RADIOCESIUM CONTAMINATION IN TERRESTRIAL PLANTS AND FUNGI NEAR A FORMER NUCLEAR REACTOR COOLING CANAL ON THE SAVANNAH RIVER SITE IN AIKEN, SC

by

Corinne Sweeney

(Under the Direction of Stacey Lance and Krista Capps)

ABSTRACT

Radiocesium (^{137}Cs) is a long-lived, hazardous byproduct of the nuclear fission process. In addition to releases from large disasters, radiocesium has also been released during weapons testing and nuclear production at US Department of Energy (USDOE) sites such as the Savannah River Site (SRS) located in Aiken, SC. Plant and fungal surveys conducted in the terrestrial environment near a former nuclear cooling canal on the SRS documented relatively high radiocesium activity concentrations, especially in ferns and fungi. Transfer from soil to plant/fungi was estimated by transfer factors. Transfer factor values near the cooling canal ranged from 0 to 313.7, well above those observed in nuclear exclusion zones, as well as other USDOE sites. Unique soil properties and cation concentrations may contribute to high bioavailability of radiocesium at the SRS. Implications of high transfer factors at the SRS include risk to higher trophic levels as well as nearby human populations.

INDEX WORDS: RADIOCESIUM, NUCLEAR ENERGY, TRANSFER FACTOR

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DEDICATION

To my parents, who have always supported and believed in me. To Vivian and Isabel, you can do anything you work hard for. And lastly, to my cat, Mila, whom I work so hard to give a better life to.

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CHAPTER 1

INTRODUCTION

The expansion of nuclear energy production and nuclear weapons testing since the 1940's has resulted in the global contamination of terrestrial and aquatic ecosystems with radionuclides. While nuclear disasters such as in Chernobyl (1986) and Fukushima (2011) have drawn public and scientific attention, nuclear fallout from weapons testing and energy production are responsible for most of the radionuclide contamination worldwide (Mackenzie 2000, UNSCEAR 1993, UNSCEAR 2000). Radiocesium (^{137}Cs) is a fission byproduct associated with nuclear energy and weapons production. It is a contaminant of particular concern due to the sheer volume produced and its relatively long half-life (~30 years). These characteristics allow for environmental persistence and sustained exposure to flora and fauna. Radiocesium emits both high-energy beta particles and gamma rays which can penetrate the skin causing DNA damage at low concentrations (ATSDR 2004, NCRPM 2007). Furthermore, researchers have not been able to fully describe the behavior and movement of radiocesium in natural environments, due to its complex chemical and physical interactions (Bell et al. 1988, Absalom et al. 1999, Nimis 1996).

Radiocesium, as with many other contaminants, enters terrestrial food chains primarily through transfer from contaminated media into basal carbon resources, such as microbes and plants (Price 2002, Woodwell 1967). Research has documented the contribution of plants and fungi to radiocesium accumulation in higher trophic level organisms (Ehlken and Kirchner 2002, Shaw and Bell 1994). Transfer factors estimate the amount of radiocesium activity in an organism of interest relative to the amount of activity in a source media. For example, they can be used to measure the amount of radiocesium activity in a plant relative to the activity in the soil where the plant is growing. Transfer factors can be used to evaluate how radiocesium moves through a food web in a particular environment (IAEA 2006, Ehlken and Kirchner 2002). Groups such as the International Atomic Energy Agency (IAEA) have published soil to plant and fungi transfer factors from across the globe, allowing scientists and managers to compare radionuclide uptake by plants and fungi across different regions and environmental conditions.

Previous research indicates that the transfer of radiocesium to plants and fungi from contaminated soil is strongly dependent on environmental characteristics, such as cation availability and soil composition (Evans and Dekker 1966, Sawhney 1964, Gyuricza et al. 2010). For example, plants depend upon potassium for cell growth, regulation of cell osmotic pressure, and enzymatic activation (White and Broadley 2000, Hu et al. 2016, Xu et al. 2020, Nieves-Cordones et al. 2016). In the absence of available potassium, plants can extract other cations from the soil (Handley and Overstreet 1961, Smolders et al. 1997). Because potassium and cesium have similar chemical properties, stable and radioactive cesium compete with potassium for plant uptake (Kobayashi et al. 2019, Adams et al. 2019). However, cesium fails to fill the essential roles potassium plays in plant development, and at high concentrations, cesium can cause cytotoxicity and reduce plant growth (Nishita et al. 1962, Rai and Kawabata 2020, White and Broadley 2000). Consequently, plants will preferentially extract potassium from soils, but may accumulate more radiocesium when potassium is limited (Zhu et al. 2002, Middelton et al. 1960). In fact, adding monovalent cations such as potassium to soil can be effective in reducing the uptake of radiocesium by plants (Seel et al. 1995, Avery et al. 1991, Handley and Overstreet 1961, Gyuricza et al. 2010).

In addition to potassium concentrations, the bioavailability of radiocesium to plants and fungi in terrestrial ecosystems is influenced by soil composition and mineralogy (Yasumiishi et al. 2021, Rai and Kawabata 2020, Poinssot et al. 1999). Higher clay content is associated with greater adsorption of radiocesium to soils (Rai and Kawabata 2020, Evans and Dekker 1966). Soils with chemical and physical characteristics that promote bioavailability of radiocesium, low exchangeable potassium concentrations, and the concentration of clays containing illite and mica, are important sources for radiocesium mobility to higher trophic levels (Sawhney 1964, Brown and Bell 1995). Additionally, characteristics such as soil pH, soil particle size, and organic matter content can also affect transfer factors (Thiry and Myttenaere 1992, Gyuricza et al. 2010, Gaso et al. 1996). Aside from soil characteristics, it is well known that transfer factors and bioaccumulation differ between plants and fungal taxa and functional groups (Pinder III and Sharitz 1978, Kammerer et al. 1994). For example, a meta-analysis conducted by Broadley et al. (1999) found dicots to have mean relative shoot cesium concentrations three times higher than monocots. Regarding mushrooms, several studies have suggested differences between radiocesium concentrations among fungal habitat types (Yoshida and Muramatsu 1994, Gaso et al. 1996, Ohnuki et al. 2016). Pinder III and Sharitz (1978) further theorize that the genetic and physiological differences between individuals is also important to consider.

Much of what we understand of the characteristics that contribute to radiocesium transfer comes from research after the Chernobyl and Fukushima nuclear disasters. These disasters have undoubtably contributed substantial amounts of radiocesium, 7.0×10^{16} Bq and 8.8×10^{15} Bq respectively, to large parts of Europe and Japan (UNSCEAR 2000, UNSCEAR 2016).

Exclusions zones were established in Ukraine, Belarus, and Japan after these accidents to mitigate risk to human populations from highly contaminated soils, crops, and air. The extreme radioactive conditions in these zones have resulted in studies relating to trophic transfer, especially to products of human consumption. Studies conducted on non-disaster sites are far less common and have historically taken place at United States Department of Energy (USDOE) locations such as the Savannah River Site (SRS) near Aiken, South Carolina. Compared to nuclear accidents, the SRS has experienced minor atmospheric and liquid radiocesium contamination releases (0.10% of Chernobyl's release and 0.81% of Fukushima's release) (Carlton et al. 1992, UNSCEAR 2006, UNSCEAR 2016). Plant and fungal data from the SRS show comparable radiocesium concentrations to samples found in nuclear disaster exclusion zones (Kaplan et al. 2005, Bryan and Canaday unpublished 2018, Yamashita et al. 2014, Grodzinskaya et al. 2003). Plant samples from the SRS typically have higher transfer factors than those published by the IAEA and other databases (IAEA 2010). In fact, transfer factors as high as 365.8 have been reported in plants from the SRS while in Chernobyl and Fukushima they are usually less than 1 (IAEA 2010, Yamashita et al. 2014, Gerzabek 1990, Hinton et al. 1999). The SRS has also reported some of the highest radiocesium concentrations in wildlife, suggesting that contaminant transfer to higher trophic levels is also important on site (Oldenkamp et al. 2013, Kennamer et al. 2017, Burger et al. 2001). Elevated radiocesium concentrations have been documented in wild boar, river otter, and largemouth bass (Oldenkamp et al. 2013, WSRC 1997, Borchert et al. 2018, Shure and Gottshalk 1975). Collectively, these data suggest radiocesium transfer from soil to basal resources at the SRS may be contributing to an increased risk of exposure to radionuclides to wildlife and human populations nearby.

Evaluating the accumulation of radionuclides at contaminated sites and evaluating the associated risks to the environment and humans can help influence future management practices and inform restoration efforts. Globally, the study of radiocesium accumulation in plants has focused on species commonly found in human diets, with particular interest in agricultural crops contaminated during large-scale accidents. The SRS has been the focus of numerous studies of organismal uptake of radiocesium, but the majority of this work has focused on animals and aquatic plants (e.g., Paller et al. 2005, Straney 1975, Seel et al. 1995). Notably, the SRS is also an ideal location to study low-level, chronic exposure to forest plants due to naturally high transfer factors and relative isolation from public activities, and preliminary data collected in 2018 suggest contamination in plants and fungi may present an environmental concern (Bryan and Canaday unpublished 2018; Table S1). The objectives of this study were to: (1) estimate how the accumulation of radiocesium varied among plants, fungi, and leaf litter collected along a gradient of soil contaminated by radiocesium, (2) compare transfer factors for plant and fungal species and functional groups, and (3) compare accumulation of radiocesium in different plant tissues (e.g., leaves, flowers, fruits, etc.).

