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Sampling design and required sample size for evaluating contamination levels of ^{137}Cs in Japanese fir needles in a mixed deciduous forest stand in Fukushima, Japan☆

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ABSTRACT

We estimated the sample size (the number of samples) required to evaluate the concentration of radiocesium (^{137}Cs) in Japanese fir (*Abies firma* Sieb. & Zucc.), 5 years after the outbreak of the Fukushima Daiichi Nuclear Power Plant accident. We investigated the spatial structure of the contamination levels in this species growing in a mixed deciduous broadleaf and evergreen coniferous forest stand. We sampled 40 saplings with a tree height of 150 cm–250 cm in a Fukushima forest community. The results showed that: (1) there was no correlation between the ^{137}Cs concentration in needles and soil, and (2) the difference in the spatial distribution pattern of ^{137}Cs concentration between needles and soil suggest that the contribution of root uptake to ^{137}Cs in new needles of this species may be minor in the 5 years after the radionuclides were released into the atmosphere. The concentration of ^{137}Cs in needles showed a strong positive spatial autocorrelation in the distance class from 0 to 2.5 m, suggesting that the statistical analysis of data should consider spatial autocorrelation in the case of an assessment of the radioactive contamination of forest trees. According to our sample size analysis, a sample size of seven trees was required to determine the mean contamination level within an error in the means of no more than 10%. This required sample size may be feasible for most sites.

1. Introduction

Once radioactive materials are artificially released into a natural environment, we need to promptly assess the contamination levels of various organisms including humans (cf. Science Council of Japan, 2011). The contamination levels of a variety of species including wild plants (Papastefanou et al., 1989; Davids and Tyler, 2003; Yoshihara et al., 2013) and animals (McGee et al., 2000; Moller et al., 2012; Hayama et al., 2013; Kubota et al., 2015; Matsui et al., 2015) were assessed after the Chernobyl and Fukushima nuclear reactor accidents.

Generically, the accumulation of pollutants into plant bodies is affected by physiological and ecological plant characteristics as well as environmental factors such as meteorological phenomena and soil types (Baker, 1983). The use of plants as a biomonitor for environmental pollution needs to understand factors that influence the accumulation of pollutants into plant body and to standardize sampling methods, sample procedure and statistical analysis (Wagner, 1993).

Spatial structures of distributions of organisms often affect the results of statistical test. In nature, organisms always have some functional spatial structures (Legendre and Legendre, 1998). The first law of geography, which is 'everything is related to everything else, but near things are more related than distant things' proposed by Tobler (1970), can apply to the spatial structure in ecology. For example, plant populations often show aggregated spatial distribution caused by endogenous factors (e.g. dispersal, spatial competition) and/or exogenous factors (e.g. the heterogeneity of environmental conditions) (Fortin and Dale, 2005). This spatial distribution pattern is known as spatial autocorrelation. According to Legendre (1993), spatial autocorrelation is loosely defined as the property of random variables taking values, as pairs of locations a certain distance apart, that are more similar (positive autocorrelation) or less similar (negative autocorrelation) than expected for randomly associated pairs of observations. When spatial autocorrelation exists among ecological data, there are some relationships among the data depending on spatial distance. Because of the

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relationships among the data, these data are not independent of each other (Legendre and Legendre, 1998) and the lack of independence among the data affect the result of statistical analysis (e.g. underestimation of the variance of the mean) (Fortin and Dale, 2005). However, if we obtain information about spatial autocorrelation, its information can assist in designing sampling methods (e.g. the distance between observations). In addition, the information can be used to adjust the underestimated variance (Cressie, 1993), and this adjustment leads to more reliable estimation of the appropriate number of samples (Petersen and Calvin, 1986). Therefore, understanding the spatial structure of observations plays an important role in good sample design and statistical analysis (Legendre et al., 2002). However, the spatial structure of radioactive contamination of forest tree species has been rarely studied.

