Article

Caesium Uptake in Mushroom — Comparison with Coexisting Elements and Effect of Ammonium Ion as a Competitor, by Laboratory Experiments Using *Hebeloma vinosophyllum* —

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An ectomycorrhizal ammonia fungus Hebeloma vinosophyllum was cultivated in the Ohta's liquid medium with Cs and different concentrations of NH_4^+ . This fungus absorbed caesium and coexisting elements (K, Ca, Mg, Zn, Cu, Fe and P) with the highly transfer factors. The highest translocation from mycelium to fruit body was observed in Cs among 8 analysed elements. The uptake of Cs might have a similar pattern to those of K and P. However, the high concentration of NH_4^+ might affect as the competitor to the uptake of both Cs and K, but not to the uptake of P. The addition of NH_4^+ affected more the uptake of Cs than that of K.

Key Words : mushroom, caesium, stable elements, potassium, ammonium ion, uptake, translocation, fruit body, mycelium, in vitro experiment

1. Introduction

After the accident of Fukushima Daiichi Nuclear Power Plant in 2011 in Japan, relatively high concentrations of radiocaesium were reported in wild mushrooms from environmental monitoring and food monitoring programs. The concentrations exceeded the food regulation (100 Bq/kg as total radiocaesium) were sometimes observed even in wood log cultivated mushroom (*Lentinula edodes*). It is quite important to understand the mechanism of radiocaesium uptake by mushrooms, in order to control and to regulate the radiocaesium concentra-

tions in mushrooms.

It is known that, mushrooms tend to accumulate caesium, including radiocaesium in nature.^{1)–3)} The concentrations of caesium and radiocaesium in mushrooms are higher than those in plants.^{2)–6)}

The Chernobyl accident resulted in the high concentration of radiocaesium in mushrooms collected in European forests.^{7)–11)} Accumulation of radiocaesium derived from atmospheric nuclear weapon testing in mushrooms was also reported in Japanese forests before the accident of Fukushima Daiichi Nuclear Power Plant.^{2)–6), 12)}

Many factors controlling the radiocaesium concentration in mushrooms such as type of forests¹³⁾, soil pH¹⁴⁾ and species of mushrooms¹⁵⁾, have been discussed. The relationship between the habitat of the mycelium and the radiocaesium concentration in the fruit body has also been studied.^{6), 16)} Some researches indicated the role of mycelium on the accumulation of radiocaesium in surface soils.^{17), 18)} Nevertheless, the detailed mechanism of radiocaesium uptake in mushrooms has not yet thoroughly known.

On the other hand, chemical behaviour of radiocaesium is expected to be similar to that of stable caesium. Rühm et al.¹⁹⁾ and Yoshida et al.^{20), 21)} summarized the relationship between radiocaesium and stable caesium in mushrooms collected from forest ecosystems. The ¹³⁷Cs/Cs ratios were fairly constant for samples collected at the same site. From these findings, they suggested the possible use of stable elements for predicting radionuclide transport.

During survey of the radiocaesium accumulation in mushrooms collected from Japanese forests, the high concentration of ¹³⁷Cs was observed in several *Hebeloma* species.⁶⁾ In laboratory experiment (in vitro), the ¹³⁷Cs accumulation by mycelium of an ectomycorrhizal ammonia fungus *Hebeloma vinosophyllum* was affected by monovalent cations, such as K⁺ and NH₄⁺, in the media.²²⁾ The uptake of ¹³⁷Cs by the mycelium of the mushroom *Pleurotus ostreatus* decreased with increasing of Cs, K or Rb concentration in the YMG media.²³⁾ Effects of coexisting elements as well as ammonium ion, however, on the radiocaesium accumulation in mushrooms, especially quantity in both mycelium and fruit bodies, have not been well examined.

Therefore, the Cs uptake from medium and

accumulation in mushroom (mycelium and fruiting bodies) were investigated by the model of fruiting on the liquid medium under the competition of NH₄⁺ in this study. The absorbing abilities of coexisting elements (K, Ca, Mg, Zn, Cu, Fe and P) by the mushroom were also investigated. Considering easily fruiting ability of *H. vinosophyllum*, it was selected as a model organism for our investigation of the Cs absorption of ectomycorrhizal fungi.

2. Experimental

H. vinosophyllum HCMUS-C2²⁴⁾ was pre-cultivated on MY agar medium [malt extract 10 g/L (Difco, Detroit, USA), yeast extract 2 g/L (Difco, Detroit, USA) and agar 15 g/L (Nacalai Tesque, Kyoto, Japan)] in a petri dish.

