Tritium Mobility in the Environment using Deuterium as an Analogue

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4 Abstract

Tritium is a radioisotope of hydrogen and a component of emissions from the nuclear industry. It is also a radioisotope of concern for human and environmental health. The near future could see an increase in tritium production as experimental fusion reactors initiate first plasma. The greatest risk pathway is human ingestion of edible plants grown near sites of tritium emissions as they can acquire high levels of organically bound tritium (OBT). Recent studies at a tritium Beta-light facility in Pembroke, Ontario, Canada characterized by tritiated hydrogen gas (HT) emissions have identified high OBT:HTO ratios that are not consistent with current tritium transfer models. This suggests that there is an unidentified physical, chemical, or biological mechanism generating OBT in plant tissue. Laboratory experiments have been undertaken using deuterium gas (D_2) as an analogue for atmospheric HT in controlled plant exposure experiments, and compared the observations with short-term exposures at the SRBT facility. While the deuterium results did not uncover a hidden pathway or enrichment mechanism, the SRBT exposures showed elevated tissue free water tritium (TFWT) in stems and leaves in the presence of atmospheric HT, and lacking HTO in both soils and surrounding air. This study proposes that hydrogenase activity in microbial communities hosted within the laminar boundary layer on the leaf surface, are responsible for HT oxidation to HTO that contributes directly to leaf waters used in photosynthesis.

5 Acknowledgments

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6 Introduction

Hydrogen is the most abundant element in the universe, and is a fundamental building block in the chemistry necessary for life on Earth. It also plays a key role in many anthropogenic processes, particularly within the nuclear energy industry. Actinide fission reactors, of which there are 438 currently operational, produce electricity, and tritium, world-wide (Starz, 2015). The Canadian design for fission reactors is based on the innovative use of hydrogen isotopes in the production of neutrons that are critical for sustaining uranium fission. All nuclear reactors require a moderator to slow the neutrons yielded by uranium and plutonium fission into the thermal range where they are effective for fission. Light water reactors (those using H_2O with large neutron-capture cross section as a moderator) are inefficient, consuming much of the neutron-flux, as hydrogen is activated to produce deuterium, therefore requiring the use of ^{235}U enriched fuel. The Canadian Deuterium Uranium (CANDU) reactor uses deuterium oxide, D_2O or heavy water as a moderator, and can therefore use natural isotopic abundance uranium fuel. However, this increased efficiency comes at the cost of enhanced production of hydrogen's only radioactive isotope; tritium.

Loss of tritium from CANDU reactors is currently not considered a risk to human health or the environment. Much is recovered and stored as titanium tritide, which is unreactive to air or water. However, some is converted to tritium gas (T_2) and used as a scintillator in electricity-free lighting applications, or Beta-lights. The use of tritium in such commercial applications results in minor emissions to the environment that must be monitored. The fate of tritiated hydrogen gas, typically referred to as HT, is similar to that of H_2, with conversion by hydrogenase enzyme activity in soils into tritiated water, HTO (Boyer *et al.*, 2009). If incorporated into biomass as organically-bound tritium (OBT), tritium can enter the food chain and potentially pose a risk to human health, as OBT has a far greater biological residence time versus HTO.

The potential for human ingestion of tritium as OBT will become more relevant in the future, as fusion facilities such as the International Thermonuclear Experimental Reactor (ITER) in Cadarache, France begin operation. ITER's first plasma is to be initiated in 2020, marking a major milestone in the advancement of renewable fusion energy. Like modern fission, tritium is a byproduct of the fusion reaction, though its production will be at a far greater scale (ITER, 2015).

Globally, there are a limited number of locations impacted by tritium emissions. Pembroke, Ontario, Canada hosts SRB Technologies, a Beta-light facility that routinely releases HT and HTO to the atmosphere. The Canadian Nuclear Safety Commission (CNSC) monitors these emissions, as well as tritium levels in the soil, water, and vegetation of the surrounding area. Recent studies have indicated an enrichment of OBT relative to HTO that conflicts with the current paradigm that OBT should be in equilibrium with tritiated water (Mihok *et al.*, 2016; CNSC, 2010). An understanding of OBT formation and mobility in edible plants is the first step in analyzing the potential risks to human and ecological health associated with elevated tritium releases.

7 Study Objectives

The objective of this study is to identify the mechanism that contributes to higher OBT versus HTO concentrations in plants exposed to atmospheric HT and HTO. As plants draw water (and nutrients) from both the soil and surrounding air, the challenge is in determining the major contributor to the plant of both tritiated species, from both compartments. Further complicating the matter, plant physiological responses to the environment are dynamic, leading to complications in identifying where and when the plant is up-taking tritium. To address these challenges, three separate investigations into tritium mobility were carried out. The first was a delineation of HTO concentrations with depth in the pore waters of soils surrounding SRB Technologies. The second was to replicate atmospheric tritium gas

exposure under controlled conditions, using deuterium gas as an analogue. The third was to bring previously unaffected potted plants to SRB Technologies for a short-term exposure in the natural environment.

Quantifying HTO and OBT with depth in the SRBT soil profiles would contribute to understanding tritium mobility following chronic atmospheric exposure of HT and HTO. As the major supplier of water and nutrients for plants, the soil regime is considered to be a critical source for HTO via root uptake.

As tritium is a radioactive isotope of hydrogen, utilization of tritiated species in the laboratory can be hindered by acquiring permits and developing the necessary protocol for working with radioactive substances. Deuterium however, is stable, and should theoretically behave similarly to both hydrogen and tritium in the environment. By using D_2 instead of T_2, the controlled laboratory experiments are simplified, while simultaneously investigating deuterium and tritium mobility, as well as the suitability for future substitutions.

Finally, while there has been historic and recent environmental tritium mobility investigations in Canada (Mihok *et al.*, 2016; CNSC, 2016), short-term exposures that consider both day and night influences on the plant are lacking. Physiological changes in plant activity associated with light and dark are thought to play a crucial role in OBT production through the beginning and cessation of photosynthesis, coupled with on-going light-independent reactions. Studying plant tritium interactions within a minimal set timeframe can address some of the finer details that are typically lost in long-term exposure experiments.

Taken together, the components of this work seek to highlight the inherent complexity in studying tritium mobility in the dynamic natural environment mingled with the interplay between macro and microscopic biological organisms and physical chemistry. The results and interpretation may

contribute to understanding the fate of current and future anthropogenic tritium, and potentially inspire new questions and motives for future research.

8 Background

8.1 Sources of Tritium in the Environment

Tritium was discovered in 1934 by Mark Oliphant, Paul Harteck, and Ernst Rutherford (Oliphant *et al.*, 1934). Luis Alvarez and Robert Cornog (1939) successfully isolated tritium and confirmed its radioactivity. Although naturally rare on Earth, tritium has become an invaluable tool since its mass anthropogenic production began in the 1950's. Today, tritium can be found illuminating watches, emergency EXIT signs, small-arms weapons sights, and can also be used as a radiotracer in biochemical and environmental studies (Boyer *et al.*, 2009).

Tritium is the only radioactive isotope of hydrogen, and has a half-life of 12.32 years (Lucas and Unterweger, 2000). As a low energy beta emitter, tritium decays into helium-3, releasing an electron with a maximum energy of 18.6 keV and an average of 5.7 keV seen in Equation [1].

[1]

$$^{3}_{1}H \rightarrow {}^{3}_{2}He^{+} + e^{-} + \overline{\nu_e}$$

Where $^{3}_{2}He^{+}$ is the stable helium decay product of tritium, e^{-} is the beta particle emission, and $\overline{\nu_e}$ represents an electron antineutrino, which dissipates the minute amount of remaining energy resulting from the neutron decay. The very low energy of the beta decay generates a maximum track length of 6 mm in air, and only 6 nm in water or organic tissue (Boyer *et al.*, 2009). The mass difference between the three isotopes (protium, deuterium, and tritium) implies that mass dependent isotope partitioning would occur naturally. While these events are common with stable isotopes like deuterium and oxygen-

18, the extremely low abundance of tritium compared to protium (1 ^{3}H per 10^{17} ^{1}H in natural waters) makes any fractionation effects at natural tritium levels negligible (Eisenbud *et al.*, 1978, McFarlane *et al.*, 1979). As a result, tritium behaves like hydrogen in the environment, and is subject to the same bulk flow and diffusion processes at natural levels (Boyer *et al.*, 2009).

The greatest contribution to naturally occurring tritium is cosmic ray bombardment in the stratosphere. Equation [2] describes a fast neutron ($^{1}_{0}n$) interacting with nitrogen to form carbon and tritium. Following production, tritium generally combines with O_2 to form water in the stratosphere (Clark and Fritz, 1997).

[2]

$$^{14}_{7}N + {}^{1}_{0}n \rightarrow {}^{12}_{6}C + {}^{3}_{1}H$$

The amount of tritium that ultimately ends up in precipitation is low. A secular equilibrium exists that balances cosmogenic production with decay and rainout. Concentrations will also vary with geomagnetic latitude, as cosmic ray bombardment is greater towards the poles. A smaller contribution is generated in the Earth's crust when lithium captures a neutron derived from the spontaneous decay of uranium and thorium, as shown in Equation [3].

[3]

$$^{6}_{3}Li + {}^{1}_{0}n \rightarrow {}^{4}_{2}He + {}^{3}_{1}H$$

Geogenic tritium moves directly into ground waters where available, with the concentration being dependent on the lithium content and rock porosity, though appreciable quantities of tritium in these instances are rare (Gascoyne and Kotzer, 1995; Clark and Fritz, 1997). The overall natural production of tritium is estimated to be between 0.15 to 0.20 kilograms per year (Boyer *et al.*, 2009).

The largest anthropogenic source of tritium on record was the result of thermonuclear bomb testing that occurred from 1951 to 1980. The Ivy-Mike detonation of 1952 initiated a period of atmospheric hydrogen bomb testing that would produce vast quantities of tritium in the stratosphere. The peak production occurred in 1962 when the USA and USSR released a combined 104 TBq (1×10^{12}) of tritium into the atmosphere (Gonfiantini, 1996). The tritium spike was observed during the spring of 1963 and became a useful tracer in many hydrology studies, as the fallout could be found migrating through terrestrial media such as soil water. Thermonuclear bomb tritium has mostly been attenuated by the oceans and groundwater, or decayed, though the oceans remain the greatest tritium sink (Clark and Fritz, 1997). While there remain latitudinal variations in tritium precipitation concentration (due to stratospheric circulation patterns and the fact that much of the testing was done in the Northern Hemisphere), the global tritium concentration in precipitation has more or less returned to pre-testing levels (Clark, 2015). The few pre-bomb measurements available indicate that the tritium concentration in the Ottawa River was approximately 15 TU (Brown, 1961).

Today, anthropogenic tritium sources are mostly limited to the nuclear power industry (Okada and Momoshima, 1993). Fuel reprocessing plants produce HTO on the order of several petabecquerels (PBq) per year while heavy water reactors, such as the CANDU reactors, release 0.1-0.5 PBq/year in liquid form, and 0.1-1 PBq/year in airborne emissions (Boyer *et al.*, 2009). Smaller commercial industries, such as medical research and design, and tritium beta-light facilities emit smaller amounts. Each modern anthropogenic tritium source is generally considered a point source that restricts tritium emissions to the surrounding area, as evidenced by reduced tritium concentrations with distance from the point of emission (CNSC, 2010). In total, anthropogenic tritium releases amount to approximately 0.06 kg per year (Belot *et al.*, 1996). In the coming decades, facilities like ITER could drastically increase this value, with ITER alone utilizing around 1.5 kg of tritium per year of the current global inventory

(approximately 20 kg) with as of yet unknown releases to the environment (Boyer *et al.*, 2009; ITER Organization, 2015).

8.2 Tritium and Human Health

Very little data is available regarding direct human health impacts associated with tritium. As a result, much of what is known is based on epidemiological studies using animals and free cell in-vitro methods. The results of these studies suggest that tritium poses a low risk to human health, with a theoretical risk of death by cancer at 6.5×10^{-2} per Sv of tritium incorporated (Straume, 1993). However, given that tritium specific dose data for humans is lacking, a degree of caution should be taken when considering tritium exposure at elevated levels.

Human exposure to tritium is by ingestion, inhalation, and direct exposure to the skin. The low energy of the beta emission cannot penetrate the dermis or the epidermis (the maximum track length of beta emission is 6 µm versus the 20-100 µm dermis thickness and 1-3 mm epidermis thickness). Therefore, for tritium to have a lasting impact, it must be present inside the human body (Okada and Momoshima, 1993). Further, 90% of absorbed tritium in the body is HTO which has a biological half-life of approximately 10 days. Any tritium in the organic form will remain longer, up to around 30 days, while a much smaller amount can remain in the fat and collagen tissues for around 450 days (Okada and Momoshima, 1993). The effective dose of each species is summarized in **Table 1**.

Table 1: Species specific tritium dosimetry (Adults)

Tritium Species	Exposure Method	Effective Dose (Sv/Bq)
HT	Inhalation	1.8×10^{-15}
HTO	Ingestion and/or Inhalation	1.8×10^{-11}
OBT	Ingestion	4.2×10^{-11}

Note: values taken from Boyer *et al.*, (2009).

The World Health Organization suggests that daily consumption of water at 10 000 Bq/L is the upper limit for water quality standards (CNSC, 2008). This is based on an annual contribution of 0.1 mSv of radiation from drinking water which accounts for less than 5% of the annual natural background dose of 2.4 mSv. Canada uses this recommendation to calculate its drinking water limit of 7 000 Bq/L.

Table 2: Tritium drinking water limits by country

Country	Tritium Limit (Bq/L)
Australia	76 103
Finland	30 000
Switzerland	10 000
Russia	7 700
Canada	**7 000**
United States of America	740
European Union	100

Note: Adapted from Standards and Guidelines for Tritium in Drinking Water, INFO-0766, CNSC, (2008).

While the toxicity of HTO is 25 000 times that of HT (Davis *et al.*, 1995), the major concern for human health is OBT. Clark *et al.*, (2010) found no evidence for systematic bioaccumulation of OBT between

trophic levels. That being said, the large variations in OBT found between compartments were likely the result of seasonal variations in tritium near the field sites in the study. The longer residence times (potentially on the order of hundreds of days) along with the greatest effective dose of the three, highlight the need for further research into OBT, especially when it comes to food crops, as those are anticipated to be the major pathway for human OBT ingestion in the future (Vichot *et al.*, 2008).