CHAPTER 2

METHODS

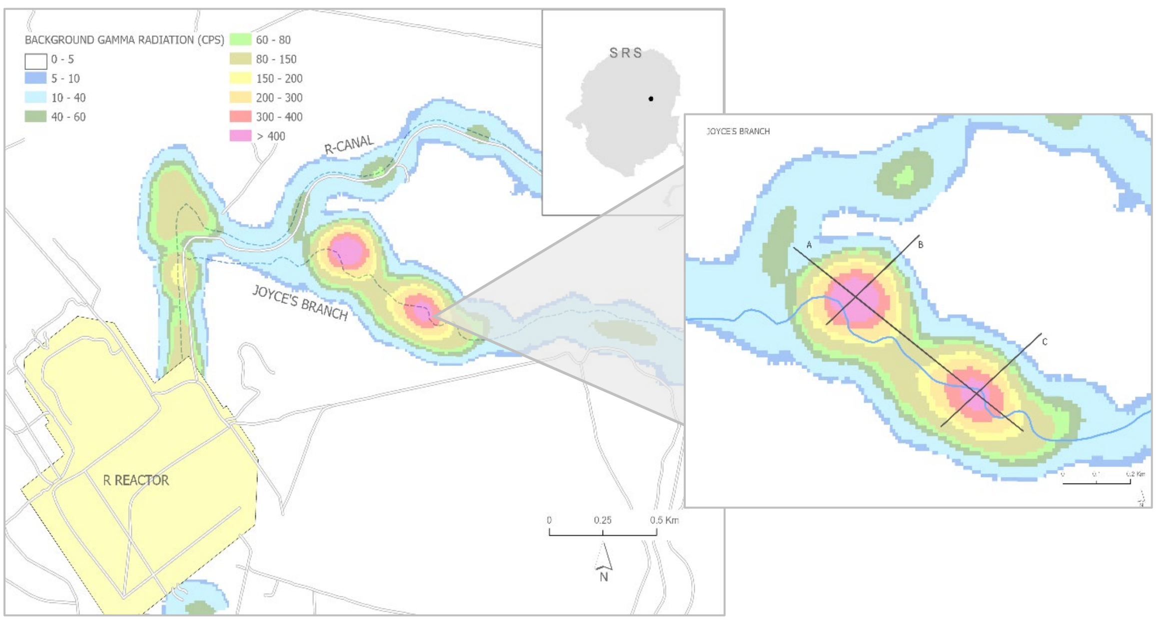
STUDY SITE

The United States Department of Energy’s Savannah River Site (SRS) was established by the Atomic Energy Commission in 1951 as part of the U.S. nuclear weapons complex (White and Gaines 2000, Garten et al. 2000). The SRS played an important role in the manufacturing of nuclear weapons prior to the end of the Cold War, primarily producing nuclear weapon products, tritium, and plutonium (Garten et al. 2000, Carlton et al. 1992). A total of five nuclear reactors were commissioned between the 1950s and the late 1980s on the SRS (Carlton et al. 1992). The primary focus of the SRS has shifted towards waste management and research since the end of the Cold War. In 1972, the SRS was established as the first National Environmental Research Park, where a wide range of ecological research and long-term studies monitor the impacts of site operations on the environment (Cairns, Jr. and Crawford 1990). The site contains more than 50 mammal, 100 reptile and amphibian, and 200 bird species and is a refuge for many at risk plant and animal species (WSRC 1997). Habitats range from upland pines to swampy bottomlands across the ~ 800 km^2 research site (White and Gaines 2000). The soils on the SRS are primarily sandy with kaolinite-rich clays and limited exchangeable potassium (Kaplan et al. 2005, Zaunbrecher et al. 2015). The SRS is located in Aiken, Allendale, and Barnwell counties, South Carolina which have a subtropical climate, an annual temperature of 17.3° C, and an annual precipitation of 113 cm (Garten et al. 2000).

R-Reactor, located on the northeast side of the SRS, was in operation from 1953 to 1964 (Carlton et al. 1992). A series of canals and retention ponds were created as a cooling system for the reactor heat exchanger (Carlton et al. 1992, Figure 1). Approximately 222.1 curies (8.2×10^{12} Bq) of radiocesium were released into the canal system and surrounding terrestrial environments throughout R-Reactor's period of operation (Carlton et al. 1992). Joyce's Branch was a discharge canal connecting the main canal (R-canal) to a retention pond (Figure 1). This canal was in use from 1953 to 1961 and experienced elevated levels of radioactive contamination from reactor effluent (Carlton et al. 1992, Whicker et al. 1990, Figure 2). In 2019, aerial surveys were conducted over the R-Reactor cooling canal system to map gamma radiation (Figure 2). The surveys were conducted from BK-117 helicopters equipped with NaI scintillation gamma radiation detectors (Radiation Solutions, Helena Valley Western Australia), RS-701 detector control consoles (Radiation Solutions, Helena Valley Western Australia) and Garmin 796 aerial GPS systems (Garmin Ltd., Olathe, K.S., U.S.A.). The gamma radiation from soil was detected at a 1 second acquisition time, at an altitude of 22.86 m above treetop (approximately 46 m from the ground), and with an energy calibration of 0 to 3,000 keV. Radiocesium net count rates were plotted onto site maps to depict the distribution of radiocesium soil contamination across sites (Figure 2, Figure 3). The results from these surveys indicated that areas of soil in the terrestrial environment surrounding Joyce's Branch had elevated levels of radiocesium (Figure 2).

Figure 1. A regional map of the R-Reactor cooling canal system at the Savannah River Site, Aiken, SC.

Figure 2. Larger image depicts a regional map of the R-Reactor canal system with plotted gamma radiation levels (cps) determined from aerial surveys. Smaller image shows the gamma radiation levels (cps) in the Joyce's Branch region and depicts the established terrestrial transects (A,B, and C).



SAMPLE COLLECTION

To evaluate radiocesium uptake in plants and fungi across a gradient of soil contamination, I established four transects. In the fall of 2020, I established a 625 m terrestrial transect (A) running parallel to Joyce's Branch canal and two 300 m terrestrial transects (B and C) perpendicular to transect A through the two areas with the highest levels of soil contamination indicated by the flyover assessments (Figure 2). I also established a fourth transect (D) that ran 600 m through the canal and the surrounding riparian vegetation.

In September and October 2020, I haphazardly placed a 1 x 1 m^2 quadrat at each sampling site every 25 m along the three terrestrial transects and every 50 m along the aquatic transect. I collected plant and fungal biomass found below 2 m height from inside the quadrat. When available, plant leaves and reproductive tissues were collected from woody species, while stems were also collected from non-woody species. I separated and bagged all samples by plant species (or lowest identified taxonomic ranking) or fungal habitat. I also collected a 10 – 40 g (dry weight) sample of leaf litter. In accordance with SRS guidelines, I sampled only above-ground plant and fungal biomass to avoid disturbance of contaminated soil. To obtain additional plants of interest and seasonal reproductive structures, I collected samples along the transect opportunistically between December 2020 and October 2021.

With assistance from the SRS Radiological Protection Department (RPD), I collected samples from the top 10 cm of soil from each sampling site along the three terrestrial transects, homogenized each sample, and provided them to RPD for initial radiation surveys. Each soil sample was then analyzed on a gasless alpha beta counting system for alpha and beta radiation levels by RPD. Samples that were below the allowable limit of 200 dpm/100 cm^2 alpha contamination and 5,000 dpm/100 cm^2 beta contamination were released for further analysis. Four samples were over the allowable limit and not released by RPD.

SAMPLE PREPARATION

I stored bagged samples in a refrigerator overnight or in a -80° C freezer (Thermo Fisher Scientific, Waltham, M.A., U.S.A.) if held longer than 24 hours before processing. I gently washed plant and fungal samples with tap water to remove any soil. After air drying, I measured the initial wet weight of each sample on a microbalance (Sartorius AG, Göttingen, Germany) then placed them in a drying oven at 50° C until they reached a constant dry weight. I homogenized the samples in coffee grinders (Hamilton Beach Brands, Glenn Allen, V.A., U.S.A.) before packing approximately 0.5 - 5 g of sample into individual 5 mL (12 mm x 75 mm) sterile, polystyrene round-bottom tubes (Corning Inc., Corning, N.Y., U.S.A.).

RADIONUCLIDE ANALYSIS

I analyzed radiocesium activity in dry tissue samples on a Packard Cobra II Model 5003 Auto-Gamma Counter (Packard Instrument Co., Meriden, C.T., U.S.A.) with a 3-inch through-hole NaI detector. The Auto-Gamma Counter was auto-calibrated daily with a radiocesium sealed source (SREL sealed source – 0113). The counting window was set to record photons between 580 and 754 keV to capture all possible radiocesium emissions (^{137}Cs = 662 KeV). I counted samples in 3600 s intervals, with sample blanks run every 5 samples. I quantified background-corrected count rates using sample blanks and an average count yield value of 0.211 to determine radiocesium activity in dry-tissue samples. I analyzed the soil samples released by RPD on the Auto-Gamma Counter using the same methods as the dry tissue samples. It should be noted that both ^{137}Cs and ^{134}Cs isotopes are analyzed during this process, however it is very unlikely that ^{134}Cs contributed substantially to the total radiocesium content in my samples (Marter 1970, Briese et al. 1975). I calculated minimum detectable concentrations (MDCs) as described in Currie (1968).

