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Factors affecting the cesium transfer factor to shiitake (*Lentinula edodes*) cultivated in sawdust medium

Masakazu Hiraide*

Abstract

The transfer factor (TF) of radioactive cesium-137 (^{137}Cs) to shiitake (*Lentinula edodes*) cultivated on bed logs varies greatly. Therefore, the present study investigated which factors affect the TF using stable cesium-133 (^{133}Cs) and sawdust medium with 5% rice bran as a model, which had similar ^{133}Cs TFs to bed-log cultivation. It was found that the Cs concentration and nutrient concentration (represented by the nitrogen concentration) concerned with the TF in the model sawdust medium. In addition, the TFs calculated using total ^{137}Cs and ^{133}Cs concentrations differed in both bed-log cultivation and the model sawdust medium cultivation, while the TFs calculated using exchangeable ^{137}Cs and ^{133}Cs concentrations were the same in sawdust medium cultivation, indicating that exchangeable Cs in the medium is the source of Cs for the fruiting body and the former difference was due to the presence of other chemical speciation of Cs that could not be absorbed. One purpose of the TF on the mushroom farm is to determine the fruiting body ^{137}Cs concentration at the start of bed-log cultivation, therefore the prediction method of TF are discussed considering the future changes of ^{137}Cs concentrations in trees.

Keywords: *Lentinula edodes*, Model sawdust medium, Radioactive fallout, Radioactive cesium-137, Stable cesium-133, Transfer factor

Introduction

The Fukushima Daiichi Nuclear Power Plant was damaged by the Great East Japan Earthquake in March 2011, resulting in radioactive materials being released into the environment and contaminating a large area of eastern Japan. These radioactive materials included an estimated 1.5×10^{16} Bq of radioactive cesium-137 (^{137}Cs), which has a half-life of approximately 30.1 years [1].

Radioactive materials are absorbed by agricultural products, so an upper limit of radioactivity in general foods has been set at 100 Bq/kg to prevent health damage in humans [2]. Fortunately, the transfer factor (TF) of ^{137}Cs to plants is generally low [3], so the effect of ^{137}Cs contamination on plants is not currently a serious issue. However, mushrooms are reported to accumulate high concentrations of ^{137}Cs [4], so the radioactive contamination of mushrooms has been a problem since the nuclear power plant accident occurred due to the long half-life of this material. To address this, provisional reference indices of 50 and 200 Bq/kg were set for mushroom cultivation in bed log and sawdust medium, respectively [5], based on ^{137}Cs TFs to the fruiting body of 2.0 and 0.5, respectively [6].

Most of the mushrooms on the market are cultivated in sawdust medium. The limit in sawdust medium is higher than that in bed log and the radioactive concentration in sawdust medium can be easily controlled within this limit, so the effect of ^{137}Cs contamination on mushrooms cultivated in sawdust medium are not currently a serious issue. Most shiitake (*Lentinula edodes*) are also cultivated in sawdust media and the

*Correspondence: hiraide@ffpri.affrc.go.jp
Department of Mushroom Science and Forest Microbiology, Forestry and Forest Products Research Institute, 1 Matsunosato, Tsukuba, Ibaraki 305-8687, Japan

remainder in bed logs. The bed-log cultivation does not require special facilities, so they are a valuable source of income in hilly and mountainous areas [7]. However, the radioactive concentration in bed log cannot be easily controlled, which needs to be addressed.

The provisional reference index for bed-log cultivation was established based on the frequency distribution of the bed log ^{137}Cs TF immediately after the nuclear power plant accident [6]. The bark of bed logs was mainly contaminated by the radioactive fallout immediately after the nuclear power plant accident, but the numbers of bed logs and trees that are directly contaminated has decreased over time. On the other hand, ^{137}Cs in the soil is being accumulating in trees via root absorption [8], resulting in the location of ^{137}Cs contamination in bed logs shifting from the bark to the wood. Consequently, the ^{137}Cs TF at the time of the accident is likely to be different from the current ^{137}Cs TF since nutrients are mainly supplied to the developing fruiting body of shiitake from the wood, including the inner bark, during bed-log cultivation [9].

Logs have an inhomogeneous physical structure and chemical composition, so the TF can vary widely in a single bed log. By contrast, sawdust medium is a homogeneous culture medium, allowing the effects of different factors on the TF to be easily assessed. However, ^{137}Cs TFs greatly differ between bed-log cultivation and sawdust medium cultivation. Therefore, to use sawdust medium as a model for bed logs, it is necessary to first establish a sawdust medium cultivation condition that can obtain a similar ^{137}Cs TF to bed-log cultivation.

TFs have been evaluated by measuring ^{137}Cs concentrations. However, the TF can vary greatly even among bed logs obtained from the same forest [10]. Moreover, a concentration of 50 Bq/kg of ^{137}Cs is equivalent to 1.56×10^{-11} g/kg based on the half-life, and the ^{137}Cs TF will vary with only a small difference in ^{137}Cs concentration. By contrast, ^{133}Cs has almost the same chemical properties as ^{137}Cs, but has already reached a steady state in nature and is present at a concentration of approximately 30 μg/kg in bed logs [11]. Consequently, the ^{133}Cs TF could be used to accurately estimate the ^{137}Cs TF. However, while ^{137}Cs adheres to the bark due to fallout and is also absorbed inside the tree through root absorption, ^{133}Cs is mainly absorbed through the roots. Cs is fixed to organic matter [12], so the available concentrations of these nuclides will differ due to their different chemical speciation, which may result in different TFs. There was a significant positive correlation between the ^{137}Cs concentration in konara oak (*Quercus serrata*) shoot and the exchangeable ^{137}Cs (^{137}Cs) concentration in the soil, which is extracted with a neutral 1 mol/L ammonium acetate solution [10]. Therefore, it is considered necessary to study the difference in behavior of exCs for both nuclides.