8.3 Tritium in the Environment

The major forms of tritium found in the environment are tritiated water (HTO), organically bound tritium (OBT), and to a much lesser extent, tritiated hydrogen gas (HT) and tritiated methane (CH_3T). Once produced naturally, or emitted through anthropogenic processes in liquid or vapor form, HTO becomes part of the biogeological water cycle, and can be present in all water pools trending towards equilibrium in the affected environment (Boyer *et al.*, 2009). HT gas and CH_3T are subject to atmospheric dilution, or in the case of HT, oxidation to HTO if depositional and environmental conditions are appropriate. If HT is converted to HTO, it will join the water cycle. **Figure 1** shows a simplified schematic of tritium mobility in the environment.

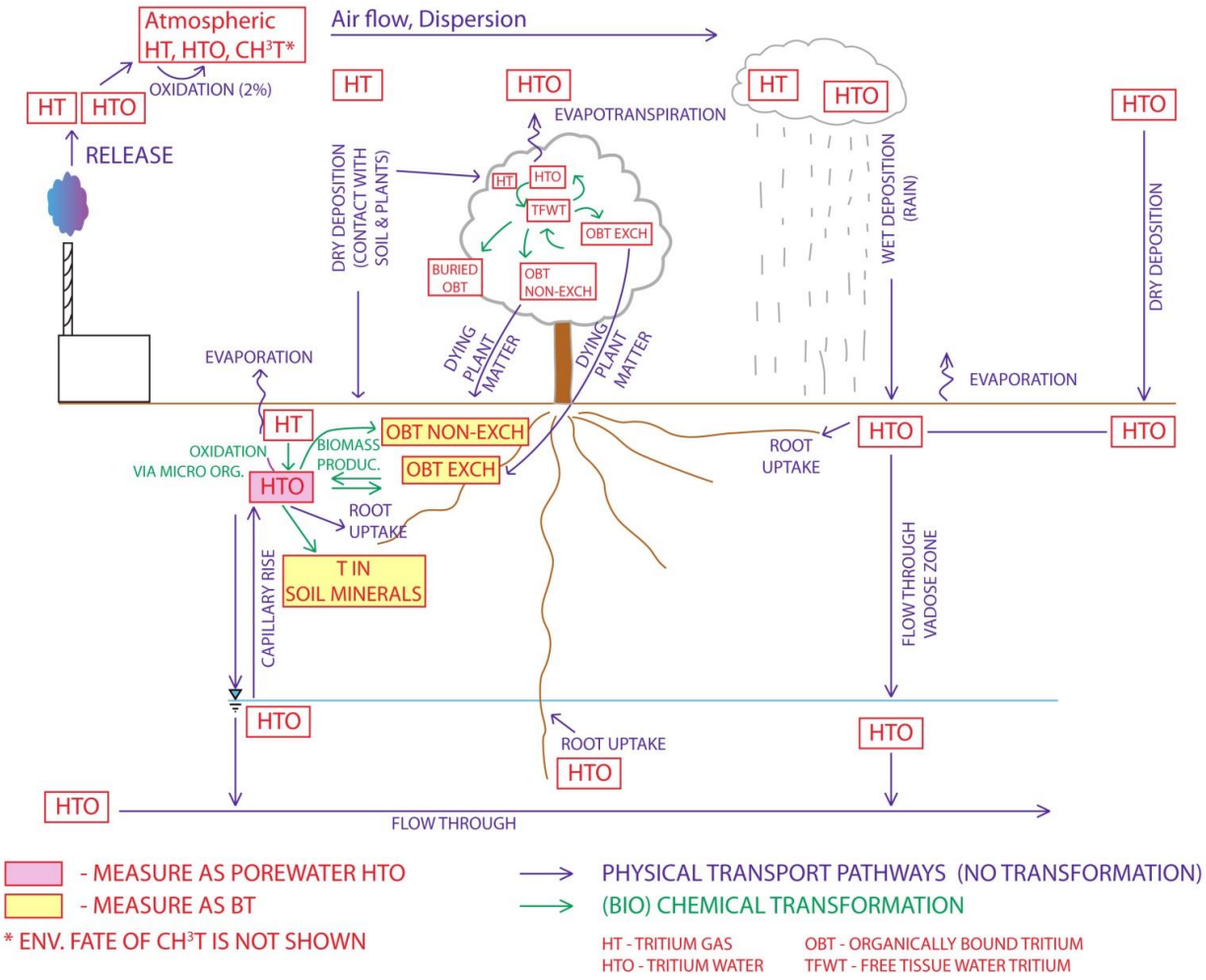


Figure 1: Tritium Cycling in the Environment The tritiated species of interest are HT and HTO due to their relative abundance in the environment compared with other species such as CH_3T. Of critical importance is the oxidation of HT to HTO in the soils, and subsequent conversion to organically bound tritium. Figure created by Alex Quesnel, Natalia Baranova, and Jude Ogwara, (2014).

If tritium is released to the atmosphere from a point source, it is dispersed and diluted before eventual deposition. Two main mechanisms deposit tritium from the atmosphere, classified as dry and wet deposition, with the latter involving precipitation events (rain, snow, fog) while the former does not. As the solubility of HT and CH_3T is very low in water, the two are controlled by dry depositional events, reaching the ground surface by gaseous advection and diffusion. HTO on the other hand, is affected by both dry and wet deposition, according to its physical variety (liquid or vapor) (Boyer *et al.*, 2009). The depositional velocities (amount of deposition per unit area divided by the atmospheric concentration) to

both soil and plants vary for HT and HTO, with HT being approximately 10 to 100 times lower than HTO (Murphy, 1993, Koarashi et al, 2001). Factors that influence the depositional velocity include meteorological condition, soil and surface properties, as well as microbial activity. For example, Noguchi *et al.*, (1995) found that cultivated soils with lower free pore volumes may have influenced the lower HT deposits the authors observed following atmospheric releases. Noguchi *et al.*, (1995) suggest that increased pore volume facilitates increased surface area for microbial oxidation of HT to HTO. Following deposition and HT to HTO oxidation, tritium is subject to transport via the same processes affecting the local water cycle. This includes runoff, evapotranspiration, diffusion and percolation to ground water, surface water flow, and eventual release into the ocean, the Earth's greatest reservoir of tritium (Murphy, 1993; Belot *et al.*, 1996; Boyer *et al.*, 2009). Throughout tritium's residence time and transit, a small percentage can be incorporated into local organics within the present medium.

8.4 Tritium in Natural Soils

The fate of tritium in soils is determined at the soil-air interface, where soil characteristics and meteorological conditions collectively influence the movement of water. HTO can evaporate, be absorbed by plant roots, or percolate deeper into the subsurface. Conversely, HT must first be oxidized to HTO by soil microorganisms, or surface mosses, before joining the HTO pool. This processes limits HT mobility in soils, with some studies suggesting that HT rarely penetrates deeper than 2.5 cm (McFarlane *et al.*, 1978; Sweet and Murphy, 1981). Soil type appears to have little effect on the rates of HT conversion to HTO (Clark *et al.*, 2010). Although, carbon content may prove to be a good indicator of H_2-oxidation activity in soils (Khdhiri *et al.*, 2015), and could potentially be used as a proxy should a relationship be observed between HT and H_2 oxidation.

Much of the HTO entering the soil is reemitted by evaporation, initially at a high rate, though it can taper off to about 1% per hour (Belot *et al.*, 1996). Residence times following acute exposures are

on the order of one to five days depending on the season, fluctuating weather conditions, as well as the physical nature of the soil profile (Kirchmann *et al.*, 1971; Koranda & Martin, 1971; Belot, 1986). Chronic deposition of HTO should lead to the development of exponential profiles with depth, as HTO is diluted, transported, or decays (Belot *et al.*, 1996).

During tritium's time in the humic layer of the soil profile, a small percentage of HTO can be converted to organic fraction as OBT. This could be the result of standard microorganism biomass production (Boyer *et al.*, 2009). The rate of OBT formation is dependent on tritium activity within the soil and can range from 0.03 to 0.05% per week (Papke and Foerstel, 1991). Adding glucose to the soil increases the rate to 0.35% per week, which supports microbial growth by providing an easily accessible carbon source (Diabaté and Strack, 1993).

As the soil tritium concentration is extremely sensitive to environmental factors, modeling tritium evolution in the subsurface is challenging, and requires extensive knowledge of how tritium behaves in the field (Jiménez-Marinez *et al.*, 2012). Three controlled, acute releases of HT to the atmosphere were carried out at Chalk River Laboratories in 1986, 1987, and 1994 (Davis *et al.*, 1995). The results of those studies confirm that HT is converted to HTO in the soil, thereby contributing to any atmospheric HTO deposited. This has a direct impact on applying a specific activity model which assumes that soil pore waters will have the same activity as air moisture (Hart, 2008). Further confounding this assumption are variations in soil characteristics, as well as fluctuations in air concentration resulting from diurnal and seasonal changes in weather conditions (Kim *et al.*, 2012). Generally, if there is a tritium point source, soil HTO will not reach equilibrium with the ambient air due to variations over time. The opposite should be true for non-point sources (Hisamatsu *et al.*, 1998; Momoshima *et al.*, 2000). Approximately 15 years after the 1994 release experiment, Kim *et al.* (2012) investigated the attenuation of tritium in soil pore water and soil OBT. The study found that the HTO

signal derived from the 1994 release was absent to 25 cm depth, while OBT was still present in the upper 5 cm of soil.

The Chalk River releases and subsequent follow up years later examined the development of a tritium signal following acute exposures representative of a hypothetical accidental release. They do not address tritium evolution at a site under regular exposure to both HT and HTO. Further, the historical Chalk River study only goes 25 cm into the subsurface. Given that future tritium facilities like ITER will be operating with regular emissions, and potentially for long periods, there is a need to investigate the effects of chronic tritium exposure in soils and at depth. Many questions remain about the nature of soil OBT, including annual variations that exceed trophic accumulation, activities that are greater than HTO, and the details of degradation (Kim *et al.*, 2012; Kim *et al.*, 2013).

8.5 The Nature of Organically Bound Tritium

Organically bound tritium is one of the critical unknowns in regards to tritium cycling in the environment and plants, as well as human health impacts. The very definition of OBT is fairly inconsistent across the literature, and only recently has there been an attempt to define OBT succinctly. Collectively, organically bound tritium is the result of natural or metabolic processes in biological organisms utilizing HTO to build biological tissues (Diabate & Strack, 1993; Belot *et al.*, 1996; Kim *et al.*, 2012; Kim *et al.*, 2013). It is the sum of two parts, known as exchangeable (E-OBT) and non-exchangeable (NE-OBT).

The distinction between E-OBT and NE-OBT is based on the nature of the chemical bonds binding tritium to the molecule. Tritium in an exchangeable position (composing E-OBT) is weakly bound to phosphorus, nitrogen, oxygen, and sulfur. In effect, E-OBT is at equilibrium with the TFWT pool and behaves the same as HTO (Galeriu *et al.*, 2008; Kim *et al.*, 2013).

Non-exchangeable OBT on the other hand, has strong bonds to carbon that are tougher to break, which increases the residence time (Diabaté and Strack, 1993; Baglan *et al.*, 2005) in the environment, and biological organisms. A second defining characteristic of NE-OBT is that it can only be removed through metabolic processes. Over time, normal metabolic processes can convert previously non-exchangeable OBT to exchangeable and vice-versa (Baumgärtner & Donhaerl, 2004; Baumgärtner, 2005). Caution must be taken not to confuse NE-OBT with 'buried tritium,' or tritium that is not bound to carbon, but otherwise unable to exchange with water or be removed by tritium-free water rinses. From an analytical point of view, it contributes to the observed NE-OBT. Boyer *et al.*, (2009) suggests that the true nature of organically bound tritium in plants and other organisms could be irrelevant, and ultimately forever debatable given the variability in tritium that is available or isolated for the multitude of general and specific reactions in biological systems.

Another challenge with respect to organically bound tritium analysis is the lack of an OBT standard for any organic tissues outside human material (Kim and Roche, 2013; Kim and Stuart, 2015). Some inter-laboratory comparisons of OBT have shown considerable variation (Workman *et al.*, 2005; Kim and Roche, 2013), thereby increasing the need for both a standardized method, and certified reference material.

For the purposes of this study, OBT will be classified as the tritium remainder following pore water/tissue free water extraction.

8.6 Tritium in Plants

Tritium is present in plants in two forms; tissue free water tritium (TFWT) and organically bound tritium (OBT). The concentration of TFWT within the plant primarily reflects the amount of HTO in the surrounding environment. This includes the plant soil, as well as the surrounding atmosphere. Weather

conditions and plant physiology have a strong influence as well, as both factors affect the plants ability to absorb HTO. OBT is less well understood, and is thought to be generated from HTO available to the plant through photosynthesis and routine metabolic processes.

8.6.1 Tritium Uptake – Soils

HTO absorbed by plants from the soil behaves similarly to H_2O. Tritiated water from the soil is absorbed through the roots, travelling through the xylem, eventually reaching the leaf. Subsequently, the majority of HTO is transpired through the stomata. This flow is maintained by an evaporation driven free-energy gradient (Murphy, 1984; Murphy, 1993). The physical properties of the soil, as well as the nature of the rooting pattern determine HTO uptake. For example, some vegetation can utilize deeper or shallower roots to absorb water, depending on the reliability of water content throughout the soil column (Williams and Ehleringer, 2000). Recent or acute tritium depositions may not be absorbed if water is being drawn into the roots from a tritium-free zone.

The residence time of plant tritium derived from soil water is dependent on the residence time of HTO in the soil, theoretically reaching equilibrium should exposure be long enough (Raney and Vaadia, 1965; Belot, 1986). Equilibration time varies along the plant structure, taking the longest in the interveinal tissue, or potentially never reaching equilibrium in vessels involved with atmospheric exchange (Raney and Vaadia, 1965). Further, HTO deposited from the atmosphere to the soil can later be reemitted, and absorbed by the plant through open stomata (Dinner *et al.*, 1980; Amano and Garten, 1991).

The second environmental tritium species of concern, HT gas, must be oxidized to HTO in the soil before being absorbed by plant roots. Previous work at SRB Technologies in Pembroke Ontario

concluded that soil OBT played little to no part in OBT production in plants. Most of the tritium in plant OBT must then be derived from HT and HTO in transpiring water or the atmosphere (Clark *et al.*, 2010).