STATISTICAL ANALYSIS

I considered observations that were calculated to have negative values for radiocesium concentrations to have no above-background activity. However, I did not exclude or substitute positive observations that fell below the MDC during analysis in order to avoid mean and variance bias in my results as indicated by Gilbert and Kennison (1981) and Newman et al. (1989). A total of 404 plant samples and 74 fungal samples were included in the analyses. Plant samples were categorized into the following plant functional groups: aquatic plants; cacti; canes; grasses, rushes, and sedges; ferns; forbs; lichens, mosses, and Spanish mosses; shrubs and trees; and woody and semi-woody vines. A full list of plant species can be found in Table S2 (Supplemental Files). Fungal samples were categorized into two groups defined by the habitats from which they were collected: fungi found growing on the ground or those found growing on decaying wood.

I conducted my statistical analysis in R version 4.2.0 (R Core Team 2022). To estimate radiocesium activity (Bq/kg dry weight) in the four soil samples that were not released by RPD, I conducted a simple linear regression (lm() function, base R, R Core Team 2022) using the beta radiation values determined by RPD and the Auto-Gamma Counter values of the released soil samples. I then extrapolated the four unreleased soil sample radiocesium activity concentrations from the fitted regression line to be used in further models. The relationship between leaf litter and soil radiocesium activity was calculated using a simple linear regression. To avoid violating normality assumptions, I added one to leaf litter, soil, and plant radiocesium activity concentrations, as well as transfer factors prior to log transforming and subsequent analysis. Transfer factors were calculated from the following equation:

$$Transfer\ Factor\ = \frac{\left(Sample\ Radiocesium\ Activity\ (Bq\ kg^{-1}\ dry\ wt.)\right)}{\left(Soil\ Radiocesium\ Activity\ (Bq\ kg^{-1}\ dry\ wt.)\right)}$$

I analyzed fungal and plant radiocesium activity (Bq/kg dry weight.) and transfer factors using a series of linear mixed models fitted by REML (function lmer(), lme4 package, Bates et al. 2015). Fungal and plant samples were analyzed separately. I removed data from transect D in my model because sediment radiocesium activities were not available at the time of the analysis. However, transect D values were still included in reporting summary statistics (Table 1, Table S2). In assessing the effects of plant functional group, plant species, and fungal habitat on radiocesium activities and transfer factors, I only included data from functional groups, species, or habitats with greater than or equal to 10 samples in the analysis. Plant reproductive tissue samples were only included in the two models that specifically looked at the effects of plant compartment on radiocesium activity concentrations and transfer factors. Only leaves/stem samples were analyzed in models that did not consider plant tissue differences.

To investigate any disparities in radiocesium activity concentrations among plant functional groups, I fitted a linear mixed model with sampling season, soil radiocesium activity, functional group, and the interactions between season and soil activity and between functional group and soil activity as fixed effects. Sampling site was included as a random intercept to account for samples collected from the same quadrat. A similar model was fitted to investigate the differences in radiocesium activity concentrations among plant species in samples that indicated contamination (sample radiocesium activity ≥ 0). Due to unequal distribution of variances among groups, I conducted the analysis of the effects of plant species only when there was evidence of sample contamination. I fit a linear mixed model to the data from contaminated samples with sampling season, soil radiocesium activity, plant species, and the interactions between season and soil activity and interactions between species and soil activity classified as fixed effects. I also included sampling site as a random intercept. To test the effects of fungal habitat on radiocesium activity concentrations, I used a linear mixed model with the fixed effects of sampling season, soil radiocesium activity, fungal habitat, and the interactions between season and soil activity and between habitat and soil activity. Sampling site was also included as a random intercept. For all models, I conducted Type II Wald Chi-square tests on the previous models using the Anova() function (car package, Fox and Wiesberg 2019) followed by post hoc pairwise comparisons using emmeans() and emtrends() (emmeans package, Lenth 2022) to estimate the significance of fixed effects and interactions in each model.

In models that predicted transfer factors, I used data from only contaminated sites. To estimate the effects of plant functional group on soil to plant transfer factors for radiocesium, I fitted a linear mixed model with sampling season and plant functional group as fixed effects along with sampling site as a random intercept to address samples collected from the same quadrat. Aquatic plant transfer factors were not calculated because sediment radiocesium activities were not available at the time of analysis. The model testing effects of plant species on radiocesium transfer factors included sampling season and plant species as fixed effects and sampling site as the random intercept. To estimate the effects of fungal habitat on radiocesium transfer factors, I used a linear mixed model including the fixed effects of habitat (ground or wood) and sampling season along with the random intercept of sampling site.

I used a linear mixed model to estimate the difference in radiocesium accumulation among plant compartments. I included data from plants from which I collected leaves/stems and reproductive tissues (flowers or fruits) from the same plant, and only from areas that experienced soil contamination (soil radiocesium activity concentration greater than 0 Bq/kg). The model included plant compartment, soil radiocesium activity, collection season, and the interactions between plant compartment and soil activity and between season and soil activity as fixed effects. Sampling site was again included as a random intercept. To estimate the difference of transfer factors among plant compartments, my model included the fixed effects of plant compartment and collection season and sampling site as the random intercept.

CHAPTER 3

RESULTS

The range of soil radiocesium activity concentrations along my transects was 0 to 13,191 Bq/kg (dry weight) with a mean of 1,420 ± 422 Bq/kg (dry weight ± SE). The higher soil values were extrapolated from the strong relationship between the RPD beta radiation values and the soil radiocesium activity concentrations estimated by the Auto-Gamma Counter ($R^2 = 0.72$, $F_{1,43} = 114.6$, p-value < 0.001). Regardless of soil radiocesium concentration, leaf litter radiocesium activity concentrations remained low, ranging from 0 to 1,344 Bq/kg (dry weight) with a mean of 197 ± 39 Bq/kg (dry weight ± SE). I found that there was a weak yet significant relationship between leaf litter and soil radiocesium activity concentrations ($R^2 = 0.44$, $F_{1,48} = 47.63$, p-value < 0.001).

In comparing radiocesium activity concentrations among plants along the established soil gradient, I found significant differences among functional groups ($X^2 = 44.53$, *df* = 5, p-value < 0.001, Figure 4a) and species ($X^2 = 192.21$, *df* = 12, p-value < 0.001, Figure 5a). The concentration of radiocesium in the soil had a significant positive impact on the radiocesium activity concentrations in both models that compared the plant functional groups ($X^2 = 97.96$, *df* = 1, p-value <0.001) and species ($X^2 = 92.41$, *df* = 1, p-value < 0.001). Across sampling sites, ferns had the highest average radiocesium activity (Table 1), though not significantly higher than canes (p-value = 0.421; Figure 4a). Among the 13 plant species I analyzed, I found a wide radiocesium activity distribution, from 0 Bq/kg to 51,249 Bq/kg (dry weight) (Table S2). The five species with the highest average radiocesium activity concentrations were fern species: netted chain ferns (*Woodwardia areolata),* cutleaf grape ferns *(Botrychium dissectum)*, Virginia chain ferns (*Woodwardia virginica)*, ebony spleenworts (*Asplenium platyneuron*), and Christmas ferns (*Polystichum acrostichoides;* Table S2). However, Eastern bracken ferns (*Pteridium aquilinum)* averaged among the lowest species (Table S2). Partridgeberries (*Mitchella repens)*, a woody vine species, had the next highest average radiocesium activity concentrations outside of the fern functional group (Table S2). Yellow jessamines (*Gelsemium sempervirens*) and muscadines (*Vitis rotundifolia*)*,* two woody vine species that were found consistently across my transects, had significantly lower average radiocesium activity concentrations than 10 out of the other 11 most common species (p-values < 0.001, Figure 5a).

The season in which I collected samples had no main effect on radiocesium activity concentrations in plant functional group or plant species models. However, there was a significant interaction between soil radiocesium activity and season in the plant functional group model, where soil radiocesium was positively associated with plant radiocesium, but only in the fall and summer sampling bouts ($X^2 = 6.72$, $df = 2$, p-value = 0.035). I saw a similar, though not statistically significant trend in the plant species model ($X^2 = 2.52$, $df = 2$, p-value = 0.283). There were no significant interactive effects of plant functional group and soil radiocesium activity ($X^2 = 5.00$, $df = 5$, p-value = 0.416) and plant species and soil radiocesium activity ($X^2 = 20.44$, $df = 12$, p-value = 0.059) on plant radiocesium activities.

Figure 3. Depicts the Joyce's Branch region with background gamma radiation levels (cps) determined from aerial surveys. Plotted points depict the plant samples collected from the three terrestrial transects. Samples are categorized by plant functional groups (icons) and radiocesium activity concentration ranges in Bq/kg dry weight (gray scale).