H. vinosophyllum inoculum disks were cut with a 4 mm-diameter cork border from the sub-peripheral regions of actively growing colonies and placed into 100 mL of the Ohta's liquid medium²⁵⁾ in a 200 mL flask, incubated at 25.0°C ± 0.5°C, in 12 hr/12 hr of the dark/light regime with a white fluorescent lamp (Hitachi, Tokyo, Japan), providing a light intensity at 4.56 μmol m⁻²s⁻¹. The pH of the medium was adjusted to 7.0 with 1 M KOH. Five flasks for each treatment were prepared.

In comparison between the Cs uptake and coexisting elements uptake, *H. vinosophyllum* was inoculated in the Ohta's liquid medium added (NH₄)₂SO₄ as a nitrogen source with the concentration 177 mg NH₄⁺/L. Concentrations and chemical forms of Cs, K, Ca, Mg, Zn, Cu, Fe and P in the medium were shown in Table 1.

In order to investigate the effect of coexisting NH₄⁺ on the uptake of Cs, K and P by *H. vinosophyllum*, (NH₄)₂SO₄ was added to the Ohta's liquid media with different concentrations of NH₄⁺ (177 mg/L, 590 mg/L and 1 770

Table 1 Concentrations and chemical forms of Cs and co-existing elements (K, Ca, Mg, Zn, Cu, Fe and P) in the medium

Element	Concentration (mg/L)	Chemical form
Cs	1	CsCl
K	1106	KH ₂ PO ₄ and KOH
Ca	13.6	CaCl ₂
Mg	97.4	MgSO ₄
Zn	0.68	ZnSO ₄
Cu	0.25	CuSO ₄
Fe	17.3	FeCl ₃
P	227.8	KH ₂ PO ₄

Table 2 Concentrations of K in media added different concentrations of NH₄⁺

Concentration of NH ₄ ⁺ in medium (mg/L)	Concentration of K in medium (mg/L)
177	1106
590	1129
1770	1145

mg/L). The concentration of K was changed in different media (Table 2) due to the pH adjustment of medium to 7 by 1 M KOH.

H. vinosophyllum formed fruit bodies after about one month of inoculation. The mycelium and the fruit bodies were collected and dried at 60°C. The dry samples were digested in Teflon™ PFA pressure decomposition vessels with acids (HNO₃, HF and HClO₄) at about 120°C for one day. Then, the samples were evaporated to dry and the residues were dissolved in 2% HNO₃.

The trace elements (Cs, Zn and Cu) were measured by ICP-MS (Agilent 7500a, Agilent Technologies Inc., Tokyo, Japan). The major elements (K, P, Ca, Mg and Fe) were determined by ICP-AES (VISTA-PRO, Seiko Instruments Inc., Chiba, Japan). Internal standards Rh, In and Bi were used to compensate for changes in analytical signals during the operation. Details of the analyses were described in Yoshida and Muramatsu.²⁶⁾

The mean values of the five flasks were calculated. The transfer factors (L/kg dry) of Cs and coexisting elements were defined as their concentrations (mg/kg dry) in dry samples (mycelium or fruit bodies) divided by their concentrations (mg/L) in the medium at the beginning of the experiment. The translocations of Cs and coexisting elements from the mycelium to the fruit bodies were determined as their concentrations in the fruit bodies divided by those in the mycelium.

3. Results and Discussion

3.1 The uptake of caesium and coexisting elements by *Hebeloma vinosophyllum*

The Cs and coexisting elements (K, Ca, Mg, Zn, Cu, Fe and P) uptakes of the mycelium and the fruit bodies were shown as concentration (mg/kg dry sample) in Table 3 and transfer factors in Fig. 1. All samples had mean transfer factors higher than 20 L/kg dry.

In the mycelium, the transfer factors were in

Table 3 Concentration (mg/kg dry sample) of Cs, K, Ca, Mg, Zn, Cu, Fe and P in mycelium and fruit bodies of *Hebeloma vinosophyllum*