8.6.2 Tritium Uptake – Atmosphere

HTO is believed to be the most common atmospheric source of plant tritium, as HT is not readily absorbed by plant tissues or dissolved in plant water (Belot, 1986). Although soil water is generally transpiring from open stomata during the day, atmospheric water can still diffuse into the leaves via the same pathway (Eisenbud *et al.*, 1978; McFarlane *et al.*, 1979). This requires that stomata pores (predominant on the underside of the leaf) remain open. Stomata are a key pathway in plant gas exchange, responsible for supplying atmospheric CO_2 to the leaf for photosynthesis, as well as releasing O_2. Their ability to remain open depends on several co-dependent variables, including light level and spectrum, temperature, relative humidity, soil water content, as well as hormonal factors. For example, if soil water content is low and the plant could run the risk of desiccation, the hormone abscisic acid (ABA) will be produced in the roots, transported to the guard cells of the stomata, and will initiate closing of the pores to prevent water lose via transpiration (Nabors, 2004). Leaves have also been known to exhibit selective closure of the stomata, with some remaining open while others close in response to low humidity (Farquhar *et al.*, 1988; Farquhar *et al.*, 1989). This would have an impact the adjacent leaf cells. **Table 3** highlights the conditions for stomatal opening or closure.

Table 3: Factors influencing stomata opening and closure

Stomata Open	Stomata Close
High Soil/Air Moisture	Low Soil/Air Moisture
Light Intensity (Daylight)	Light Intensity (No Daylight)
Moderate Temperatures	High Temperatures

Should conditions be appropriate, the stomatal pores will open, and atmospheric gas (including HTO) can diffuse into air cavities that generally represent 15% to 40% of the leaf interior (Nabors, 2004). Once inside the leaf, HTO vapor can become part of the bulk leaf water pool, diffuse to other parts of the plant, evaporate, or participate in primary sugar production to generate OBT (McFarlane *et al.*, 1979; Murphy, 1993).

It must be noted that rarely would a plant be exposed solely to soil tritium, or atmospheric tritium. Granted, an exception would be if the only contribution came by root absorption of soluble OBT, however if this occurs it remains a mystery (Kim *et al.*, 2013). The dynamic nature of the field ensures that conditions and circumstances affecting tritium uptake are constantly shifting. Consider that both plant tritium uptake pathways are heavily influenced by the relative humidity of the surrounding air. High relative humidity will reduce evaporative demand, thereby limiting the strength of the transpiration stream to the point that foliar uptake of HTO becomes the primary source (assuming tritium is present in both soil and atmosphere simultaneously) (Raskob, 1995; Diabaté and Strack, 1997). The opposite is possible in conditions of lower relative humidity, which trend towards soil HTO being the primary tritium source. However, it is debatable as to which pathway contributes more tritium overall to the TFWT pool. Raskob (1995) found foliar uptake of HTO to be four times higher than the soil route. It is possible that both pathways operate simultaneously to some degree, as tritium is deposited to the soil

(and reemitted) or absorbed by plant leaves (Boyer *et al.*, 2009). Murphy (1993) suggests that the tritium concentration in plants will be a combination of both sources under field conditions, as precipitation cannot reach equilibrium with HTO vapor before reaching the surface (Amano and Kasai, 1988; Belovodski *et al.*, 1997).

8.6.3 Organically Bound Tritium in Plants

In plants, the main mechanism for NE-OBT formation is the utilization of HTO during photosynthesis (Diabaté & Strack, 1993; Belot *et al.*, 1996). Similarly to hydrogen, tritium labels primary photosynthates, or simple carbohydrates, which are subsequently metabolized into larger, more complex molecules like proteins, lipids, and amino acids (Diabaté and Strack, 1993; Kim *et al.*, 2013). As only 0.06-0.3% of hydrogen in plants is utilized for organics, the actual amount of tritium available for conversion to OBT is likely very small (Murphy, 1990).

OBT formation from HTO can be broken down into light dependent reactions (photosynthesis) and light independent reactions (such as the Calvin Cycle). Photosynthesis occurs in the thylakoid membranes of the chloroplasts, which are most commonly found in leaf tissue. The light reactions are initiated when a photon activates a chlorophyll *a* pigment known as P680, which transfers an electron to a primary electron acceptor, being a pheophytin molecule. Together, the chlorophyll *a* and pheophytin compose Photosystem II of the 'Z-scheme' that describes the light reactions. The ejected electron in the chlorophyll *a* is replaced after photolysis of water splits an H_2O molecule into two electrons, two H^+, and one atom of oxygen. The previously ejected electron is transferred through a series of oxidation-reduction reactions in the electron transport chain, losing energy along the way, until it neutralizes the positively charged chlorophyll *a* (P700) of Photosystem I. Another photon will release an electron from P700, initiating a second electron transport chain that ultimately reduces $NADP^+$ to $NADPH + H^+$ (or

potentially T^+). The ATP and NADPH generated in these light reactions are later used in the light-independent reactions. **Figure 2** describes the Z-scheme of photosynthesis in plants.

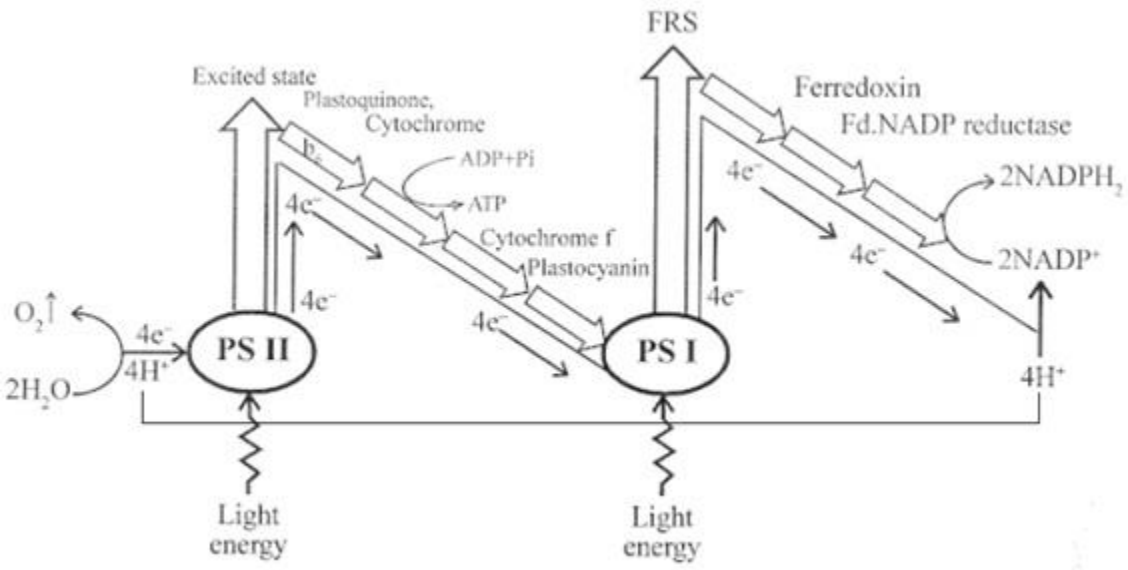


Figure 2: The 'Z-scheme' of Photosynthesis Two photosystems in series are activated by light to facilitate the photolysis of water and production of ATP and NADPH necessary for the light-independent reactions. Note that protons from split water are later bound to $NADP^+$ to form $NADPH_2$. (Adapted from Reece *et al*., (2011)).

As long as the ATP and NADPH generated during photosynthesis are present, the light independent reactions of the Calvin cycle can proceed. However, cellular stockpiles of these products last only a few seconds to minutes, so it cannot proceed throughout long periods of darkness (Nabors, 2004). The Calvin cycle takes place in the stroma of the chloroplast, and begins with an ATP molecule energizing the Ribulose-5-phosphate (Ru5P) to Ribulose-1,5-biphosphate (RuBP). The enzyme rubisco facilitates the fixing of CO_2 to RuBP, thus forming a short-lived six-carbon molecule that separates to form two molecules of 3-phosphoglycerate (PGA). ATP molecules then energize the PGA into 1,3-Bisphosphoglycerate (BPG), which are subsequently reduced by NADPH into Glyceraldehyde-3-phosphate (G3P). Following three turns, a G3P molecule leaves the cycle to be indirectly used in various reactions, including the formation of glucose, respired to CO_2 and H_2O, and the formation of sucrose for

transport throughout the plant. The key step for tritium incorporation occurs as BPG is reduced into G3P, as seen in **Figure 3** (Boyer *et al.*, 2009).

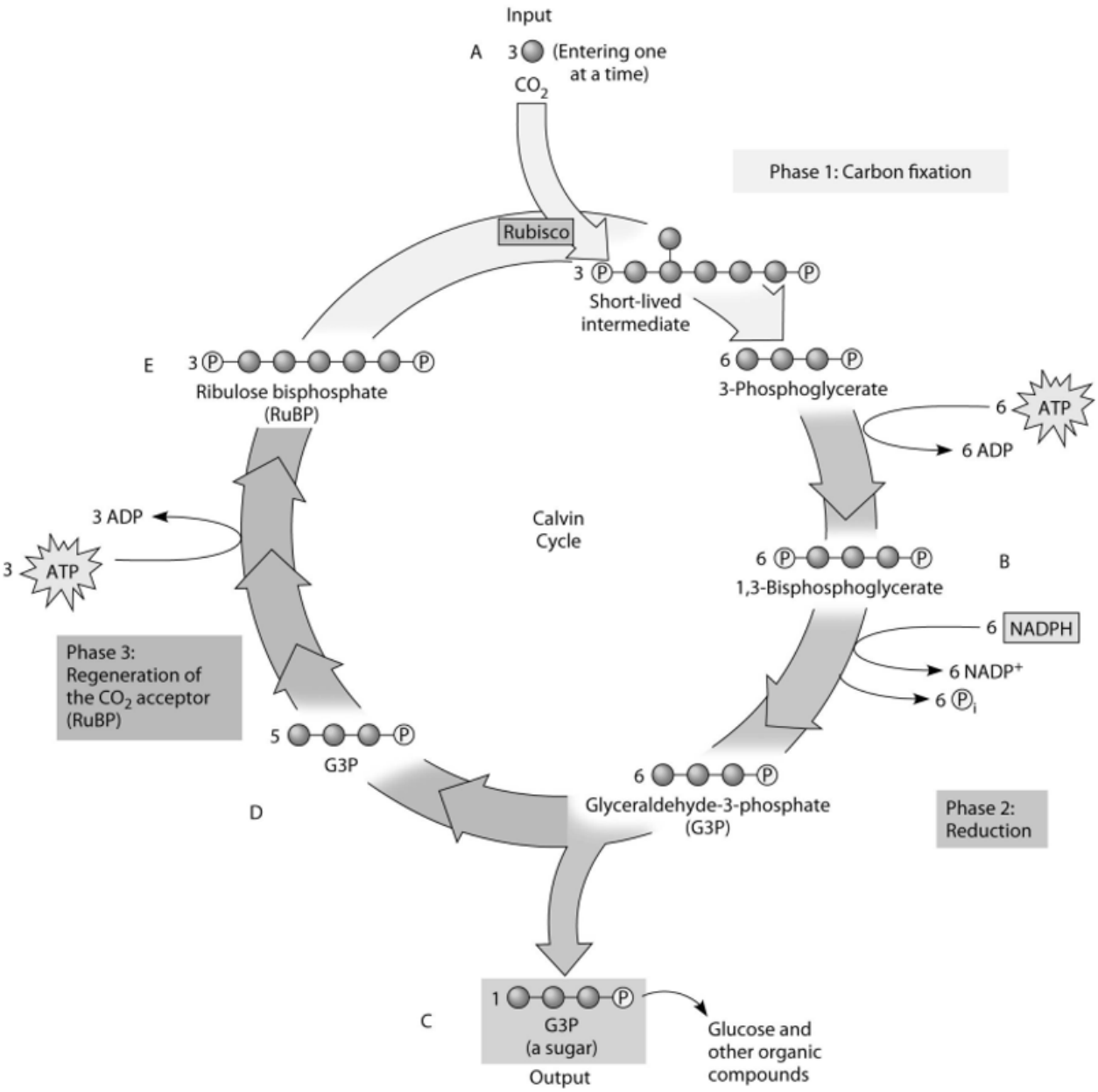


Figure 3: The Calvin Cycle Products of the light-dependent ($NADPH_2$ and ATP) are used in the light-independent reactions of the Calvin cycle to form the precursors of simple sugars like glucose and sucrose. These primary photosynthates can be distributed to all locations of the plant, where they can be stored or utilized for energy or mass production. From Reece *et al.*, (2011).

Regardless of light level, tritium can integrate into plant organics through routine metabolic processes, such as oxidative respiration and hydrogenation (Belot, 1986; Diabaté & Strack, 1997). The dynamics of OBT formation in the absence of light are poorly understood (Boyer *et al.*, 2009; Kim *et al.*, 2013). However, compared to OBT generation via photosynthesis, the night time rates tend to be one-third to one-tenth of that during the day (Strack *et al.*, 2005; Galeriu *et al.*, 2013). Further complicating the issue is the fact that these same metabolic reactions responsible for growth and general plant upkeep, can strip OBT from non-exchangeable positions, though the process is slow (Boyer *et al.*, 2009; Kim *et al.*, 2013).

Previous work conducted near several tritium facilities in Ontario, Canada, found that plant OBT was often enriched over plant HTO in the stems and stocks (Clark *et al.*, 2010). Produce, being fruiting bodies or tubers, from these plants showed the opposite relationship, with HTO routinely exceeding OBT. These observations confirm that OBT levels can vary within the plant, dependent on which organ is being considered. For example: prior to tuber or fruit development, resources produced during photosynthesis are allocated to root and stem growth. Later in the season, translocation of primary photosynthates can shift to fruiting bodies (Diabaté & Strack, 1997; Kim *et al.*, 2013). Variations in seasonal HT (and thus, HTO) are thought to reflected in the growing patterns of these plants, with resources, and therefore OBT, being to allocated to different plant components during growth (Clark *et al.*, 2010). The study further concluded that soil OBT played little to no part in OBT production in plants. Most of the tritium in plant OBT must then be derived from HT and HTO in transpiring water or the atmosphere (Clark *et al.*, 2010).