When investigating environmental pollution such as aerial and soil pollution, tree leaves are often used as biomonitors (Sawidis et al., 1995; Moreno et al., 2003; Tomašević et al., 2004; Maher et al., 2008). In the case of radioactive contamination, many studies have been conducted examining the accumulation of radiocesium (^{137}Cs) in leaves, especially conifer needles. In this study, we investigated the contamination levels in needles of Japanese fir trees (*Abies firma* Sieb. & Zucc.) contaminated by ^{137}Cs released from the Fukushima Daiichi Nuclear Power Plant (FDNPP) to clarify the spatial structure of the radioactive pollution of this species. *A. firma* is a common large evergreen coniferous tree species of forests in Fukushima. It is a shade-tolerant species whose saplings are abundant in shaded understory. This paper addresses following objectives; (1) to clarify the concentration of ^{137}Cs in current year needles of this species 5 years after the FDNPP accident; (2) to examine whether spatial autocorrelation exists among trees contaminated with ^{137}Cs; and (3) to estimate the number of samples that were necessary and sufficient (i.e. required sample size) to assess the contamination level of this tree species using the results of spatial analysis. The final goal of this study is to provide information about sampling methods for the assessment of contaminated forest trees.

2. Materials and methods

2.1. Study sites

The study was conducted in a deciduous broadleaf (*Quercus crispula* and *Cornus macrophylla*) and evergreen coniferous (*Pinus densiflora* and *A. firma*) mixed forest located in Soma, Fukushima Prefecture (N37°45′41, E140°47′52) (Fig. 1). In a preliminary survey, two plots (20 m × 20 m) set in the forest showed that a tree density of 2825 trees (over 5 cm in DBH) ha^{-1} and basal area of 45.5 m^2 ha^{-1}. 24 tree species were recorded in the plots. The plots were dominated by *Q. crispula* and *A. firma* accounting for 32.7% and 12.8% of total tree number, respectively. The trees of *Q. crispula*, *Quercus serrata*, *P. densiflora* and *A. firma* made up canopy layer at about 20 m from the ground. No bare land was located in the forest. Soil types of the study site is brown forest soil. The forest was located approximately 45 km northwest of the FDNPP. According to the nearest meteorological observation point (approximately 12 km southwest of the study site), the average annual precipitation from 1981 to 2010 was 1361.1 mm and the average annual temperature from 1981 to 2010 was 10.0 °C (Japan Meteorological Agency, 2016). According to the results of the third airborne monitoring survey conducted from 31 May to 2 July in 2011, the initial deposition of ^{137}Cs around the study site was 100–300 kBq/m^2 (MEXT, 2011).

2.2. Samples and ^{137}Cs measurements

The sampling was carried out in March 2016. First, we selected 40 saplings varying in height from 150 cm to 250 cm. We recorded the coordinates of each tree by a level survey using a compass and a laser rangefinder (TruePulse 200, Centennial, Colorado, USA) and measured the tree height with a measuring tape.

Previous studies have commonly reported a large inter-individual variability of ^{137}Cs in the same tree species. For example, Ertel and Ziegler (1991) reported that the needles of *Picea abies* (L.) Karst which was most highly contaminated with ^{137}Cs had contamination levels two orders of magnitude higher than the least contaminated ones in a southeast Bavarian forest in 1985. A large intra-individual variability in an individual tree is one of the factors that a large inter-individual variability is generated. For example, in the case of evergreen coniferous species, the contamination levels of needle-leaves in an individual tree showed a large variation between branches depending on the age of branches (Sombre et al., 1994), the size of trees (Thiry et al., 2002) and the height at which the branch was located (Yoshihara et al., 2014). Generally, a large variation among environmental samples masks the effect being examined by statistical tests and lessens the power of statistical tests (Cohen, 1988). Therefore, we used a sampling method designed to exclude the factors causing a large intra-individual variability (tree size, branch age and branch height) to decrease the variation between individuals. Branch growth in *A. firma* species occurs over spring and summer, sprouting from the ends of the previous year's growth. Buds are produced from summer to winter and remain dormant until branch growth starts again the following spring. This growth pattern allows us to identify branch age by measuring the number of nodal units. We sampled a lateral branch of the annual trunk that had grown in 2015 to standardize the sampling branch age (current year branches) and position on an individual tree. At the same time, we removed the litter layer and collected soils at a depth of 0–5 cm within a 15 cm radius from the base of trees using a soil core sampler (φ50 × 51 mm in height, 100 ml in volume). One soil sample was taken per tree. Sampled branches were oven-dried at 65 °C for 72 h and needles were removed from the branches. The needles were pulverized using a powdering machine (Tube Mill control, IKA Japan K.K., Osaka, Japan) and packed into a plastic container (U-9 container, 50 ml). Soil samples were oven-dried at 80 °C for 72 h and sieved using a 2 mm mesh sieve to remove plant roots and pebbles. After that, soil samples were packed into a plastic container (U-9 container, 50 ml).