The ultimate goal of this research was to re-evaluate the ^{137}Cs TF to shiitake cultivated in bed logs. To achieve this, a sawdust medium cultivation condition that gave a similar TF to that of bed-log cultivation was first established using ^{133}Cs. The factors that affect the TF were then clarified by cultivating shiitake in the model sawdust medium. Finally, a method for predicting the fruiting body ^{137}Cs concentration in bed-log cultivation was discussed.

Materials and methods

Strains

Two commercial shiitake strains were purchased, which are referred to as Strain 1 and Strain 2, respectively, hereafter in consideration of reputational damage. Strain 1 and Strain 2 are mainly used for sawdust medium cultivation and bed-log cultivation, respectively.

Bed-log cultivation for estimating the sampling position of bed log for accurate TF measurement

Konara oak bed logs that were approximately 11 cm in diameter and 90 cm long and had been inoculated with Strain 2 approximately 2 years previously were purchased from a grower. A hole with a diameter of 1 cm was made in the wood (depth = 3.2 cm) in the longitudinal center of each bed log, and antibiotic assay filter paper (49005010; ADVANTEC Co., Ltd., Tokyo, Japan) impregnated with a CsCl solution containing 1.0 mg ^{133}Cs was placed in the bottom of the hole. The hole was then sealed with Japanese beech (*Fagus crenata*) sawdust. Three bed logs were prepared for the experiment. The bed logs were soaked in tap water for approximately 16 h, and maintained at 15 °C and a relative humidity of ≥ 90% under fluorescent lamp irradiation for fruiting body development. Each fruiting body was harvested when the cap was approximately 80% open, and the point on the bed log where the fruiting body developed was recorded. Because the stipes of shiitake cultivated in bed-log cultivation are cut off before the ingredients are analyzed [13], the stipe was discarded and only the cap was sliced, dried at 105 °C, and milled with a Millser (IFM-620DG; Iwatani Co., Tokyo, Japan). The concentration of ^{133}Cs in each cap was determined by subtracting 0.59 mg/kg from the measured value, which equated to the cap ^{133}Cs concentration in an untreated bed log. The cap ^{133}Cs concentrations at the sampling locations are shown as a contour graph using SigmaPlot version 14.0 (Systat Software, Inc., California, USA), with the circumferential direction of the bed log aligned along the *x*-axis, the axial direction of the bed log aligned along the *y*-axis, and the perforated portion is represented by (0, 0).

Bed-log cultivation for estimating the ^{137}Cs TF and the ^{133}Cs TF

The same kind of konara oak bed logs were purchased from the same grower as described above, and the fruiting bodies were developed and harvested, the point on the bed log where the fruiting body developed was recorded, and the caps were treated in the same manner as described above. Once fruiting body development was completed, each bed log was cut radially 3 cm axially from the recorded point, and the resulting disc was then cut axially along the line connecting the center of the bed log and the place 5 cm circumferentially away from the recorded point (Fig. 1). The bark and wood were separated from the resulting wedge-shaped blocks and dried at 105 °C. The wood was then crushed with a cutting mill (P-15; Fritsch Japan Co., Ltd., Kanagawa, Japan) and both the bark and wood were milled separately with the Millser.

Sawdust medium cultivation for estimating the effect of medium ^{133}Cs concentrations on the cap ^{133}Cs concentrations

Commercially available Japanese beech sawdust and rice bran were used as the sawdust medium. Sawdust and rice bran were mixed at a ratio of 75:25 (w/w), and the sawdust medium ^{133}Cs concentration was adjusted to 5 levels [control, 0.50, 0.10, 0.15, and 0.20 mg/kg (w/w)] using CsCl. After adjusting the water content to 65% (w/w), a 1 kg mixture was packed into a plastic bag and autoclaved at 121 °C for 90 min. Three sawdust media were prepared for each CsCl concentration. The sterilized sawdust media were inoculated with the mycelia of the both strains that had been cultivated for 2 weeks in 40 mL SMY liquid medium [10 g/L sucrose, 10 g/L malt extracts (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan), and 4 g/L yeast extracts (Oxoid Ltd., Hampshire, England)] and cultured at 22 °C and 70% relative humidity in the dark for 16 weeks [14]. The fruiting bodies that developed were then treated in the same manner as described above for bed-log cultivation. Once fruiting body development was completed, the sawdust medium was dried at 105 °C, crushed with a pulverizer (V-360; HORAI Co., Ltd., Osaka, Japan), and milled with the Millser.

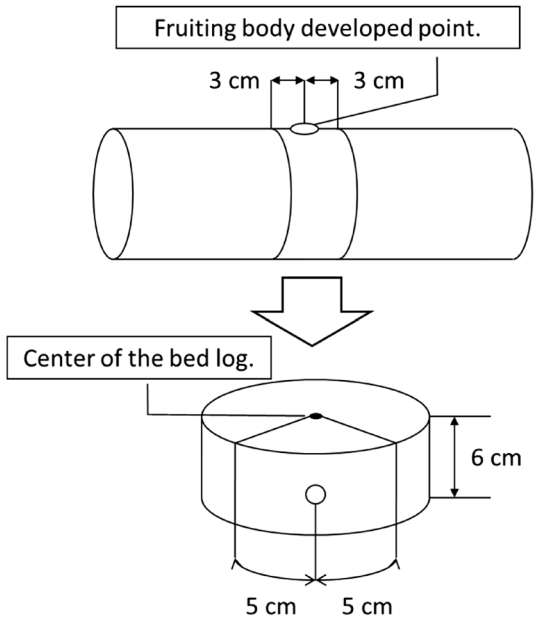

Fig. 1 Sampling method for analyzing Cs concentration in bed log at the point where fruiting body developed

Sawdust medium cultivation for estimating the effect of the nutrient concentration on TF

Japanese beech sawdust and rice bran were mixed at 95:5, 90:10, 85:15, 80:20, 75:25, and 70:30 (w/w), and the ^{133}Cs concentrations in these 6 media were adjusted to 0.20 mg/kg (w/w) using CsCl. The cultivation was performed in the same manner as described above for sawdust medium cultivation.