Following up on this research, Mihok *et al.*, (2016) found that the OBT/HTO ratio was actually higher in stems and leaves watered with rain and tap water (low tritium content) versus those watered with high tritium well water. HTO and OBT activities were expectantly high plants and soils watered with

high tritium water, and relatively low in rain and tap watered samples. These results were derived from a long-term exposure of garden produce at the SRB Technologies facility, where various plants (including potatoes, swiss chard, and sod grass) were sampled throughout the growing season. Daily variations and their effects on OBT formation were therefore indiscernible given the length of the study.

8.7 Deuterium and Oxygen-18

Given that tritium behaves like hydrogen in the environment, this study hypothesized that deuterium could be used as an analogue for tritium in laboratory experiments. As tritium is naturally abundant in trace quantities, kinetic and equilibrium isotope effects commonly seen in deuterium are absent. However, where tritium concentrations exceed natural quantities such as at SRB Technologies, these same isotope effects, or other unidentified mechanisms, could be serving to partition tritium similarly to deuterium.

8.7.1 Deuterium and Oxygen-18 in the Hydrogeological Cycle

At nearly every stage of the hydrogeological cycle, there exists potential for the isotopic signature of water to be altered. For example, evaporation of water will select for lighter isotopes in the vapor phase, while rain water will be enriched relative to that of the vapor from which it was derived. On a global scale, a relationship has been identified between the $\delta^{18}O$ and deuterium signatures of meteoric waters by Craig (1961). Craig's findings indicate that the isotopic signature of meteoric waters is predictable, as characterized by Equation [4].

[4]

$$\delta\,^{2}H = 8.13\delta^{18}\mathrm{O} + 10.8$$ (Rozanski *et al.*, 1993)

From this equation, the Global Meteoric Water Line (GMWL) can be generated that describes how colder regions are generally characterized by enrichment in the light isotopes of both oxygen and

deuterium. Conversely, warm regions trend towards enrichment in the heavy isotopes. The GMWL is actually a global average, built from smaller scale regions that can each be described by a Local Meteoric Water Line (LMWL). For example, the LMWL for the Ottawa region is described in **Equation [5]**.

[5]

$$\delta\,^{2}H = 7.6\delta^{18}O + 6.5 \text{ (Fritz } \textit{et al.}\text{, 1987)}$$

The GMWL is the result of fractionation during condensation of water vapor in the air, while Raleigh distillation is responsible for the variation in LMWLs (Clark and Fritz, 1997). Closed inland basins can differ by as much as 200‰ on account of evaporative enrichment, while ocean waters can vary in isotopic composition due to salinity levels (Dansgaard, 1964). Groundwater in the Ottawa region will share the isotopic composition of the mean weighted annual precipitation (Clark and Fritz, 1997). Water available for plant uptake will therefore generally have similar isotopic compositions as precipitation, groundwater and soil water.

8.7.2 Deuterium and Oxygen-18 in Plants

The deuterium and ^{18}O isotopic signature of deep soil water should be consistent with that of the annual mean of precipitation (Clark and Fritz, 1997). Near the surface however, evaporation can lead to isotopic enrichment of soil water. Evaporation is generally limited to the top 10 to 20 cm (Marshall *et al.*, 2008) Variations resulting from season to season can also occur, as melting snow cover allows for percolation of isotopically lighter waters to deeper layers. Soil water should have a direct impact on the tissue free water, and by extension, the plant organics.

No isotopic fractionation occurs during water uptake from the soil to the plant, or during transport through the xylem (Dawson and Erhleringer, 1993). As a result, xylem water should reflect the isotopic composition of the soil water surrounding the roots, and presumably, localized precipitation

(Marshall *et al.*, 2008). When soil water reaches an evaporation site in the plant, such as the leaves, there is opportunity for evaporative enrichment (Gonfiantini *et al.*, 1965; Wershaw *et al.*, 1970; White *et al.*, 1985; Dawson & Ehleringer, 1993).

Changes in the isotopic composition of leaf water resulting from evaporative enrichment are poorly understood. One assumption is that there are distinct isotopic heterogeneities resulting from the different water pools in the leaf, known as the apoplastic and symplastic pools (Yakir *et al.*, 1990; Leuenberger, 1998). The apoplastic pool (composed mostly of the cell water surrounding the stomata) contains water that is directly exposed to transpiration, and therefore, evaporative enrichment. Vein water, which can account for less than 2% to 5% of leaf water, should maintain the same signal as the source water (Parkhurst, 1982). Water in the symplastic pool, composing approximately 69% of leaf water, is where the majority of the cell's biochemical reactions take place, and exchanges slowly with the apoplastic pool (Yakir, 1990; Leuenberger, 1998). The isotopic composition of the symplastic pool is therefore influenced by photosynthesis, with some exchange between the other pools. Bulk leaf water analysis will therefore represent an integrated signal of unaltered source water, that which is exposed to evaporative enrichment, and metabolic reactions, though it should be noted that that there is likely to be variation between species in the relative proportions of water in each pool (Leuenberger, 1998).

Another model for leaf water heterogeneity was proposed by Farquhar and Lloyd (1993) and suggests that leaf water enrichment will decrease with distance from the evaporation site, and diffusion and advection from the transpiration site will be dictated by the transpiration rate. At higher rates of transpiration, isotopically enriched water will fail to diffuse throughout the leaf, or plant, as it is being replaced too quickly by soil water migrating to the leaf (Leuenberger, 1998).

The most commonly applied and altered quantitative model with respect to leaf water enrichment is the Craig-Gordon model which describes isotope effects resulting from ocean water

evaporation. It has frequently been modified to describe hydrogen and oxygen isotope enrichment at the leaf surface (Flanagan *et al.*, 1991). Essentially, the model allows for both equilibrium (phase change from liquid to gas) and kinetic (diffusion driven) effects. Among the models assumptions are that the air in the intercellular spaces is saturated, and that liquid water and vapour present are at the same temperature. Further, it takes into account both diffusion through the stomata, and the boundary layer at the leaf surface. The leaf boundary layer is typically a zone adjacent to the leaf surface where turbulence is quite low. As a result, gas concentrations and diffusion rates are different near the surface versus the surrounding air. The model is known for routinely overestimating evaporative enrichment, as it does not account for the different water pools. Roden and Ehleringer (1999) suggest that these discrepancies are a result of not considering atmospheric vapour, thus rendering the assumption that water vapour is in equilibrium with soil water invalid.

Regardless of the qualitative or quantitative model, it has been noted that water isotope fractionation in the leaf is heavily dependent on the transpiration rate, which is in turn dependent on the ambient relative humidity. In all cases, subsequent back diffusion and translocation of enriched evaporative leaf water occurs in the stems via the phloem (Farquhar & Lloyd, 1993). This can alter the bulk stem water signatures as both unchanged soil water in the xylem will be mixed with phloem sap, producing a mixture of both enriched leaf water with that of enriched soil water (Flanagan *et al.*, 1991).

8.7.3 Deuterium and Oxygen-18 Uptake from the Atmosphere

Water vapour present in the air can be absorbed by gas diffusion through the stomata. This is often overlooked in studies of leaf isotopic composition. Roden and Ehleringer (1999) investigated the effects of creating a non-equilibrium environment for plants, where the water vapour was isotopically different than soil waters. Using the modified Craig-Gordon model of Flanagan *et al.*, (1991), they found that leaves leaf water responded quicker to atmospheric water vapour than soil water. Leaves reached

steady-state with atmospheric water vapour within 2-3 hours of exposure, versus the 24 hours required with soil water. Of course, the response time was heavily dependent on vapour pressure gradients, with lower gradients inducing longer response times. Therefore, atmospheric water vapour could be a driving factor in leaf water isotopic composition, which would have implications the isotopic composition of plant organics.

8.7.4 Deuterium and Oxygen-18 in Plant Organics

Hydrogen from water is transferred to carbohydrates during photosynthesis, selecting against deuterium quite heavily (Hoefs, 2009). Autotrophic carbohydrate metabolism produces a deuterium fractionation of -120‰ to -171‰ (Yakir and DeNiro, 1990). Hydrogen isotope signatures are also compound specific, with some lipids showing depletions of up to 300‰ (Hoefs, 2009). Conversely, secondary heterotrophic metabolism can produce enrichments of between +144‰ to +166‰ (Yakir and DeNiro). Much of what is known about hydrogen isotopes in plant organic tissues is based on plant cellulose. Seventy percent of hydrogen in cellulose is bound to carbon and is non-exchangeable (Epstein *et al.*, 1976). Plant organic material has an oxygen-18 composition that reflects the source water and the evaporative changes induced on the leaf water during photosynthesis (Farquhar *et al.*, 2000; Barbour et al, 2000). Typically, oxygen fractionates only through water/carbonyl reactions, enriching by an average of +27‰ (Yakir and DeNiro, 1990).

8.8 Microbial Oxidation of Atmospheric Hydrogen and Tritium Gas

8.8.1 Oxidation in Soils

HT conversion rate measurements, normalized to hydrogen, have been measured at approximately 200 nmol/g per hour, suggesting that HT conversion functions the same as H_2 oxidation (Guo and Conrad, 2008; CNSC, 2010). The ability of hydrogenotrophs to oxidize elemental hydrogen

requires the ambient atmospheric mixing ratio to be above 0.5 ppmv (Ichimasa *et al.*, 1999). Therefore, it has been suggested that soil HT oxidation is dominated by abiontic hydrogenase enzymes, produced from lysed bacterial cells in the soil. These enzymes have been shown to oxidize hydrogen gas at faster rates with lower levels (Guo and Conrad, 2008). Conversely, Constant *et al.*, (2011) found that high affinity H_2-oxidizing bacteria play the dominant role, with abiontic hydrogenase contributing weakly to the overall activity, at a mere 2.5 %. Further, it was hypothesized that abiontic enzymes would not only need to accumulate in the soil following cell lysis, but they would also need to remain partnered to a functioning electron transport chain. Ten days after inoculation of sterile soils, there was still no abiontic activity in the Constant *et al.*, (2011) study. Regardless of whether abiontic enzymes, or living bacterial cells are responsible for hydrogen and tritium gas oxidation, the soil media likely plays a pivotal role in tritium availability for plants.

8.8.2 Oxidation in Plants

Despite the fact that HT appears to play little direct role in plant tritium concentrations, HT has been shown to be oxidized to HTO in small amounts by some vegetation. Using in-vitro methods, Ichimasa *et al.*, (1999) found that mosses and lichens were 50 to 500 times more adept at oxidizing HT to HTO when compared with pine needles. Pine needles themselves were capable of oxidation rates 1/10000 to 1/1000 of the top 0-5 cm of surface soils (Ichimasa *et al.*, 1999). However, analysis of several herbaceous plant varieties showed HT-oxidizing activities associated with leaves, though 2 to 4 orders of magnitude lower relative to the soil. These results were consistent with previous findings that HT oxidation on leaves was low (Belot, 1986; Spencer and Dunstall, 1986; Ichimasa *et al.*, 1989). Ichimasa *et al.* (1999) found that there are some exceptions to the rule when considering herbaceous plant leaves. *Phalaris arundinacea*, a perennial tussock grass, had an HT-oxidation activity of 0.0405 +/- 0.0129 relative to soil, far greater than other varieties. HT-oxidation activity associated with plants extends to

the roots as well, where rates are comparable to the soil (Ichimasa *et al.*, 1999). The origin of the HT conversion ability in plants is believed to be the same as soil; a result of microbial oxidation activity, and their hydrogenase enzymes, adhered to the surface of the leaves and roots (McFarlane, 1978; Belot, 1986; Spencer and Dunstall, 1986; Ichimasa *et al.*, 1999).

The boundary layer between the surface of the leaf and the atmosphere is generally characterized by laminar flow. HTO produced at the leaf surface may then diffuse through the stomata and contribute to the overall plant HTO concentration. The leaf boundary layer is also believed to have a negligible impact on hydrogen isotope fractionation (though the opposite is true for oxygen-18) (Flanagan *et al.*, 1991). Given that the HT-oxidation ability of soils is far greater than the plant, soil pore waters are often believed to be the main source of HT uptake by plants by conversion to HTO (Belot, 1986; Amano *et al.*, 1995; Ichimasa *et al.*, 1999).

9 Field Site

SRB Technologies (Canada) Inc. is a tritium light production facility located at 320-140 Boundary Road in Pembroke Ontario, Canada. The facility produces several tritium beta-light products, ranging from EXIT signs to gun sights, and has been processing imported gaseous tritium since 1990. As part of routine daily operations, HT and HTO are sporadically released at high velocity into the atmosphere via two stacks located at the building's western corner (SRBT correspondence). The tallest stack, called the 'Rig Stack' is 11.86 m tall measured from the ground surface. The second stack is slightly shorter and known as the 'Bulk Stack.' The two stacks ventilate different sections of the facility and are active during working hours. SRBT utilizes a real time monitoring system of total tritium stack emissions however it cannot distinguish between different tritium species. A bubbler system is analyzed on a weekly basis to determine the different contributions of HT and HTO. **Figure 4** and **Figure 5** below show Beta-light

production in action at the SRB Technologies facility. Tritium is encapsulated into vials that can, on occasion, break during production.