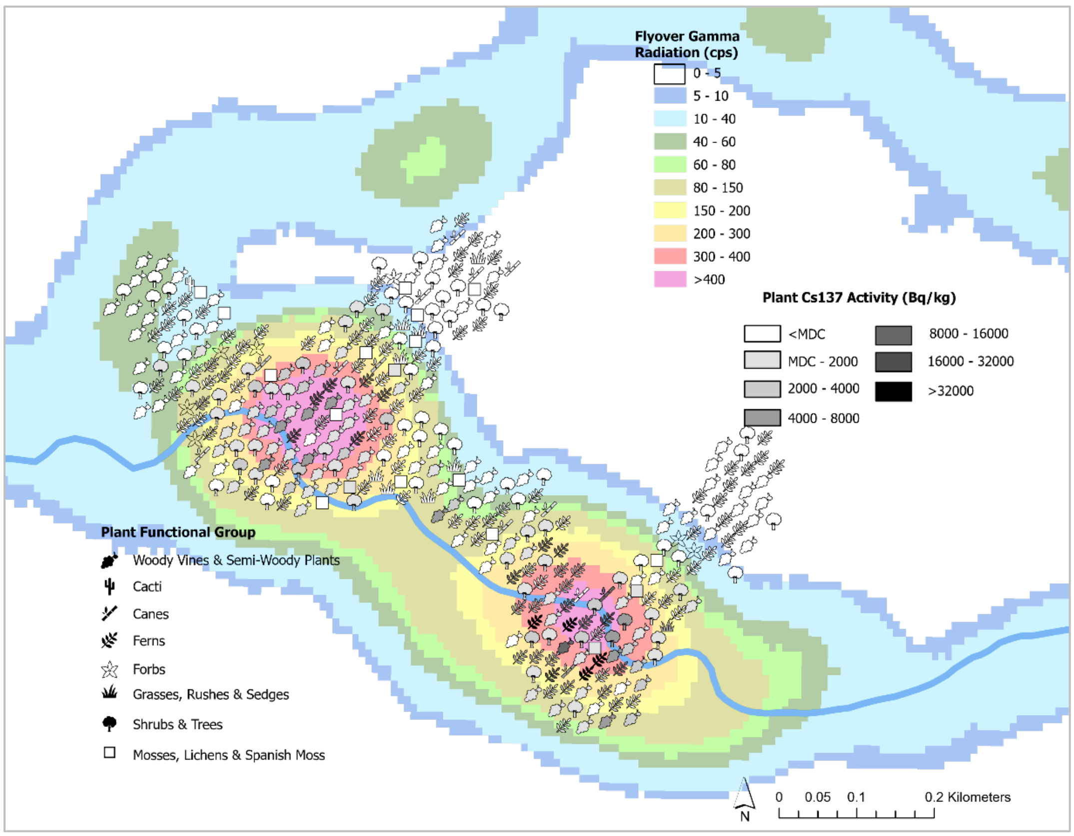


Figure 4. Comparison of log transformed (a) radiocesium activity concentrations (Bq/kg dry weight) and (b) soil to plant/fungi transfer factors among plant functional groups and fungi habitats. Plant and fungi samples were analyzed separately. Plant functional groups and fungi habitats sharing the same letter were not significantly different in a posthoc pairwise comparison. Plant functional groups indicated by * were not included in the statistical analysis due to insufficient sample numbers but visualized here for comparison. Transfer factors for aquatic plants were not calculated because sediment samples were not available at the time of analysis. Plant functional groups were depicted by the following abbreviations: Aquatic plants (AQUA); Ferns (FERN); Canes (CANE); Grasses, rushes, and sedges (GRASS); Lichens, mosses, and Spanish Mosses (LICH/MOSS); Shrubs and trees (TREE); Woody vines and semi-woody plants (WOODY); Cacti (CACTI); and Forbs (FORB). Fungal habitats were depicted by the abbreviations GROUND for fungi growing out of the ground and WOOD for fungi growing on decaying wood.

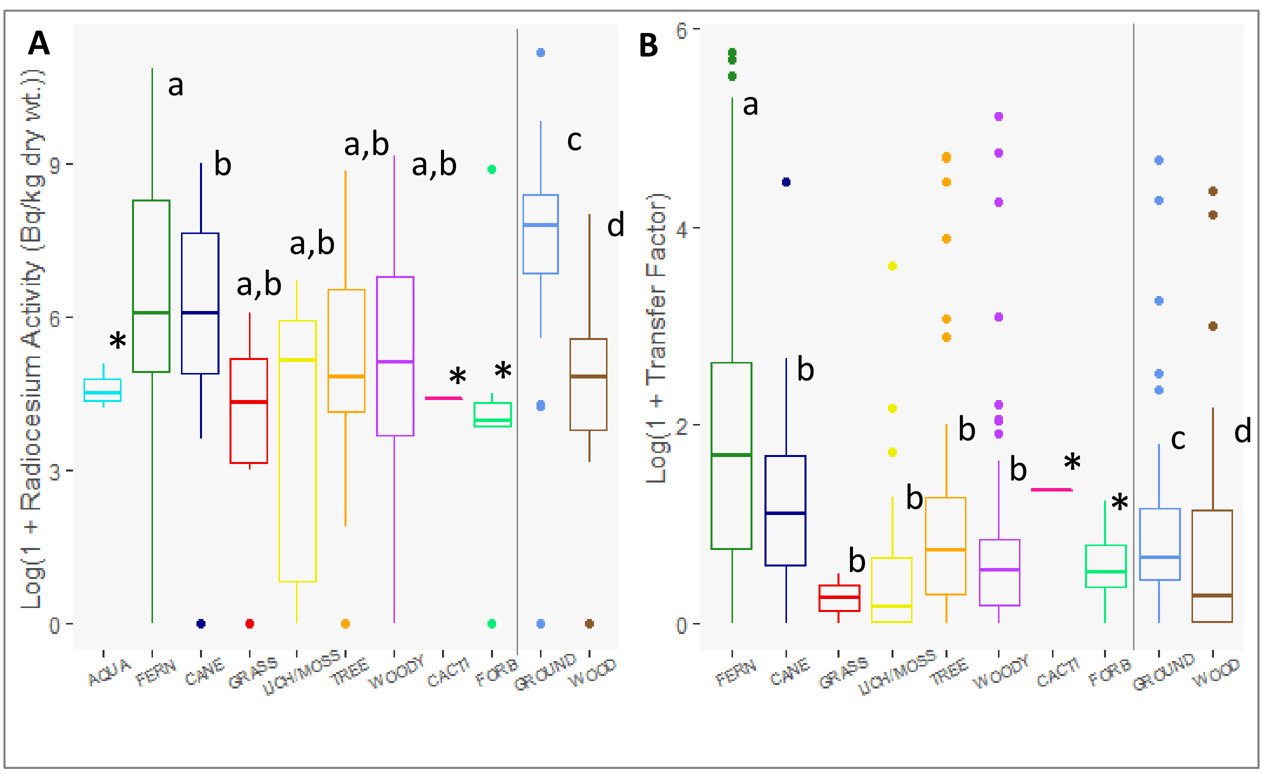


Table 1. Radiocesium Activity (Bq/kg dry wt) in Plant and Fungal Samples

Plants

Plant Functional Group	Radiocesium activity (Bq/kg dry wt) N	Range	Mean+/- SE	Mean MDC	% Below MDC	Transfer Factor Range	Mean +/- SE
Aquatic Plants*	3	66 - 158	105 +/- 27	294	100%	-	-
Cacti*	1	-	79	1,250	100%	-	2.8
Canes	13	0 - 8,189	1,740 +/- 790	389	38.5%	0 - 84.5	9.8 +/- 6.9
Ferns	114	0 - 51,249	4,627 +/- 870	645	50.9%	0 - 313.7	19.8 +/- 6.0
Forbs*	4	0 - 340	204 +/- 81	631	100%	0 - 62.8	19.8 +/- 14.5
Grasses, Sedges, and Rushes	17	0 - 7,122	526 +/- 413	424	82.3%%	0 - 2.4	0.8 +/- 0.4
Lichens, Mosses, and Spanish Mosses	24	0 - 814	242 +/- 54	435	75%	0 - 36.0	2.8 +/- 1.8
Shrubs and Trees	89	0 - 6,967	755 +/- 151	434	55%	0 - 110.8	7.0 +/- 2.6
Woody Vines and Semi-Woody Plants	98	0 - 9,395	745 +/- 139	366	56.1%	0 - 166.9	5.6 +/- 2.6

Fungi

Fungal Habitat	Radiocesium activity (Bq/kg dry wt) N	Range	Mean+/- SE	Mean MDC	% Below MDC	Transfer Factor Range	Mean +/- SE
Ground	45	0 - 70,143	5,311 +/- 1,695	344	11.6%	0 - 106.0	6.2 +/- 2.9
Wood	26	0 - 2,929	313 +/- 120	299	73.1%	0 - 76.7	7.6 +/- 4.1
Other*	1	-	1,720	324	0%	-	0.6

* Indicates plant functional groups that were not included in statistical analysis, due to insufficient sample number

