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Efficient sampling of shiitake-inoculated oak logs to determine the log-to-mushroom transfer factor of stable cesium

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Background. Stable cesium (Cs-133) naturally exists in the environment whereas recently deposited radionuclides (e.g., Cs-137) are not at equilibrium. Stable cesium has been used to understand the long-term behavior of radionuclides in plants, trees and mushrooms. We are interested in using Cs-133 to predict the future transfer factor (TF) of radiocesium from contaminated logs to shiitake mushrooms in Eastern Japan. However, the current methodology to obtain a representative wood sample for Cs-133 analysis involves mechanically breaking and milling the entire log (excluding bark) to a powder prior to analysis. In the current study, we investigated if sawdust obtained from cutting a log along its length at eight points is as robust but a faster alternative to provide a representative wood sample to determine the TF of Cs-133 between logs and shiitake. **Methods.** Oak logs with ready-to-harvest shiitake fruiting bodies were cut into nine 10-cm discs and each disc was separated into bark, sapwood and heartwood and the concentration of Cs-133 was measured in the bark, sapwood, heartwood, sawdust (generated from cutting each disc) and fruiting bodies (collected separately from each individual disc), and the wood-to-shiitake TF was calculated. **Results.** We found that the sawdust-to-shiitake TF of Cs-133 did not differ ($P = 0.223$) compared to either the sapwood-to-shiitake TF or heartwood-to-shiitake TF, but bark did have a higher concentration of Cs-133 ($P < 0.05$) compared to sapwood and heartwood. Cs-133 concentration in sawdust and fruiting bodies collected along the length of the logs did not differ ($P > 0.05$). **Discussion.** Sawdust can be used as an alternative to determine the log-to-shiitake TF of Cs-133. To satisfy the goals of different studies and professionals, we have described two sampling methodologies (Methods I and II) in this paper. In Method I, a composite of eight sawdust samples collected from a log can be used to provide a representative whole-log sample (i.e., wood and bark), whereas Method II allows for the simultaneous sampling of two sets of sawdust samples—one set representing the whole log and the other representing wood only. Both methodologies can greatly reduce the time required for sample collection and preparation.

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Abstract

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Methods. Oak logs with ready-to-harvest shiitake fruiting bodies were cut into nine 10-cm discs and each disc was separated into bark, sapwood and heartwood and the concentration of Cs-133 was measured in the bark, sapwood, heartwood, sawdust (generated from cutting each disc) and fruiting bodies (collected separately from each individual disc), and the wood-to-shiitake transfer factor (TF) was calculated.

Results. We found that the sawdust-to-shiitake TF of Cs-133 did not differ ($P = 0.223$) compared to either the sapwood-to-shiitake TF or heartwood-to-shiitake TF, but bark did have a higher concentration of Cs-133 ($P < 0.05$) compared to sapwood and heartwood. Cs-133 concentration in sawdust and fruiting bodies collected along the length of the logs did not differ ($P > 0.05$).

Discussion. Sawdust can be used as an alternative to determine the log-to-shiitake TF of Cs-133. To satisfy the goals of different studies and professionals, we have described two sampling methodologies (Methods I and II) in this paper. In Method I, a composite of eight sawdust samples collected from a log can be used to provide a representative whole-log sample (i.e., wood and bark), whereas Method II allows for the simultaneous sampling of two sets of sawdust samples—one set representing the whole log and the other representing wood only. Both methodologies can greatly reduce the time required for sample collection and preparation.

Introduction

Over 25 million logs are used annually for shiitake mushroom production in Japan (Ministry of Agriculture, Forestry & Fisheries, 2017). Prior to the nuclear reactor accident at the Fukushima Daiichi Nuclear Power Plant in 2011, Fukushima prefecture was a major source of trees used for shiitake log-cultivation (Miura, 2016). As a result of the accident, the Japanese government restricted radiocesium (Cs-134 and Cs-137) in general foods, including mushrooms cultivated on logs, to ≤ 100 Bq/kg fresh weight (FW) (Ministry of Health, Labour and Welfare, 2012). The transfer of radiocesium from fallout-contaminated logs to shiitake, known as the transfer factor (TF), was determined to be 0.43, with 90% of samples having a TF of less than 2 (Forestry Agency, 2012). A provisional limit of radiocesium allowed in logs for mushroom cultivation was then determined to be 50 Bq/kg FW (i.e., radiocesium in logs (Bq/kg) = 100 Bq/kg FW / 2) (Ministry of Agriculture, Forestry & Fisheries, 2012). Because shiitake mycelia colonizes sapwood (Tokimoto, 2005), it is presumed to obtain the majority of its nutrients exclusively from this part of a log. However, the most contaminated part of a log immediately after the accident was the bark and not the inner wood (Kuroda, Kagawa & Tonosaki, 2013). In the coming years, it is believed that radiocesium will be absorbed through the bark into the wood of trees (Mahara *et al*., 2014; Wang *et al*., 2018) and be taken up through the roots from contaminated soil (IAEA, 2006) giving rise to higher concentrations of radiocesium within logs. For example, it has been modelled that the maximum Cs-137 concentration in xylem wood (120 Bq/kg) will occur in 2039, compared with 6 – 47 Bq/kg found in contaminated oak sapwood in 2012 (Mahara *et al*., 2014). Although cultivating shiitake from oak logs grown in contaminated regions of Eastern Japan is currently not recommended, the forestry industry needs more clarity about the long-term trend of radiocesium transfer from contaminated logs to shiitake.

Stable cesium originates from the mineral component of soil, and therefore naturally exists within trees (Mahara *et al*., 2014), and can be used to understand the long-term behavior of radiocesium (Rühm *et al.*, 1999; Yoshida *et al.*, 2004; Karadeniz & Yaprak, 2007). However, a more efficient sampling method is required to obtain a representative wood sample for Cs-133 analysis because the current in-house methodology is both time and labor intensive (Fig. 1A). By cutting a log at multiple points along its length and collecting the sawdust for Cs-133 analysis would greatly facilitate the sampling of a larger number of logs; a method involving fewer steps would also minimize cross-contamination (Keith *et al*., 1983). Sawdust samples have been used previously to measure Cs-133 (Yoshida *et al*., 2004) and radiocesium concentrations in standing trees (Yoshida *et al*., 2004; Schell *et al*., 1996).