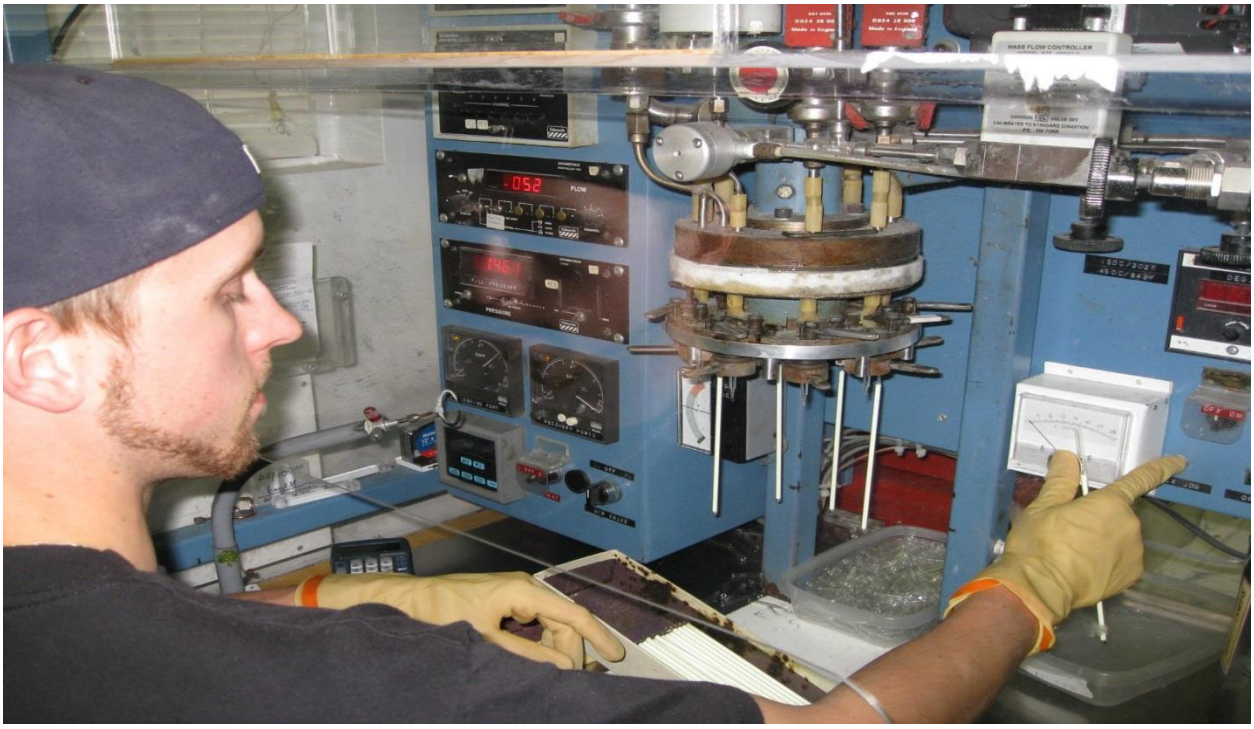


Figure 4: Tritium Beta-light production at SRB Technologies, Pembroke, Ontario, Canada.

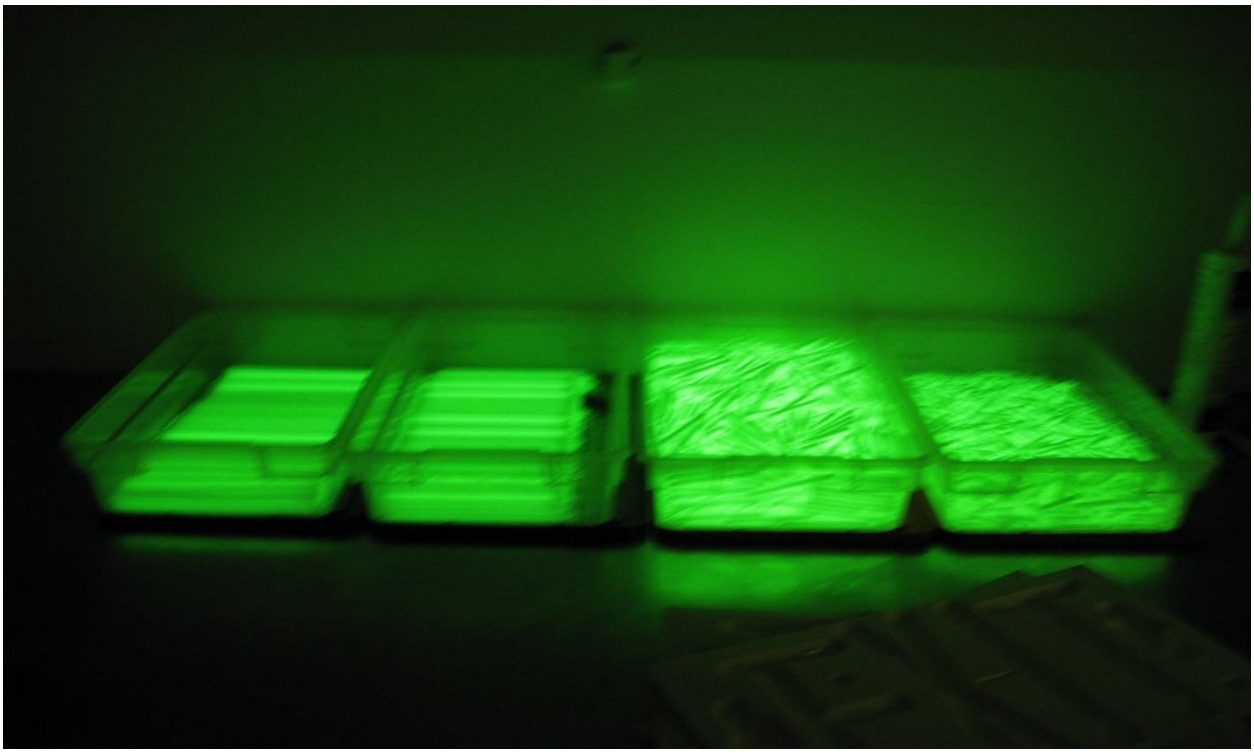


Figure 5: Tritium encapsulated in Beta-light vials at SRB Technologies, Pembroke, Ontario, Canada.

In 2007, SRB Technologies was temporarily shut down by order of the Canadian Nuclear Safety Commission due to exceeding allowable levels of tritium in the environment. An on-site monitoring well had a tritium concentration of 60 000 Bq/L, believed to be the result of water dripping from the emissions stack. Operations resumed in July 2008. As required by the CNSC operating license, an Environmental Monitoring Program (EMP) has been employed by SRB Technologies. This includes routine passive air sampling around the facility and in Pembroke, as well as both onsite and residential well monitoring (CNSC, 2010).

The area immediately surrounding SRBT is primarily zoned as industrial. Much of the surrounding area has been reworked for various purposes and natural vegetation is generally sparse. A farm field can be found to the west and south-west, opposite Upper Valley Road. The bulk of Pembroke is located to the north, north-east, and north-west of the facility, with the nearest residential zone

located about 250 km north. The Muskrat River is approximately 400 m to the east and discharges 2.5 km north into the Ottawa River.

Soils in the area have been described by Gillespie *et al.* (1964) in the Ontario Soil Survey. The area is dominated by clay silt and silt clay mixtures with small, scattered regions of silt and sand. In some modified locations there are thin layers of topsoil and the occasional gravel fill. Generally, the soils demonstrate poor drainage.

In terms of local hydrogeology, Pembroke lies completely within the Ottawa Valley Clay Plain, which generally consists of deep (>30 mbgs) and shallow (<15 mbgs) groundwater flows derived from the Madawaska Highlands moving from south-west to north-east into the Ottawa River (Golder, 2003). However, localized hydrogeology varies in the region due to the influence of topography and surface waters. SRBT, being in close proximity to the Muskrat and Ottawa rivers, can be considered a discharge zone. Recharge in the area is quite low, with 0-100 mm/year. The clay and silt overburden is the result of Champlain Sea deposits and there is a small, 2-4 m thick sand and gravel layer just above the bedrock surface throughout Pembroke. The overburden has low hydraulic conductivity, ranging from10^{-6} to 10^{-10} m/s.

An INTERA (1994) study of the property adjacent to SRBT found that groundwater flow is generally eastward towards the Muskrat River. Mean water table depths are approximately 4.5 mbgs with hydraulic conductivity values of less than 10^{-6} m/s. The Gillespie (1964), INTERA (1994), and Golder (2003) studies are confirmed by comprehensive hydrogeological assessments by EcoMetrix (2006, 2008).

10 Data Collection

10.1 Field Data Collection

Two cores were taken with an open face hand auger (diameter: 1.5 inch, length: 19 cm) at two locations on the SRBT property on June 20 2013 (**Figure 6**). Samples were taken from the middle of the auger sample to prevent soil mixing, placed in Ziploc freezer bags, and immediately put on ice for transport. Sediment samples were frozen and stored at the University of Ottawa the same day until ready for tritium pore water extraction by cryogenic distillation, and organically bound tritium extraction by combustion.

Figure 6: SRBT Core Locations, Pembroke Ontario Locations of SRBT auger cores, hand augured, and sampled for pore water and organically bound tritium.

Seven potted plants, previously grown in a growth chamber at the University of Ottawa were transported to the SRBT property and left for a 24-hour exposure period. In four hour intervals, a single plant was separated into leaves, stems, and potted soil, put on ice and frozen for transport. Leaf samples included any flower bodies present, while stems samples included the roots. The plants were watered with background level tritium water the morning before arriving on site, and were not watered during exposure.

A set of soil samples were prepared by placing nutriMIX HP growth media into Isojars, and wetted with background level tritium water. One group was brought on site unsealed, while another group was left sealed and without access to the ambient air. A third group was outfitted with a 6 cm 3/8" silicone tube diffuser impermeable to polar gases. The diffuser was capped at one end by silicone caulking and attached to a needle at the other, also sealed by silicone caulking. The needle was inserted through the septa of the Isojar, thereby allowing for HT diffusion into the soil encased within. The three groups of soils (each consisting of one open, one sealed, and one diffuser soil) were sampled every 5 hours. The same experiment was replicated in the growth chamber, with D_2 substituted for atmospheric tritium.

Both the plants and soil sets were placed approximately 50 m south-west from the SRBT stacks. Atmospheric HTO and HT measurements were taken every 3 hours using the active tritium air sampler. This same portable sampler was used to measure atmospheric HDO and HD in the growth chamber experiments.

10.2 Laboratory Data Collection

Potted Arugula plants were grown from seed in nutriMIX HP growth media, contained inside a Conviron E15 growth chamber (**Figure 7**). Growing conditions were chosen to optimize arugula growth

while attempting to replicate average summer seasonal temperatures in Pembroke, Ontario during the growing season. Relative humidity was maintained at approximately 60%, with 15 hours of light at 20°C and 9 hours of dark at 13°C. A timed hydroponic drip system was used to water the plants three times a week for one minute from a water reservoir (**Figure 7**). The watering schedule was not intended to replicate precipitation events. The reservoir, being a 94 liter storage plastic storage tote, was initially filled with tap water, and replenished as needed. The tote was lidded and sealed as best as possible to prevent isotopic changes to the water reservoir while still allowing for pump line access. The water was sampled before each experiment. The chamber settings were adjusted with each deuterium gas exposure trial (in accordance with light and dark exposure times) and no watering took place during exposure save for immediately before the beginning of the trial. Watering before trial initiation ensured that the soils were flushed with water of known isotopic composition. Deuterium gas (Sigma-Aldrich 99.8% D 45L Lecture Bottle) was delivered by diffusion through PVC tubing at an approximate rate of 2.6 cc/hour (**Figure 9**). After exposure, the arugula plants were separated from their respective soils, and divided into leaf and stem samples. Leaf samples included any flowering bodies if present, while stem samples included the roots. The soil, leaf, and stem samples were frozen prior to water extraction of tissue free and pore waters.

Figure 7: Growth Chamber Conviron growth chamber with water reservoir supplying the plants inside. Drainage is discharged into the white barrel.

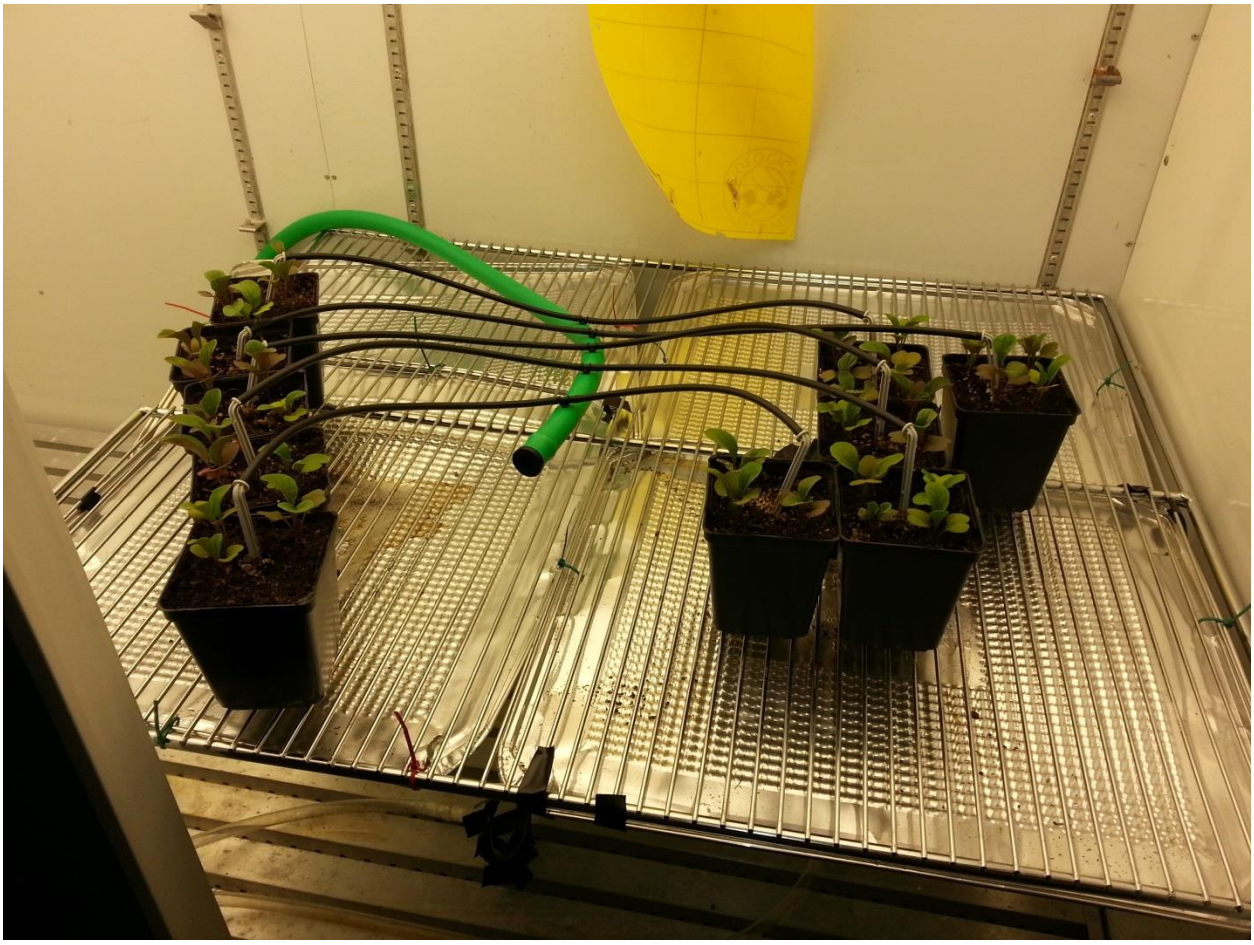


Figure 8: The scheduled hydroponic drip system provided water to the plants three times a week to maintain consistent growing conditions. Note that there are several seedlings per pot. This was later changed to increase leaf volume, as plants grown in close proximity led to reduced growth.