Element	Mycelium	Fruit bodies
Cs	$2.33 \times 10^1 \pm 1.10 \times 10^0$	$5.02 \times 10^1 \pm 1.10 \times 10^0$
K	$2.90 \times 10^4 \pm 2.48 \times 10^3$	$4.95 \times 10^4 \pm 5.18 \times 10^3$
Ca	$6.68 \times 10^2 \pm 1.38 \times 10^2$	$6.00 \times 10^2 \pm 1.25 \times 10^2$
Mg	$2.13 \times 10^3 \pm 3.09 \times 10^2$	$2.25 \times 10^3 \pm 2.77 \times 10^2$
Zn	$7.04 \times 10^1 \pm 2.32 \times 10^1$	$5.96 \times 10^2 \pm 1.24 \times 10^1$
Cu	$4.63 \times 10^1 \pm 6.80 \times 10^0$	$1.29 \times 10^1 \pm 2.70 \times 10^0$
Fe	$1.46 \times 10^3 \pm 3.07 \times 10^2$	$5.88 \times 10^2 \pm 1.38 \times 10^2$
P	$8.34 \times 10^3 \pm 3.65 \times 10^2$	$1.31 \times 10^4 \pm 2.23 \times 10^3$

Mean $\pm$ standard deviation

Fig. 1 The transfer factors of Cs, K, P, Ca, Mg, Fe, Zn and Cu in *Hebeloma vinosophyllum* biomass (mycelium and fruit bodies). Mean $\pm$ standard deviation (vertical bar).

order of $\text{Cu} > \text{Zn} > \text{Fe} > \text{Ca} > \text{P} > \text{K} > \text{Cs} > \text{Mg}$. The transfer factors of Cu and Zn were markedly higher than 100 L/kg dry.

In the fruit bodies, the different order of transfer factors were observed as $\text{Zn} > \text{P} > \text{Cu} > \text{Cs} > \text{K} > \text{Ca} > \text{Fe} > \text{Mg}$. The transfer factor of Zn was the highest (87.6 L/kg dry). These results showed that *H. vinosophyllum* tended to highly accumulate Zn, P, Cu and Cs in fruit bodies with the transfer factors higher than 50 L/kg dry.

The highest translocation from the mycelium to the fruit bodies (2.2) was observed in Cs. The translocations of Cs, K and P were much higher than 1, while those of Ca, Mg and Zn were approximately equal to 1. The translocations of Cu and Fe from mycelium to fruit bodies were lower than 1 (Table 4).

Cs, K and P concentrations in the fruit bodies were higher than those in the mycelium, while Ca and Mg concentrations in the fruit bodies approximated to those in the mycelium. Con-

Table 4 Translocation of Cs, K, P, Ca, Mg, Fe, Zn and Cu in *Hebeloma vinosophyllum* from mycelium to fruit bodies

Element	Translocation from mycelium to fruit bodies
Cs	2.2
K	1.7
Ca	0.9
Mg	1.1
Zn	0.8
Cu	0.3
Fe	0.4
P	1.6

versely, Fe, Zn and Cu concentrations in fruit bodies were lower than those in the mycelium.

These results indicated that the translocations of Cs and coexisting elements from the mycelium to the fruit bodies in *H. vinosophyllum* had three different patterns. Cs, K and P were mostly translocated to fruit bodies while most of Fe, Zn and Cu were kept in the mycelium. Ca and Mg were not translocated from the mycelium to the fruit bodies. The results also showed that in *H. vinosophyllum*, the uptake of Cs might have a similar pattern to that of K and P.

The accumulation of Fe, Zn and Cu in the fruit bodies with the high transfer factors (34.3, 87.6 and 51.7 L/kg dry, respectively) in *H. vinosophyllum* also implies that this fungus would be a suitable bioindicator of the environmental heavy metal pollution. Besides, in the control of heavy metal in ecosystems, the keeping of Fe, Zn and Cu in the mycelium would be attended.

Ban-nai et al.^{4), 22)} reported that in *H. vinosophyllum* fruit bodies, the transfer factors of ¹³⁷Cs and ⁸⁵Zn were 21 and 15 – 30, respectively. Whereas, those of Cs and Zn in the fruit bodies in this study are 50.2 and 87.6, respectively. According to Duff and Ramsey²⁷⁾, some conditions such as pH, organic matter content and pre-

ferred growth medium probably have the effects on the accumulation of Cs as well as other radionuclides by mushroom. Therefore, our results might be caused by the higher fruiting ability which derived from the suitable concentration of NH₄⁺ for this ammonia fungus (unpublished data).

3.2 The effect of NH₄⁺ concentrations on the caesium, potassium and phosphorus uptake by *Hebeloma vinosophyllum*

The results of the above experiment showed that in *H. vinosophyllum*, the uptake of Cs might have a similar pattern to that of K and P. Therefore, the effect of NH₄⁺ concentrations on the uptake of Cs, K and P were investigated.

The transfer factors of Cs and K in the mycelium and the fruit bodies decreased according to the increment of NH₄⁺ concentration while that of P varied with different NH₄⁺ concentrations (Fig. 2A – C). It means that the high concentration of NH₄⁺ might affect to the uptake of both Cs and K as the competitor but not to that of P in *H. vinosophyllum*.

