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

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**A B S T R A C T**

We estimated the sample size (the number of samples) required to evaluate the concentration of radiocaesium ($^{137}\text{Cs}$) 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 $^{137}\text{Cs}$ concentration in needles and soil, and (2) the difference in the spatial distribution pattern of $^{137}\text{Cs}$ concentration between needles and soil suggest that the contribution of root uptake to $^{137}\text{Cs}$ in new needles of this species may be minor in the 5 years after the radionuclides were released into the atmosphere. The concentration of $^{137}\text{Cs}$ 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.

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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., 2008). In the case of radioactive contamination, many studies have been conducted examining the accumulation of radionuclides in leaves, especially conifer needles. In this study, we examined whether spatial autocorrelation exists among trees from the Fukushima Daiichi Nuclear Power Plant (FDNPP) to clarify the contamination level of this tree species using the results of airborne monitoring surveys (Legendre et al., 2002). However, the spatial structure of radioactive contamination of forest trees has been rarely studied.

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 m² 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 samples were oven-dried at 80°C and sieved using a powdering machine (Tube Mill control, IKA Japan K.K., Osaka, 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 137Cs concentration was converted to that on the sampling date based on the physical decay rate.

2.3. Statistical analysis

The concentration of 137Cs 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 137Cs concentrations from sampled tree needles and soils for spatial autocorrelation using Moran’s I statistics. The Moran’s I statistic at...
distance class \(d\) \((I(d))\) is calculated from the following equation (1):

\[
I(d) = \frac{1}{\sum_{i=1}^{n} \sum_{j=1}^{n} w_{ij}(d)(x_i - \bar{x})(x_j - \bar{x})}{\frac{1}{n} \sqrt{\sum_{i=1}^{n} (x_i - \bar{x})^2}},
\]

where \(w_{ij}(d)\) is the distance class connectivity matrix that indicates whether a pair of sampling locations are in distance class \(d\). \(x_i\) and \(x_j\) are the values of the \(^{137}\text{Cs}\) concentrations \((x)\) at sampling location \(i\) and \(j\), and \(W(d)\) is the sum of \(w_{ij}(d)\) \((\text{Fortin and Dale, 2005})\). Values for the Moran’s \(I\) statistics run from \(-1\) to \(+1\) \((\text{Fortin and Dale, 2005})\). Positive values indicate positive autocorrelation, and negative values show negative autocorrelation. If the value is close to 0, there is no significant autocorrelation \((\text{Fortin and Dale, 2005})\). 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 \((\text{R Core Team, 2016})\). We calculated the sample size required \((n_{req})\) using the equation by \text{Petersen and Calvin (1986)} as follows:

\[
n_{req} = \frac{t_{0.95}^2 s^2}{D^2},
\]

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 \(^{137}\text{Cs}\) concentrations 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 \((\text{Fortin and Dale, 2005})\). 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 \((\theta)\) following \text{Cressie (1993)}. The correction factor is expressed by \(I(d)\) (Moran’s \(I\) statistics) as:

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

Then equation (2) was modified as:

\[
n_{req} = \frac{t_{0.95}^2 \theta s^2}{D^2}. (4)
\]

3. Results

3.1. \(^{137}\text{Cs}\) concentration in needles and soil

The maximum (minimum) values of \(^{137}\text{Cs}\) 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 \(^{137}\text{Cs}\) 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 \(^{137}\text{Cs}\) 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 \(^{137}\text{Cs}\) concentration in needles and soil \((\text{Pearson’s correlation coefficient} r = 0.217, p = 0.179)\).

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 \(^{137}\text{Cs}\) concentration in needles \((\text{Fig. 3a})\) 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 \((\text{Fig. 3b})\). The pattern of Moran’s \(I\) spatial correlogram of the \(^{137}\text{Cs}\) concentration of needles showed a quite different pattern from that of soils \((\text{Fig. 3a and b})\).

3.2. The number of required samples

Because the \(^{137}\text{Cs}\) concentration in needles showed a significant
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.
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, further investigations are needed to clarify them.

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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