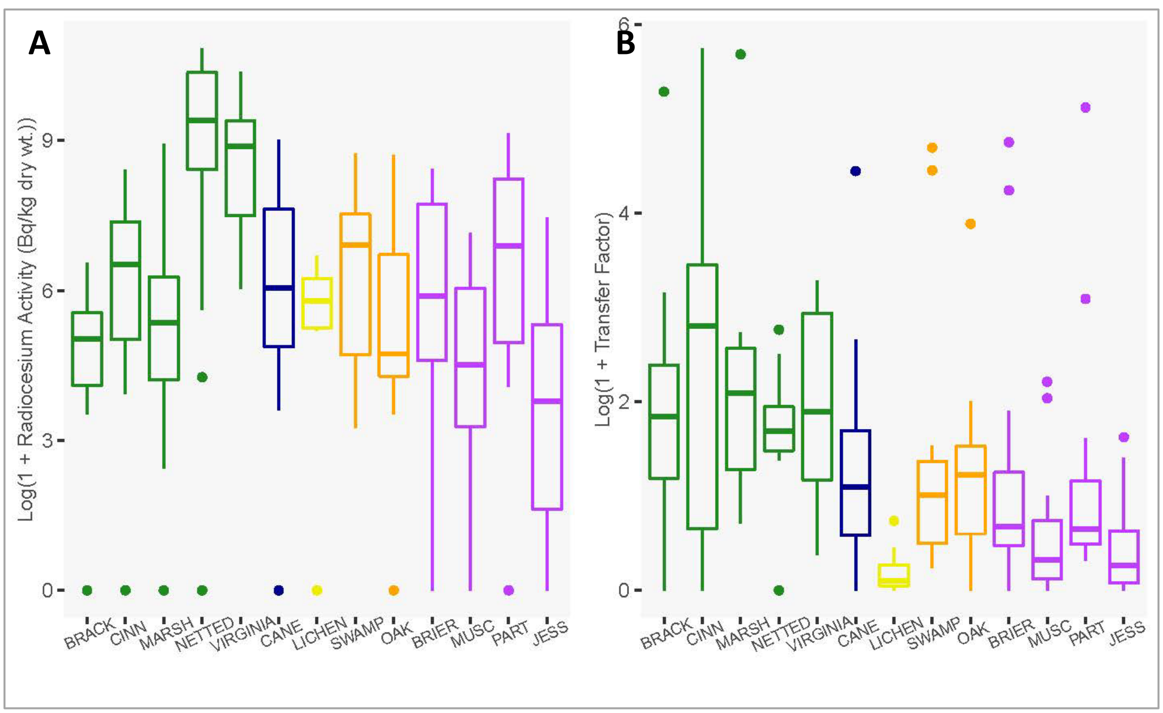
MDC is the minimum detection concentration calculated for each sample (Currie 1968)

Fungal radiocesium activity differed between ground and wood habitats (X^2 = 19.78, *df* =1, p-value < 0.001), with fungal samples collected from the ground exhibiting significantly higher radiocesium activity concentration than fungal samples collected from decaying wood (p-value <0.001; Table 1, Figure 4a). Unlike plants, soil radiocesium activity did not significantly predict fungal radiocesium activity (X^2 = 1.95, *df* = 1, p-value = 0.163). The was no significant difference in fungal radiocesium activities between samples collected in the spring and the summer (X^2 = 3.30, *df* = 1, p-value = 0.069). The interactions between soil radiocesium activities and habitat (X^2 = 1.24, *df* = 1, p-value = 0.265) and between soil radiocesium activity and season (X^2 = 0.49, *df* = 1, p-value = 0.485) also did not significantly predict fungal radiocesium.

To estimate the movement of radiocesium from soil to plants across contaminated sites, I also calculated and compared transfer factors among species and functional groups (Figure 5). Transfer factors differed significantly among plant functional groups (X^2 = 44.53, *df* = 5, p-value < 0.001), with ferns having a significantly higher transfer factor (p-value < 0.001) than any other functional group (Table 1, Figure 4b). Lichens, mosses and Spanish mosses and grasses, sedges and rushes had the lowest transfer factors (Table 1). While forbs as a group only included four samples and thus were not included in my statistical models, forbs had an equally high average transfer factor to that of ferns (Table 1). Transfer factors were significantly different between the 13 plant species I compared (X^2 = 119.47, *df* = 12, p-value < 0.001, Figure 5b). Spotted wintergreens and blue huckleberries averaged high transfer factors, although less than 10 samples were collected for both species, so they were not included in the statistical models (Table S2). Lichens and Spanish moss transfer factors averaged among the lowest out of the 47 species (Table S2). Plant transfer factors were not impacted by collection season at either the functional group ($X^2 = 0.27$, $df = 2$, p-value = 0.872) or species ($X^2 = 0.18$, $df = 2$, p-value = 0.916) level.

I found that fungal habitat had a significant effect on soil to fungi transfer factors (X^2 = 8,91, $df = 1$, p-value = 0.003; Figure 4b) while season did not ($X^2 = 2.05$, $df = 1$, p-value =0.153). My model indicated that mushrooms collected from the ground had significantly higher transfer factors than mushrooms collected from decaying wood (p-value = 0.005). However, my mean transfer factor for ground mushrooms was slightly lower than the mean of wood fungi (Table 1). In order to better understand this relationship, I created a second model, a non-parametric Generalized Additive Model (GAM; gam() function, package mgcv, Wood 2011), and removed the assumption of normality. Post hoc comparisons from my GAM model echoed my results from the linear mixed model, showing ground mushrooms to have a significantly higher mean log transfer factor than wood fungi (p-value = 0.001). I believe the discrepancy between my model outputs and mean transfer factor values can be attributed to two outlier values in my wood fungi dataset. Both samples are from the same sampling site which has a very low soil contamination value (2.3 Bq/kg dry weight). Therefore, the fungi radiocesium activity concentrations of 383 and 264 Bq/kg (dry weight) result in very large transfer factor values even though the sample radiocesium activity concentrations were low themselves.

Figure 5. Comparison of log transformed (a) radiocesium activity concentrations (Bq/kg dry weight) and (B) soil to plant transfer factors among plant species. Only species with greater than or equal to 10 samples were included. Plant species were depicted by the following abbreviations: Bracken fern (BRACK); Cinnamon fern (CINN); Marsh fern (MARSH); Netted chain fern (NETTED); Virginia chain fern (VIRGINIA); Giant cane (CANE); Lichens (LICHEN); Swamp bay (SWAMP); Oaks (OAK); Greenbriers (Brier); Muscadine (MUSC); Partridgeberry (PART); and Yellow jessamine (JESS). Bar color corresponds with the plant functional groups depicted in Figure 4.



I found no significant difference in radiocesium activities between plant compartments ($X^2 = 0.40$, df = 2, p-value = 0.822). Similar to my other plant models, soil radiocesium activity concentrations did significantly predict the radiocesium activity concentrations of the plant samples regardless of plant compartment type ($X^2 = 34.36$, df = 1, p-value < 0.001). There was no significant difference among plant compartments over collection seasons ($X^2 = 1.18$, *df* = 2, p-value = 0.555) and there were no significant interactions between season and soil radiocesium activity ($X^2 = 5.80$, *df* = 2, p-value = 0.055) and between plant compartments and soil radiocesium activity ($X^2 = 1.18$, *df* = 2, p-value = 0.555). I found no significant difference in transfer factors among plant compartments ($X^2 = 0.19$, *df* = 2, p-value = 0.908), or between seasons ($X^2 = 0.10$, df = 2, p-value = 0.953).

Comparisons between radiocesium activities and transfer factors from Joyce's Branch to those observed in the Chernobyl and Fukushima nuclear exclusion zones are expressed in Table 2.

Table 2. Comparision of plant radiocesium activites and transfer facters between Joyce's Branch, the Chernobyl nuclear exclusion zone, and the Fukushima nuclear exclusion zone

Sampling Location	Year Collected	Highest Plant Sample ID	Highest Plant Functional Group	Highest Radiocesium Activity Concentration (Bq/kg dry wt)	Transfer Factor Range	Mean Soil Radiocesium Concentration (Bq/kg dry wt)	Source
SRS (Joyce's Branch)	2020-2021	Netted Chain Fern (*Woodwardia areolata*)	Ferns	51,249	0 - 313.7	1,420	-
Chernobyl Exclusion Zone	1992-1993	Lady Fern (*Athyrium filix-femina*)	Ferns	192,712	0.03 - 1.81	104,765	Lux et. al 1995
Fukushima Exclusion Zone	2012	Asian Lady Fern (*Athyrium yokoscense*)	Ferns	18,200	0.006 - 0.703	12,184	Yamashita et al. 2014

CHAPTER 4

DISCUSSION

The results from this study documented, large variation in radiocesium accumulation and transfer from soil to plants and fungi. Differences among functional groups, species, and habitats provide the basis for future research to be conducted on taxa and tissues with the potential of contributing to trophic transfer. In the following paragraphs, I will address the plant and fungi groups I found to be potential vectors of radiocesium trophic transfer and further discuss the implications.