Our goal in this study was threefold. First, we tested if the sawdust-to-shiitake TF of Cs-133 was comparable to the sapwood-to-shiitake and heartwood-to-shiitake TF. Second, if differences were observed between wood and sawdust TFs, we wanted to know if it was due to a heterogeneous distribution of Cs-133 across heartwood, sapwood and bark. As Cs-133 is not an essential element for plant growth (Taiz *et al*., 2015), little is known about its distribution within trees. However, because of the similar chemical properties of cesium and potassium, it is believed Cs-133 could be translocated to the growing tissues of a tree (Wyttenbachl *et al*., 1995) such as the bark. A higher concentration of Cs-133 in the bark (i.e., the denominator in the equation to calculate the TF) would result in an underestimation of the sawdust-to-shiitake TF. Third, we determined the Cs-133 concentration in sawdust and fruiting bodies along the length of the logs. The distribution of Cs-133 will have implications for the number of sawdust and fruiting bodies samples that need to be collected to represent the entire log and mushroom crop, respectively. Based on the findings of this study, we propose two different sampling methodologies using sawdust to determine the log-to-shiitake TF of Cs-133.

Materials & Methods

Experimental design

Ten oak logs and their ready-to-eat shiitake fruiting bodies (= *Lentinula edodes*) were sampled. Each log was cut at *ca.* 10-cm intervals along its length (n = 9 discs/log). The sawdust produced from cutting each disc (n = 8 sawdust samples/log) and fruiting bodies present on each disc (n = 9 fruiting body samples/log) were collected and analyzed for Cs-133 concentration (Fig. 1B). For five of the ten logs, each 10-cm disc was separated into bark (n = 9 samples/log), sapwood (n = 9 samples/log) and heartwood (n = 9 samples/log) and also analyzed for Cs-133 concentration.

Oak logs and shiitake mushroom samples

Quercus serrata `konara` is the most common oak species grown in Eastern Japan and commonly used for shiitake cultivation in Japan. Logs with shiitake mushrooms were supplied by a grower located in Ibaraki Prefecture, Japan. This grower purchases up to 200,000 logs per year from oak suppliers throughout Japan. The grower reported that the logs used in the current study were sourced from trees felled in either December 2016 or January 2017 and allowed to dry for 1 month before inoculation. Logs were inoculated with multiple 2-cm plug spawns in either January or February 2017. Fruiting occurred in a humidity- and temperature-controlled environment with logs stored in a horizontal orientation. To minimize the influence of possible confounding factors affecting Cs-133 distribution in logs and fruiting bodies, all logs used in this study were (1) from the same species of oak (*Q. serrata*), (2) sourced from the same region (Sano-city, Tochigi Prefecture), (3) inoculated with the same strain of shiitake (F103, Fujishukin Co., Ltd., Japan), (4) managed similarly from the point of inoculation to fruiting, and (5) producing their first crop of fruiting bodies which were ready to collect and eat on the day of sampling. Selected logs needed to have at least one fruiting body present at each 10-cm interval along its length.

Collecting logs and shiitake fruiting bodies

Logs and fruiting bodies were collected in March 2018. For each log, 10-cm intervals along its length were demarcated and labelled. Fruiting bodies growing on each 10-cm disc were collected, the bottom 1 cm of the stipe was removed with a knife to eliminate any attached pieces of bark and then the fruiting bodies were placed into factory-new paper bags. When sampling was complete, logs and fruiting bodies were transported to the laboratory.

Sample preparation

Fruiting bodies

Fruiting bodies from each 10-cm disc were weight and thinly sliced (*ca.* 2 – 3 mm) before being placed loosely on a polypropylene mesh, dried overnight at 60 ºC and then at 105 ºC for 1 day and reweighed to determine the dry weight (DW). The use of a mesh avoided the fruiting bodies adhering to the inside of the paper bags while drying and the initial drying at 60 ºC ensured the sliced fruiting bodies were not too hard prior to milling. The dried fruiting bodies were milled using an Iwatani mill to a powder and placed in an air-tight factory-new plastic bags which in turn were placed into a larger air-tight plastic bag containing silica gel sachets.

Sawdust, wood and bark

The weight, length and diameter of each log was recorded. Discs were cut from each log using a circular saw (model LS1500, Makita, Japan) and the sawdust was collected using a dust collector connected to a vacuum. The wet and dry weight of each sawdust, wood and bark samples were recorded and their DW determined. Each sawdust sample was placed into a separate pre-labelled factory-new plastic container, dried for 1 day at 60 ºC and at 105 ºC until the dry weight became constant (*ca.* 1 day). To determine DW of wood and bark, each disc was first dried at 60 ºC for 2 – 3 days and then at 105 ºC until the weight became constant (*ca.* 3 days), and then separated into the bark, sapwood and heartwood parts using a hammer and chisel. We distinguished the heartwood from the sapwood by the darker color of the heartwood. Bark, sapwood and heartwood samples were crushed using Horai V-360 mill (Japan) and all parts (including sawdust) were milled to a powder using an Iwatani mill. Samples were placed into air-tight factory-new plastic bags which were then placed into a larger air-tight plastic bag containing silica gel sachets.

Cs -133 analyses

To keep the number of samples manageable and to focus our sampling efforts where variation in Cs-133 concentration was likely to be high (i.e., within logs) rather than where it was like to be low (i.e., between analytical replicates of individual samples), multiple wood, bark and fruiting body samples were collected from each log and no analytical replicates were used. To confirm the soundness of this decision, five randomly selected samples representing the materials in this study were digested in duplicate and the Cs-133 concentration measured. Cs-133 concentration between these duplicate digested samples was very low compared to the variation observed within logs (see Table 1); for example, the mean (±SD) concentration of Cs-133 in the duplicate heartwood, sapwood, bark, sawdust and fruiting body samples were 1.06±0.007, 1.40±0.006, 1.61±0.021, 1.36±0.001 and 2.64±0.001 $\log_{10}$ µg/kg DW, respectively.