Sawdust medium cultivation for estimating the effect of the cultivation period on TF

Japanese beech sawdust and rice bran were mixed at a ratio of 95:5 (w/w), and the ^{133}Cs concentrations in the resulting sawdust media were adjusted to 0.20 mg/kg (w/w) using CsCl. The cultivation was performed in the same manner as described above for sawdust medium cultivation, but cultivation periods were set for 5 levels (16, 20, 24, 28, and 32 weeks).

Sawdust medium cultivation for estimating the differences between the ^{137}Cs TF and ^{133}Cs TF, and the differences between strains

Radioactive contaminated sawdust and rice bran were mixed at a ratio of 95:5 (w/w). The radioactive sawdust was collected from a contaminated site in Fukushima prefecture in July 2011 and consisted of mainly hardwood (unknown species). The cultivation was performed in the same manner as described above for sawdust medium cultivation, but the cultivation period was 28 weeks.

Elemental analysis

The total ^{137}Cs (to^{137}Cs) concentrations in the bark and wood samples were measured by filling 20-mL vials (6000477; PerkinElmer Japan Co., Ltd., Kanagawa, Japan) with each sample and measuring the to^{137}Cs concentration twice per sample for 1 h each using a gamma counter (2480 WIZARD^2; PerkinElmer Japan

Co., Ltd.). The to^{137}Cs concentration in the sawdust medium was measured by placing each sample in a 0.7-L Marinelli beaker or U-8 container, depending on the sample volume, and using a high-purity germanium detector (GEM20-70; SEIKO EG&G Co., Ltd., Tokyo, Japan). The cap ^{137}Cs concentrations in the bed-log cultivation were measured by mixing each sample cap with a cap of known ^{137}Cs concentration (6.3 Bq/kg) to give a total amount of approximately 9 g, because each sample volume was too small to measure with the gamma counter. The ^{137}Cs concentration in the resulting mixture was then measured in the same manner as described above using the gamma counter and the cap ^{137}Cs concentration was calculated from its weight ratio in the mixed sample. The cap ^{137}Cs concentrations in the sawdust medium cultivation were measured by enclosing each sample in a U-8 container and using the high-purity germanium detector, as described above.

To determine the ex^{137}Cs concentration in the sawdust medium, 10 mL of 1 mol/L ammonium acetate solution adjusted to pH 7.0 with acetic acid or ammonia solution was added to approximately 1 g of each sawdust sample. The mixture was then swirled for 16 h with a rotating incubator (RT-50; TAITEC Corp., Saitama, Japan) and centrifuged at 5000 × *g* for 10 min. The resulting supernatant was filtered through a 0.50-μm hydrophilic polytetrafluoroethylene (PTFE) membrane filter, and the ex^{137}Cs concentration was measured three times per sample for 2 h each using the gamma counter, as described above.

To measure the total ^{133}Cs (to^{133}Cs) concentration in the all samples, 14 mL of concentrated nitric acid (Ultrapure-100; KANTO CHEMICAL Co., Inc., Tokyo, Japan) was added to approximately 0.5 g of sample in the lower container of an ECO-PRE (OD-98-100; ACTAC Co., Kanagawa, Japan), and 6 mL of 5% nitric acid was added to the trap part. Digestion was then carried out by repeating nine cycles of 210 °C for 20 min and 100 °C for 25 min. The resulting solution was filtered through the same PTFE membrane filter, and the to^{133}Cs concentration was measured using an inductively coupled plasma mass spectrometer (ICP-MS) (7700x; Agilent Technologies International Japan Ltd., Tokyo, Japan). The cap cultivated in the bed-log cultivation was mixed with supplemental sample for measuring the cap ^{137}Cs concentration, and the supplemental cap ^{133}Cs concentration was 0.32 mg/kg, so the cap ^{133}Cs concentration in the bed-log cultivation was calculated as described above.

To measure the exchangeable ^{133}Cs (ex^{133}Cs) concentration in the sawdust medium, the ex^{133}Cs was extracted as described above for ex^{137}Cs, and measured as described above using ICP-MS.

The nitrogen (N) concentration in the sawdust medium was measured with an NC analyzer (Sumigraph NC-22F; Sumica Chemical Analysis Service Ltd., Osaka, Japan) using approximately 20 mg sample.

Calculation

The sawdust medium Cs concentrations were changed depending on the cultivation stages, so the TFs based on the medium Cs concentration at the start of cultivation (TF_{SC}), before fruiting body development (TF_{BD}), and after fruiting body development (TF_{AD}) were calculated as follows:

$$TF_{SC} = \frac{\text{Fruiting body Cs concentration}}{\text{Medium CS concentration at the start of cultivation}}, \quad (1)$$

$$TF_{BD} = \frac{\text{Fruiting body Cs concentration}}{\text{Medium Cs concentration before fruiting body devrlopment}} = \frac{\text{Fruiting body Cs concentration}}{\frac{\text{Medium Cs amount after fruiting body development} + \text{fruiting body Cs amount}}{\text{Medium weight after fruiting body development} + \text{fruiting body weight}}}, \quad (2)$$

$$TF_{AD} = \frac{\text{Fruiting body Cs concentration}}{\text{Medium Cs concentration after fruiting body development}}. \quad (3)$$

Since there were two kinds of Cs concentration (toCs concentration and exCs concentration) and two kinds of Cs (^{137}Cs and ^{133}Cs), they were reported as to^{137}Cs TF, to^{133}Cs TF, ex^{137}Cs TF, and ex^{133}Cs TF, respectively. The amount of fixed Cs (fxCs) was calculated as follows:

$$\text{Fixed Cs amount} = \text{total Cs amount} - \text{exchangeable Cs amount.} \quad (4)$$

The exCs solubilization ratio was calculated as follows:

$$\text{exCs Solubilization ratio} = \frac{\text{fxCS}_{\text{SC}} - \text{fxCs}_{\text{AD}}}{\text{fxCs}_{\text{SC}}}, \quad (5)$$

where fxCs_{SC} and fxCs_{AD} were the fxCs amounts at the start of cultivation and after harvesting fruiting body, respectively. The wet weight-based TF was calculated as follows:

$$\text{Wet weight} - \text{based TF} = \frac{\frac{\text{Fruiting body Cs concentration}}{1-\frac{\text{Fruiting body water content (\%)}}{100}}}{\frac{\text{Medium Cs concentration}}{1-0.12}}, \quad (6)$$

because the water content of the culture medium is specified as 12% [15]. The significance level was set to 0.05.