The ^{137}Cs concentration in samples was determined using a low background germanium detector (GEM30-70, Seiko EG & G Co., Ltd., Tokyo, Japan). The detection efficiency of gamma-rays are reported separately (Shizuma et al., 2016). The measuring time was 10,000 s for each sample. Measurement uncertainty averaged under 10% for all samples. The ^{137}Cs concentration was converted to that on the sampling date based on the physical decay rate.

2.3. Statistical analysis

The concentration of ^{137}Cs in needles and soil were both transformed to a natural logarithm to meet the criteria of normal distribution (Kolmogorov-Smirnov Test, needles: $p = 0.072$, soil: $p = 0.2$). We calculated the sample size required from the sample variance as follows. We tested the spatial patterns of ^{137}Cs concentrations from sampled tree needles and soils for spatial autocorrelation using Moran's I statistics. The Moran's I statistic at

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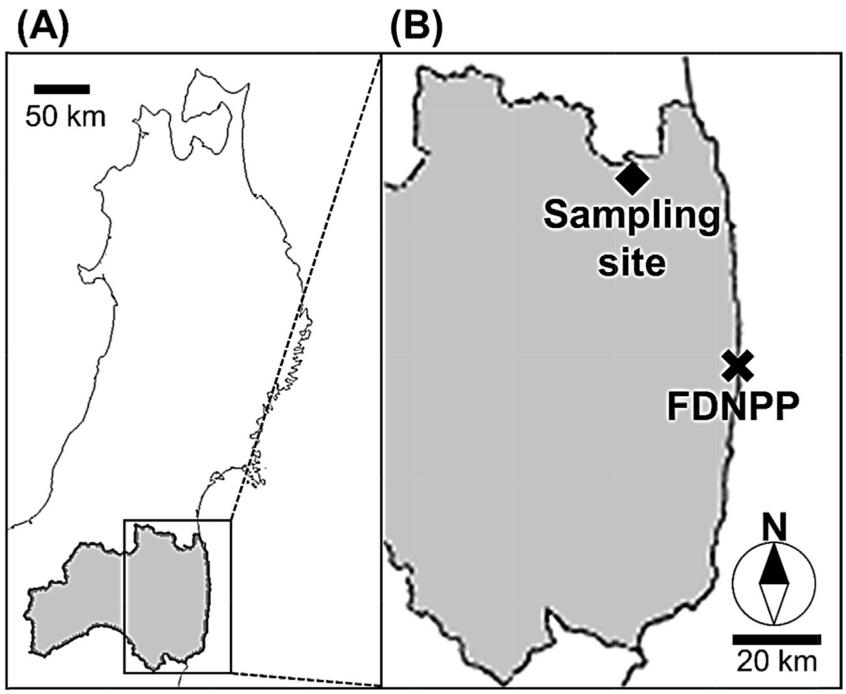

Fig. 1. Maps showing the location of the sampling site and the Fukushima Daiichi Nuclear Power Plant (FDNPP), Fukushima, Japan. Gray parts represent Fukushima Prefecture.

distance class d ($I(d)$) is calculated from the following equation (1):

$$I(d) = \frac{\left(\frac{1}{W(d)}\right)\sum_{i=1}^{n}\sum_{j=1}^{n} w_{ij}(d)(x_i - \overline{x})(x_j - \overline{x})}{\frac{1}{n}\sqrt{\sum_{i=1}^{n}(x_i - \overline{x})^2}}, \quad (1)$$

where $wij\,(d)$ is the distance class connectivity matrix that indicates whether a pair of sampling locations are in distance class d, xi and xj are the values of the ^{137}Cs concentrations (x) at sampling location i and j, and $W\,(d)$ is the sum of $wij\,(d)$ (Fortin and Dale, 2005). Values for the Moran's I statistics run from −1 to +1 (Fortin and Dale, 2005). Positive values indicate positive autocorrelation, and negative values show negative autocorrelation. If the value is close to 0, there is no significant autocorrelation (Fortin and Dale, 2005). Fifty distance classes with 2.5 m distance intervals were used. The significance of the Moran's I statistics were obtained by a randomization test of 5000 permutations. This analysis was carried out using the software package R version 3.3.2 (R Core Team, 2016).