Fruiting bodies, log parts and sawdust samples (*ca.* 0.3 g) were digested in 10 ml of 60% HNO_3 (Wako, Japan) using the Multiwave 3000 digester (PerkinElmer Anton Paar, Austria); samples were digested at 600 w for 40 min at ≥ 120 °C (30 bars of pressure). The digestates were diluted 1-in-5 with Milli-Q water (Merck, Germany) and filtered (Advantec 0.2 µl cellulose acetate filters; Toyo Roshi Kaisha Ltd, Japan) prior to Cs-133 analysis. The Cs-133 content was measured by inductively coupled plasma-mass spectrometry (ICP-MS, NexION 350S, PerkinElmer, Waltham, MA, USA) with indium (2 ppb) as the internal standard. To minimize variation between ICP-MS runs, wood, bark, sawdust and fruiting bodies of individual logs were analyzed in the same run. At the time of ICP-MS analysis, a subsample (0.5 g) of each sample was oven dried at 105 °C for 48 h for residual moisture determination. For quality control purposes, the National Institute of Standards and Technology standard reference material 1575a

(Pine needles) was included in each analysis. Average precision for Cs-133 was 9.7%. Cs-133 values were determined on a DW basis unless stated otherwise.

Data analysis

The heartwood-to-shiitake and sapwood-to-shiitake transfer factors (TF) were determined between each disc and the fruiting bodies growing on that disc (Eq. 1), unless stated otherwise.

$$\text{Transfer factor} = \frac{\text{Cs} - 133 \text{ concentration in fruiting bodies}}{\text{Cs} - 133 \text{ concentration in heartwood or sapwood}} \qquad (1)$$

The mean Cs-133 concentration in fruiting bodies growing on each pair of discs that produced a sawdust sample was used to calculate the sawdust-to-shiitake TF (Eq. 2).

$$\text{Transfer factor} = \frac{\text{Average Cs} - 133 \text{ concentration in fruiting bodies from disc pair}}{\text{Cs} - 133 \text{ concentration in sawdust from disc pair}} \qquad (2)$$

Cs-133 concentration values were $\log_{10}$ transformed to better conform to normality. A one-way ANOVA followed by the Tukey post-hoc test was used to compare TF values based on heartwood, sapwood, and sawdust. A similar analysis was used to compare Cs-133 concentration between heartwood, sapwood and bark and, between fruiting bodies growing on each disc and cuts containing sawdust along the length of the logs.

To demonstrate that collecting fruiting bodies from four of the nine discs (per log) would be representative of the shiitake crop on a log, Cs-133 concentration in fruiting bodies growing on odd- (i.e., 1, 3, 5, 7, 9) and even-numbered discs (i.e., 2, 4, 6, 8) were compared with the paired sample t-test. To demonstrate that the whole-log TF and wood-only TF can be determined simultaneously, the TF based on sawdust collected from odd-numbered cuts (i.e., 1, 3, 5, 7) was compared with sawdust from even-numbered cuts (i.e., 2, 4, 6, 8) (eq. 3) using the paired sample t-test.

$$\text{Transfer factor} = \frac{\text{Average Cs} - 133 \text{ concentration in fruiting bodies from a log}}{\text{Average Cs} - 133 \text{ concentration in odd} - \text{ or even} - \text{numbered sawdust samples}} \quad (3)$$

Correlations coefficients of Cs-133 concentrations in parts of the log, sawdust and shiitake fruiting bodies and their level of significance was determined. All statistical analyses were carried out using the SPSS version 25 for Mac (IBM SPSS Statistics, NY, USA). Values were deemed significantly different when $P < 0.05$.

Results

The mean and standard deviation (SD) in weight, length and diameter of the ten logs was 5 ± 1.0 kg, 94 ± 0.9 cm and 9 ± 1.0 cm, respectively. The logs and fruiting bodies had a DW of 601 ± 41.4 and 109 ± 6.8 g/kg, respectively. There were on average 36 fruiting bodies per log.

The sawdust-to-shiitake TF was numerically lower ($P > 0.05$) than both the sapwood-to-shiitake TF and heartwood-to-shiitake TF. The mean concentration of Cs-133 in the sapwood and heartwood was lower ($P = 0.025$ and $P = 0.034$, respectively) than in the bark (Table 1). Along the length of the log, Cs-133 concentration in the sawdust (Fig. 2A) and fruiting bodies (Fig. 2B) did not differ ($P > 0.05$) between cuts and discs, respectively. Cs-133 concentration in fruiting bodies collected from odd- (2.72 $\log_{10}$ µg/kg) and even-numbered discs (2.71 $\log_{10}$ µg/kg) did not differ ($P = 0.233$) (Fig. 2C) and the sawdust-to-shiitake TF from odd- (TF = 22.6) and even-numbered (TF = 23.1) cut positions did not differ (P = 0.684) (Fig. 3A). Cs-133 concentration in heartwood, sapwood, bark, sawdust and fruiting bodies were positively intercorrelated (P < 0.05; Table 2).

Discussion

Stem wood and bark have been reported to be chemically heterogeneous (Harju *et al*., 1996, 2002; Saarela *et al*., 2005), which would imply a rigorous sampling strategy is required when sampling logs for Cs-133. The current in-house methodology to ensure that shiitake fruiting bodies and wood samples for inorganic elemental analysis are representative involves cutting logs into nine 10 cm-discs, discarding the bark and mechanically breaking the wood into smaller pieces (i.e., crush and mill), and then compositing the wood from three discs to provide a total of three subsamples per log for Cs-133 analysis (Fig. 1A). Similarly, prior to cutting the log, all fruiting bodies are collected from each set of three discs, sliced and milled to provide three subsamples per log. Although this sampling method does provide a representative sample for analysis, it is very time-consuming and labor intensive to process samples. In studies that require a larger number of logs to determine the log-to-shiitake TF of Cs-133 for example, a more efficient methodology was required.