RADIOCESIUM UPTAKE IN VASCULAR PLANTS

My research at Joyce's Branch resulted in a wide range of radiocesium uptake by different plants and fungi. In plant functional groups, ferns stood out as radiocesium hyperaccumulators. Some fern species have previously been established as hyperaccumulators of heavy metals, including arsenic (Tiwari et al. 2013, Srivastava et al. 2006, Iskan et al. 2019). Specifically, at Joyce's Branch, two species of the *Woodwardia* genus, the netted chain fern and Virginia chain fern, accumulated especially high levels of radiocesium. My results correspond with previous studies conducted on the SRS. Kaplan et al. (2005) also observed that netted chain ferns accumulated high levels of radiocesium in a riparian zone within the R-Reactor cooling canal system. Additionally, Kaplan et al. (2001) and Knox et al. (2008) found netted chain ferns to take up high concentrations of cobalt, cerium, chromium, thorium, and uranium on the SRS, suggesting netted chain ferns accumulate high levels of various contaminants. Netted chain ferns and Virginia chain ferns are primarily found in eastern North America, so I was not able to directly compare radiocesium uptake from the Chernobyl and Fukushima nuclear zones in these species (Smith 1793, Cranfill 1983). However, studies conducted by Yamashita et al. (2014) and Lux et al. (1995) that examined radiocesium uptake in plants and fungi in nuclear exclusion zones allow us to compare uptake among functional groups (Table 2). Soil samples from the Joyce's Branch transects were typically much lower than those found in the nuclear exclusion zones of Fukushima and Chernobyl (Yamashita et al. 2014, Takata et al. 2013, Lux et al. 1995; Table 2). However, as was previously reported at the SRS, bioaccumulation of radiocesium in plant samples was uniquely high at Joyce's Branch given the level of soil contamination present (Hinton et al. 1999, Kaplan et al. 2005, Sharitz et al. 1975, Briese et al. 1975). In fact, Joyce's Branch ferns reach comparable levels of radiocesium activity concentrations to those found in the Fukushima nuclear exclusion zone one year after the disaster (Yamashita et al. 2014; Table 2). Additionally, in the Chernobyl nuclear zone which averaged soil radiocesium activity concentrations approximately 73 times higher than Joyce's Branch soil sample concentrations, Lux et al. (1995) found the highest plant radiocesium activity in a lady fern (*Athyrium filix-femina)* to be only 3.7 times higher than Joyce's Branch netted chain ferns (Table 2). In all three studies, ferns accumulated the highest radiocesium activity concentrations of the plant samples collected, noting that this pattern is not unique to the SRS (Yamashita et al. 2014, Lux et al. 1995). In a controlled experiment, Tyson et al. (1999) found bracken ferns internally relocated ^{134}Cs to meristems and developing organs, effectively internally recycling ^{134}Cs at the end of each growing season. The long lifespan and clonal life cycle of bracken ferns may contribute to this phenomenon (Tyson et al. 1999). Further research should be conducted on other long-lived, clonal species, such as netted chain ferns, to better understand this potential mechanism for contaminant accumulation. The natural histories of other plant taxa and functional groups may also shed light on the ability to extract radiocesium from soils. Factors such as water and potassium requirements, and root structure and depth couldl help explain disparities between radiocesium uptake (Yang et al. 2005, Sytar et al. 2021, Rascio and Navari-Izzo 2011, Zhu and Smolders 2000). For example, muscadines and yellow jessamines had consistently low radiocesium activity concentrations at Joyce's Branch, regardless of soil contamination level. As opposed to some other plant species, the roots associated with these woody vine species may have been too shallow to penetrate the soil horizons containing most of the contamination (Olien et al. 1993, Dearing 1947, Putz and Mooney 1991). My data provides the basis for additional questions to be asked about how plant physiology contributes to radiocesium uptake, which taxa may pose a risk for radiocesium transfer to higher trophic levels, as well as what plants could be used for bioremediation applications.

RADIOCESIUM UPTAKE IN FUNGI AND NON-VASCULAR PLANTS

Previous studies conducted in Chernobyl and Fukushima impacted areas have found fungi, mosses, and lichens to also be bioaccumlators of radiocesium (Marović et al. 2008, Sloof and Wolterbeek 1992, Dowdall et al. 2005). Grodzinskaya et al. (2003) found fungi samples as high as 17,117,000 Bq/kg (dry weight) in the Chernobyl exclusion zone in 1993. Fungal samples from the preliminary study, as well as this study conducted at Joyce's Branch, were the highest radiocesium activities found on site so far (Bryan and Canaday unpublished 2018; Table S1). Interestingly, I found no correlation between fungal and soil radiocesium activity concentrations. These findings correspond with those found in Borio et al. (1990). However, my broad categorizations of fungal habitats may oversimplify this relationship. While I combined all fungal samples growing out of the ground into one category, in actuality, several fungal functional groups can be classified by these loose standards (Bödeker et al. 2016, Webster and Weber 2007). Saprotrophic fungi decompose dead plant and animal materials. While they often observed on decaying wood, free-living saprotrophs can also occupy leaf litter. Conversely, mycorrhizal fungi form symbiotic relationships with plants in the soil (Webster and Weber 2007). Therefore, free-living saprotrophic and mycorrhizal fungi may appear in the same habitats, despite access to different radiocesium sources (Bödeker et al. 2016, Webster and Weber 2007, Nimis 1996, Ohnuki et al. 2016). Several studies have found that mycorrhizal fungi accumulate radiocesium at higher concentrations than saprotrophs (Barnett et al. 1999, Komatsu 2019, Yoshida and Muramatsu 1994). Additionally, mycelium depth plays a large role in the availability of radiocesium taken up by fungi (Yoshida et al. 1994, Baeza et al. 2005). Classifying both free-living saprotrophs and mycorrhizal fungi as ground fungi may have confounded my ability to interpret the significance of soil radiocesium activity on accumulation in differing fungal groups. Further classifications of fungal habitat as wood, litter layer, surface layer, and below surface layer, such as was done in Yoshida et al. (1994) could have been useful in understanding the disparities of radiocesium uptake by different fungal groups. Although similar trends were observed in fungal samples between nuclear exclusion zones and Joyce's Branch, lichens and mosses at Joyce's Branch contained among the lowest radiocesium activities across the transects. In arctic locations impacted by Chernobyl fallout, Dowdall et al. (2005) found mosses accumulated the most radiocesium, followed by lichens, and then vascular plants. The difference between my results and those from nuclear disasters may be due to the mode of contamination dispersal. While much of the radionuclide contamination from Chernobyl and Fukushima was dispersed through atmospheric fallout, contamination at Joyce's Branch was primarily from effluent ultimately settling into sediments and soils (Carlton et al. 1992, UNSCEAR 2000, UNSCEAR 2016). Vascular plants typically accumulate contaminants through their roots, while mosses and lichens, lacking roots, are more competent at removing contaminants from the atmosphere (Nimis 1996, Garty 2001, Mazzoni et al. 2012).

RADIOCESIUM PLANT AND FUNGAL TRANSFER FACTORS

While radiocesium activity concentrations provide information regarding the quantity of radiocesium in a given biotic or abiotic sample, transfer factors can provide more ecologically relevant information such as the bioavailability of radiocesium to biota from contaminated media (Ehlken and Kirchner 2002, IAEA 2010). Higher transfer factors can indicate bioavailable concentrations of radiocesium to basal carbon resources or the effectiveness of those resources to remove radiocesium from the contaminated media. Both of which are useful in predicting further transfer to higher trophic levels. Similar to my observations in radiocesium activity concentrations, transfer factors at Joyce's Branch were highest in ferns. Interestingly, while netted and Virginia chain ferns had the highest radiocesium activity concentrations, cinnamon ferns (*Osmunda cinnamonea*) and Eastern marsh ferns (*Thelypteris palustris)* had the highest average transfer factors. Many of my chain fern samples were collected from sampling sites with the highest soil radiocesium concentrations. My combined radiocesium activity and transfer factor data presents the basis for further questions. Including whether or not *Woodwardia* species might be outcompeted in areas of lower contamination, while they are capable of surviving in areas of high contamination. Or, if cinnamon ferns and Eastern marsh ferns are efficient at removing radiocesium from contaminated soils yet may not be able to sustain high levels of radiocesium in their tissues. The data from Joyce's Branch has provided essential starting points for future research.