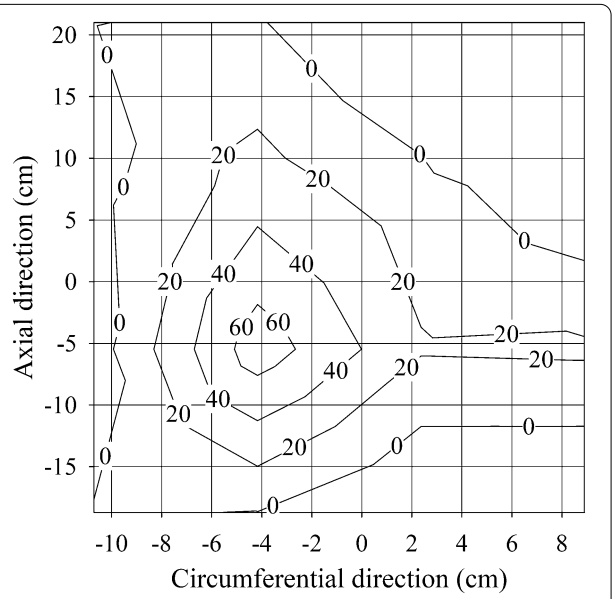

Fig. 2 Distribution of cap ^{133}Cs concentration of shiitake (*Lentinula edodes*) in bed-log cultivation ($n=16$). ^{133}Cs solution was injected to a perforation depth of 3.2 cm. The cap ^{133}Cs concentration (mg/kg) was obtained by subtracting the cap ^{133}Cs concentration in shiitake cultivated on a control bed log (0.59 mg/kg). The coordinates (0, 0) indicated the perforated portion

Results

Relationship between the fruiting body Cs concentration and the distance from the source of cesium

A sufficient number of fruiting bodies required to draw contour graphs was only obtained from one of the three bed logs examined. The cap ^{133}Cs concentrations at locations where fruiting bodies developed ($n=16$) are shown in Fig. 2. A mountain-shaped contour graph with vertices near (0, 0) was observed. It can clearly be seen that the cap ^{133}Cs concentration decreased with increasing distance from the point where ^{133}Cs was applied. In addition, the fruiting body absorbed ^{133}Cs from a distance of about 20 cm in an axial direction and about 10 cm in a circumferential direction.

Both nuclides $_{\text{to}}$Cs TF_{AD} in bed-log cultivation

Relationships between the cap Cs concentration and bed log Cs concentration and toCs TF_{AD} in the bed-log cultivation are shown in Table 1. There was no significant correlation between the cap ^{137}Cs concentration and the bark to^{137}Cs concentration [Spearman's rank order correlation (ρ) = 0.14, $P=0.37$, $n=43$]. However, there was a significant positive correlation between the cap ^{137}Cs concentration and the wood to^{137}Cs concentration ($\rho=0.41$, $P<0.01$, $n=43$). In contrast, there was no significant correlation between the cap ^{133}Cs concentration and the wood to^{133}Cs concentration ($\rho=-0.09$, $P=0.56$, $n=43$). Furthermore, when comparing the toCs TF_{AD} of the wood, the to^{133}Cs TF_{AD} was significantly higher than the to^{137}Cs TF_{AD} (t-test: $P<0.01$; Table 1).

Units of the ^{137}Cs and ^{133}Cs concentration were Bq/kg and mg/kg, respectively. The toCs TF_{AD} was referred

Table 1 Relationships between the cap Cs concentration and bed log Cs concentration and toCs TF_{AD} in the bed-log cultivation

Nuclide	^{137}Cs					^{133}Cs		
Part	FB	Bark		Wood		FB	Wood	
	Conc	Conc	TF_{AD}	Conc	TF_{AD}	Conc	Conc	TF_{AD}
Average	74.3	5.4	11.4	12.2	7.4[a]	10.2	0.62	19.4[b]
CV	0.65	0.61	0.87	0.65	0.80	0.38	0.22	0.57
ρ		0.14		0.41*			− 0.09	

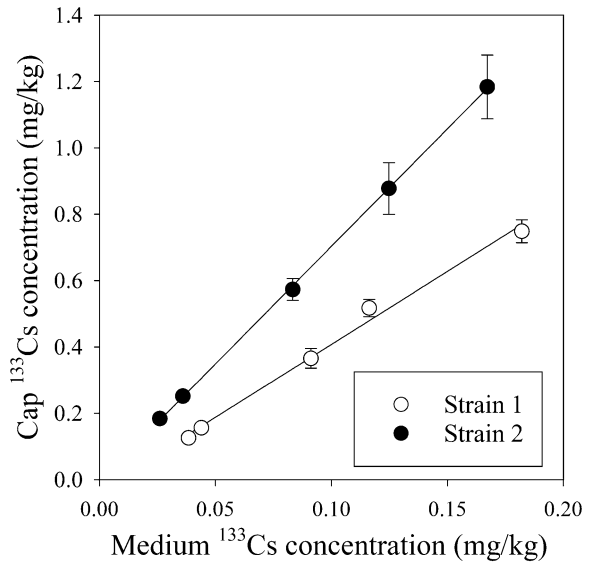

Fig. 3 Relationship between the ^{133}Cs concentrations in the cap and sawdust medium. The error bars above and below each point indicated the standard deviation ($n=3$)