Transfer factor (TF) of Cs-133 between logs and shiitake

The average sawdust-to-shiitake TF of Cs-133 was 23 (on a DW basis) and 4.2 (on a FW basis). To the best of our knowledge, this is the first report of the TF of Cs-133 between logs and shiitake, although the TF of radiocesium was reported to be 0.43 between semi-dried logs (12% moisture content) and fresh fruiting bodies (Forestry Agency, 2012). One reason the TF of Cs-133 is higher than the TF of radiocesium (on a FW basis) is because the log-to-shiitake TF of radiocesium was determined within 1 year of the nuclear accident when only the bark was contaminated (Mahara *et al*., 2014), whereas Cs-133 was shown to be distributed through all parts of a log in this study (Table 1), and is likely to be more available for uptake.

In the current study, we found that the TF based on Cs-133 concentration in heartwood, sapwood and sawdust was not significantly different. However, heartwood and sapwood had a lower concentration of Cs-133 than bark, concurring with the findings of Wang *et al.* (2018) in 45 – 52-year-old oak trees. Even though the TF of Cs-133 based on sawdust without bark was not directly compared to wood (i.e., sapwood and heartwood combined) in this study, the numerically lower TF value based on sawdust reflects the higher concentration of Cs-133 in the bark. For shiitake cultivation, logs from trees aged *ca.* 15 – 25 years are used, and therefore it was important to confirm if Cs-133 distribution between heartwood, sapwood and bark showed a similar trend as found in older oak trees.

Sampling of the whole log using sawdust

The concentration of Cs-133 in sawdust and fruiting bodies along the length of the logs did not differ significantly and there were no unusually high concentrations in any one sawdust sample or in fruiting bodies from any one disc in individual logs (Table 1). The natural variation in Cs-133 concentration that was evident in sawdust (Fig. 2A) and fruiting bodies (Fig. 2B) can be overcome by collecting multiple samples per log during sampling. In the current study, the average concentration in eight sawdust samples was used to obtain an estimate of Cs-133 concentration in each log. An alternative approach would be to collect a number of sawdust samples per log at pre-determined locations (eight was found to be satisfactory and convenient in the current study) and then mix these samples to produce one representative sample for the whole log. Comparing Cs-133 concentration in fruiting bodies collected from odd- and even-numbered discs, we found no significant difference between these two sets of discs (Fig. 2C). With approximately four fruiting bodies on each 10-cm disc in the current study, our data suggest that collecting 16 fruiting bodies from four discs would be sufficiently representative of the shiitake crop growing on an individual log. However, if fruiting bodies were collected randomly along the length of a log to ensure at least one fruiting body per disk, 12 fruiting bodies would likely suffice. The methodology described above will be hereafter known as the whole-log sampling method (Method I) and has been illustrated in more detail in Fig. 2D-E.

Factors to consider when sampling logs and areas where further research is required

Source of Cs-133 in shiitake fruiting bodies

Unless proven otherwise, we believe that bark should be included as part of the growth substrate of shiitake when determining the TF of Cs-133. Although Tokimoto (2005) reported that shiitake obtains its nutrients from the wood component of logs, Matsumoto *et al.* (1992) showed that a fruiting body may also acquire some of its nutrients from the inner bark. For example, the amount of some individual elements (e.g., Mg in the inner bark, Na, Fe and Cu in the inner bark and sapwood) increased beneath a fruiting body during the early stages of its development, and N, P and K decreased with the maturation of the fruiting body (Matsumoto *et al.*, 1992). There is also a notable decline in yield with each successive crop of mushrooms produced on a log (Bratkovich, 1991) which is likely due to one or more essential nutrients becoming limited (Tokimoto, 2005) and therefore, shiitake may be sourcing specific nutrients from both the wood and the bark depending on their availability and accessibility during fruit body development. Vane, Drage & Snape (2006) found that shiitake will degrade the bark of oak logs if allowed to grow for an extended period of time.

Yoshida & Muramatsu (1997) and Rühm *et al.* (1999) reported the importance of identifying the soil layer from which certain species of fungi predominantly take up radiocesium, as it could lead to either an overestimation or underestimation of TFs (Rühm *et al*., 1999). In the case of shiitake, research is needed to determine what proportion of Cs-133 in fruiting bodies is sourced from the wood and bark of logs, and if this proportion changes during consecutive shiitake harvests. The availability of Cs-133 for uptake by shiitake from wood and bark would also need to be determined, as previously demonstrated between soil layers and mushrooms (Baeza, Guillén & Bernedo, 2005).

Thickness of oak bark

Bark comprises 10 – 20% of woody vascular plants (Vane, Drage & Snape, 2006) and this percentage range can affect the accuracy of the sawdust-to-log TF of Cs-133 when comparing logs cut from different tree species or trees of different age. In the current study, bark represented 19% of the total log DW but contained 38% of the total Cs-133 on a DW basis. This discrepancy was due to a 2.6-fold higher Cs-133 concentration in bark compared to wood. For researchers contemplating carrying out field trials relating to Cs-133 or radiocesium, they should consider using logs with approximately the same proportion of bark (on a DW basis) across different treatments.

Requirements of different professionals

When using Cs-133 as a proxy element to understand the long-term behavior of Cs-137, plant and mushroom physiologist may prefer to use a TF of Cs-133 based on wood only. Whereas in Japan, the government requires researchers in the food industry to report the TF of Cs-133 based on the whole log. These preferences are likely based on either tradition or belief of where shiitake sources its Cs-133 rather than from empirical evidence.

The three factors discussed above highlight the need for a more comprehensive and flexible sampling methodology. Below, we describe a second sampling methodology (Method II) to help satisfy the goals of different studies and professionals.

