SRNL-STI-2019-00314

Assimilation and Transport of Organic Bound Tritium in an Irrigated Pine Forest

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ABSTRACT

The speciation of radioactive tritium (T) in a naturally-established subtropical loblolly pine forest that has been irrigated with highly-contaminated pond water for the last 20 years is reported. This irrigation project was created to limit the underground transport of a tritium-rich plume which also contains low levels of toxic organics, metals and radionuclides such as carbon-14 (^{14}C) from a nearby low-level waste burial ground. The levels of tritiated water (HTO) in the wood cores were not influenced by recent irrigation activities. However, the tritium levels in the last 20 years of tree growth were more than 3-fold higher than that of tritium in the older growth. This was due to recent irrigation with organic-bound tritium (OBT)-rich water and subsequent accumulation of high levels tritium as OBT relative to tissue HTO. High levels of pond irrigation water OBT resulted from biogenic processes that converted HTO to OBT. Data for ^{14}C that were acquired for some of the forest materials indicated that the processes controlling the movement and accumulation of ^{14}C in this system are somewhat different than that of tritium. Spectroscopic characterization of tree core tissue of <20 years in age found no explanation for the unusually wide dark growth rings. It was concluded that the trees were over-irrigated based on results from other published studies with wood from severely-flooded areas. Although HTO is indeed toxic to biota, OBT represents a relatively greater hazard to biota because it can be bioaccumulated and retained for long periods of time in living tissues.

INTRODUCTION

Anthropogenic radioactive tritium (T, half-life of 12.3 years) has been released into the environment by nuclear activities for the last 75 yrs. Tritium is produced naturally in the upper atmosphere by cosmic ray interactions with nitrogen (N) and oxygen (O) at levels of 0.15 to 0.2 kg year^{-1} or 5 to 7 x 10^{16} Becquerels (Bq; as in Lebaron-Jacobs et al., 2009; cited by Calmon and Garnier-Laplace, 2010). This level of natural production is low relative to known anthropogenic tritium releases of up to several hundred kilograms of tritium annually depending on the type of source term.[1] Some examples of these source terms include but are not limited to: nuclear reactors, nuclear weapons, the tritiated light source industry (which uses tritium to create luminous dials and emergency lighting), the radiopharmaceutical industry and a variety of nuclear wastes from anthropogenic activities. Anthropogenic tritium inputs can vary with geographical location and hemisphere as well as with time or release event.[1]

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Tritium presents a potential biological hazard when it interacts with biota because hydrogen (H) is an essential element that occupies numerous molecules that are essential for life. Tritium levels in biota usually reflect local atmospheric tritium levels because biota cannot exclude the ^{3}H isotope which is found in tritiated water or "HTO". Transfer of tritium upwards in a food chain generally results in the depletion of tritium in biological tissue[2,3,4] and with some conversion of HTO to organic bound tritium (OBT) forms.[5] The conversion of HTO to OBT through plant photosynthesis, respiration, and the active transport to make proteins, lipids, carbohydrates and many other necessary molecules for existence is thought to be a slow process.[6,7] But under specific circumstances, tritium can become accumulated as OBT by biota when it is released in the environment as persistent and "technologically enhanced" tritiated organic compounds,[8,9] when the organisms have access to a tritiated food source,[10] and when tritium becomes absorbed as a tritium-rich vapor onto plant tissue.[11,12,13]

OBT can be defined based on how this form of tritium interacts with biological systems. Several researchers have delineated exchangeable organically-bound tritium (E-OBT) from non-exchangeable organically-bound tritium (NE-OBT) in their reviews and studies with biota.[5,6,12,13,14,15] They have defined E-OBT as being tritium bound to either O or N whereas NE-OBT was defined as tritium bound to C but the terminology for defining OBT varies widely in the literature. These authors collectively classify tritiated water in tissues as "tissue free water tritium" (TFWT). In this report due to the complexity of terminology, TFWT in environmental material will be referred to as HTO (as in Melintescu and Galeriu, 2017); OBT will be defined as NE-OBT or E-OBT when the analytical results support the assignment of NE-OBT and/or E-OBT; otherwise, "OBT" will collectively signify all types of OBT.[16]

Tree rings have occupied considerable research interest due to their potential to be a metric of historic tritium releases.[17,18,19,20,21] Cellulose-based tritium is thought to be a highly stable form of OBT within the tree and comparisons between cellulose tritium and wood tritium have produced similar results.[21] The preservation of tritium release levels in wood rings can be related to tree species. For example, in 1996 to 2008 OBT levels in aspen tree rings were sampled near SRB (emergency lighting) Technologies, Ontario, Canada and were found to partially reflect historic tritium levels in emissions whereas 1993 to 2008 OBT levels in poplar tree rings at Darlington Nuclear Generating Station in Ontario did not reflect known emissions of tritium.[19] Measured OBT (as cellulose) in tree rings of pine and eucalyptus successfully reflected atmospheric water vapor monitoring data after the release of tritium-labeled organic compounds that were made for biomedical studies.[17] In addition to T, carbon-14 (^{14}C) is another radionuclide that is sometimes found in contaminated areas and its levels have been measured in tree rings.[22,23,24,25,26] These studies suggest tree ring ^{14}C levels can be fairly representative of historic ^{14}C releases from nuclear reactors as well as from nuclear weapons testing.

Large volumes of contaminated groundwater were generated from prior Cold War activities at U.S. Dept. of Energy Sites. They are a challenging and expensive problem to manage and disposition. Some groundwater contaminants can be successfully pumped and treated—as in the case of some toxic organic compounds, which can be decomposed through various physical and chemical means. Some radioactive contaminants like tritium that have a fairly-short half-life become suitable candidates for other treatment approaches that slow or limit the release of the tritium to the environment. At the U.S. Department of Energy's Savannah River Site (SRS), the subsurface movement of tritium and ^{14}C in highly-contaminated groundwater plume has been successfully curtailed for the last 20 years due to a "pump, holding pond and irrigation" approach at the Mixed Waste Management Facility (MWMF).[27] The MWMF uses the tritiated groundwater for irrigation and the facility was designed so that the tritiated water would move through irrigated forest trees and eventually transpired and released to the atmosphere. The current study examines the incorporation and movement of tritium and ^{14}C in an established subtropical pine forest which has been irrigated with tritium- and ^{14}C-contaminated water for the last 20 years.

EXPERIMENTAL

1. MWMF Operational Data

The MWMF is at the SRS in Aiken, SC, USA and the MWMF comprises about 0.29 km^2 of the greater 500 km^2 area at SRS. The SRS was primarily rural farmland, floodplain and pine and hardwood forest before becoming a federal nuclear site in 1950 as part of the Cold War.[27] The SRS is inaccessible to the public; it has limited vehicle traffic constrained to ~12,000 employees. For the last 70 years, the SRS has consisted of mostly managed forested, pond and swamp floodplain areas which have attracted a large diversity of wildlife—including many federally-endangered and threatened animal and plant species. Approximately 10% of the SRS land is used for industrial nuclear fuel cycle activities.