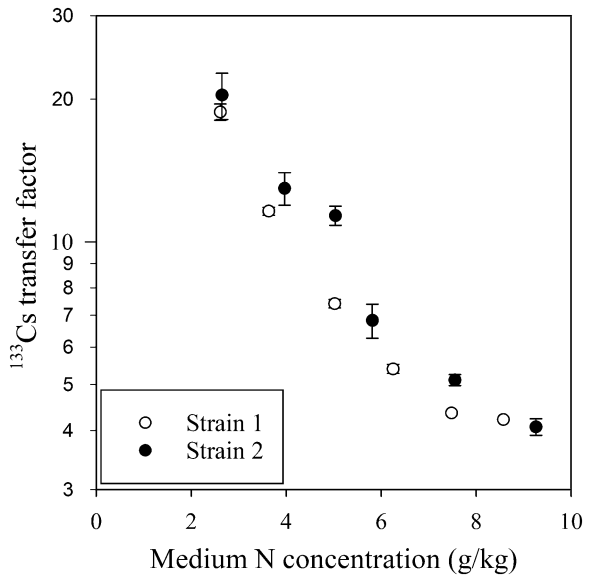

Fig. 4 Relationship between the ^{133}Cs transfer factor and the N concentration in the sawdust medium. The ^{133}Cs transfer factor was based on the sawdust medium Cs concentration after fruiting body development. The error bars above and below each point indicated the standard deviation. $n=1$ at an N concentration of 8.6 g/kg (rice bran addition rate = 30%) for Strain 1; $n=2$ at an N concentration of 5.0 g/kg (rice bran addition rate = 15%) for Strain 2; and $n=3$ for all other points

to Eq. 3. Conc., and CV indicated ^{137}Cs or ^{133}Cs concentration and coefficient of variation, respectively. The ρ indicated Spearman's rank order correlation coefficient between the cap Cs concentration and the bark or wood Cs concentration. The different superscript letters indicated significant difference as determined by *t*-test and * indicated that the *P* value was below the significance level (0.05) ($n=48$ fruiting bodies).

Relationship between ^{133}Cs concentration in the cap and sawdust medium

There were significant positive correlations between the sawdust medium ^{133}Cs concentrations and the cap ^{133}Cs concentrations for both strains [Strain 1: $y=4.40x-0.03$, Pearson correlation coefficient (r) = 0.99, $P<0.01$; Strain 2: $y=7.09x$, $r=1.00$, $P<0.01$; Fig. 3]. There were no significant correlations between the cap yields and the medium ^{133}Cs concentrations for either strains (Strain 1: $\rho=0.23$, $P=0.40$; Strain 2: $\rho=-0.01$, $P=0.97$).

Effect of the nutrient concentration in the sawdust medium on the to^{133}Cs TF_{AD}

There were significant negative correlations between the to^{133}Cs TF_{AD} and the sawdust medium N concentrations for both strains (Strain 1 and 2: $\rho=-1.00$, $P<0.01$; Fig. 4). When the N concentration of the sawdust medium was 2.6 g/kg (rice bran addition ratio = 5%), the ^{133}Cs TF_{AD} for Strain 1 and 2 with were 18.8±0.7 and 20.4±2.3, respectively. The water contents of the fruiting bodies for Strain 1 and 2 with the same sawdust media adjusted an N concentration of 2.6 g/kg were 93.9±0.2 and 90.5±2.5%, respectively. Therefore, the to^{133}Cs TF_{AD} for Strain 1 and 2 on a wet weight basis were 1.9±0.5 and 2.2±0.4, respectively (Eq. 6).

Effect of the cultivation period in the model sawdust medium on to^{133}Cs TF_{AD}

No fruiting bodies were obtained from Strain 2 with cultivation periods of 16 to 20 weeks, so only data for cultivation periods of 24–32 weeks were included in the

Table 2 Relationships between to^{133}Cs TF_{AD}, cultivation period, cap yield, and cap number for Strain 1 and 2

Strain		Cultivation period	Cap yield	Cap number
1	to^{133}Cs TF_{AD}	− 0.53*	− 0.51*	− 0.49
	Cultivation period		0.45	− 0.04
	Cap yield			0.49
2	to^{133}Cs TF_{AD}	− 0.57	− 0.36	− 0.18
	Cultivation period		0.00	0.63
	Cap yield			0.47

Table 3 TFs in the model sawdust medium cultivation

Strain	TF_{SC}				TF_{BD}				TF_{AD}			
	toCs		exCs		toCs		exCs		toCs		exCs	
	^{137}Cs	^{133}Cs	^{137}Cs	^{133}Cs	^{137}Cs	^{133}Cs	^{137}Cs	^{133}Cs	^{137}Cs	^{133}Cs	^{137}Cs	^{133}Cs
1	4.4 [a*] (0.2)	8.6 [a*] (0.1)	14.6 [c*] (0.8)	11.0 [c*] (0.2)	2.9 [e*] (0.2)	5.6 [e*] (0.1)	6.9 [g, m*] (0.4)	7.0 [g, n] (0.3)	3.2 [i*] (0.3)	7.0 [i*] (0.1)	9.2 [k] (0.8)	9.5 [k] (0.4)
2	6.8 [b*] (0.2)	13.9 [b*] (0.4)	22.3 [d*] (0.6)	17.9 [d*] (0.5)	3.3 [f*] (0.1)	6.5 [f*] (0.3)	7.7 [h, m*] (0.3)	7.6 [h, n] (0.3)	3.5 [j*] (0.1)	7.8 [j*] (0.4)	9.7 [l] (0.6)	9.5 [l] (0.3)