Simultaneous sampling of the whole log and wood only using sawdust

Using data from the present study, we simulated sampling 10 logs using sawdust to determine the sawdust-to-shiitake TF between even- and odd-numbered cuts of the whole log (i.e., bark and wood) and found no significant difference (Fig. 3A). Although we did not repeat a similar analysis for wood-only sawdust samples, we believe that the significantly positive intercorrelation of Cs-133 concentration between heartwood, sapwood and bark (Table 2) would indicate that the sawdust-to-shiitake TF between even- and odd-numbered cuts of wood-only samples would also not differ. Using this information, we showed that four sawdust samples per log can be used to represent either a whole-log or a wood-only sample. Method II involves collecting four sawdust samples per log at pre-determined locations (i.e., 1, 3, 5 and 7) and mixing these samples to produce one representative sample of the whole log. The bark is then removed from the discs and another four samples are collected at pre-determined locations (i.e., 2, 4, 6 and 8); these four samples are mixed to produce one representative sample of wood only

(Fig. 3B-C). As per method I, *ca.* 16 fruiting bodies should be collected randomly along the length of each log and composited. In Table 3, we provide some of the likely advantages and disadvantage of Methods I and II, as well as their potential uses in the field.

Conclusions

Collecting multiple sawdust and fruiting body samples per log is a robust and efficient method to provide a representative sample to determine the TF of Cs-133 from logs to shiitake. The use of sawdust will greatly reduce the time for sample collection and preparation, and this will facilitate sampling a larger number of logs in Eastern Japan to predict the future TF of radiocesium from contaminated logs. To further refine the two proposed sampling methodologies discussed, it would be important to measure the proportion of Cs-133 in shiitake fruiting bodies originating from bark and wood and its availability in each part.

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Table 1(on next page)

Transfer factors (TF) of Cs-133 and Cs-133 concentration in oak log parts, sawdust and shiitake fruiting bodies (n = 5 or 10 logs)

[1]TF values were calculated using non-$\log_{10}$transformed Cs-133 data expressed on a dry weight basis

[2]n = 5 logs

[3]The sawdust-to-shitake TF was 4.2 when expressed on the fresh weight basis

[4] Standard error of the mean

Mean values in columns in bold font with a common superscript letter do not differ significantly as determined with the Tukey post-hoc test. The mean values of logs in each column without a superscript letter were excluded from the statistical analysis.

Table 1:

Transfer factors (TF) of Cs-133 and Cs-133 concentrations in oak log parts, sawdust and shiitake fruiting bodies (n = 5 or 10 logs).

Log ID	Transfer factor[1]			Cs-133 concentration (log_{10} µg/kg dry weight)		
	Mean	SD	Range	Mean	SD	Range
Heartwood (n = 9 discs/log)						
1	40	10.4	20 – 55	1.3	0.12	1.1 – 1.5
2	23	4.8	13 – 28	1.8	0.15	1.6 – 2.1
3	24	8.4	15 – 43	1.4	0.10	1.3 – 1.6
4	32	9.7	13 – 48	1.1	0.17	0.9 – 1.5
5	30	7.5	20 – 42	1.1	0.08	1.0 – 1.2
Mean ± SD[2]	**29 [a] ± 6.9**			**1.3 [a] ± 0.27**		
Sapwood (n = 9 discs/log)						
1	31	6.6	20 – 41	1.4	0.08	1.3 – 1.5
2	22	5.8	13 – 29	1.8	0.16	1.6 – 2.1
3	25	7.6	18 – 43	1.4	0.07	1.3 – 1.5
4	29	6.0	18 – 36	1.1	0.12	1.0 – 1.4
5	27	7.5	16 – 38	1.2	0.11	1.0 – 1.4
Mean ± SD[2]	**27 [a] ± 3.3**			**1.4 [a] ± 0.25**		
Bark (n = 9 discs/log)						
1	-	-	-	1.9	0.06	1.8 – 2.0
2	-	-	-	2.1	0.13	2.0 – 2.4
3	-	-	-	1.8	0.08	1.6 – 1.9
4	-	-	-	1.6	0.06	1.5 – 1.7
5	-	-	-	1.7	0.05	1.6 – 1.8
Mean ± SD[2]	-			**1.8 [b] ± 0.18**		
Sawdust (n = 8 cuts/log)						
1	27	3.7	22 – 31	1.4	0.05	1.4 – 1.5
2	22	3.4	16 – 26	1.7	0.09	1.7 – 2.0
3	24	3.9	18 – 30	1.4	0.04	1.3 – 1.5
4	26	2.9	22 – 31	1.2	0.05	1.1 – 1.2
5	19	4.9	14 – 29	1.3	0.10	1.1 – 1.4
6	21	6.8	16 – 37	1.6	0.11	1.3 – 1.7
7	27	5.7	21 – 37	1.1	0.09	1.0 – 1.2
8	26	7.8	14 – 38	1.0	0.14	0.8 – 1.2
9	23	3.8	21 – 29	1.6	0.05	1.5 – 1.6
10	15	2.8	11 – 20	1.3	0.08	1.1 – 1.4
Mean ± SD[2] (logs 1 – 5)	**24 [a] ± 3.3**			1.4 ± 0.22		
Mean ± SD[3](logs 1 – 10)	23 ± 4.0			1.4 ± 0.23		
Fruiting bodies (n = 9 discs/log)						
1	-	-	-	2.9	0.06	2.8 – 3.0
2	-	-	-	3.1	0.05	3.0 – 3.2
3	-	-	-	2.8	0.06	2.7 – 2.9
4	-	-	-	2.6	0.07	2.4 – 2.7
5	-	-	-	2.6	0.04	2.5 – 2.6
6	-	-	-	2.9	0.02	2.8 – 2.9
7	-	-	-	2.5	0.04	2.5 – 2.6
8	-	-	-	2.4	0.06	2.3 – 2.5
9	-	-	-	2.9	0.04	2.5 – 2.6
10	-	-	-	2.5	0.05	2.4 – 2.5
Mean ± SD[2] (logs 1 – 5)	-	-	-	2.8 ± 0.22		

Mean ± SD (logs 1 – 10)	-	-	-	2.7 ± 0.23
SEM[4]	1.31			0.08
Significance	0.223			0.016

[1] TF values were calculated using non-log_{10} transformed Cs-133 data expressed on a dry weight basis

[2] n = 5 logs

[3] The sawdust-to-shitake TF was 4.2 when expressed on the fresh weight basis

[4] Standard error of the mean

Mean values in columns in bold font with a common superscript letter do not differ significantly as determined with the Tukey post-hoc test. The mean values of logs in each column without a superscript letter were excluded from the statistical analysis.