The MWMF is located within an area identified as the Low Level Radioactive Waste Disposal Facility (LLRWDF), which resides between the F- and H-Area Separations Facilities where rainwater infiltration has spread tritium contamination from the LLRWDF into the groundwater. With tritium concentration ranging from <740 to 370,000 Bq L^{-1} (based on conversion of Ci L^{-1} to Bq L^{-1}), this plume once flowed toward a small stream called Fourmile Branch (**Fig. S1**).[28] It was estimated that ~167 million L of surface water were discharging annually from the seepage channel.[29] To reduce tritium contamination of Fourmile Branch, a small sheet pile dam was constructed in the year 2000 over the seepage area. Since late 2000, groundwater from this plume has been pumped into this 10.22-million-L holding pond. As part of a ~20-yr old phytoremediation project, an irrigation system was constructed to pump and distribute the tritiated pond water to an adjacent ~0.18 km^2 naturally forested area. The pond also contains low levels of ^{14}C (up to 40.7 Bq L^{-1}), technetium(Tc)-99 and iodine(I)-129, various chlorinated organics, acetone, and heavy metals which occur as groundwater plume co-contaminants (see **Table S1**). [Prior to 2000, the pine trees in this area received rainwater as their water source. With shallow root zones of ~1 m in depth these pine trees which thrive in acidic sandy soil were unexposed to the groundwater plume that was ~60 m below].[30]

Irrigation with the holding pond water began in March 2001 and offsite atmospheric releases of tritium from the trees are estimated to be less than 0.004 mrem yr^{-1} (0.00004 mGray yr^{-1}). The area includes natural and managed stands of loblolly pine (*Pinus taeda*) and some hardwoods (oaks and sweet gum). The MWMF trees are irrigated by a series of risers and sprinkler heads that are ~5 m on a square grid with a flow rate of 2.0 L min^{-1}.[31] Average tritium pond water concentrations fluctuate based on ambient conditions (such as rainfall, heat and humidity) range from ~5,920 to 573,500 Bq L^{-1}. In the last decade, annual total irrigation rates averaged 39.4 million L yr^{-1} with an average total tritium delivered to plots of 7,681 TBq yr^{-1} (from 2003 to 2011).[32] The pond water level is also maintained by a mechanical evaporator, which resides near the water's edge and operates during periods of high precipitation. The pond supports local wildlife, such as small (~1 m) alligators, amphibians, several smaller reptiles, fish, insects, aquatic plants and birds using it for a water source and for food.

The management of the irrigation of MWMF trees is based on the soil moisture deficit, which is the difference between evapotranspiration and net precipitation relative to irrigation and rainfall. Most watering occurs in the late spring and summer. Although the MWMF trees range in age from <1 to 100 years, the current study focuses on a naturally-forested area with the oldest trees that have received tritiated irrigation water since the MWMF (pond and facility) was established 20 years ago (see map on **Fig. S1**). Comprehensive water quality and contaminant analyses for unfiltered water samples between Jan. 2002 and Dec. 2017 were provided by the SRS's Accelerated Closure Projects (ACP) (see **Table S1**).

2. Field Collected Samples

Tree cores, soil, leaf litter and bark were collected from irrigation plots at the MWMF phytoremediation project. Tree cores were collected in April and August of 2017 from loblolly pine trees using a Haglöf increment borer with a 0.515 cm diameter. Two cores from each tree were collected at standard breast-height. Tree girth (diameter breast height) and height measurements were made for each sampled tree. Leaf litter was collected near the trees and the underlying acidic sandy loam soil (derived from the Coastal Plain and consists of the Fuquay-Blanton-Dothan Association) was sampled to a depth of 15 cm.[33] Bark was collected by peeling from the tree at locations above and below the irrigation spray height. All samples were individually bagged, approved for free release by SRS health physics personnel, and frozen at -80°C until analysis. Unfiltered pond water that is used for the irrigation of the trees was collected in April 2017. Bark from a mature loblolly pine in an uncontaminated rural area 25 km northeast of the MWWF was also collected.

3. Sample Preparation, Tritium and ^{14}C Extraction and Measurement

To collect all isotopes of H including tritium from the various biological matrices the Raddec Pyrolyser-6 Trio™ combustion furnace (Raddec International Ltd., United Kingdom) was used. This combustion furnace extracts tritium and CO_2 from various sample matrices. Before extraction, the samples were removed from a -80°C freezer, cut into small pieces (and homogenized as needed without allowing the samples to completely thaw) and placed in a sample boat. Wet weights (0.7 to 4.3 g) of material were measured and the material was used for extraction. Sample masses were higher (e.g. 4.3 g) for extractions with intact core material. Soil samples had 2-g wet weights. Bark, leaf litter and most of the 10-yr increment tree cores weighed ~1-g but some of the core samples weighed up to 2.5 g.

For determination of tissue HTO, NE-OBT and E-OBT, sequential steps were required to isolate each extracted fraction whereas total tritium by complete combustion was performed separately (see **Table S2** for details). For sequential fraction studies, collection of tissue HTO began with a slow ramp heating of the sample up to 150°C. The liberated HTO was collected using a bubbler in 0.1 M HNO_3. For collection of E-OBT, tritium-free or "dead" water was added to the dried sample while in the quartz sample boat at room temperature. The sample boat was sealed to limit evaporation and exchange with the ambient room air. After 72 h on a shaker table, the water was decanted and collected for tritium analysis. The sample boat was then placed in a drying oven at 150°C for 15 minutes (min) prior to collection of NE-OBT (in 0.1 M HNO_3). The remaining material and the sample boat then underwent a slow ramp up to 600°C for complete sample combustion by the Pyrolyser.

For tritium analysis, 8 mL from the recovered tritium fractions was mixed with 12 mL of Ultima Gold liquid scintillation cocktail and counted by liquid scintillation counting (LSC) using a Quantulus 1220 (PerkinElmer). For ^{14}C analysis, the Pyrolyser was used to liberate CO_2 from the samples via decomposition (see **Table S2** for details).[34,35] The liberated CO_2 was then trapped in Carbo Sorb E (an organic alkali solution of 3-methoxypropylamine) trapping solvent (PerkinElmer Inc., Packard BioScience B.V., Groningen, The Netherlands) and ^{14}C was quantified by LSC using the Quantulus in the Carbo Sorb E cocktail after addition of Permafluor E. Estimated net activity rates have been reported in Bq g^{-1} (of fresh weight material) correcting for matrix effects. A series of blanks were run between sample sets to quantify any carryover, which was determined to be minimal from these studies. Decay corrections were applied to account for the time difference between extraction and the completion of the counting of tritium by LSC.

4. Spectroscopic Characterization

The barks, tree cores, soil and pond water were characterized "as is" using Fourier Transform Infrared Reflectance (FTIR) spectroscopy with a Nicolet 7600 FTIR Spectrometer (Thermo Electron Corp.). The spectrometer was equipped with an attenuated total reflectance (ATR) accessory with a diamond crystal at a 45° angle of incidence. Spectra were collected from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. 128 scans were averaged to obtain the final spectra. The spectra were baseline corrected and an ATR correction was made using a sample refractive index of 1.5.

RESULTS and DISCUSSION

1. Tritium in Pond Water, Tree, Soil and Leaf Litter

Table 1 shows that the intact tree core material from a single tree consisted of mostly HTO and NE-OBT. Analyses of five sister tree core sections in a 100-yr old tree were also conducted at 20-yr growth (ring) intervals. The tritium speciation in the oldest four sections were dominated by HTO and a lesser amount of NE-OBT and in contrast, the NE-OBT levels in the most recent 20-yr old core material were nearly twice that of older material. Additionally, the total tritium (by summation of each extracted tritium fraction) in this most recent section was nearly twice that of older core material. The NE:OBT to E:OBT ratio in this 20-yr old material was higher relative to the older material or that of the intact core.

Table 1. Analytical data for the fractions of HTO, E-OBT, and NE-OBT measured in the MWMF samples, total tritium and ^{14}C; the ^{14}C analyses were performed on cores from neighbor trees and not those used for tritium analyses. Values are reported as Bq per gram of fresh weight material.