TF_{SC}, TF_{BD}, and TF_{AD} were referred to Eqs. 1, 2, and 3, respectively. to^{137}Cs, ex^{137}Cs, to^{133}Cs, and ex^{133}Cs were indicated total ^{137}Cs, exchangeable ^{137}Cs, total ^{133}Cs, and exchangeable ^{133}Cs, respectively. The to^{137}Cs, the ex^{137}Cs, the to^{133}Cs, and the ex^{133}Cs concentrations in the sawdust medium at the start of cultivation were 297 Bq/kg, 104 Bq/kg, 35.3 µg/kg, and 27.1 µg/kg, respectively. The values in parentheses indicated the standard deviation. The same superscript letters indicated the comparison groups. *: $P < 0.05$

analysis. The relationships between to^{133}Cs TF_{AD}, cultivation period, cap yield, and cap number for Strain 1 and 2 are shown in Table 2. There was a significant negative correlation between the to^{133}Cs TF_{AD} and the cultivation period and between the to^{133}Cs TF_{AD} and the cap yield for Strain 1. However, there was no significant difference among the to^{133}Cs TF_{AD} for Strain 1 (one-way analysis of variance, $P=0.15$). Moreover, there was no significant correlation between the cultivation period and the cap yield for Strain 1 ($P=0.08$). Furthermore, there was no significant correlation among the to^{133}Cs TF_{AD}, the cultivation period, the cap yield, and the cap number for Strain 2 (Table 2).

to^{133}Cs TF_{AD} was referred to Eq. 3. The numbers indicated the Spearman's rank order correlations ($n=3$ sawdust medium bags per strain). * indicated that the P value was below the significance level (0.05).

Differences in TFs between ^{137}Cs and ^{133}Cs in the model sawdust medium

There were significant differences between the to^{137}Cs TF_{SC} and to^{133}Cs TF_{SC} for both strains (t-test: $P<0.01$ for all contrasts; Table 3, a and b) and between ex^{137}Cs TF_{SC} and ex^{133}Cs TF_{SC} for both strains (t-test: $P<0.01$ for all contrasts; Table 3, c and d). There was also a significant difference between the to^{137}Cs TF_{BD} and to^{133}Cs TF_{BD} for both strains (t-test: $P<0.01$ for all contrasts; Table 3, e and f) and between the to^{137}Cs TF_{AD} and to^{133}Cs TF_{AD} for both strains (t-test: $P<0.01$ for all contrasts; Table 3, i and j). However, there was no significant difference between the ex^{137}Cs TF_{BD} and the ex^{133}Cs TF_{BD} for either strain (t-test: Strain 1: $P=0.57$, Table 3, g; Strain 2: $P=0.40$, Table 3, h) and between the ex^{137}Cs TF_{AD} and the ex^{133}Cs TF_{AD} for either strain (t-test: Strain 1: $P=0.62$, Table 3, k; Strain 2: $P=0.61$; Table 3, l).

Table 3 shows that the toCs and exCs TF_{SC} of both nuclides are higher than the toCs and exCs TF_{BD} of both nuclides and the toCs and exCs TF_{AD} of both nuclides, indicating that Cs concentrations of both nuclides and both types in the model sawdust media increased during cultivation. The medium weights of Strains 1 and 2 decreased by 41.8 ± 1.5 and 57.2 ± 0.4%, respectively, from the start of cultivation until harvesting the fruiting bodies. Furthermore, the amount of fx^{137}Cs in the medium decreased by 18.5% for Strain 1 and 18.0% for Strain 2 from the start of cultivation until all the fruiting bodies were harvested, and the amount of fx^{133}Cs also decreased, albeit by a lesser amount (3.3% and 8.2%, respectively) (Table 4).

Differences in TF_{BD} between the strains

The ex^{137}Cs TF_{BD} for Strain 1 was significantly lower than that for Strain 2 (t-test, $P=0.03$, Table 3, m). In contrast, there was no significant difference in the ex^{133}Cs TF_{BD} between the strains (t-test, $P=0.07$, Table 3, n). Therefore, clear strain differences were not found between the strains. Furthermore, there was no significant difference in the ex^{137}Cs solubilization ratio between the strains (t-test, $P=0.76$, Table 4, a), nor was there a significant difference in the ex^{133}Cs solubilization ratio (t-test, $P=0.22$, Table 4, b).

Discussion

Model sawdust medium

When the sawdust medium N concentration was 2.6 g/kg (rice bran addition ratio = 5%), the to^{133}Cs TF_{AD} for Strain 1 and 2 on wet weight bases were close to the TFs that led to the provisional reference index value of 2.0 in bed-log cultivation (Fig. 4) [5]. In addition, the to^{133}Cs TF_{AD} for Strains 2 in sawdust medium cultivation at an N concentration of 2.6 g/kg was almost the same as the to^{133}Cs TF_{AD} for Strains 2 in bed-log cultivation (Fig. 4, Table 1). These results indicated that the sawdust medium with an N concentration adjusted to 2.6 g/kg could be used as a model for bed logs with respect to

Table 4 Mass balance (existence rate, %) of ^{137}Cs and ^{133}Cs in the model sawdust medium

		Medium				Cap Cs		Unknown Cs	
		fxCs		exCs					
		^{137}Cs	^{133}Cs	^{137}Cs	^{133}Cs	^{137}Cs	^{133}Cs	^{137}Cs	^{133}Cs
Start of cultivation	Sawdust	70.7	22.3	29.3	75.4				
	Rice bran	0	0	0	2.2				
	Total	70.7	22.3	29.3	77.7*				
After development	Strain 1	52.2	19.0	28.0 (26.2 [a] ± 3.4)	52.5 (15.1 [b] ± 10.3)	11.2	21.5	8.6	7.0
	Strain 2	52.7	14.1	30.0 (25.4 [a] ± 2.0)	62.8 (37.1 [b] ± 23.7)	9.0	18.4	8.3	4.7

The fixed Cs (fxCs) amount and exchangeable Cs (exCs) solubilization ratio were referred to Eqs. 4 and 5. The numbers in parentheses indicated the exCs solubilization ratio and its standard deviation. *Total value did not match due to the rounding of values. The same letters indicated the comparison groups and there was no significant difference between them according to t-test

TF. A ^{133}Cs concentration in the sawdust medium up to about 0.20 mg/kg had no effect on cap yield. Therefore, the ^{133}Cs concentration of the model sawdust medium was set to 0.20 mg/kg to clarify the behavior of TF. There was no significant relationship between the cultivation period and to^{133}Cs TF_{AD} during the tested period in the sawdust medium cultivation (Table 2). However, the yield of Strain 1 tended to increase with an increasing cultivation period (Table 2). Based on these findings, the cultivation period was set to 28 weeks for harvesting a sufficient yield of fruiting bodies. By the way, since Strain 1 and 2 are mainly used for sawdust medium cultivation and bed-log cultivation, respectively, it is likely that Strain 2 took longer to develop fruiting bodies than Strain 1 in the sawdust medium cultivation.