Table 2 (on next page)

Correlation of Cs-133 concentration between parts of oak logs, sawdust and shiitake fruiting bodies (n = 5 logs)

*P<0.05, **P<0.01

Table 2:

Correlation of Cs-133 concentration between oak log parts, sawdust and shiitake fruiting bodies (n = 5 logs).

	Heartwood	Sapwood	Bark	Sawdust
Sapwood	0.982**			
Bark	0.878*	0.952*		
Sawdust	0.939*	0.974**	0.965**	
Fruiting bodies	0.938*	0.982**	0.971**	0.947*

*P<0.05, **P<0.01

Table 3(on next page)

Sampling methodologies to determine the transfer factor (TF) of Cs-133 between logs and shiitake

[1] between methods I and II and compared to the in-house sampling method using wood (Fig. 1A).

[2] the number of logs that should be sampled will depend on the kind of information required by the investigation.

[3] the proportion of Cs-133 in shiitake fruiting bodies originating from bark and wood is not known, nor its availability for uptake by shiitake from each part.

Table 3:

Sampling methodologies to determine the transfer factor (TF) of Cs-133 between logs and shiitake.

Sampling methodology	Advantages[1]	Disadvantages[1]	Potential use[2]
Method I – whole log	• Lower sampling costs – no need to remove the bark before cutting • Suitable for large sample sizes • Sampling a larger number of logs ensures the sample mean TF ($\bar{x}$) will be closer to the population mean TF (μ) (cf central limit theorem)	• On an individual log basis, the TF may be slightly underestimated because of a higher Cs-133 concentration in bark[3]	• Surveys to determine the TF throughout a region/country • Field trials
Method II – whole log & wood only	• Comprehensive – likely to satisfy the requirements of different studies and professionals who need to know the TF based on either the whole-log, wood-only samples or both materials • From a food safety perspective, it would be important to know the upper limit of radiocesium transfer to shiitake based on wood-only samples[3]	• Higher sampling costs associated with removing the bark from each log • Higher analytical costs associated with analysing triplicate samples for both the whole log and wood only • Less suitable for large sample sizes because of higher sampling and analytical costs	• Studies that require the TF to be reported based on both the whole-log and wood-only samples

[1] between methods I and II and compared to the in-house sampling method using wood (Fig. 1A).

[2] the number of logs that should be sampled will depend on the kind of information required by the investigation.

[3] the proportion of Cs-133 in shiitake fruiting bodies originating from bark and wood is not known, nor its availability for uptake by shiitake from each part.

Figure 1(on next page)

Sampling and preparing a representative wood and sawdust sample for Cs-133 analysis.

(A) In-house sampling method using wood. (B) The current study sampling method using sawdust. Fruiting bodies were collected prior to cutting the logs in both methods.