Matrix	**HTO**		**E-OBT**		**NE-OBT**		**Sum of Tritium Fractions**	**OBT: HTO**	**NE-OBT: E-OBT**	**Total Tritium Bq g^{-1}**	**Total ^{14}C Bq g^{-1}**
	Bq g^{-1}	**%**	**Bq g^{-1}**	**%**	**Bq g^{-1}**	**%**	**Bq g^{-1}**				
Tree Core Intact (n=2): 1918-2016	0.74±0.01	56	0.13±0.003	10	0.44±0.01	33	1.31±0.03	**0.77**	3.24	ND	ND
Tree Core Sections (n=2): 1918-1938	0.85±0.02	51	0.22±0.005	13	0.61±0.01	36	1.69±0.03	**0.98**	2.75	ND	ND
1939-1958	1.12±0.02	53	0.23±0.005	11	0.77±0.02	36	2.11±0.04	**0.89**	3.38	ND	ND
1959-1978	0.75±0.02	43	0.23±0.005	13	0.77±0.02	44	1.75±0.04	**1.34**	3.34	ND	0.13±0.02 (n=1)
1979-1998	1.11±0.02	50	0.32±0.007	14	0.80±0.02	36	2.22±0.05	**1.01**	2.52	ND	0.16±0.02 (n=1)
1999-2016	0.90±0.02	23	0.33±0.007	8	2.76±0.06	69	4.00±0.08	**3.43**	8.30	ND	0.15±0.02 (n=1)
Tree Cores (n=6) 1986-1996	ND	-	ND	-	ND	-	ND	-	-	ND	0.14±0.03 (n=1)
1996-2016	1.38±0.04^{A}	19	0.85±0.03^{B}	11	5.16±0.08^{B}	70	7.38±0.14	**4.36**	6.07	8.67±0.17 (n=6)	0.19±0.03 (n=1)
Pondwater (n=2)	75.26±1.12	69	Total Suspended OBT: 33.67±0.68 (31% suspended OBT)				108.93±1.8	**0.45**	ND	ND	ND
Lower Bark (n=4)	0.35±0.02^{A}	10	0.65±0.02^{A}	16	2.83±0.08	74	3.82±0.12	**9.94**	4.35	4.90±0.11 (n=5)	0.37±0.08 (n=2)
Upper Bark (n=4)	0.41±0.02	8	0.60±0.02	16	2.95±0.09	76	3.96±0.12	**11.60**	4.92	4.77±0.13 (n=5)	0.18±0.02 (n=1)
Leaf Litter (n=4)	0.50±0.01	10	0.37±0.01^{B}	8	4.10±0.08^{A}	82	4.97±0.11	**8.94**	11.08	4.50±0.11 (n=6)	0.16±0.03 (n=2)
Surface Soil (n=3)	0.47±0.01	42	0.14±0.01^{A,B}	8	0.57±0.02^{A,B}	50	1.18±0.04	**1.51**	4.07	0.55±0.02 (n=6)	0.09±0.02 (n=2)

Note: HTO (^{A} p-value = 0.03), E-OBT (^{A} p-value = 0.04), (^{B} p-value = 0.001), NE-OBT (^{A} p-value = 0.02, ^{B} p-value = 0.001); ND: Not determined; Sample size "n" is listed in left heading or noted otherwise. The OBT:HTO ratios equate to the sum of each OBT amount divided by the HTO amount in the tissue. Pondwater OBT values are for suspended OBT; dissolved OBT was not quantified so the total OBT are conservative.

Examinations with recent 20-yr old sections of cores from other neighboring trees provided more detail on the behavior of the tritium as well as mass balance information for extracted tritium relative to total tritium by sample combustion (**Table 1**). NE-OBT was the most common tritium species in the extracts of these most recent 20-yr core sections but the sum of the tritium fractions was typically 1.3 Bq g^{-1} less than the total tritium as determined by combustion. Our preliminary studies with tree core tissue concurred with the literature that period of 72 h[5,36] would be sufficient to isotopically-exchange H for tritium in E-OBT (unpublished SRNL data). But there was the potential for some loss of tritium despite efforts to contain the tritium vapor during the H-T exchange during this 72-h period. The percent difference in the total tritium measured by the sample combustion relative to the summed total of the HTO, E-OBT, and NE-OBT fractions indicates that there are variations for each matrix but summed values of tritium in these fractions in bark, leaf litter and tree cores were within 25% of each other (**Table 1**). A standard amount of 1 g of material (in most cases and when possible in this study) was found to work well for the sample matrices. In summary, if the perceived ~1.3 Bq g^{-1} deficit were added to the E-OBT values for the tree core material, the findings regarding elevated OBT:HTO ratios in these samples would not change and the NE-OBT:E-OBT ratio would still be mostly driven by NE-OBT.

The pond water contained ~109 Bq tritium g^{-1} and about 31% of that was OBT based on analysis of Whatman #10 filter paper material that was used to filter the pond water. Due to the large particle size of the filter, these estimates of pond water OBT are conservative because we did not quantify the amount of dissolved OBT that was present. The upper and lower pine bark (**Table 1**) had similar NE-OBT levels and OBT:HTO ratios. A one-way ANOVA with Tukey's test of means revealed no statistically-significant differences between total tritium levels in the barks that were collected from above or below the irrigation spray line (p=0.81). It is conceivable that tritium is absorbed by the lower tree bark as wet droplets and as a moist vapor. Mihok et al. (2016) observed similar enrichments of OBT in agricultural plants relative to tissue HTO—particularly for above ground portions of plants relative to roots. They concluded that the source of the OBT enrichment was tritium-containing irrigation water that had absorbed to above ground portions (i.e., bark) of the plants. The upper MWMF pine tree bark had a slightly higher OBT:HTO ratio than the lower bark, which supports the evaporation and deposition of tritium vapor as being contributing to the accumulation of tritium as OBT. One difference in our study and that of Mihok et al. (2016) is that the source term of water to the MWMF trees contained a considerable amount of OBT in addition to HTO. Dissolved or suspended forms of OBT in the irrigation water could eventually become part of the OBT fraction as primarily NE-OBT.

Leaf litter, which was comprised of mostly pine needles, had tritium levels that were statistically-similar to that of the tritium in the upper or lower bark. Like the bark, the leaf litter tritium was also enriched in NE-OBT and the total tritium by combustion was 0.7 Bq tritium g^{-1} less than the sum of tritium extracts from the litter tritium analyses. [This difference is a recognized tradeoff that could be due to sample heterogeneity; because an extended physical processing of the sampled materials to make them more homogeneous was avoided to minimize losses of tritium prior to analysis.] The tritium in the leaf litter most likely reflects the levels of tritium in the pine needles before they fell as well as the latent uptake of HTO and OBT uptake from the evaporation of the irrigation water that deposited on the forest floor litter.

The mean total tritium level in the soil was statistically different and much lower relative to the other sample matrices (*p*=<0.001; **Table 1**). The total tritium (by summation of each OBT fraction) in the soils was less than that of the tree and leaf litter material and the level of soil NE-OBT represented ~50% of the total tritium activity. This soil is characterized as a sandy loam and it has a characteristically low organic content due to the absence of overlying green leafy vegetation that dies back annually and the lack of root mass material. Again, differences in soil sample heterogeneity and the selected minimization of sample physical homogenization could explain why total combustion levels are lower than that of the summed tritium fractions. The soil E-OBT levels were significantly different from the E-OBT levels measured in tree cores (*p*=0.001) with nearly six times as much E-OBT in the tree core matrix relative to the soil.

A one-way ANOVA with Tukey's test of means found that the HTO collected from each matrix was compared at the *p*=0.05 level. The population means for each matrix between trees were not significantly different for upper and lower bark, leaf litter, and soil and the HTO levels in the tree cores were marginally significant as compared to lower bark (*p*=0.04). The results are consistent with other previously mentioned observations that tritium is bound to cellulose and our studies conclude that tritium is found mainly as NE-OBT in bark. The adhesion of tritium to the bark may be another mechanism of tritium accumulation in addition to leaf litter and tree wood in this system.

Trees from the sampling area had a mean diameter at breast height of 35.4 cm (range: 13 to 54 cm) and were on average 23 m tall. The calculated Pearson's correlation coefficient showed a moderate correlation for HTO values that decreased with decreasing tree diameter and although the correlation of -0.61 is small, it suggests that younger (~20-year old) trees have lower HTO levels than older trees. **Table 1** reveals that younger MWMF trees have more OBT relative to HTO than the older trees. This could be expected because the young trees have a proportionately longer lifetime of exposure to a water source that is rich in OBT than the other much older trees. The HTO can absorbed by the plant (via root or tissue uptake) but HTO undergoes little or slowed incorporation into organic matter.[13,36,37] The HTO can be transpired readily by the tree but the OBT that is absorbed by the tree (through above ground physical contact or uptake through the roots) has few if any rapid paths for removal from the tree. This is in great contrast to HTO, which can be transpired and released to the atmosphere. Non-living plant matter can also participate in tritium uptake via tritiated moisture and this is likely occurring at the MWMF forest.[38] Momoshima et al. (2006) discovered that dried cedar needles participate in D_2O uptake (D_2O was used as a surrogate for HTO).[38] Although some details about the amount of surface area exposure for the live vs. dead leaf material are not provided, their work suggests that dead plant tissues are a potential tritium sink. They note that the uptake and release of D_2O was rapid relative to that of living tissue and attribute this behavior to a greater number of opened stoma in dead relative to living leaves.