Besides, *H. vinosophyllum* formed fruit bodies in NH₄⁺ 177 mg/L, 590 mg/L and 1 770 mg/L (Figs. 3, 4). The highest dry weight of the fruit bodies was obtained at 177 mg NH₄⁺/L (Fig. 3 A and Fig. 4). The immature fruit bodies which

Fig. 2 The transfer factors of Cs(A), K(B) and P(C) in *Hebeloma vinosophyllum* biomass (mycelium and fruit bodies) under different NH_4^+ concentrations. Mean $\pm$ standard deviation (vertical bar).

had unexpanded cap without gills formed at 1770 mg/L NH_4^+ (Fig. 3 C).

Muramatsu et al.²⁸⁾ and Baeza et al.²⁹⁾ reported the influence of the maturity of fruit bodies on radionuclide uptake. Our results showed that the concentration of NH_4^+ might affect to the fruit body formation of *H. vinosophyllum*. Hence, the uptake of Cs and K in *H. vinosophyllum* could depend on the condition of the fruit body formation.

According to Yoshida and Muramatsu²⁾, Cs

was not correlated with K in mushrooms collected from a pine forest in Tokai-mura, Ibaraki, Japan ($r=0.37$). They suggested that the mechanism of Cs uptake was different from that of K uptake. In this study, the ratios of Cs and K concentrations in both the mycelium and the fruit bodies decreased when the concentration of NH_4^+ increased (Fig. 5). This result indicates that the addition of NH_4^+ affected more the uptake of Cs than that of K, suggesting that a part of Cs might be taken up by similar

Fig. 3 Fruit body formation of *Hebeloma vinosophyllum* in different NH_4^+ concentrations. A : 177 mg/L, B : 590 mg/L and C : 1 770 mg/L. Bar = 10 mm.

Fig. 4 Dry biomass of *Hebeloma vinosophyllum* (mycelium and fruit bodies) in different NH_4^+ concentrations. Mean $\pm$ standard deviation (vertical bar).

Fig. 5 The ratios of Cs and K concentrations in mycelium and fruit bodies of *Hebeloma vinosophyllum* in different NH_4^+ concentrations. Mean $\pm$ standard deviation (vertical bar).

mechanism as NH_4^+ rather than K. The result provides one of the possible explanations why Cs was not correlated with K in the field observation by Yoshida and Muramatsu²⁾.

Steiner et al.³⁰⁾ and Harbhajan³¹⁾ discussed mycorrhizal fungi using as bioremediation in the control of radionuclides in forest ecosystems. Vinichuk et al.³²⁾ investigated the effect of arbuscular mycorrhizal fungi in radiocaesium uptake by plants. As a putative ectomycorrhizal fungus³³⁾, *Hebeloma vinosophyllum* would be an excellent model fungus for the investigation of caesium accumulation in ectomycorrhizal fungi. The further research is required to understand the uptake of caesium among *H. vinosophyllum* mycelium, fruit bodies and its ectomycorrhizal host plant *Pinus* spp. and *Quercus* spp. Belonging to ammonia fungi³⁴⁾, this fungus occurs at high frequency after urea treatment to the forest floor. Therefore by urea application to the forest floor and using ectomycorrhizal symbiosis of *H. vinosophyllum* – *Pinus* spp. and/or *Quercus* spp., the efficient control of radiocaesium in the field could be expected.

4. Conclusion

The ectomycorrhizal ammonia fungus *Hebeloma vinosophyllum* absorbed caesium and co-existing elements (K, Ca, Mg, Zn, Cu, Fe and P) with the high transfer factors. These eight analyzed elements were translocated from mycelium to fruit bodies in three different patterns. Cs, K and P were mostly translocated to the fruit bodies while most of Fe, Zn and Cu were kept in the mycelium. Ca and Mg were not translocated between the mycelium and the fruit bodies. The highest translocation from the mycelium to the fruit bodies was observed in Cs among eight analysed elements.

The uptake of Cs might have a similar pattern to those of K and P. However, the high concentration of NH_4^+ might also affect as the competitor to the uptake of both Cs and K, but not to that of P. Besides, the addition of NH_4^+ affected more the uptake of Cs than that of K. All these results contribute to clearer understanding the caesium uptake by ectomycorrhizal fungi under competition of coexisting elements in the environment. *H. vinosophyllum*

would be an excellent model fungus for the investigation of caesium accumulation in ectomycorrhizal fungi in laboratory condition (in vitro).

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