Factors affecting the TF

There were significant positive linear correlations between the cap ^{133}Cs concentrations and the sawdust medium ^{133}Cs concentrations (Fig. 3). A similar relationship has been reported for oyster mushroom (*Pleurotus ostreatus*) [14], then it is considered that the cap Cs concentration is proportional to the Cs concentration in the medium if the Cs concentration in the medium is uniform. On the other hand, the to^{137}Cs concentration at the point where fruiting bodies developed were varied (Table 1), which indicated that the distribution of ^{137}Cs concentration in a single bed log also varied. Therefore, the cap ^{133}Cs concentration in bed-log cultivation is considered to be affected by the concentration of the ^{133}Cs source and the distance from the source within about 20 cm in an axial direction and about 10 cm in a circumferential direction (Fig. 2). A change in enzyme activity has also been observed in bed log about 17 cm in an axial direction from the point where the fruiting body developed [9], indicating that not only nutrients, but also Cs is absorbed at a considerable distance from the point where the fruiting body developed. The bed log Cs concentration at the point where the fruiting body developed would have the greatest influence on the cap Cs concentration, but the conventional method of calculating TF uses the ^{137}Cs concentration of the entire bed log [15]. Therefore, the toCs TF_{AD} was measured using the bed log Cs concentration at the point where fruiting body developed. There was a significant positive correlation between the cap ^{137}Cs concentration and the wood to^{137}Cs concentration, but no significant correlation between the cap ^{137}Cs concentration and the bark to^{137}Cs concentration, although the bark to^{137}Cs concentration showed as much variation as the wood to^{137}Cs concentration, as indicated by their similar coefficients of variation (CVs) (Table 1). On the other hand, one reason for the lack of significant correlation between the cap ^{133}Cs concentration and the wood ^{133}Cs concentration may be because the bed log ^{133}Cs concentrations were not sufficiently varied to make a significant correlation since the CV is 0.22 (Table 1). Therefore, bark to^{137}Cs is considered to have less effect on to^{137}Cs TF_{AD} than wood to^{137}Cs. Furthermore, a clear linear relationship has not been obtained even between the cap ^{137}Cs concentration and the wood ^{137}Cs concentration since the ρ is 0.41 (Table 1). These results indicated that there are other factors affecting the toCs TF_{AD} besides Cs concentration. Many factors will affect the toCs TF_{AD} in bed-log cultivation, making it difficult to elucidate the most important ones. Therefore, these factors were examined using the model sawdust medium.

By varying the nutrient contents of the sawdust media, the difference in to^{133}Cs TF_{AD} between bed-log cultivation and sawdust medium cultivation is thought to be caused mainly by the difference in nutrient concentration of the substrate (Fig. 3). However, rice bran contains various nutrients [16], so it is unclear which component affected the TF_{AD}. N is the most consumed nutrient component in sawdust medium [17], so the nutrient concentration was represented as N concentration. Since kunugi oak (*Q. acutissima*) is estimated to have a higher N concentration than konara oak [18], the use of kunugi oak may reduce the to^{137}Cs TF_{AD}.

There was a significant negative correlation between the to^{133}Cs TF_{AD} and the cap yield for Strain 1 but not for Strain 2 (Table 2). As the yield of the cap changes, the amount of Cs transferred to the cap changes, and as a result, the cap Cs concentration is likely to change. How the cap Cs concentration changes is thought to depend on the nature of the strain.

The strain difference is considered to be caused by the difference in the amount of exCs transferred from the medium to the fruiting body and the difference in the amount of dissolved Cs which is fixed in the medium during cultivation. I speculate that the former corresponds to exCs TF_{BD} and the latter corresponds to exCs solubilization ratio. However, there was no clear difference in the ex^{137}Cs TF_{BD} and ex^{133}Cs TF_{BD} between the strains (Table 3). Moreover, there was no significant difference in the ex^{137}Cs and the ex^{133}Cs solubilization ratios between the strains (Table 4). From these results, no strain difference was evident between the strains. In this experiment, two kinds of strains for sawdust medium cultivation and bed-log cultivation were used in order to obtain strain differences, but it is presumed that the differences between the strains were not large enough to obtain clear significant differences. However, the details of this are unknown, so further study is required.

On the other hand, the lack of significant differences in the ex^{133}Cs solubilization ratio between the strains may be due to the large standard deviation (Table 4). The ^{133}Cs present in the tree is absorbed from the soil by root

absorption, and the absorbed Cs may be bound to intracellular lignin and other substances, some of which may be surrounded by various substances such as cellulose and hemicellulose, and therefore many types of enzymes may be required to solubilize ^{133}Cs. The diversities of these enzyme activities among strains have been shown [19, 20], and because Cs is not an essential element for shiitake, it is hypothesized that ^{133}Cs was not solubilized at a constant rate. The ^{137}Cs concentration in trees is increasing [21], and the variations in ex^{137}Cs solubilization rate may further vary cap ^{137}Cs concentration.

Cs concentration leading to accurate TF

There were significant differences between the to^{137}Cs TF_{AD} and to^{133}Cs TF_{AD} in the bed-log cultivation (Table 1) and the model sawdust medium cultivation (Table 3), but no difference between ex^{137}Cs TF_{AD} and ex^{133}Cs TF_{AD} in the model sawdust medium cultivation (Table 3). Mechanism of Cs migration from the bed log to the fruiting body has been proposed to be intracellular transport through mycelium and the extracellular transport via the inter-hyphal cavity [22]. In either case, ionic Cs is migrated from the bed log to the fruiting body, suggesting that exCs acts as a Cs supply source for the fruiting body. Soil organic matter contains readily soluble and sparingly soluble Cs [12, 23], and the sawdust medium also contained exCs and fxCs, which corresponds to soluble and sparingly soluble Cs, respectively (Table 4). However, the TF is usually calculated using toCs TF [15], so it is considered appropriate to use exCs concentration rather than toCs concentration for calculating the accurate TF.