Fiévet et al. (2013) examined the ratios of OBT to HTO in biota from coastal marine areas that were exposed to tritium releases in nuclear facility effluents.[39] They found that the OBT:HTO ratios in biota were near 1.0 although there were deviations. In general, they concluded that the conversion of HTO to OBT is considerably slow. They concluded that the OBT to HTO ratios should be near 1.0 in the absence of considerable isotopic fractionation, non-steady state “transient” conditions such as during and after acute accident releases and when the form of tritium that is released is high in OBT relative to HTO. All sampled MWMF matrices that were exposed to tritiated irrigation water had data that indicated the equilibrium with the HTO and the H from the irrigation water had been reached and that OBT was being further enriched in these materials. Older tree wood (core) materials that were not formed prior to when the irrigation commenced had OBT:HTO levels that were generally closer to 1. We did not measure the HTO content in air but we suspect that the MWMF system is not at equilibrium with the air since most of the HTO is being taken up through the roots and bark rather than the stomata, leading to longer residence times than for HTO exchange through the leaves as in prior work.[1,40] The HTO values measured in the various matrices represented a minor fraction of the total tritium that was measured in the sampled bark, leaf litter and soil materials (**Table 1**).

2. Carbon-14 in Pond Water, Tree, Soil and Leaf Litter

The ^{14}C levels in the MWMF samples are listed in **Table 1**. All plant litter, tree core and bark samples had higher ^{14}C levels than that of the nearby soil, much like that observed for tritium in these materials. If ^{14}C were a tracer for the carbon-based OBT in the irrigation water, the retention of ^{14}C in the bark supports the accumulation and immobilization of OBT. This would certainly be the case for the lower bark and it is conceivable that any organic forms of ^{14}C would also become transported to the upper bark after deposition by water vapor. This potential behavior of organic ^{14}C is consistent with that of the organic forms of tritium (such as OBT) in this study as previously discussed.

The potential use of ^{14}C as a tracer for inorganic C in the pine bark can be also considered. Dissolved $CO_{2(g)}$ and HCO_3^- are the primary forms of dissolved inorganic C and ^{14}C in the pH 6.05±0.69 irrigation water, which has a low carbonate alkalinity and low calcium concentration (**Table S1**; see Drever 1988 for more info.).[41] The situation regarding inorganic C behavior is complex because the pine bark is moderately acidic (pH ~4 to ~5.5) and the forms of inorganic C that can be found in pine bark include oxalate ($C_2O_4^{2-}$), calcium oxalate (CaC_2O_4) and carbonate minerals.[42] One substantial difference between the lower and upper bark is that the lower bark is routinely wet from sprinkler irrigation throughout the year and the upper bark is not. Differences in moisture could alter the bark surface chemistry such as the cation exchange capacity and surface pH. The continued application of irrigation water to the bark surface may promote retention of inorganic C (^{14}C) in contrast to the drier upper bark but this is only a theory. The chemical speciation of ^{14}C in the MWMF samples were determined in this study. There were no steps to selectively leach or analyze surface deposits of inorganic C to better assess the two types of bark environments. The FTIR and ATR (a surface sensitive reflectance IR technique) studies (data not shown) that were performed on the bark (upper and lower) were inconclusive. The results did not provide information on inorganic C speciation determinations such as whether any carbonate or oxalate were present. This was because the peaks (that were found to be unique to lower and upper bark) were very weak and near the threshold of detection. Additionally, there were few if any consistencies in the spectra for the lower and upper barks from the two MWMF trees that were examined. A follow-on study would help elucidate the behavior of organic and inorganic forms of C that exist in the irrigation water and the bark MWMF samples.

The levels of ^{14}C in the youngest 20-year old core section were not significantly greater than that of the older core sections for the same tree so the trend of enrichment that was observed for tritium was not as apparent for ^{14}C. Triplicate samples from a 30-year old tree permitted the analysis of ^{14}C in 10- and 20-year increments (**Table 1**). Despite limited data, some enrichment of ^{14}C during the most recent 20 years of wood growth occurs relative to the initial 10 years of oldest growth (0.19±0.03 vs. 0.14±0.03 Bq g^{-1}, respectively). Despite the small sample size and associated elevated analytical error in the MWMF ^{14}C tree ring data, these results for the MWMF trees differ in studies with pine and cedar tree rings. These studies with pine and cedar rings observed decreasing ^{14}C [as $\Delta^{14}C$(‰)] levels with decreasing plant age as described by Xu et al. (2015).[24] Bomb pulse ^{14}C from nuclear testing activities contributed large amounts of bomb pulse ^{14}C in the late 1960s but the levels of ^{14}C in the youngest MWMF tree core tissues are indicative of another more recent source of ^{14}C which we attribute to irrigation with ^{14}C-contamined pond water. It has been demonstrated that in the absence of newer sources of ^{14}C contamination, the levels of ^{14}C in more recent tree ring tissue and the atmosphere should continue to decrease with time due to the radioactive decay of ^{14}C to stable ^{14}N and intensive atmospheric mixing[22,26] but we do not observe this in the tree tissues that formed during the ~20-year irrigation period.

The ^{14}C data for the tissue from these younger MWMF tree cores indicate that ^{14}C is not highly subject to the same level of enrichment as the tritium. Additionally, some of the forms of ^{14}C that enter the tree and reside in the upper bark, wood and needles may be more labile and mobile (relative to tritium) as they become utilized by the tree or lost by other processes as previously discussed. Yet there are other interpretations. Differences between the assimilation between tritium and ^{14}C have been noted in the literature and they appear to be related to the source term of these isotopes.[17,22,23] Environmental ^{14}C often undergoes dilution by C-containing material that is old and depleted in ^{14}C (including that of anthropogenically-released ^{13}C-enriched fossil fuels)[24] and this makes the study of ^{14}C in the environment as well as comparisons between the behavior of tritium and ^{14}C difficult. Additional studies are clearly warranted here. For example, $^{12/13}C$, D/H and N isotopic studies in addition to tritium and some more extensive ^{14}C analyses would reveal more about the dynamics of H, C, and N in this system.

3. Tree Ring Morphology and FTIR Characterization Studies

Many of the pine trees cores from the MWMF displayed large late wood bands (**Fig. 1**). These large bands were indicating a longer winter growth period with more water uptake in their last 20 years of growth. This type of behavior was not observed in older wood tissue (prior to the year 2000) from the same MWMF trees. This phenotypic behavior for the tree cores was unusual and it seemed possible that the health of the MWMF trees could be adversely affected. FTIR studies revealed that the wood consisted of typical lignin, (hemi-)cellulose, and polysaccharides (**Table S3**).

Fig. 1. Images of two 0.515-cm diameter pine tree cores from the MWMF sampling area. Above: a core from young tree showing wide late wood bands that are indicative of a long growth season and excessive water exposure. Below: a core portion from an older MWMF pine tree whose growth occurred before the irrigation began and show a more normal phenotype for growth rings.

It was anticipated these analyses would reveal detectable differences in wood chemistry that could be indicative of a change in the health of the trees during the last 20 years. Young pines are reported to be difficult to establish in some parts of the MWMF due to poor soil quality and water retention (USDA Forest Service, personal communication). Additionally, several of the sweet gum and maple tree cores that we originally collected from the MWMF showed evidence of wood rot (particularly in their most recent growth) so they could not be used in this study. There was also the possibility that low levels of hazardous organics in the water (listed in **Table S1**) were contributing to the rotting and decay of the younger wood. FTIR studies did not detect any of these toxic compounds or unusual anthropogenic-type decomposition products of these compounds. There were differences between early and late wood where the early wood had more absorbance at 1,700 cm^{-1} (due to the carbonyl-OH IR stretch in lignin, homo and hemi cellulose); **Table S3**) than that of the late wood. This behavior is normal and attributed to differences in density as the lighter early wood is formed more rapidly during times of fast spring growth as compared to the darker late wood, which is denser due to its slow formation.[43]

The FTIR spectroscopic studies did not provide a clear explanation for why the late rings were darker and larger during the last 20 years. If divalent Fe were being oxidatively-precipitated as Fe (oxy)hydroxides (which are dark in color) during the irrigation period, peaks in the FTIR spectra for the wood that were indicative of Fe-O or Fe-O-H bends/stretches would have been discernable.[44] However, recent studies have shown that desert shrubs and trees exhibit similar ring darkening behavior after receiving off-normal flooding events.[45] Larger late wood bands that coincide when irrigation was most prevalent during spring and summer relative to small early wood bands during the fall and winter. Vaganov and researchers (2005) indicate that tree ring morphology is highly complex because it is a function of temperature, nutrient, tree location (relative to forest border), elevation and growing season but in general, tree ring widths are wider when precipitation is high as opposed to low.[46] It is possible that many of MWMF trees have been receiving excessive irrigation during their 20-year lifetime within the MWMF phytoremediation operation.