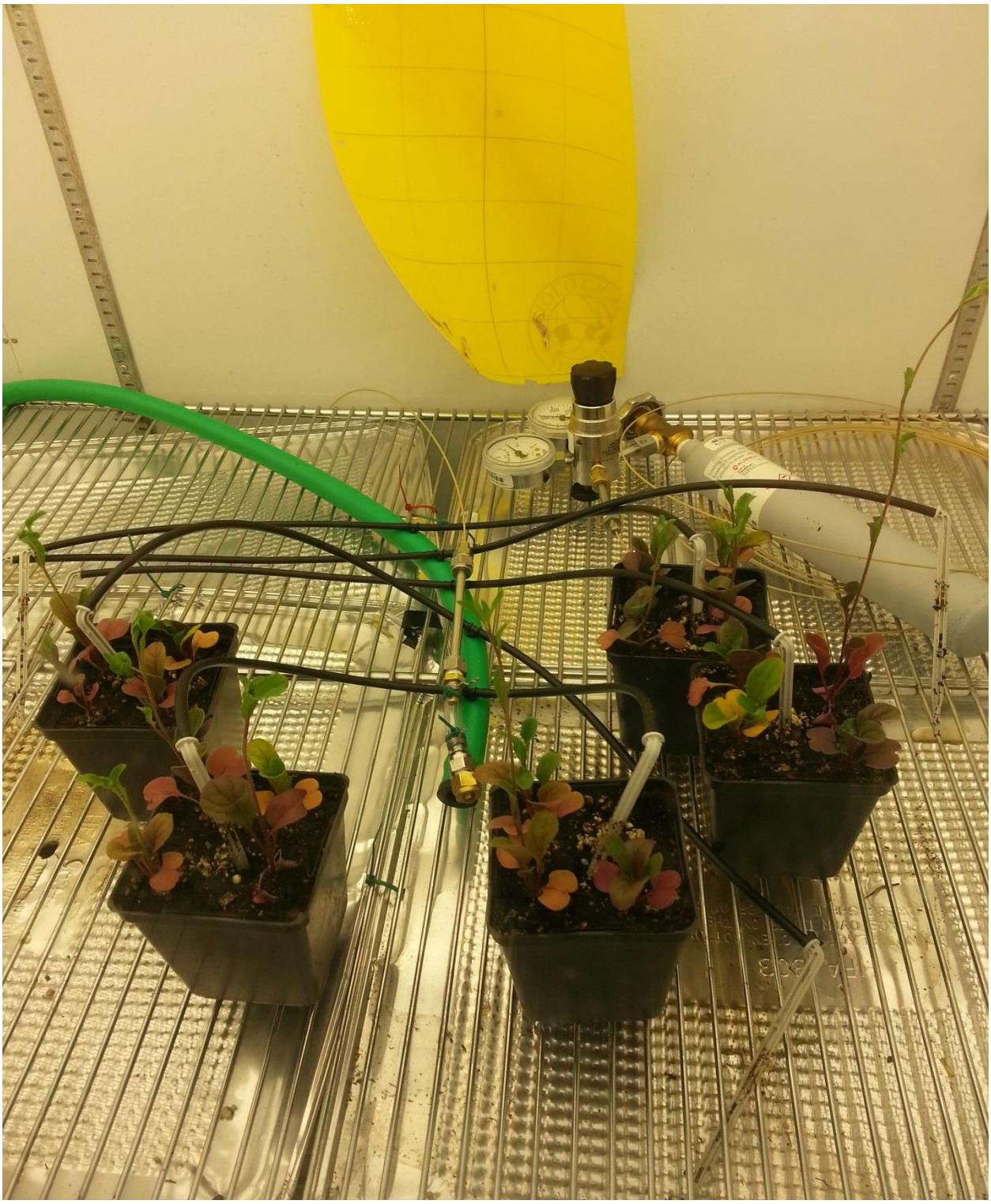


Figure 9: Deuterium Gas Delivery Mechanism D_2 gas is delivered by diffusion through soft PVC tubing from a compressed gas tank.

10.3 Analytical Methods

10.3.1 Atmospheric Deuterium, Oxygen, and Tritium

Deuterium and oxygen-18 measurements of the air inside the growth chamber were gathered using a custom air bubbler originally designed for atmospheric tritium measurements (**Figure 10**) (Lapp, 2012). The sampler is capable of capturing both HDO or HTO and HD or HT atmospheric signals. Ambient air is pumped and bubbled at measured flow rates, typically 15 mL per minute, into an EPA vial filled with water where the deuterium signature of the air equilibrates with the starting water. The air is then pumped into a heated glass chamber containing a platinum metal honeycomb catalyst capable of converting water into HD or HT. A second EPA vial equilibrates with the air leaving the catalyst chamber. The water samples were then prepared for low-level liquid scintillation counting or Los Gatos Research Triple Isotope Water Analyzer (LGR) analysis as required.

The LGR is capable of measuring $^{2}H/^{1}H$, $^{18}O/^{16}O$, and $^{17}O/^{16}O$ in fresh, clean, waters. One milliliter of sample was pipetted into a glass vial for the analysis. Samples are first heated at approximately 85°C for isotopic analysis of the water vapour. Each sample is injected 10 times, with the first two injections being run to reduce any memory effects between samples.

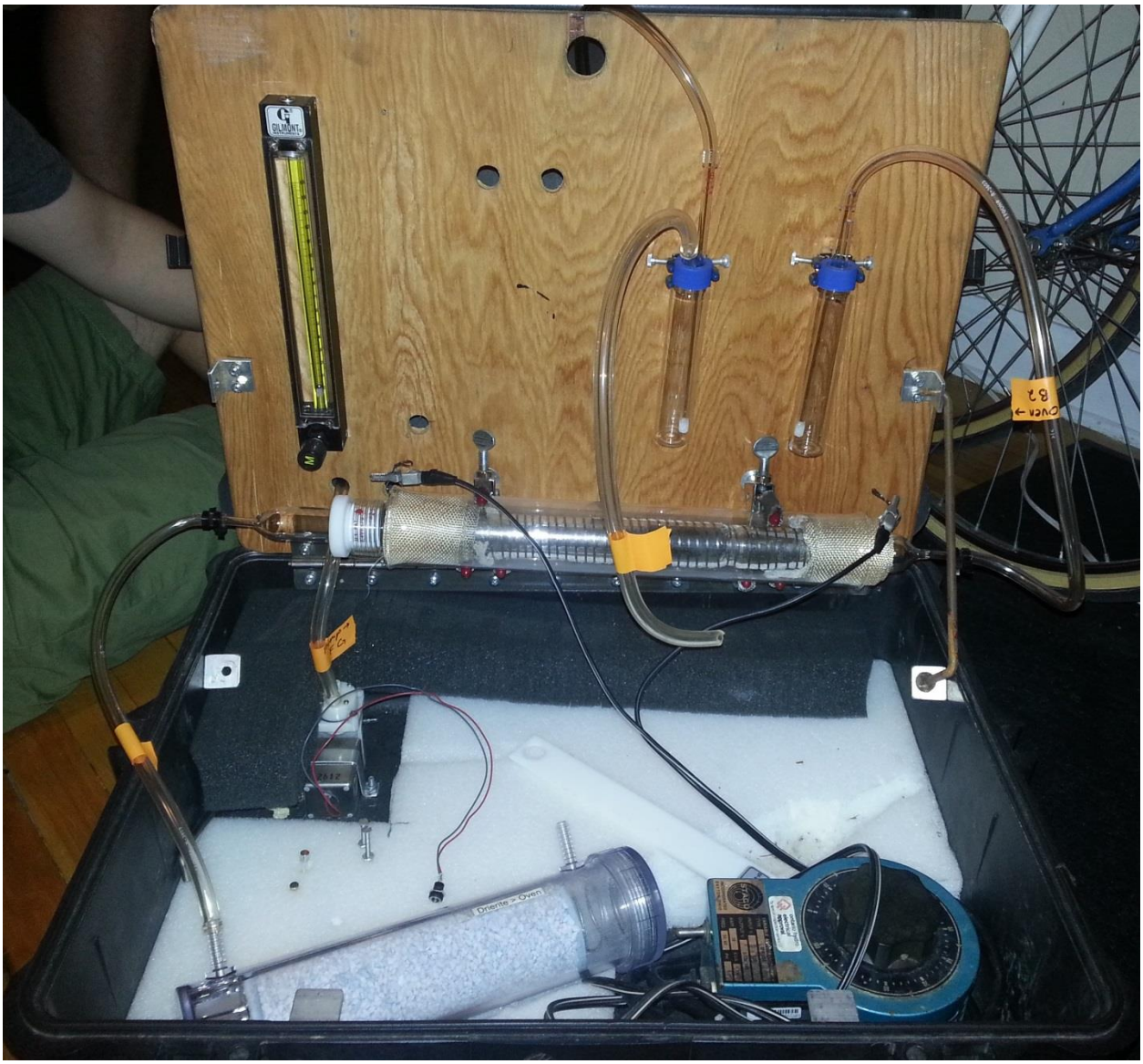


Figure 10: Custom air sampler used to actively measure HT and HTO, or HD and HDO in the immediate atmosphere. Air is pumped into the first bubbler (left), where isotopic equilibrium is reached with the vial water. Subsequent air flow leads to a dessicator, then to the heated glass catalyst chamber, where tritium/deuterium gas is converted to HTO/HDO. Leaving the catalyst, the air is pumped into the second bubbler (right), where it equilibrates with vial water.

10.3.2 Plant Tissue Free Water and Soil Pore Water – Tritium

Frozen plant and soil samples were placed into glass vials and weighed prior to being sealed onto a custom glass extraction line. The sample vials were frozen in liquid nitrogen so no sample would

be lost as the line was evacuated. Pressure within the line was reduced to 10^{-1} mbar, at which time the sample traps were submerged in liquid nitrogen, and heat was applied to the frozen sample vials.

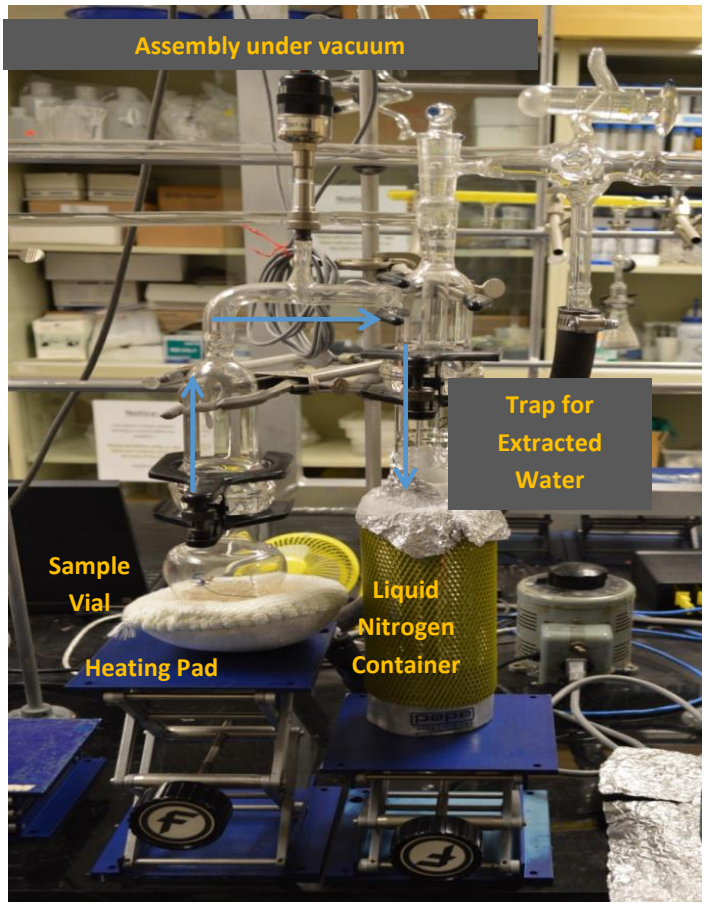


Figure 11: The sample is heated (left) after the sample vial is submerged in liquid nitrogen to evacuate the chamber. Water vapour is trapped in the liquid nitrogen trap (right) and prepared for analysis.

Extracted waters were thawed, and 10 mL was placed in a scintillation vial, along with 10 mL of Ultima Gold scintillation cocktail. Samples were counted in a Perkin Elmer Quantulus low-level liquid scintillation counter at the University of Ottawa (**Figure 12**). Upon completion of the extraction, plant and soil samples were placed in an oven at 60°C until weight constancy. Generally, plant materials showed no weight loss during the post-extraction drying, suggesting that all of the tissue water was removed. Samples were then ready for OBT analysis if necessary.

Figure 12: Low-level liquid scintillation counter at the University of Ottawa.

10.3.3 Plant Tissue Free Water and Soil Pore Water – Deuterium

When considering the extraction of tissue and pore waters for deuterium analysis, smaller sample sizes were used to ensure a complete extraction of all sample water. This was to prevent any isotope fractionation induced during the procedure. Extracted waters were filtered if necessary prior to hydrogen and oxygen isotope analysis on a Gasbench Delta Plus XP Isotope Ratio Mass Spectrometer.

Deuterium and oxygen-18 analysis required 0.2 mL of sample water, pipetted into an Exetainer. Copper metal chips and activated carbon removed any contamination by dissolved hydrogen sulfide and organics. Samples were left for three days prior to oxygen analysis, at which point the Exetainers were flushed with 2% CO_2 in helium gas and left to equilibrate at room temperature for a minimum of 18 hours. For deuterium analysis, Hokko beads were added to the Exetainers prior to a flush with 2% H_2 in He. Samples were left to equilibrate at room temperature for a minimum of 1.5 hours. The same sample was used for both oxygen and hydrogen isotope analysis.