We calculated the sample size required (n_{req}) using the equation by Petersen and Calvin (1986) as follows:

$$n_{req} = t_{0.95}^{2} s^{2} \Big/ D^{2}, \quad (2)$$

where $t_{0.95}$ is the Student's t statistic with n-1 degrees of freedom at the 95% probability, s^2 is the sample variance of the ^{137}Cs concentration in needles, and D is the specified error limit. D denotes the allowable 95% confidence interval of the mean. In this study, we estimated the n_{req} to be the D smaller than or equal to 10% and 20% of the sample mean. When a positive spatial autocorrelation exists, the observed sample variance is known to be underestimated compared with the true variance (Fortin and Dale, 2005). The underestimation leads us to accept a smaller n_{req} than really exists. We corrected the underestimation of observed sample variance when a significant positive autocorrelation was found as follows. First, we calculated the correction factor (θ) following Cressie (1993). The correction factor is expressed by $I\,(d)$ (Moran's I statistics) as:

$$\theta = \left[1 + 2\frac{I(d)}{1 - I(d)}\left(1 - \frac{1}{n}\right) - 2\frac{I(d)^2}{(1 - I(d))^2}\,\frac{1 - I(d)^{n-1}}{n}\right] \quad (3)$$

Then equation (2) was modified as:

$$n_{req} = t_{0.95}^{2}\theta s^{2} \Big/ D^{2} \quad (4)$$

3. Results

3.1. ^{137}Cs concentration in needles and soil

The maximum (minimum) values of ^{137}Cs concentrations in the needles of lateral branches of annual trunk of Japanese fir and soil under the sampled trees were 7020 (156) Bq/kg-DW and 38,100 (3940) Bq/kg-DW, respectively. The geometric mean ^{137}Cs concentration in the needles was 1530 Bq/kg-DW, whereas in the soil the mean was 10,500 Bq/kg-DW. The standard deviation of ^{137}Cs concentration in needles and soil ranged from 738 to 3190 Bq/kg-DW and from 6340 to 17,600 Bq/kg-DW, respectively. There was no significant correlation between the ^{137}Cs concentration in needles and soil (Pearson's correlation coefficient $r = 0.217$, $p = 0.179$).

The locations and contamination levels of needles taken from target trees and soil are shown in Fig. 2. The Moran's I correlogram of the ^{137}Cs concentration in needles (Fig. 3a) showed strong significant positive autocorrelation at the distance class from 0 to 2.5 m (Moran's I autocorrelation coefficient, $I\,(d) = 0.375$, $p < 0.01$). In addition, 6 other significant autocorrelations were found. The Moran's I of soil showed no significant spatial autocorrelation at the 0–2.5 m distance class (Fig. 3b). The pattern of Moran's I spatial correlogram of the ^{137}Cs concentration of needles showed a quite different pattern from that of soils (Fig. 3a and b).

3.2. The number of required samples

Because the ^{137}Cs concentration in needles showed a significant

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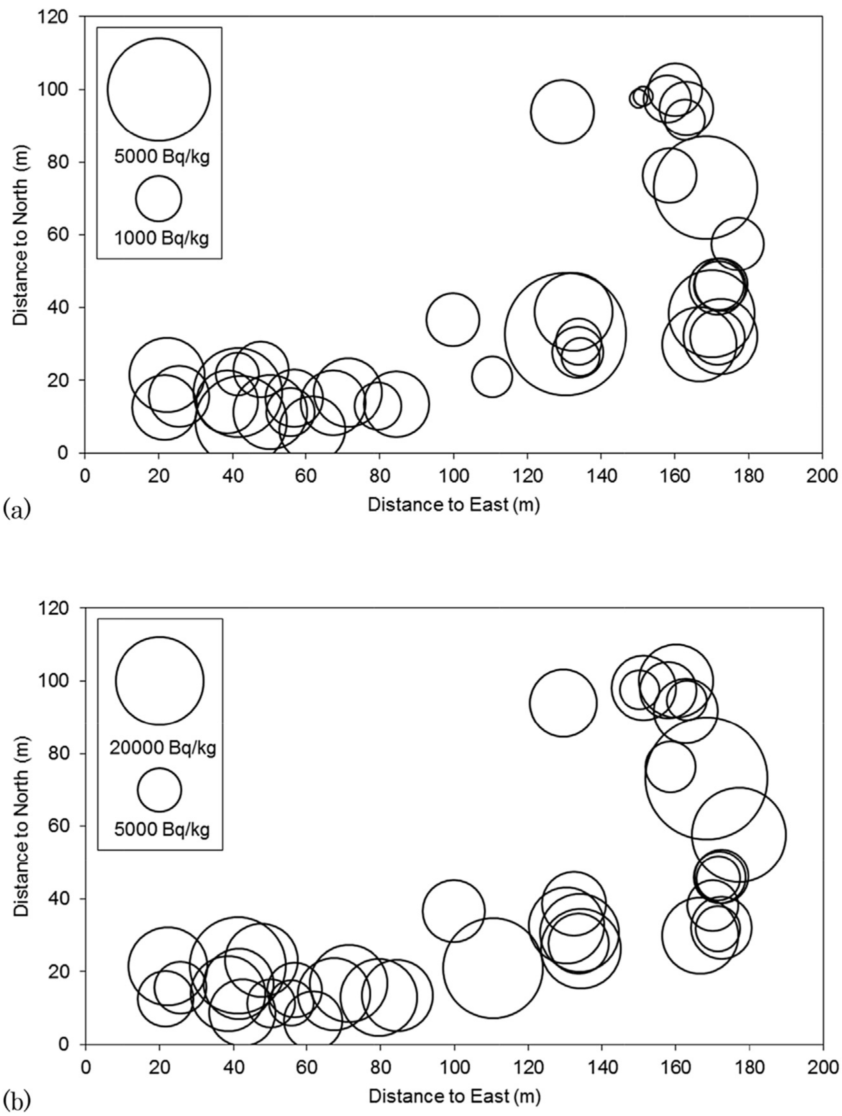