Soil to plant/fungi radiocesium transfer factors have been collected by many domestic and international groups such as the USDOE, the International Atomic Energy Agency (IAEA), and the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) in order to compare radiocesium movement into aquatic and terrestrial food webs across contaminated sites worldwide. While the majority of collected transfer factors have been for agricultural species, typical transfer factors range from 0.001 to 5 in temperate zones (IAEA 2010). In comparing plant taxa where transfer factors have not yet been experimentally or observationally established, the IAEA uses a default transfer factor of 0.1 (IAEA 2010). Similar to previous SRS studies that looked at plant transfer factors in aquatic systems, I found terrestrial plant and fungal samples at Joyce's Branch to have uniquely high transfer factors (Pinder III and Sharitz et al. 1978, Hinton et al. 1999, Kaplan et al. 2005). Joyce's Branch plant transfer factors were as high as 313.7, while fungal samples were as high as 106. In comparison, even in highly contaminated areas such as the Chernobyl and Fukushima nuclear zones, Lux et al. (1995) and Yamashita et al. (2014) found soil to plant transfer factors ranging from 0.03 to 1.81 and 0.006 to 0.703 respectively (Table 2). Although there are lower concentrations of radiocesium in the soils at SRS than exclusions zones, I can infer from the high transfer factors on the SRS that there is more bioavailable radiocesium for plants and fungi to extract. When comparing the transfer factors at the SRS to those of two additional USDOE nuclear facilities, Hanford site in Washington and Oak Ridge Reservation in Tennessee, you can observe again that SRS transfer factors are distinctively high (Auerbach and Crossley 1958, Dahlman and Van Voris 1976, Cataldo et al. 1978, Schreckhise et al. 1993). The Hanford site and Oak Ridge Reservation typically report radiocesium soil to plant transfer factors well below 1 (Auerbach and Crossley 1958, Dahlman and Van Voris 1976, Cataldo et al. 1978, Schreckhise et al. 1993). However, as previously observed in the literature, transfer factors at Joyce's Branch varied widely within functional groups and even species (Ehlken and Kirchner 2002, Guillén et al. 2017). As others have suggested, transfer factor ranges are large for a number of reasons. First, transfer factors are highly site and situation dependent (Shaw and Bell 1994). They can only be accurately applied when the exact conditions from the original study are met. As indicated with my fungal dataset, very low radiocesium activities can result in the highest transfer factors if the soil radiocesium level is low. Second, variation is more likely to occur in forested areas than agricultural plots due to the complexities of soil composition, geography, hydrology, and vegetation density in forested areas (Thiry and Myttenaere 1993). Soil composition, minerology, and potassium concentration heterogeneity among sampling locations can result in widely varying efficacy of soil to plant radiocesium transfer (Evans and Dekker 1966, Sawhney 1964, Kobayashi et al. 2019, Gyuricza et al. 2010). Additionally, bioavailability to basal resources strongly depends on whether root and mycelial depth corresponds with the vertical migration of radiocesium in soils (Thiry and Myttenaere 1993, Yoshida et al. 1994, Baeza et al. 2005). As transfer factors are currently calculated, indirect transfer from decaying materials to saprotrophic fungi, and redistribution of contaminants to plant roots through mycorrhizal relationships, are two factors that are not accounted for (Yoshida et al. 1994, Baeza et al. 2005, de Boulois et al. 2008, Ohnuki et al. 2016). Ehlken and Kirchner (2002) further suggest that components such as soil microbes and rhizosphere processes can have large impacts that have not yet been thoroughly explored. Contrary to other studies that found little or no correlation, at Joyce's Branch I found plant radiocesium activity concentrations were significantly influenced by soil radiocesium concentrations (Sharitz et al. 1975, Wirth et al. 1994). This suggests that plant radiocesium concentrations cannot always be inferred from transfer factors at sites where radiocesium is less bioavailable (Bell et al. 1988, Absalom et al. 1999). Several models have suggested including some additional factors such as soil composition and potassium concentrations to better predict radiocesium uptake in the future (Shaw and Bell 1989, Absalom et al. 1999). However, data for these additional parameters may not always be available, especially in areas of widespread contamination. Therefore, transfer factors can be useful for the small amount of inputs required to obtain a value. While transfer factors may provide limited reliability in predicting sample radiocesium activity concentrations given soil concentrations, they can be effective in comparing radiocesium bioavailability and movement between locations.

ENVIRONMENTAL CONDITIONS AT THE SAVANNAH RIVER SITE

The observed high radiocesium activity concentrations and transfer factors in plants and fungi samples from Joyce's Branch indicate that there are potential environmental characteristics at the SRS that promote uptake from contaminated media (Zaunbrecher et al. 2015, Kaplan et al. 2005, Carlton et al. 1992). The soils of the Southeast U.S. are described as highly weathered kaolinitic clays that are inefficient at retaining potassium levels to support crop needs without fertilization (Mikhailova et al. 2019, Evans et al. 1983, Dahlman et al. 1975). At the SRS there is a high percentage of sand relative to clay (NCRP 2007). Notably, Evans et al. (1983) found that over 80% of the clay in two retention ponds connected to the R-Reactor cooling canal system contained the mineral kaolinite and less than 10% contained the mineral illite. Clays containing sufficient minerals such as illites can act as radiocesium sinks through fixation between mineral layers (Sawhney 1964, Poinssot et al. 1999). Comparatively, kaolinitic clays experience much higher rates of desorption and greater bioavailability to plants roots and fungal hyphae (Dahlman et al 1975). Evans et al. (1983) also found the sediments of these retention ponds to have high ion exchange capacity. Consequently, the soils of the southeast U.S., and specifically SRS, contain several properties that increase the likelihood of plant and fungal uptake of radiocesium compared to other sites. Conversely, the soils of the Oak Ridge Reservation are silty and contain large amounts of clay with illite and vermiculite (Coobs and Gissel 1986, Garten et al. 2000). Hanford site soils are also characterized by clays with micaceous minerals (Zachara et al. 2002). The strong sorptive properties of Oak Ridge and Hanford soils are reflected in their low plant to soil transfer factors (Auerbach and Crossley 1958, Dahlman and Van Voris 1976, Cataldo et al. 1978, Schreckhise et al. 1993). While the contaminated areas resulting from the Chernobyl and Fukushima nuclear disasters are too large to produce comprehensive soil and mineral composition comparisons, studies within these contaminated areas have found similar responses of radiocesium uptake to clay and mineral content (Koarashi et al. 2012, Bunzl et al. 1995).

ENVIRONMENTAL AND HUMAN HEALTH IMPLICATIONS

While the unique environmental conditions at the SRS have and will continue to allow for extensive research on radionuclides, there are very large environmental and human health consequences for those living in proximity to the site. Predictively, high radiocesium concentrations and bioavailability to basal carbon resources can result in bioaccumulation in higher trophic levels at the SRS (White 2003). Elevated radiocesium activities observed in wild boars, game fish, and wading birds on the site support this prediction (Oldenkamp et al. 2016, Kennamer et al. 2017, Paller et al. 2005). Additionally, many of these high trophic level organisms have the ability to leave nuclear exclusion zones and USDOE contamination boundaries. Deer and other game animals can enter into hunting areas, extending environmental and human risk beyond these established barriers. While my results indicated no difference between plant vegetative and reproductive tissues, observed trends in certain species reinforce my strong beliefs that more comprehensive research regarding this question is needed. Specifically because many migratory wildlife taxa, such as deer and birds, include plant reproductive tissues in their diets (Lay 1965, Levey 2009). Given more extensive and consistent seasonal sampling, I believe that plant reproductive tissues could indicate a potentially significant pathway of radiocesium trophic transfer. A comprehensive understanding of radionuclide movement and fate at sites with high contaminant bioavailability is essential for predicting current and future environmental and human health risks.

CONCLUSION

As countries move towards green energy alternatives and energy independence, we can expect to see an increase in reliance on nuclear energy (UNSCEAR 2016). Consequently, it is important to better understand the movements of environmentally persistent nuclear fission byproducts, such as radiocesium, to make better management and restoration decisions for past and future nuclear processes. The majority of radionuclide research has focused on taxa consumed by humans (IAEA 2010). For example, research was conducted on agricultural plants and animals in the areas affected by the Chernobyl nuclear disaster in 1986 in order to mitigate exposure to human populations (Krouglov et al. 1997, Karlén et al. 1995, Green and Dodd 1988). However, radiocesium contamination is not limited to large disasters. Nuclear weapons testing and energy production at USDOE sites have been the primary source of contamination ((Mackenzie 2000, UNSCEAR 1993, UNSCEAR 2000). Research has been conducted on radiocesium at the SRS for over 60 years. However, research has tended to focus on animals and studies conducted on plant uptake of radiocesium at the SRS have primarily been conducted in aquatic or riparian environments (Pinder III and Sharitz et al. 1978, Hinton et al. 1999, Kaplan et al. 2005, Kennamer et al. 2017, Paller et al. 2005). Since the 2011 Fukushima nuclear disaster, focus on cesium/radionucleotide contamination has shifted more towards non-human food webs including research on terrestrial plants (Yamashita et al. 2014, Sakai et al. 2021). My study is one of the first to look at radiocesium accumulation and transfer in a wide range of terrestrial plants and fungi along a contaminated soil gradient outside of a nuclear disaster zone. What I can infer from my findings at the SRS is both troubling and informative. Nuclear disaster zones are not the only contaminated sites that pose environmental and human health risks. Given the right environmental conditions, high radiocesium activity concentrations and transfer factors can still result from even moderately contaminated soils. The impact environmental characteristics, such as soil composition, minerology, and potassium content, can have on whether or not radiocesium will remain bound to soil or will become available to biota, can be exemplified by large differences in transfer factors observed among USDOE sites. While the environmental conditions of Hanford site and Oak Ridge have seemingly mitigated radiocesium transfer into aquatic and terrestrial ecosystems, the environmental conditions at SRS have instead promoted radiocesium bioavailability (Auerbach and Crossley 1958, Dahlman and Van Voris 1976, Cataldo et al. 1978, Schreckhise et al. 1993). Worldwide, reliance on nuclear energy is predicted to continue to grow (UNSCEAR 2016). Informative data of a site's potential for radionuclide trophic transfer is necessary for implementing future nuclear management and restoration efforts. As the US and other countries consider available locations for establishing new nuclear facilities, I strongly suggest that along with geographic location and socioeconomic parameters, that decision-makers consider the aforementioned environmental conditions in order to mitigate future nuclear production environmental and human health risks.