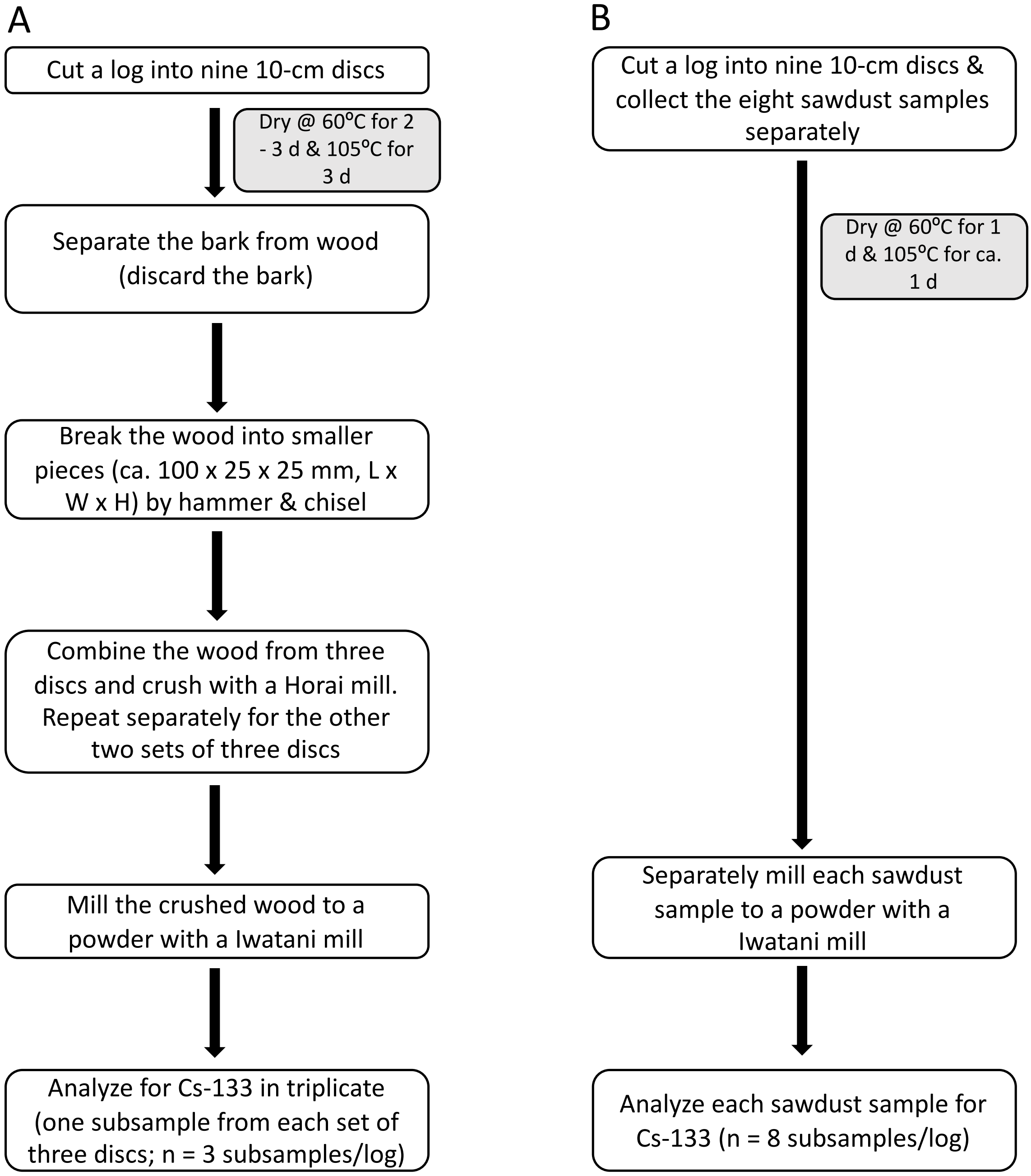

Figure 1. Sampling and preparing a representative wood and sawdust sample for Cs-133 analysis. (A) In-house sampling method using wood. (B) The current study sampling method using sawdust. Fruiting bodies were collected prior to cutting the logs in both methods.

Figure 2 (on next page)

Development of a whole-log sampling methodology (Method I)

(A) Cs-133 concentration (mean ± SD) in sawdust collected from eight positions along the length of the logs (n = 10 logs). The concentration in sawdust did not differ between cut positions (standard error of the mean (SEM), 0.03 log_{10}µg/kg dry weight (DW); $P > 0.05$, one-way ANOVA). (B) Cs-133 concentration (mean ± SD) in fruiting bodies collected from nine discs along the length of the logs (n = 10 logs). The concentration in fruiting bodies did not differ between discs (SEM, 0.02 log_{10}µg/kg DW; $P > 0.05$, one-way ANOVA). (C) Cs-133 concentration (mean ± SEM) in fruiting bodies collected from odd- and even-numbered discs (n = 10 logs). (D) Locations where logs were cut in the current study and cut positions proposed for Method I. (e) An overview of Method I.