Fig. 2. The locations of sampled trees of *A. firma* in the study area (n = 40). The size of circles shows (a) the concentration of ^{137}Cs in current year needles of lateral branches of annual trunk growing in 2015, (b) the concentration of ^{137}Cs in soil (0–5 cm) under the trees. Note that the size of circles shows different concentration of ^{137}Cs between (a) and (b).

positive autocorrelation at the distance class of less than 2.5 m, we corrected the observed variance of the ^{137}Cs concentration in needles to calculate the required sample sizes. After correction, the required sample sizes were calculated as seven and two for allowable margins of error of 10% and 20% in the sample mean at the 95% probability level, respectively.

4. Discussion

The contamination levels of ^{137}Cs, 5 years after the FDNPP accident, in *A. firma* sapling current year needles that sprouted in 2015 were shown in Fig. 2. Kato et al. (2015) reported that the concentrations of ^{137}Cs in throughfall and stemflow were low about 2 years after the FDNPP accident, especially in a mixed broad-leaved stand. We thus assumed that the possibility of external contamination and absorption from the surface of needles in this study was low. From the results that (1) there was no correlation between the ^{137}Cs concentration in sampled needles and soil, and (2) there was a difference in the spatial distribution pattern of the ^{137}Cs concentration between needles and soil, we deduce that the contribution of root uptake to the concentration of ^{137}Cs in new needles in this study may be small 5 years after the radionuclides were released into the atmosphere. Nishikiori et al. (2015) also indicated that there was a small contribution of root uptake to contamination in new leaves of *Cryptomeria japonica* that sprouted in 2012 in forest located approximately 160 km southwest of the FDNPP, and that ^{137}Cs in new leaves was translocated from other tree parts. We conclude that the ^{137}Cs concentration in new needles in the present study may be largely explained by the translocation of radiocesium within the tree body.

As far as we know, this is the first study to examine the geostatistical characteristics of ^{137}Cs in trees. Our study showed a strong positive spatial autocorrelation of ^{137}Cs in trees at the distance class within 2.5 m. Although the underlying mechanism creating the spatial autocorrelation remains unclear, a spatial autocorrelation over a short distance might be applicable to other tree species growing in a similar environment. Spatial autocorrelation affects accurate sampling in that the presence of a significant positive autocorrelation violates the independence of observations, resulting in an underestimation of the variance of sampling means