4. Incorporation of Tritium as OBT with Comparisons of OBT:HTO Ratios in Other Biota

The FTIR studies with the pond water filtrate showed that the filtrate is also rich in polysaccharide, cellulose and lignin. Polysaccharides are natural byproducts of living organisms and they are secreted by plants at the roots to assist with obtaining nutrients. They are water insoluble compounds that can consist of starch, chitin, glycogen, arabinoxylans, cellulose, and pectin. Microbes and fungi can potentially degrade and obtain energy from polysaccharides. It is conceivable that the interactions between microbes, fungi, amphibians, insects and plants result in considerable re-cycling of tritium in not only polysaccharides but as other water-soluble components as well. This effect would probably be more difficult to isolate in waters with much lower levels of tritium contamination. But the high levels of tritium in this contaminated pond are likely to exchange tritium for H in water as well as in other compounds such as sugars, amino acids and other cellular structural components in the trees. This type of enhanced tritium dynamic behavior could explain the elevated levels of NE-OBT relative to HTO in the tissue—particularly after a period of two decades. The evaporation of tritiated pond water at the pondwater's edge is likely to facilitate the transfer of HTO and OBT (both dissolved and suspended) to the biota at the water's edge may contribute to the pond dissolved and suspended organic tritium levels.

The considerably high levels of OBT relative to HTO that were found in biota from the present study were compared with published OBT:HTO values for biota (**Table 2**). It was evident that nearly every study must be considered individually but some generalizations could be made. Biota from low or background levels of tritium contamination had somewhat high OBT:HTO ratios, such as those in milk (up to 4.58) and in vegetables and fruits from tritium-contaminated areas (**Table 2**). Low OBT:HTO tissue ratios in the range of 0.57 to 1.3 are observed for rice (based on cited 1980s work by Hiramatsu and co-workers).[13] These values were attributed to the rice being an inundated crop for most of its existence and so the crop is more in equilibrium with the flood water HTO rather than atmospheric HTO. Soil OBT:HTO ratios are sometimes higher in uncontaminated areas relative to that in ours, so elevated OBT:HTO ratios in soil are not necessarily indicative of elevated tritium contamination. However OBT:HTO ratios of up to 1,400 have been observed in soils from a highly-contaminated area and biota can acquire OBT:HTO ratios of ~20 in laboratory tritium exposure studies and >13 in field studies.[11] These high OBT:HTO ratios exceed those in our study but the OBT:HTO ratios for the bark and leaf litter in our study represent the upper tier of elevated OBT:HTO as summarized in **Table 2**.

Reported conclusions about the behavior of tritium with plants are often unique to each study and the understanding of OBT behavior in biota remains a challenge. There are often differences in how each study approaches and reports OBT speciation determinations. A few studies report TFWT, NE-OBT, E-OBT and total OBT levels in plant tissue and other studies focus mostly on the isolation of cellulose-based tritium in wood.[17] Le Goff et al. (2014) take a different approach and employ a correction factor for NE-OBT:HTO tissue ratios in vegetation and other biological matrices which can result in 5 to 50% higher NE-OBT:HTO values. These corrections (which are supposed to account for isotopic fractionation) cannot be applied when NE-OBT is not reported, but in our study, such a factor would only increase the OBT:HTO ratios.[47] Another issue that has been addressed in the recent literature is the need for more consistency in the quantification of NE-OBT and E-OBT amongst researchers[48] as well as related concerns over the inadvertent loss of

NE-OBT when tritium is exchange with “dead water” to obtain E-OBT values.[5] Issues like these can certainly explain some of the variation that is observed in the literature and make comparisons between studies difficult.

CONCLUSION

The present study reveals that suspended and dissolved tritiated molecules in a pond that is used to irrigate a forest are most likely influencing the retention of tritium in the OBT compartment of trees. Because the pond is physically accessible to wildlife, it is biologically active due to the presence of algae, insects, birds, reptiles, other forms of biota and subtropical climate. After several years, these relationships promote the incorporation, release and recycling of organic forms of anthropogenic ^{14}C and T. Such interactions explain the biological accumulation of OBT as a persistent organic contaminant by the forest biota and tree litter during the operation of the MWMF.

Our ability to observe the incorporation of ^{14}C in this system was hindered due to the comparatively lower levels of ^{14}C relative to tritium and primarily, the limited sample size. A closer examination into the integration and cycling of organic tritium and ^{14}C into the pond and MWMF ecosystem was not the focus of this study but more studies have been proposed to examine the movement and speciation of these two radionuclide elements at this location. These studies would be complimented by data from other stable isotope measurements (with C, N and H).

It can be challenging to anticipate the impact of natural processes on the intended function of groundwater pump and irrigate systems for the disposal of large volumes of tritium-contaminated waters. The present study reveals that tritium is being accumulated as OBT in the pine forest trees and that the trees are being over-irrigated with the tritium-rich pond water. Although it was thought that tritium would become released by the trees as HTO through evapotranspiration, it was not anticipated that a third of the tritium in the holding pond would change to an organic form that behaved like a persistent organic pollutant. This is evidenced by the high OBT:HTO ratios in the pine forest biota relative to prior published OBT:HTO ratios in biota from other tritium-contaminated areas. This study describes a unique circumstance where a portion of the chemical speciation of tritium in an irrigation water source became a strong influence on the retention of a biologically-more toxic form of tritium by trees.

There are no conflicts of interested to declare.

Table 2. Summary of HTO and OBT measurement data as well as OBT:HTO ratios for environmental samples.

Material	HTO and OBT Levels in Bq L^{-1} or as Noted	OBT:HTO or Other Ratios and Information	Location
Algae		0.67 to 0.91	Ref.[49]
Algae	HTO: 22,578±244; OBT: 2,922±430 Bq g^{-1}	2.62; from a highly contaminated lake	White Oak Lake, TN, USA[50]
Algae		0.785	Ref.[49]
Algae	HTO: 6,000; OBT: ~2,750	~2.18; from a contaminated lake	Perch Lake, Canada[51]
Aquatic plants	HTO: 1,500; OBT: 1,000	0.49 to 0.78	
Barley and rice		1 to ~11; highest ratios at low tissue HTO levels	Ref.[16]
Bass muscle	HTO: 15,168; OBT: 6,156 Bq g^{-1}	1.69: from a highly contaminated lake	White Oak Lake, TN, USA[50]
Bluegill muscle	HTO: 15,281; OBT: 6,959 Bq g^{-1}	1.48; from a highly contaminated lake	
Bird (tree swallow)	HTO mean: 557±145; range: 372 to 802; OBT mean: 831±60; range: 753 to 909	1.49; from a contaminated lake	Perch Lake, Canada[51]
Cabbage and persimmon		0.23 to ~0.8; highest ratios at low tissue HTO levels	Ref.[16]
Carrot	HTO: 2.41 to 3.55; OBT: 7.53 to 12.1	2.11 to 5.02; low level tritium contamination	Russell, Ontario, (Ont.) Canada[19]
Cattail stem	HTO: 13,371; OBT: 2,920 Bq g^{-1}	2.46	White Oak Lake, TN, USA[50]
Cattail top	HTO: 837 to 6,884; OBT: 579 to 3,490	0.51 to 0.81; from a contaminated lake	Perch Lake, Canada[51]
Cattail bottom	HTO: 980 to 10,200; OBT: 536 to 3,295	0.27 to 0.87	
Clam flesh	HTO mean: up to 5,000; OBT mean: up to 1,000	0.72 to 1.21; increasing with time	
Fish flesh	HTO: ~4,000; OBT: ~1,500	0.90 to 1.11; increasing with time	
Fish	HTO: 5.93±1.11; OBT: 23.33±1.85	3.90	USA[4]
Fish	HTO: 20,568; OBT: 6,900 Bq g^{-1}	1.97; a highly contaminated lake	White Oak Lake, TN, USA[50]
Fruits and vegetables	HTO: 7.60 to 224; OBT: 40.3 to 234	0.9 to 15.4; sampled near a tritium processing facility	SRB Tech., Ont., Canada[19]

