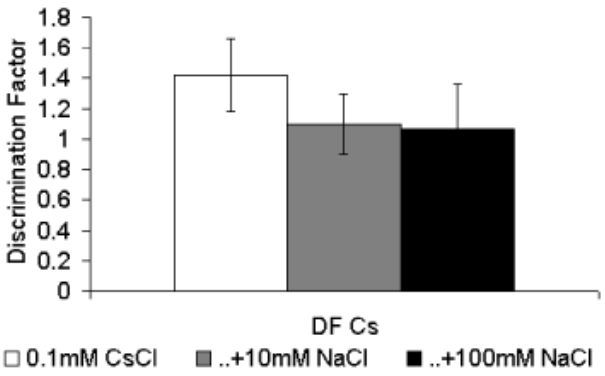

Fig. 4. Discrimination factor (DF), caesium to potassium, in respect to caps/stipes at 10 mM and 100 mM Na^+ (2nd crop).

The obtained results confirm the possibility of using mushrooms for soil bioremediation [5]. The proven high ability of *Pleurotus eryngii* for caesium hyperaccumulation during its growth on artificial medium proves importance of using this macromycete in bioremediation of caesium-contaminated organic waste. The results will serve to create the first model of transfer of caesium from the growing medium to mushrooms with exclusion of a variable characteristics of medium, which will focus interpretation of the physiological properties of the investigated species.

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