10.3.4 Plant Tissue – Oxygen and Deuterium Analysis

Following tissue free water extraction, plant materials were oven dried to ensure that all water had been removed during the extraction. The samples were then ground to a powder in a ball mill in preparation for analysis of plant organic deuterium and oxygen isotopes using a Delta Plus XP Isotope Ratio Mass Spectrometer equipped with a TC/EA Vario Elementar Pyro Cube. Some plant material was also analyzed for H-C-N-S elemental ratios using an Elementar Micro Cube, to test for anomalies in plant growth.

Approximately 0.4 mg (hydrogen analysis) and 0.3 mg (oxygen analysis) of dried and powdered plant material was weighed into silver capsules and placed in the carousel of a Thermo-Finnigan Elementar Vario Pyro Cube in preparation for pyrolysis (**Figure 13**). Helium gas purged any air present as

the sample fell into a graphite crucible held at 1450°C. Gas products were reduced to CO and/or H_2 then separated by a chemical column that traps the CO, while the H_2 was carried through by helium. Subsequent heating of the column released the CO before introduction into the IRMS.

Approximately 5 mg of dried and powdered plant material was weighed into tin capsules and then loaded into an Elementar Micro Cube elemental analyzer. Samples were combusted in oxygen at 1800°C, and pushed through a series of oxidizing/reducing columns to generate N_2, CO_2, H_2O, and SO_2. The gases were trapped in an adsorption column and released separately into the thermal conductivity detector.

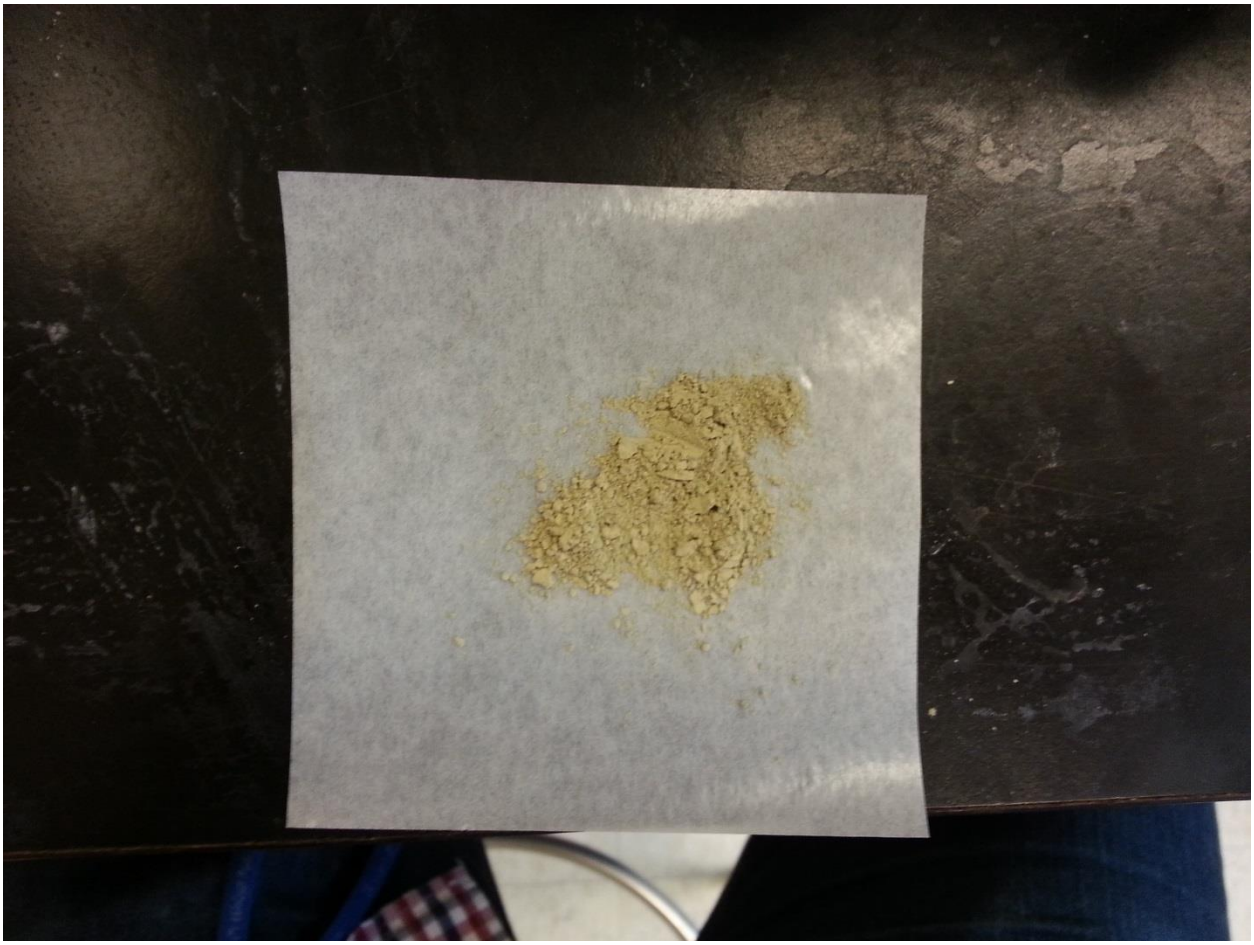


Figure 13: Plant material after grinding in a ball mill, in preparation for organics isotope analysis.

10.3.5 Soil Organically Bound Tritium Extraction

Approximately 30 g of dried soil sample was weighed into a 25 cm long glass boat with a diameter of 18 mm. The samples were then combusted at 800°C using two Lindberg clamshell tube furnaces capable of holding a 25 mm diameter quartz glass tube. Combustion products were moved at 200 mL/minute by carrier gas (dry compressed air) from the first combustion furnace to a second catalyst furnace where copper oxide filings heated to 750°C in the presence of pure oxygen catalyzed the combustion products into H_2O and CO_2. The oxygen was introduced at 100 mL/min in the opposing direction to carrier gas flow via a glass inlet stem in the catalyst oven (**Figure 14**).

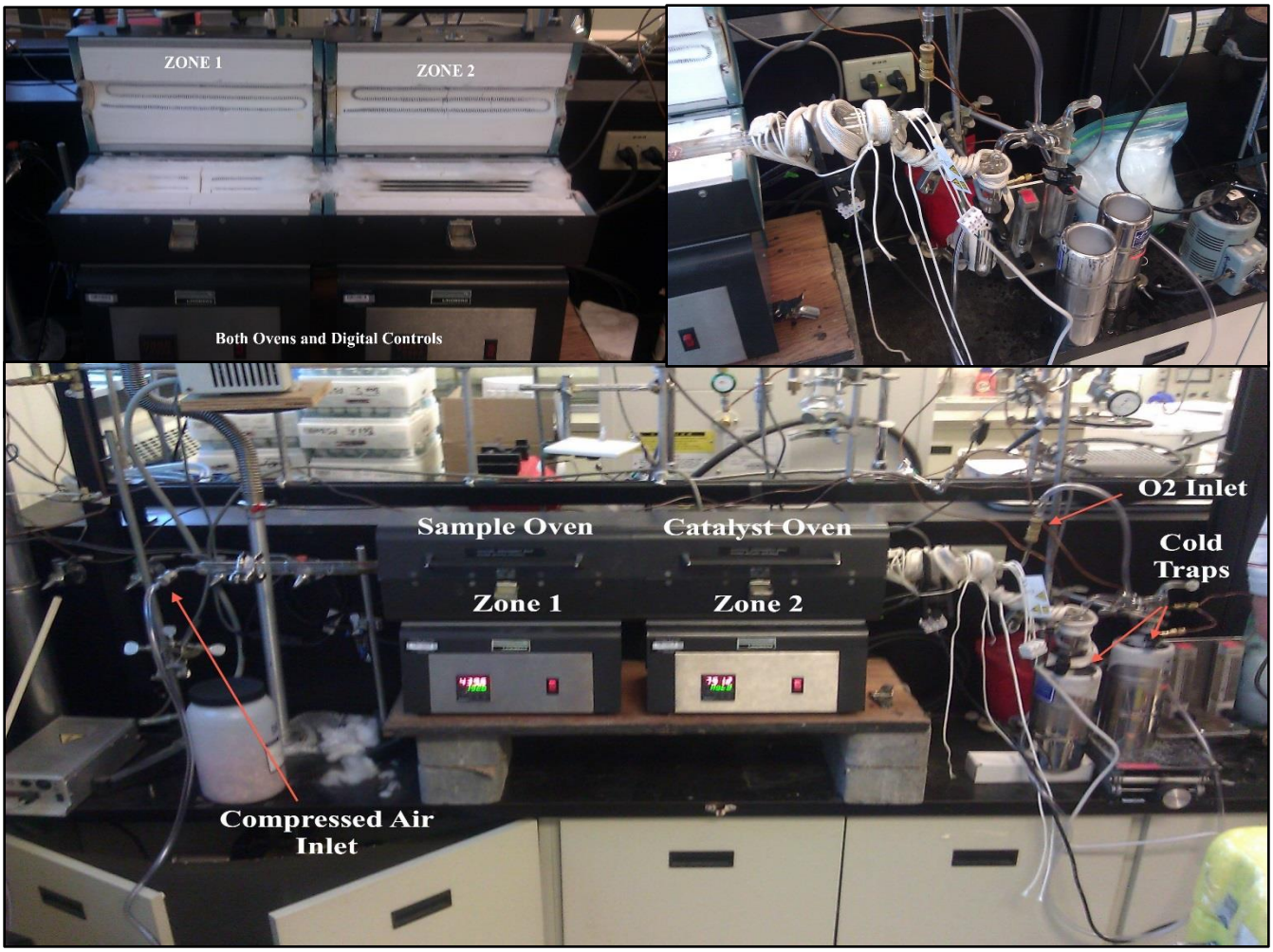


Figure 14: Custom tube furnace apparatus for soil OBT extraction. The soil sample is placed in the sample oven where it is combusted at 800°C in the presence of dry compressed air, which pushes the products into the catalyst oven, flushed with oxygen. HTO produced is captured in a liquid nitrogen-ethanol slurry.

The H_2O (and HTO if present) was frozen in two glass traps immersed in 99% ethanol slurry cooled by liquid nitrogen to approximately -90°C. Extracted water was thawed and transferred to 20 mL scintillation vials and topped up to 10 mL using tritium-free water. Ten milliliters of Ultima Gold scintillation cocktail was added before the vials were counted in a Perkin Elmer Quantulus 1220 low-level liquid scintillation counter at the University of Ottawa. Tritium concentrations, generated in Bq/L from the Quantulus, are reported in Bq/kg of soil using Equation [6][6

[6]

$$T_{\text{wet soil}} = \text{Measured T x } \frac{m_{\text{extracted water}}}{m_{\text{dry soil}}} \text{ x } (1 - \theta_{\text{soil}})$$

10.3.6 Plant Organically Bound Tritium

For plant OBT analysis, the masses of the plant samples obtained were too small for analysis by combustion and decay counting. Therefore, the samples were encapsulated under vacuum to allow for ^{3}He ingrowth resulting from the decay of tritium. Dried plant materials were finely chopped and placed into copper tubes sealed at one end. Copper filings were used to hold the sample in place before the tubes were placed on a vacuum line, and evacuated to 10^{-1} mbar. The tubes were crimp sealed and are currently undergoing ^{3}He ingrowth. After the ingrowth period, the samples will be analyzed on a Helix SFT noble gas mass spectrometer at the University of Ottawa. It is capable of measuring tritium concentrations of 0.006 Bq/g, assuming a 100 day ingrowth period of a 5 g sample.

Figure 15: Helium ingrowth preparation line. Samples are packed tightly into copper tubing, then the air is evacuated in each tube using a diffusion pump. At the appropriate vacuum pressure, the tubes are crimped and left to accumulate helium from tritium decay.

11 Results

11.1 SRB Technologies Soil Profiles

Figure 6 in **Section 9** shows the locations of two cores taken at the SRB Technologies field site in Pembroke Ontario. SRBT Core 1 (maximum depth: 117.5 cm) was augured from the north-west manicured grass boulevard while SRBT Core 2 (maximum depth: 121 cm) was augured south-west in a previously reworked field overgrown by long grasses. Both cores were almost completely dominated by light brown clay silt throughout the profile.

In the uppermost 30 cm, SRBT Core 1 contained layers of organic rich top soil littered with small roots, eventually transitioning into higher silt and clay contents with depth. SRBT Core 2 lacks this top soil layer, with twigs and roots littering the top silt-clay layers. Moisture content increased with depth in SRBT Core 1 (minimum 14% H_2O; maximum 27% H_2O) while remaining fairly consistent in SRBT Core 2 (average 25% H_2O).

Pore water tritium concentrations in both SRBT Core 1 and 2 decreased slightly from 580 Bq/L and 458 Bq/L respectively at the ground surface until approximately 22 cm depth (**Figure 16** and **Figure 17**). Below 22 cm, pore water tritium concentrations increased with depth, peaking at 1173 Bq/L in SRBT Core 1 and 1739 Bq/L in SRBT Core 2. Organically bound tritium concentrations drop rapidly from the surface in both cores. SRBT Core 1 has a maximum concentration of 143 Bq/kg at the ground surface and drops to below 2 Bq/kg at 117.5 cm. SRBT Core 2 has a maximum concentration of 297 Bq/kg at the surface and drops below 2 Bq/kg at 53 cm depth.