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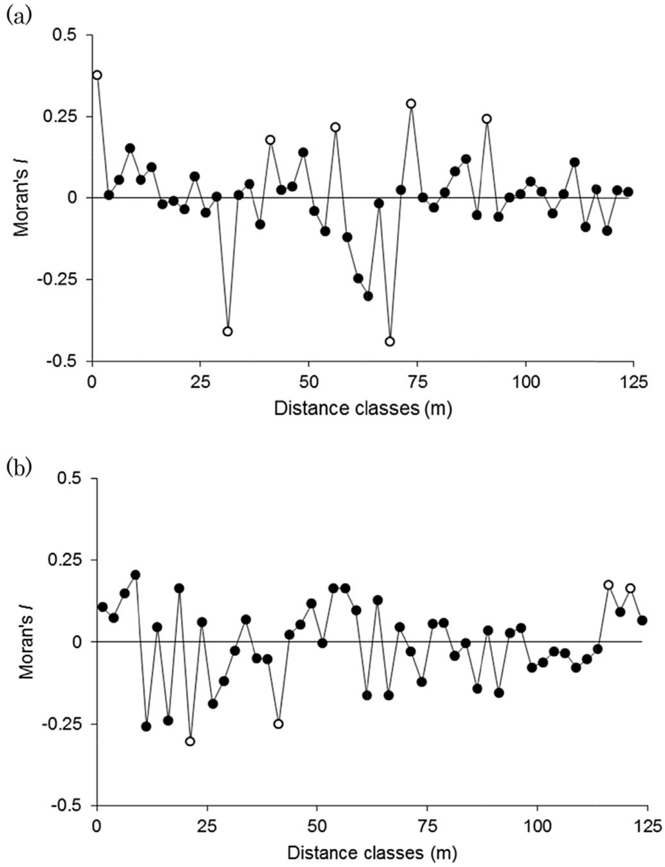

Fig. 3. Spatial correlograms of the concentration of ^{137}Cs in (a) needles of lateral branches of annual trunks, and (b) soil under the sampling trees. Open circles show significant Moran's *I* statistics ($p < 0.01$) and closed circles show nonsignificant ones. Significance of Moran's *I* statistics was obtained by the randomization test of 5000 permutations. Distance class is at intervals of 2.5 m. Straight line shows Moran's $I = 0$.

(Fortin and Dale, 2005). According to Fortin and Dale (2005), it is important to understand the characteristics of spatial autocorrelation before the start of research. This understanding will make it possible to design sampling methods that avoid spatial autocorrelation, or to reduce the effect of spatial autocorrelation on following analyses. They suggest, therefore, that a pilot study for the design of sampling is required. However, the assessment of radioactive contamination of the environment is often urgent (Science Council of Japan, 2011). Therefore, the best sampling methods would be designed to allow the analysis of a spatial pattern of radioactive contamination of forest trees at the same time that an assessment of contamination levels of forest trees is conducted. For example, the locations of trees are recorded to examine whether a significant positive spatial autocorrelation is present in a study plot. If a significant positive spatial autocorrelation is detected, an observed sample variance can be corrected using a correction factor calculated using Moran's *I* statistics. Our results showed six additional significant autocorrelations in the ^{137}Cs concentration in needles in some distance classes. However, the processes underlying these autocorrelations are unclear. Further investigations are needed to clarify them.

The accumulation of ^{137}Cs into tree leaves is affected by various factors. Yoshihara et al. (2014) reported that the concentration of ^{137}Cs in leaves differed between leaves near the top of the tree and those in the lower parts of the tree. In addition, the concentrations of ^{137}Cs in leaves were affected by the leaf age (Sombre et al., 1994; Yoshida et al., 2004). If the sampling design ignores these factors, the variance of ^{137}Cs between trees may become unnecessarily large. A large variation between individuals may be reduced by a well-designed sampling method that excludes factors caused by a large intra-individual variability (Fisher, 1966). It is therefore necessary to standardize the characteristics of sampled branches for the assessment of radioactive contamination of forest trees. This allows us to minimize variation generated by poor sampling methods, resulting in a small variance between trees. According to our sample-size analysis based on the observed variance between trees, the sample sizes required to fall within the 95% confidence limit of the population mean of ^{137}Cs concentrations, with allowable margins of error of 10% and 20% of the population mean, were seven and two, respectively. These sample sizes would be feasible for many case studies. Additional studies are needed to test if our results are applicable to other forest stands and other tree species.

We found the spatial structure of radioactive contamination of trees growing in a mixed deciduous broadleaf and evergreen coniferous forest. In the present study, however, the results were derived from only one forest stand. Several studies reported that the dynamics of ^{137}Cs in forest ecosystems depend on the type of forest stand (Melin et al., 1994; Goor and Thiry, 2004; Komatsu et al., 2016). Thus, based on the present analysis, further investigations are needed to clarify the spatial structure of contamination of various forest stands, including stands in plantation and natural forests. Additionally, using these results, it is necessary to develop methods of scaling up from a stand-level evaluation to a forest-level evaluation in assessing the overall contaminated forest areas in future work.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2017.02.023.

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