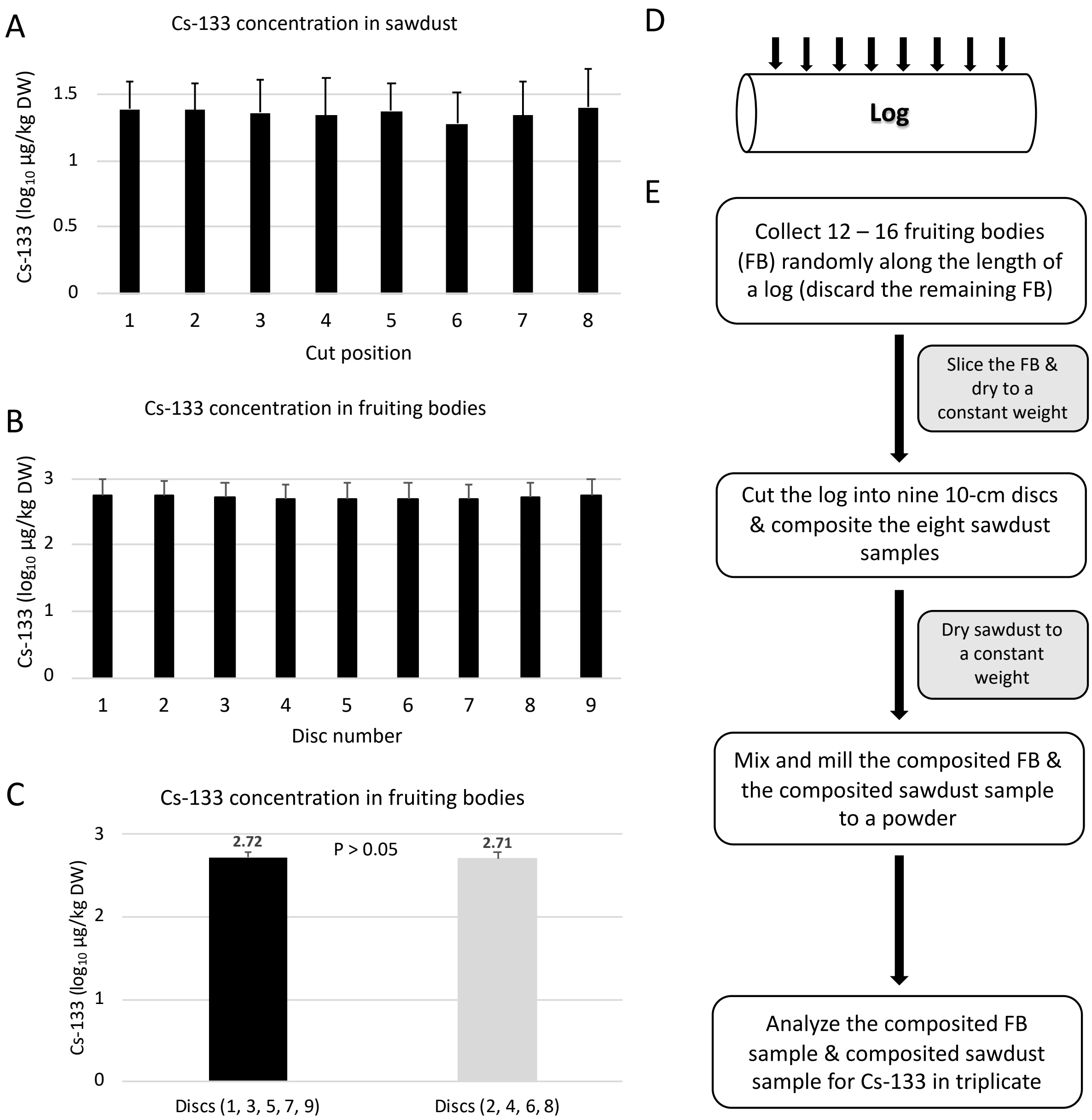

Figure 2. Development of a whole-log sampling methodology (Method I). (A) Cs-133 concentration (mean ± SD) in sawdust collected from eight positions along the length of the logs (n = 10 logs). The concentration in sawdust did not differ between cut positions (standard error of the mean (SEM), 0.03 $\log_{10}$ µg/kg dry weight (DW); $P > 0.05$, one-way ANOVA). (B) Cs-133 concentration (mean ± SD) in fruiting bodies collected from nine discs along the length of the logs (n = 10 logs). The concentration in fruiting bodies did not differ between discs (SEM, 0.02 $\log_{10}$ µg/kg DW; $P > 0.05$, one-way ANOVA). (C) Cs-133 concentration (mean ± SEM) in fruiting bodies collected from odd- and even-numbered discs (n = 10 logs). (D) Locations where logs were cut in the current study and cut positions proposed for Method I. (e) An overview of Method I.

Figure 3 (on next page)

Development of a whole-log and wood-only sampling methodology (Method II)

(A) The transfer factor (TF) of Cs-133 between the odd- and even-numbered whole-log sawdust samples; error bars are standard error of the mean (n = 10 logs). The TF was calculated based on Eq. 3 (see method section). (B) Locations where logs were cut in the current study and cut positions proposed for Method II. (C) An overview of Method II. It is recommended that 16 fruiting bodies are collected before cutting, and then mixed, milled and analyzed in triplicate for Cs-133.

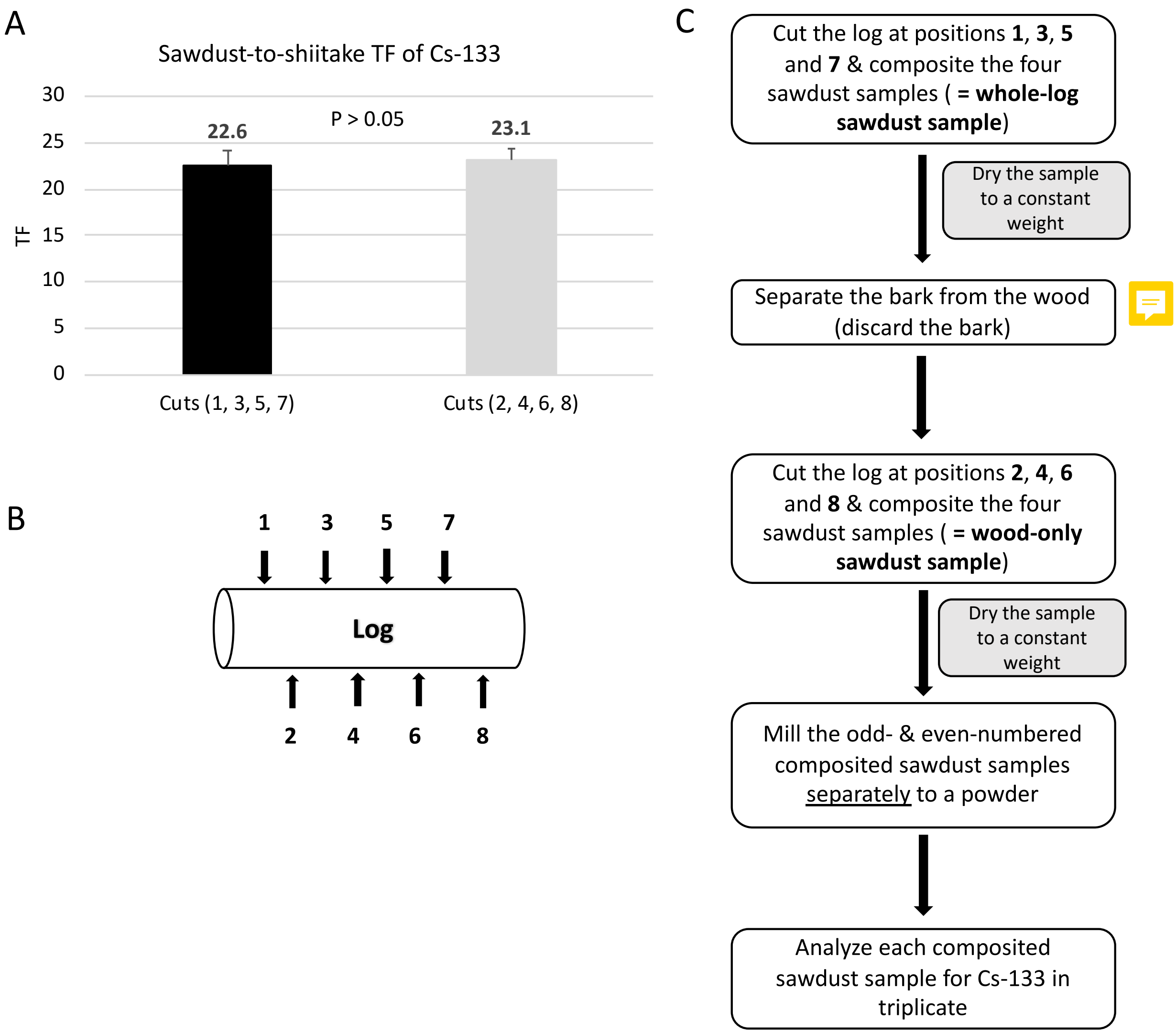

Figure 3. Development of a whole-log and wood-only sampling methodology (Method II). (A) The transfer factor (TF) of Cs-133 between the odd- and even-numbered whole-log sawdust samples; error bars are standard error of the mean (n = 10 logs). The TF was calculated based on Eq. 3 (see method section). (B) Locations where logs were cut in the current study and cut positions proposed for Method II. (C) An overview of Method II. It is recommended that 16 fruiting bodies are collected before cutting, and then mixed, milled and analyzed in triplicate for Cs-133.