There are two methods for calculating TF in bed-log cultivation, one is TF_{SC} and the other is TF_{AD} [15]. However, there was difference between TF_{SC} and TF_{AD} in the model sawdust medium cultivation (Table 3). The sparingly soluble Cs is presumed to be bound to lignin or tannin [12], which can be degraded by wood-rotting shiitake [24]. The fxCs concentrations of both nuclides decreased in the sawdust medium cultivation (Table 4), which indicated that exCs concentrations for both were increasing from the start of the cultivation to the fruiting body development, along with the decrease in the model sawdust medium weight. Therefore, TF_{AD} is considered more appropriate than TF_{SC}. The to^{137}Cs TF_{AD} of wood was over 10 (Table 1), and the cap ^{137}Cs existence rates in Strain 1 and 2 were 9.0 and 11.2%, respectively, in the model sawdust medium cultivation (Table 4). If the TF_{AD} is sufficiently small, the Cs concentration in the sawdust medium can be regarded as constant, but the TF_{AD} of shiitake was large, and the amount of Cs in the sawdust medium after fruiting body development was reduced as compared with that before fruiting body development (Table 4). In order to obtain an accurate TF, it is considered necessary to consider the amount of Cs taken up by the fruiting body from the medium. Therefore, TF_{BD} is considered more appropriate than TF_{AD}.

When calculating the mass balance of sawdust medium, unknown ^{137}Cs and ^{133}Cs accounted for a few percent of the to^{137}Cs and to^{133}Cs, respectively, after harvesting fruiting bodies (Table 4). Only caps were analyzed in this experiment, therefore it is possible that the unknown Cs amount was equivalent to the amount of Cs in the stipe. However, ^{137}Cs is mainly accumulated around the edge of the cap in the fruiting body [25], and it is unlikely that the stipe would contain 26%–93% Cs of the cap Cs. However, the details of this are unknown, so further study is required.

Calculation method of TF in bed-log cultivation

What is desired on the mushroom farm is to predict the fruiting body ^{137}Cs concentration from the to^{137}Cs concentration of the log at the start of cultivation. For the purpose, it is necessary to find the relationship between to^{137}Cs TF_{SC} and ex^{137}Cs TF_{BD}. However, it is difficult to obtain accurate exCs concentrations because the Cs concentrations in the current bed logs vary even within a single bed log. Furthermore, ex^{137}Cs TF_{BD} in bed-log cultivation is considered to be affected by the nutrient concentration as well as the sawdust medium. Nutrients such as N, potassium (K), and phosphorus have been reported to be likely to be deficient during bed-log cultivation [18], and ^{137}CsTF in wild mushrooms has been reported to decrease with increasing concentrations of exchangeable K in the soil [26]. It is necessary to clarify the effects of the concentrations of these nutrients on ex^{137}Cs TF_{BD} for clarifying the relationship between the to^{137}Cs TF_{SC} and ex^{137}Cs TF_{BD}. However, even if the relationship between the concentrations of these nutrients and ex^{137}Cs TF_{BD} becomes clear, the concentrations of these nutrients in the logs vary with the time of tree felling [27, 28]. The upper limit of to^{137}Cs concentration in logs capable of producing less than 100 Bq/kg of fruiting bodies may be different from the current value when taking into account the variation in the concentration of these nutrients in the log. In addition, it seems practically impossible to measure these nutrient concentrations for each lot of logs and determine the suitability of each lot of logs.

As more time passes since the nuclear power plant accident, fewer directly contaminated trees will be used as bed logs, with newly planted and sprouted coppice trees increasingly being used in their place. However, the

^{137}Cs concentrations in the wood are gradually increasing [21], and the ^{137}Cs concentrations will reach a steady state in the future as currently is the case for ^{133}Cs concentration. At that time, the relationship between to^{137}Cs TF_{SC} and ex^{137}Cs TF_{BD} and the relationship between to^{133}Cs TF_{SC} and ex^{133}Cs TF_{BD} may match. Therefore, it is considered necessary to determine the relationship between the to^{133}Cs TF_{SC} and ex^{133}Cs TF_{BD} so that fruiting body ^{137}Cs concentration can be predicted from the wood to^{137}Cs concentration in the future.

Conclusions

The cap Cs and sawdust medium Cs concentrations were in direct proportion to each other. Moreover, Cs that could not be absorbed by shiitake was remained in the medium, highlighting the importance of using the exchangeable Cs concentration to measure the transfer factor. Furthermore, since the exchangeable Cs concentration in the sawdust medium increased during cultivation, it is desirable to measure transfer factor just before fruiting body development. The Cs transfer factor was negatively correlated with the nutrient concentration (represented by the N concentration) in the sawdust medium. From these results, the exCs concentration and nutrient concentration were key factors affecting Cs transfer factor in the sawdust medium cultivation, and therefore it is likely that they are also key factors affecting Cs transfer factor in bed-log cultivation.

Abbreviations

CV: Coefficient of variation; exCs: Exchangeable Cs; fxCs: Fixed Cs; $fxCs_{AD}$: Fixed Cs amount after harvesting fruiting body; $fxCs_{SC}$: Fixed Cs amount at the start of cultivation; ICP-MS: Inductively coupled plasma mass spectrometer; PTFE: Polytetrafluoroethylene; TF: Transfer factor; TF_{AD}: Transfer factor based on the medium Cs concentration after fruiting body development; TF_{BD}: Transfer factor based on the medium Cs concentration before fruiting body development; TF_{SC}: Transfer factor based on the medium Cs concentration at the start of cultivation; toCs: Total Cs.

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Authors' contributions

MH designed and performed the experiments, analyzed the data, and wrote the manuscript. The author read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the author on reasonable request.

Competing interests

The author declares that there are no competing interests.

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