Material	HTO and OBT Levels in Bq L^{-1} or as Noted	OBT:HTO or Other Ratios and Information	Location
Fruits and vegetables	HTO: 19.6 to 1,010; OBT: 23.6 to 170	0.37 to 4.80; sampled near a tritium processing facility	Shield Source Inc., Ont., Canada[19]
Fruits and vegetables	HTO: 5.90 to 21.10; OBT: 6.80 to 26.40	0.48 to 2.61; sampled near nuclear reactor	Darlington, Ont., Canada[19]
Fruits and vegetables	HTO: 4.3 to 20.7; OBT: 3.3 to 17	0.51 to 2.67; sampled near a nuclear reactor	Gentilly-2, Canada[19]
Himalayan balsam	HTO: 0.064 to 0.084 Bq g^{-1}; NE-OBT: 0.028 to 0.039 Bq g^{-1}	NE-OBT:HTO: 0.447 to 0.468	Mohelno Valley, Czech Republic[52]
Lettuce	HTO: 7.4±1.5; OBT: 25±1.9	3.40	USA[4]
Pine and spruce needles	HTO mean: 46±15; range: 21 to 82; OBT mean: 66±15; range: 38 to 97	Range: 0.9 to 2.1; mean: 1.5	Japan[53]
Pine bark	HTO: ≤0.41 Bq g^{-1}; OBT: ≤3.55 Bq g^{-1}	9.94 to 11.60; OBT was mostly NE-OBT	This study.
Pine cores	HTO: ≤1.38 Bq g^{-1}; OBT: ≤6.01 Bq g^{-1}	0.89 to 4.36; OBT was mostly NE-OBT	
Pine needle litter	HTO: 0.50 Bq g^{-1}; OBT: ≤4.47 Bq g^{-1}	8.94; OBT was mostly NE-OBT	
Milk (cow)	HTO: 9.25±1.48; OBT: 17.04±1.85	1.8	USA[4]
Milk (cow)	HTO: 1.27 to 1.92; OBT: 5.17 to 5.82	1.69 to 4.58	Russell, Ont., Canada[19]
Mussels, fish and snails	HTO: 11.98 to 16.52 (TU); OBT: 13.2 to 72.9 (TU)	1.1 to 4.86; sampled near a nuclear reactor	Hungary[54]
Mussels, fish seaweed and crustaceans	HTO: up to ~30; OBT: up to 10	Mean: 1.2±0.5	Cotentin Peninsula Coast, France[39]
Plants	HTO: 75 to 3,000; OBT: 90 to 31,002	0.4 to 15.1	Ont., Canada[11]
Plants	HTO: 15 to 95; OBT: 105 to 450	Up to 13 in whole bean plants	Pembroke near SRB Tech, Canada[11]
Rice		0.57±0.12 to 1.3±0.3	Japan; cited 1980s work by Hiramatsu and co-workers.[13]
Root vegetable	OBT: 27.8±1.9	3.9	USA[4]
Sediment	HTO mean: 8.14±1.5; OBT mean: 129.6±14.8	15.9; 0 to 25 cm depth	New York, USA[4]
Sediment	HTO range: 1,320 to 12,550; OBT range: 490 to 2,970	0.23 to 0.50 in 2003; 0.41 to 0.60 in 2013	Perch Lake, Canada[51]

Material	HTO and OBT Levels in Bq L^{-1} or as Noted	OBT:HTO or Other Ratios and Information	Location
Soil	HTO: 7.9 to 18.4; OBT: 1.8 to 28.9	0.18 to 1.94; sampled near a nuclear reactor	Darlington, Ont., Canada[19]
Soil	HTO: 4.3 to 15.6; OBT: 3 to 16.2	0.26 to 4.15; sampled near a nuclear reactor	Gentilly-2, Canada[19]
Soil	HTO mean: 3,200; HTO range of 430 to 118,000; OBT mean: 705; OBT range: 18,018 to 13,200,000	Median: 80, ranging from 20 to 1,400; sampled near a tritium processing facility	Peterborough, Canada[11]
Soil	HTO: 2.9 to 3.2; OBT: 8.5 to 10.4	2.66 to 3.53; near low level tritium contamination	Russell, Ont., Canada[19]
Soil	HTO: 4.30 to 122; OBT: 14.9 to 1,010	3.5 to 9.9; sampled near a tritium processing facility	SRB Tech., Ont., Canada[19]
Soil	HTO: 0.47 Bq g^{-1}; OBT: $\leq$0.71 Bq g^{-1}	1.51; OBT mostly NE-OBT	This study.
Stinging nettle	HTO: 0.050 Bq g^{-1}; NE-OBT: 0.024 Bq g^{-1}	NE-OBT:HTO: 0.47; sampled near a nuclear reactor	Mohelno Valley, Czech Republic[52]
Soil water	HTO: 6.67±1.5 to 7.7±1.5; OBT: 92.60±3.7 to 107±3.7	11.9 to 14.7	New York, USA[4]
Sediment pore water	HTO: 3.9±0.6 to 26±3; OBT: 10±3 to 23±3 (for surface material) and HTO: 2.6±0.5 to 4.1±0.7; OBT: <1.0 to 13±3 (for core material)	1.7±0.2 to 3.1±0.3 (for surface material) 0.6±0.1 to 3.6±1.1 (for core material)	Loire River Estuary, France[55]
Wheat		0.005 to ~20; ratio increased with exposure time	Ref.[16]

ACKNOWLEDGEMENTS

This project was conducted in conjunction with work accomplished under Contract No. DE-AC09-08SR22470 with the U.S. Dept. of Energy (DOE). This work was also supported by U.S. DOE funding from the SRNL Laboratory Directed Research and Development (LDRD) Program, the National Nuclear Security Administrations' Nuclear Safety Research and Development (NSR&D), and Office of Defense Nuclear Nonproliferation Research and Development—NA-22 (Grant No. DE-FG52-05NA27035) Programs. We appreciate the input from the four reviewers of this work.

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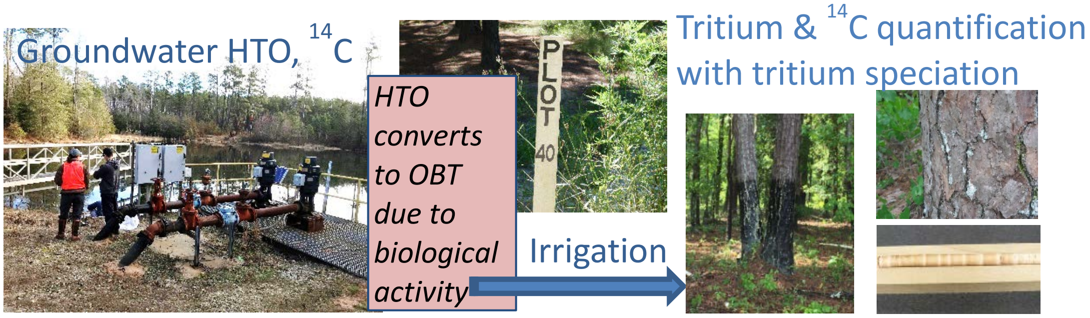
After 2 decades of irrigation with groundwater from a holding pond: OBT and ^{14}C are retained in the recent wood growth whereas HTO levels remained constant with wood age.