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SUPPLEMENTAL MATERIALS

Table S1. Preliminary Plant and Fungi Sampling at Joyce's Branch (Bryan and Canaday unpublished 2018)

Plants

Plant Functional Group	Plant Species		N	Radiocesium activity (Bq/kg dry wt) Range	Mean+/- SE
Ferns	Cinnamon Fern	*Osmunda cinnamomea*	14	876 - 40,172	8,690 +/- 12,869
	Southern Grape Fern	*Sceptridium biternatum*	1	-	43,450
	Chain Fern	*Woodwardia spp.*	2	13,363 - 21,292	17,328 +/- 5,607
Woody Vines and Semi-Woody Plants	Muscadine (Leaves/Stems)	*Vitis rotundifolia*	11	336 - 2,032	1,086 +/- 557
	Muscadine (Fruit)	*Vitis rotundifolia*	1	-	3,524

Fungi

Fungal Habitat	N	Radiocesium activity (Bq/kg dry wt) Range	Mean +/- SE
Ground	6	9,449 - 207,897	109,625 +/- 81,383
Wood	2	1,241 - 7,847	4,544 +/- 4,671

Table S2. Radiocesium Activity (Bq/kg dry wt) in Plant Species

Plant Functional Group	Plant Species		N	Radiocesium activity (Bq/kg dry wt)				Transfer Factor	
				Range	Mean+/- SE	Mean MDC	% Below MDC	Range	Mean +/- SE
Aquatic Plants	American Bur-reed*	*Sparganium americanum*	2	90 - 158	124 +/- 34	310	100%	-	-
	Duckweeds*	*Lemna sp.*	1	-	66	246	100%	-	-
Cacti	Eastern Prickly Pear*	*Opuntia humifusa*	1	-	79	1,250	100%	-	2.8
Canes	Giant Cane	*Arundinaria gigantea*	13	0 - 8,189	1,740 +/- 703	389	38.5%	0 - 84.5	9.8 +/- 6.9
Ferns	Eastern Bracken Fern	*Pteridium aquilinum*	36	0 - 84	174 +/- 27	522	97.2%	0 - 197.7	14.6 +/- 8.4
	Christmas Fern*	*Polystichum acrostichoides*	4	0 - 3,483	2,560 +/- 858	621	25%	0 - 17.4	8.7 +/- 4.9
	Cinnamon Fern	*Osmunda cinnamomea*	16	0 - 4,535	1,186 +/- 373	716	56.3%	0 - 313.7	52.3 +/- 26.5
	Fern Fiddlehead*	Unidentified	1	-	723	336	0%	-	43.1
	Cutleaf Grape Fern*	*Botrychium dissectum*	1	-	15,977	932	0%	-	-
	Eastern Marsh Fern	*Thelypteris palustris var. pubescens*	11	0 - 7,625	1,410 +/- 814	561	54.5%	1.0 - 294.0	42.6 +/- 36.0
	Netted Chain Fern	*Woodwardia areolata*	15	0 - 51,249	18,112 +/- 4,289	648	20%	0 - 14.9	5.5 +/- 1.0
	Ebony Spleenwort*	*Asplenium platyneuron*	16	186 - 7,046	3,021 +/- 569	973	18.8%	0.1 - 4.8	1.2 +/- 0.6
	Virginia Chain Fern	*Woodwardia virginica*	14	414 - 32,124	9,986 +/- 2,710	745	7.1%	0.5 - 25.7	10.5 +/- 2.9
Forbs	Butterfly Milkweed*	*Asclepias tuberosa*	1	-	341	565	100%	-	12.2
	Creeping Lespedeza*	*Lespedeza repens*	1	-	0	493	100%	-	0
	Spotted Wintergreen*	*Chimaphila maculata*	2	144 - 329	237 +/- 92	892	50%	4.2 - 62.9	33.4 +/- 29.3
Grasses, Sedges, and Rushes	Sedges*	*Carex sp.*	2	0 - 330	165 +/- 165	943	50%	-	0
	Rushes*	*Juncus sp.*	4	0 - 427	196 +/- 89	303	75%	-	-
	Slender Woodoats*	*Chasmanthium laxum*	2	88 - 7,122	3,605	160	50%	1.4 - 2.4	1.9 +/- 0.5
	Switchgrass*	*Panicum sp.*	1	-	0	1,878	100%	-	0
	Whip Nutrush*	*Scleria triglomerata*	1	-	51	266	100%	-	0.67
	Other*	No ID	7	19 - 175	80 +/- 24	236	100%	-	0.65
Lichens, Mosses, and Spanish Mosses	Lichens	-	10	0 - 814	366 +/- 91	377	50%	0 - 1.1	0.3 +/- 0.1
	Mosses*	-	9	0 - 777	224 +/- 81	548	88.9%	0 - 36.0	6.5 +/- 4.3
	Spanish Mosses*	*Tillandsia usneoides*	5	0 - 87	26 +/- 17	347	100%	0 - 0.6	0.2 +/- 0.2

Table S2. Radiocesium Activity (Bq/kg dry wt) in Plant Species

Plant Functional Group	Plant Species		N	Radiocesium activity (Bq/kg dry wt) Range	Mean+/- SE	Mean MDC	% Below MDC	Transfer Factor Range	Mean +/- SE
Shrubs and Trees	American Beautyberry*	*Callicarpa americana*	5	0 - 3,002	731 +/- 575	400	60%	0 - 2.5	0.9 +/- 0.4
	American Holly*	*Ilex opaca*	8	0 - 4,526	1,193 +/- 552	320	25%	0.1 - 2.3	0.7 +/- 0.4
	Black Cherry*	*Prunus serotina*	1	-	382	136	0%	-	0.5
	Blue Huckleberry*	*Gaylussacia frondosa*	5	0 - 6,967	1,482 +/- 1,374	265	60%	0 - 110.8	26.3 +/- 21.5
	Coastal Doghobble*	*Leucothoe axillaris*	1	-	149	147	0%	-	-
	Deerberry*	*Vaccinium stamineum*	5	6 - 220	79+/- 37	381	100%	0.5 - 3.5	2.0 +/- 1.5
	Elliott's Blueberry*	*Vaccinium elliottii*	1	-	67	165	100%	-	-
	Flowering Dogwood*	*Cornus florida*	1	-	90	332	100%	-	-
	Hickories*	*Carya sp.*	1	-	29	139	100%	-	1.7
	Large Gallberry*	*Ilex coriacea*	6	84 - 674	374 +/- 77	160	16.7%	0.4 - 17.1	5.1 +/- 2.5
	Oaks	*Quercus sp.*	16	0 - 6,019	805 +/- 405	194	56.3%	0 - 47.9	5.6 +/- 3.3
	Pines*	*Pinus sp.*	10	0 - 3,877	1,079 +/- 467	1,440	60%	0 - 0.8	0.2 +/- 0.1
	Sassafras*	*Sassafras albidum*	5	0 - 120	55 +/- 21	747	100%	0 - 3.0	1.7 +/- 0.6
	Smooth Adler*	*Alnus serrulata*	1	-	118	199	100%	-	-
	Southern Bayberry*	*Myrica caroliniensis*	1	-	668	194	0%	-	0.2
	Swamp Bay	*Persea palustris*	14	25 - 2,712	712 +/- 237	188	35.7%	0.3 - 108.7	13.3 +/- 11.9
	Sweetgum*	*Liquidambar styraciflua*	3	44 - 60	51 +/- 5	111	100%	0.7	0.7
	Sweetleaf*	*Symplocos tinctoria*	2	0 - 94	47 +/- 47	3,006	100%	0 - 2.5	1.3 +/- 1.3
	Winged Elm*	*Ulmus alata*	1	-	1,866	171	0%	-	0.7
Woody Vines and Semi-Woody Plants	Alabama Supplejack*	*Berchemia scandens*	1	-	40	142	100%	-	1.0
	Black Raspberry*	*Rubus occidentalis*	6	45 - 1,674	657 +/- 257	416	50%	0.2 - 6.8	1.9 +/- 1.1
	Greenbriers	*Smilax sp.*	21	0 - 4,597	1,253 +/- 320	603	47.6%	0 - 115.1	11.0 +/- 6.8
	Muscadine	*Vitis rotundifolia*	26	0 - 1,274	319 +/- 76	152	50%	0 - 8.1	1.1 +/- 0.4
	Partridgeberry	*Mitchella repens*	14	0 - 9,395	1,971 +/- 700	666	35.7%	0.4 - 166.7	16.6 +/- 13.8
	Yellow Jessamine	*Gelsemium sempervirens*	28	0 -1,721	243 +/- 83	235	75%	0 - 4.1	0.8 +/- 0.2

* Indicates plant functional groups that were not included in statistical analysis, due to insufficient sample number
MDC is the minimum detection concentration calculated for each sample (Currie 1968)