ENVIRONMENTAL SIGNIFICANCE STATEMENT

Tritium is a radioactive anthropogenic contaminant. Most environmental tritium exists as tritiated water, which is hazardous to biota but the biological incorporation of organic bound tritium (OBT) presents an overlooked and understudied hazard in tritium-contaminated areas. This study examines tritium chemical speciation in biota at a “pump and treat” facility, where a pine forest was irrigated with tritium-rich groundwater for the last 20 years. It was anticipated that tritiated water would eventually be released by the trees through evapotranspiration with little bioaccumulation but substantial retention of tritium as OBT occurred in the pine wood (cores), bark and needles. The groundwater holding pond became an unanticipated OBT source to the pine forest, which was extensively irrigated with that tritium-contaminated pondwater.

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SRNL-STI-2019-00314

SUPPORTING INFORMATION

Assimilation and Transport of Organic Bound Tritium in an Irrigated Pine Forest

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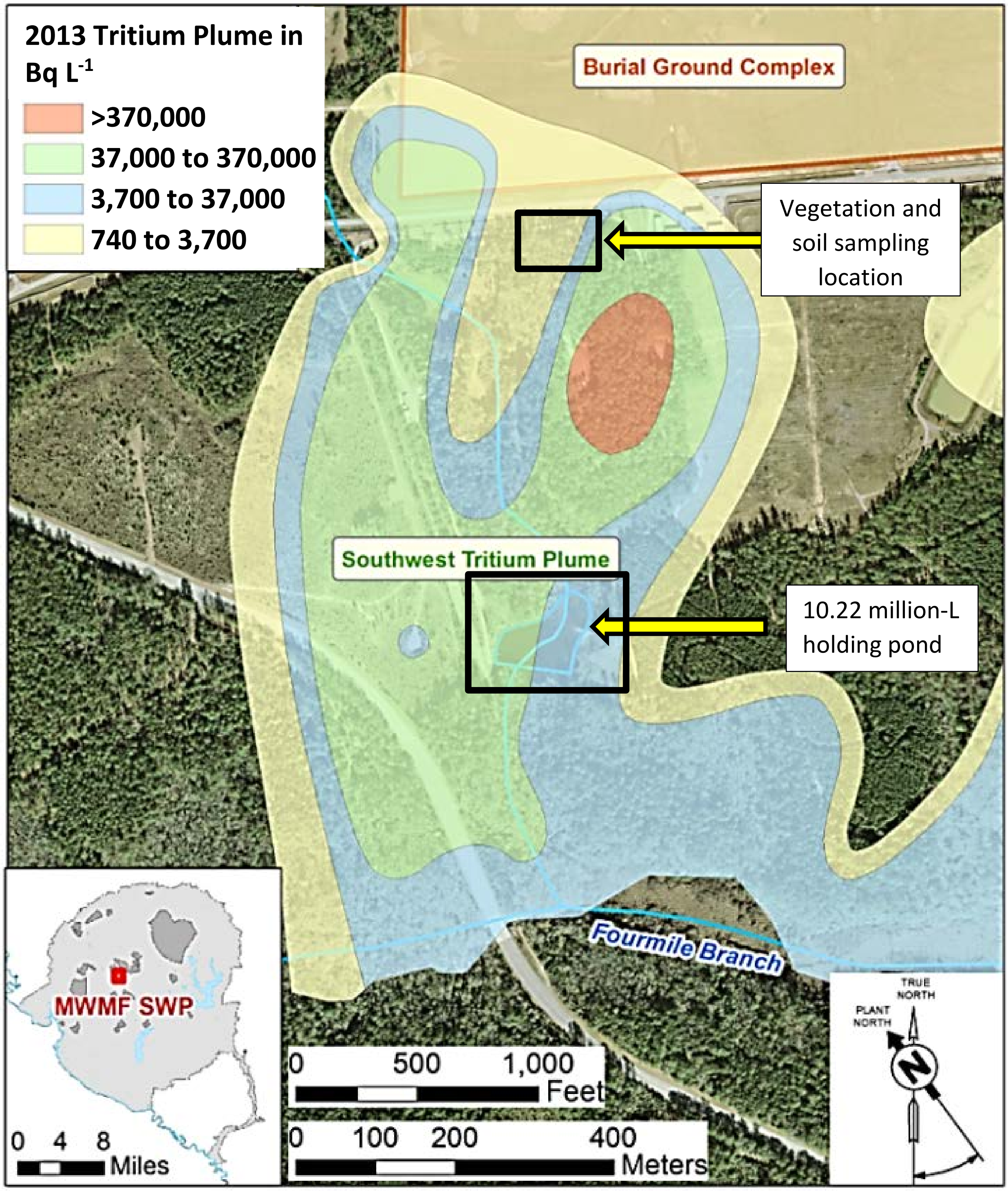
Fig. S1. The MWMF forest lies true north of the holding pond, which is used for the irrigation of the MWMF forest trees—Adapted from SRNS (2014).[1]



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[1] Savannah River Nuclear Solutions (SRNS), Mixed Waste Management Facility Groundwater Remediation. Fact Sheet. Updated on 09/22/2014, Aiken, SC 2014.

Table S1. Typical contaminant and water quality values for MWMF irrigation pond water.*

Component	Method	Range	Units	Comments
1,2-dichloroethylene	EPA8260B	0.29 to 20	µg L^{-1}	Most of these compounds are RCRA and CERCLA listed.
1,4-dioxane		3.7 to 20		
Acetone		2.3 to 9.21		
cis-1,2-dichloroethylene		2.5 to 20		
Tetrachloroethylene		0.057 to 1.1		
Trichloroethylene		0.89 to 10.0		
Ethylene	AM20GAX	0.048 to 0.12	µg L^{-1}	
Methane		≤1,300		
Nitrate	EPA9056 & 300.0	0.30 to 0.38	mg L^{-1}	
Nitrate-nitrite as N	EPA353.1	0.08 to 0.31		
Redox potential (E_h)		+84 to +305	mV	
pH		4.6 to 9.5		Average: 6 to 6.5
Specific conductance		27 to 348	µS cm^{-1}	Average: 30 to 40
Total $CaCO_3$ alkalinity		0 to 80	mg L^{-1}	Average: 3 to 9
Total dissolved solids	EPA160.1	46 to 50		
Total organic carbon	EPA9060	9.4 to 10.0		
Total organic halogens	EPA9020B	0.023 to 0.042	µg L^{-1}	
Turbidity		0.9 to 48.8	NTU	
Temperature		4.3 to 34.8	°C	
Aluminum	EPA6010B	36.4 to 37.8	µg L^{-1}	
Barium	EPA6020A	36.8 to 110		
Calcium	EPA6010B & 6020A	1,900 to 2,330		
Cobalt	EPA6010B, 6020A & B	2.00 to 2.04		
Copper	EPA6020A & B	≤1.39		
Iron	EPA6010B & 6020A	2,520 to 6,380		
Magnesium	EPA6010B	1,270 to 1,600		
Manganese		29.8 to 323		
Nickel	EPA6010B, 6020A & B	0.854 to 1.86		
Potassium	EPA6010B	971 to 1,620		
Selenium		3.5 to 5.0		
Strontium	EPA6020A	≤12.1		
^{14}C	LSC	Up to 40.7	Bq L^{-1}	
Gross alpha	Alpha counting	Up to 0.15		
^{129}I	LSC	0.02 to 0.31		
Non-volatile beta	LSC	0.01 to.0.73		
Total radium	EPA903.0MOD	0.04 to 0.09		
^{99}Tc	Radiometric counting	Up to 1.89		
Tritium	LSC	5,923 to 574,074		Average: ~54,000,000

*Data provided by the SRS ACP as part of their 2012 to 2017 monitoring studies. NTU: Nephelonic Turbidity Units.

Table S2. Analysis protocols for various biological sample matrices. HTO, E-OBT and NE-OBT were extracted sequentially whereas total tritium was isolated in by complete sample combustion. Total OBT extraction (E-OBT plus NE-OBT) from water occurred via the NE-OBT step (only) on Whatman #10 filtrate.

Matrix	HTO	E-OBT	NE-OBT	Total Tritium from Complete Combustion
Bark, leaf litter	Heat to 150°C at a ramp rate of 2.5°C min^{-1} and hold for 30 min.	Rinse at room temp. with tritium-free water for 72 h.	Heat to 300°C at a ramp rate of 5°C min^{-1}; continue to 600°C at 7°C min^{-1} and hold 60 min collect tritium in 10 mL 0.1 M nitric acid (HNO_3) in bubbler trap.	Heat to 155°C at a ramp rate of 3.5°C min^{-1} and hold for 12 min at air flow of 0.2 L min^{-1}; Heat to 600°C at 5°C min^{-1} at oxygen (O_2) flow of 0.2 L min^{-1} to facilitate combustion and hold for 30 min collected in 10 mL 0.1 M HNO_3 in bubbler trap.
Tree cores			Heat to 600°C at 5°C min^{-1} and hold 60 min collect tritium in 10 mL 0.1 M HNO_3 in bubbler trap.	
Soil			Heat to 300°C at 5°C min^{-1}; continue to 600°C at 7°C min^{-1} and hold 20 min; continue to 900°C at 10°C min^{-1} and hold 15 min; collect tritium in 10 mL 0.1 M HNO_3 in bubbler trap.	Heat to 155°C at 3.5°C min^{-1} and hold for 12 min at air flow of 0.2 L min^{-1}; Heat to 600°C at 5°C min^{-1} at O_2 flow of 0.2 L min^{-1} and hold for 30 min; Heat to 900°C at 8°C min^{-1} with a 20-min hold collect in 10 mL 0.1 M HNO_3 in bubbler trap.

Matrix	Total ^{14}C as CO_2 by Complete Combustion
Any	A second bubbler trap was used after tritium collection for $^{14}CO_2$ capture. The liberated $^{14}CO_2$ is trapped in Carbo Sorb E scintillation cocktail, which was counted after the addition of 10 mL of Permafluor E.

Table S3. FTIR assignments made for tree core material based on peak matching with reference databases and literature.

Wavelength (cm^{-1})	Band Assignment	Band Origin
805	Glucomannan[A]; vibration (vibr) of mannan in hemicellulose (hemiC), and C-H out-of-plane bending in phenyl rings[B]	Polysaccharide (polyS)[A,B]
825	C-H out-of-plane bending in guaiacyl units[B]	Lignin (lgn)[B]
853-860	C-H out-of-plane in position 2, 5 and 6 of guaiacyl units[A,B]	lgn[A,B]
892-900	Aromatic (arom) C-H out-of-plane deformation (deform)[C]; anomeric C-groups, C_1-H deform, ring valence vibr[C]; C-H deform of beta-glycosidic linkages in cellulose (cel)[B]; anti-symmetric out-of-phase stretching (strh) in pyranose ring[D]; C_1 vibr[E]	cel/hemiC/pectin[C], polyS[A,B]
945	O-H out-of-plane deform in carboxylic acid (R–COOH)[B]	Terpenoids[B]
960	C-H out-of-plane deform in lgn[B]	lgn[B]
985	C-O strh in cel[B]	polyS[B]
998-1005	C-O strh in cel[A,B] and hemiC[A]	polyS[A,B]
1024-1035	C-O strh[C]; C-O deform in primary alcohols (R-OH), unconjugated (unconj.) C=O strh, arom C-H in-plane deform in lgn, C-O and C-C strh and CH_2 rocking in cel[A]; C-O strh in primary R-OH in cel[B]; C-O-C deform[D]; C-O strh vibr[E]	hemiC[A,C,E], cel[A,C], lgn[A,B,E] polyS[A,B],
1055-1060	C-O strh vibr of cel and hemiC[A]; C-O strh of secondary ROH[B]; C-O strh vibr[E]	polyS[A,B], cel/hemiC[E]
1101-1106	Ring asymmetric (asym) valence vibr[C]; C-O and C-C strh and CH_2 rocking in cel[A]; C-O-C strh in cel and hemiC[B]	polyS[A-C]
1128	Syringyl lgn and C-O, C-H deform in syringyl lgn, C-O strh[D]	
1140	Guaiacyl lgn and C-O, C-H deform in guaiacyl lgn, C-O strh[D]	
1155-1160	C-O-C symmetric (sym) strh[C]; C-O-C asym strh vibr in cel and hemiC[A,B]; C-O-C asym strh in cel I and II[E]	cel/hemiC[C], polyS[A,B]
1185	C-O strh in cel[B]	polyS[B]
1200-1205	O-H in-plane bending in cel I and II[A]	polyS[A]
1221-1230	Syringyl ring and C-O strh[C]; C-C, C-O, and C=O strh, acetyl and carboxylic vibr in xylan[A]; O-H vibr in guaiacyl ring, C-C, C-O and C=O strh in lgn[B]	lgn[A,C], xylan[C], polyS[A]
1264-1270	Guaiacyl ring plus C=O strh[A,C]; acetyl and carboxylic vibr in xylan[A]; C-O vibr in guaiacyl rings[B]; syringyl ring breathing and C-O strh in lgn and xylan[E]	Guaiacyl-lgn[C], polyS[A], lgn[A,B]
1315-1317	CH_2 rocking vibr[C]; CH_2 wagging in crystalline cel[B]	cel[C], polyS[B]
1320-1330	Phenolic O-H, syringyl and guaiacyl (ring) condensed, C=H vibr in polyS[A]; C-H of methyl (Me) groups in methoxy of amorphous cel[B]; C_1-O vibr in syringyl derivatives, C-H in-plane bend in cel I and II[E]	lgn/polyS[A]
1360-1372	C-H deform vibr[C]; phenolic O-H, aliphatic C-H strh in CH_3, not in O-Me, C-H vibr in polyS[A]; C-H deform in cel and hemiC[B]; aliphatic C-H strh in Me and phenolic OH[E]	cel[C], lgn[A], polyS[A,C]
1385	C-O strh in cel and hemiC[B]	
1405	C=O in carboxylic groups in R–COOH and esters[B]	Terpenoids[B]
1420-1430	Arom skeletal (skl) vibr with C-H in-plane deform in lgn and polyS[A]; C-H asym deform in methoxyl, arom skl vibr, lgn[B]; arom ring and CH, benzene (benz) skl with C-H deform[D] and strh[E]	lgn[A,B], polyS[A]
1450-1453	C=C and C-H bond, O-H in-plane deform[C]	lgn, hemiC[C]

1460-1470	C-H deform, asym in -CH_3 and -CH_2-[A]; C-H asym deform in methoxyl for lgn, asym in -CH_3 and CH_2 in pyran for hemiC[B]; C-H deform[D]; CH_2 deform strh in lgn and xylan[E]	lgn[A,B], polyS[A]
1500-1515	C=C strh of arom skl vibr[C]; arom skl vibr[A,E]; C=C strh of arom ring, C=O bond vibr in extractants[B]; arom/benz ring strch vibr[D]	lgn[A-C]
1590	Skl vibrations from the C-C[B]	lgn[B]
1593-1610	Arom skl and C=O strh vibr[A,C]; C=O strh conjugated (conj) to arom ring and carboxylic groups of lgn, R–COOH, esters[B]; arom/benz ring strh vibr[D]	lgn[A,C]
1635	Absorbed O-H and conj C-O in polyS[B]	
1640	C=O strh vibr in conj carbonyl of lgn[E]	
1655-1675	C=O strh on conj *p*-subst aryl ketones[A]; absorbed O-H and conj C-O in polyS[B]	lgn[A]
1690	C=O vibr in carboxylic group in resin acid[B]	Terpenoids[B]
1709-1750	C=O strh of acetyl and carbonyl groups[C]; C=O strh in unconj ketones and ester groups (usually polyS origin), conj aldehydes and R–COOH absorb ≤1700 cm^{-1} [A]; C=O carbonyls in ester and acetyl groups in xylan[B]; in aldehydes and acids, unconj C=O and ketone strh[D,E]	hemiC[C], lgn[A], polyS[A,B]
2850-2970	C-H strh, CH_2, CH-, and CH_3[D]	
3400	O-H strh, O-H of R-OH, phenols, and acids[D]	

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