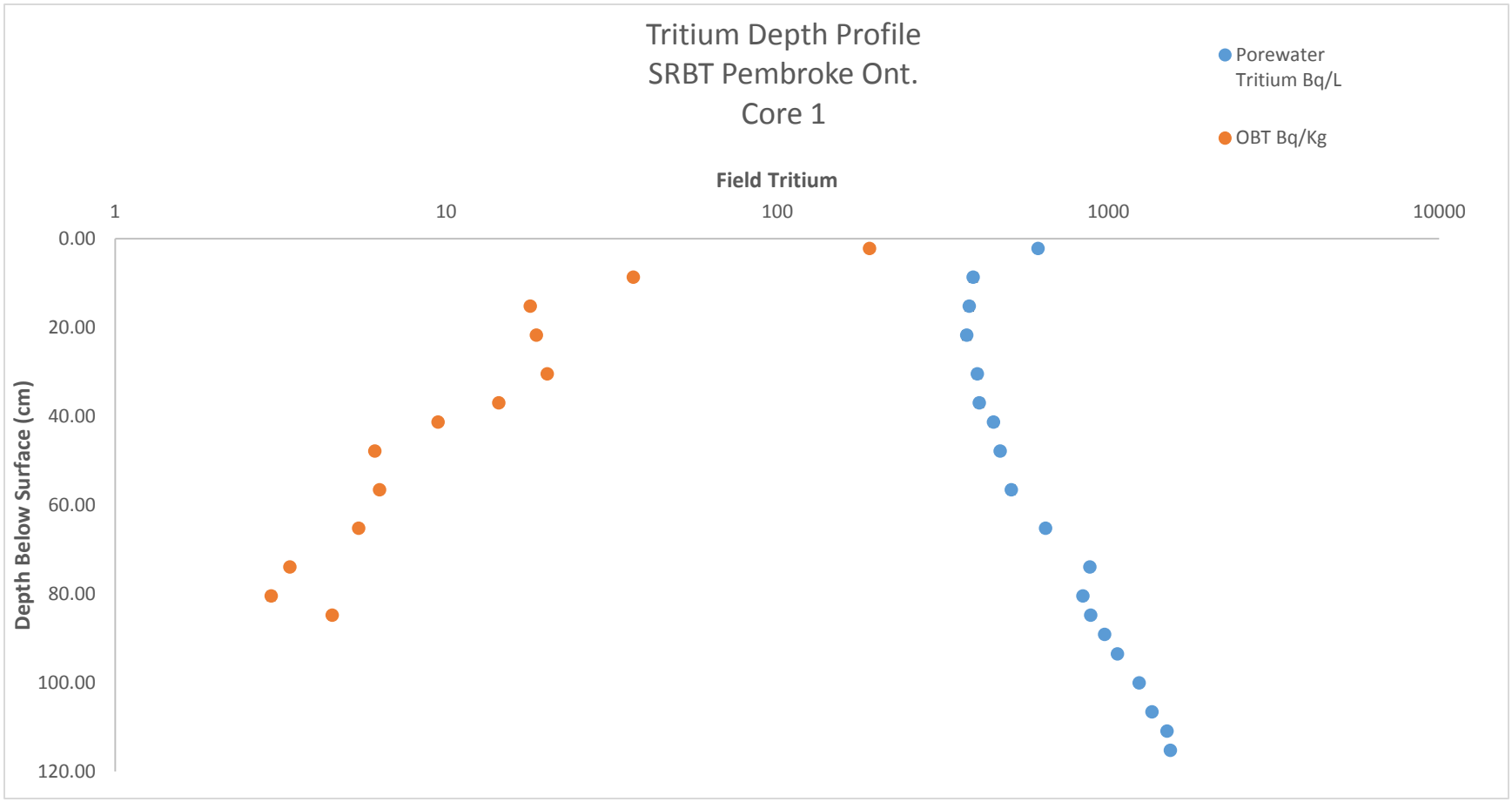


Figure 16: Pore water tritium and OBT concentrations with depth in SRBT Core 1. OBT is presented in Bq/kg while pore water tritium is presented in Bq/L. Note that a logarithmic scale was used to compare the two measurements.

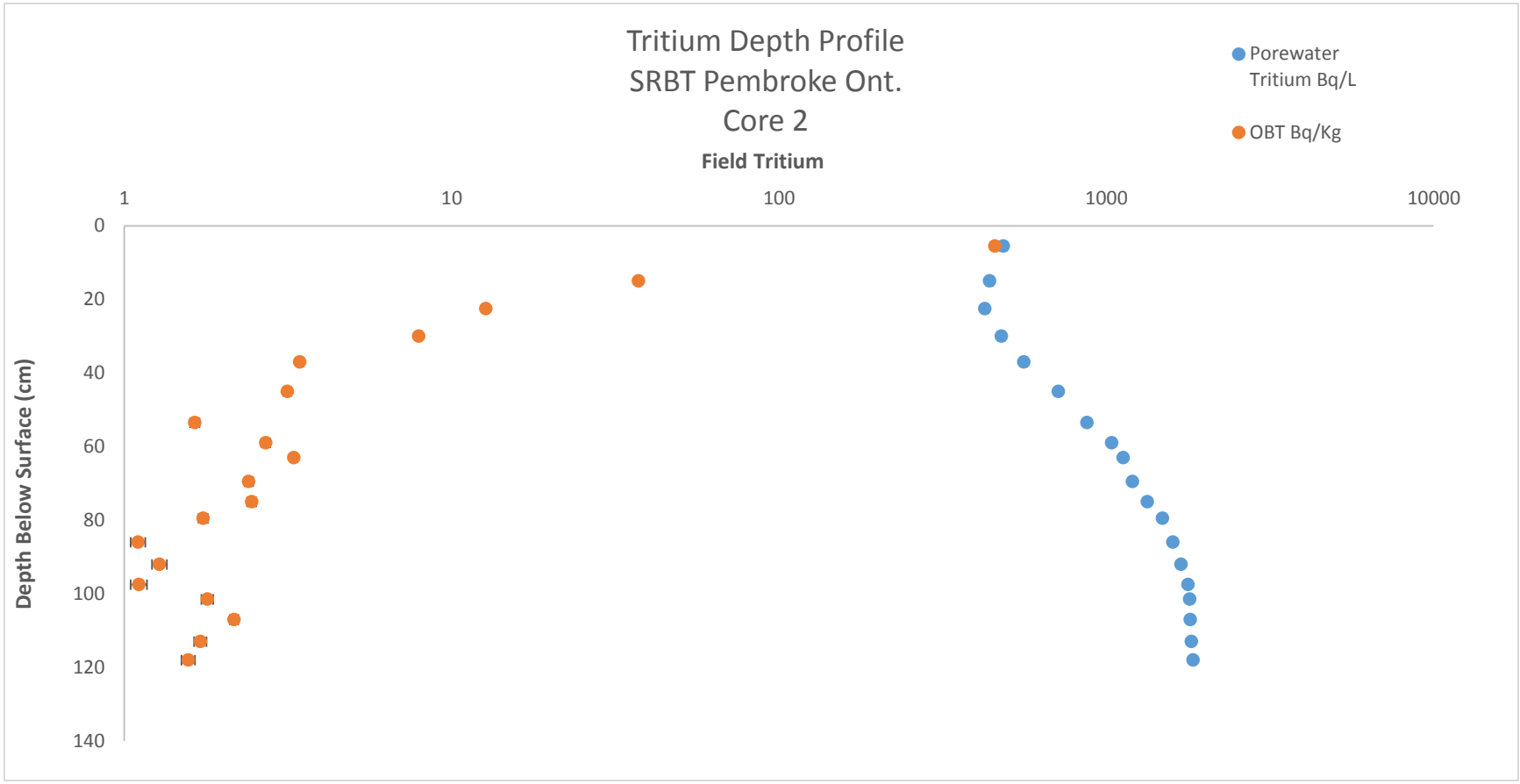


Figure 17: Pore water tritium and OBT concentrations with depth in SRBT Core 2. OBT is presented in Bq/kg while pore water tritium is presented in Bq/L. Note that a logarithmic scale was used to compare the two measurements.

While OBT levels are largely similar throughout the soil profiles in both cores, pore water tritium is consistently higher in SRBT Core 2 versus SRBT Core 1. A third background sediment core was augured in Russell Ontario, approximately 150 km south-west of Pembroke. The maximum depth of this core is 80 cm, on account of groundwater limiting the effectiveness of the auger and plug collection. However, the lithology is similar to the SRBT cores, being mostly clay and silt throughout the profile. The background core showed no remarkable tritium concentration in either the pore water or organically bound fractions. Maximum pore water tritium was 3.5 Bq/L at 35 cm depth, while maximum OBT concentrations were 0.4 Bq/kg at 11 cm (**Figure 18**). Loss on ignition measurements were carried out on SRBT Core 1 only, and indicate low organic matter content within the soils that decrease slightly with depth). The highest organic matter contents of 6% to 7% were within the organic rich soil layers of the top 30 cm. Below this threshold, the organic matter content ranged from 2% to 5%. Loss on ignition measurements were only taken up to a mid-point interval depth of 89 cm in SRBT Core 1.

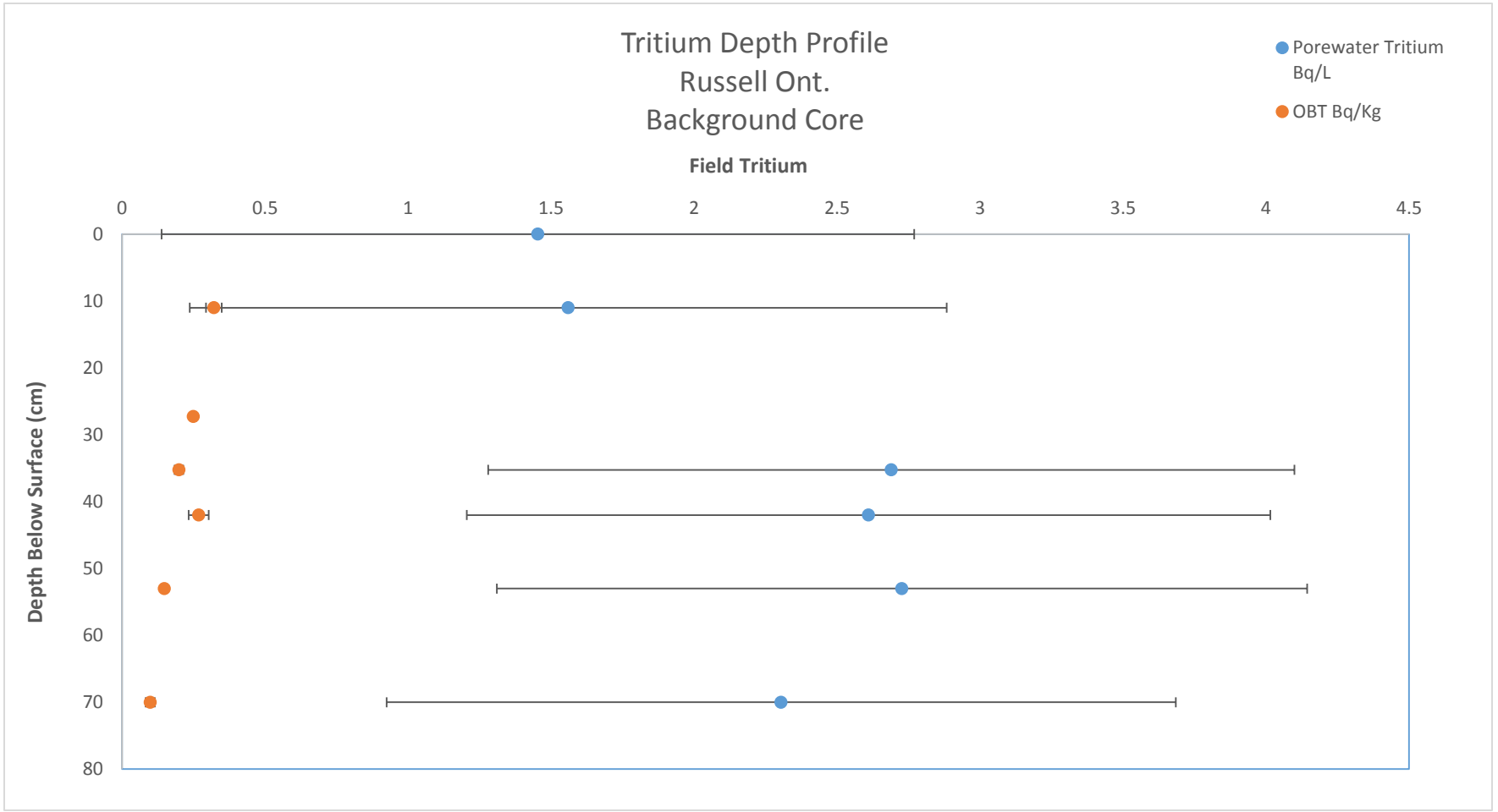


Figure 18: Pore water tritium and OBT concentrations with depth in the Background Core taken in Russell, Ontario. OBT is presented in Bq/kg while pore water tritium is presented in Bq/L. Note that a logarithmic scale was used to compare the two measurements.

11.2 Deuterium Exposure Experiments

In total, there were three deuterium exposure experiments (The Experimental Control, Trial 1, and Trial 2). Each experiment had an Environmental Control, being a potted arugula plant grown with the exposed plants, but removed from the growth chamber and left at room temperature and humidity for the duration of the experiment. Environmental Controls were used to observe differences in plant response due to changing environmental conditions. Relative humidity and temperature are different between the growth chamber and the office in which the Environmental Control was kept. The Experimental Control was a group of plants grown in the growth chamber, and sampled without exposure to deuterium to form a baseline for comparison purposes.

11.2.1 Experimental Control Light and Dark Experiments

Soil pore waters in the Experimental Control show no obvious deuterium enrichment over controls in both light and dark conditions (**Figure 19**). Starting Waters used to flush the soils have a ∂D of -69.2‰ and a ∂^{18}O of -10.76‰. The soil pore waters either show no deuterium enrichment, or show depletion over the starting water, particularly with respect to the dark experiments (**Table 4**). Note that measurements of the starting water for the dark experiments were unavailable, therefore the Starting Water from the Light Experiments was used. The Dark Experiment pore waters have the greatest range of values in both deuterium and oxygen-18, as well as the largest variation from both the assumed Starting Waters and Environmental Control. The Light Experiment soil pore waters, including the Light Experiment Control, deviated only slightly from the Starting Water, though one sample (-39.5‰) did show a +29.7‰ enrichment versus the Starting Water, and a +26.2‰ enrichment over the Environmental Control. The ^{18}O signature changed consistently by enrichment across Light and Dark Experiments, including the Environment Controls, by average enrichments of +5.05‰.

Table 4: Deuterium and oxygen-18 measurements of soil pore waters in the Experimental Control Light and Dark experiments.

Experimental Control Soil Pore Water					
Starting Water		**Light**		**Dark**	
δD (‰)	$\delta^{18}O$(‰)	δD (‰)	$\delta^{18}O$(‰)	δD (‰)	$\delta^{18}O$(‰)
-69.2	-10.6	-39.5	-4.9	-61.7	-4.37
		-58.8	-5.13	-65	-6.36
		-64.9	-6.2	-84.2	-6.43
				-90.3	-10.4
Environmental Control Soil		-65.7	-4.56	-47.5	-3.08

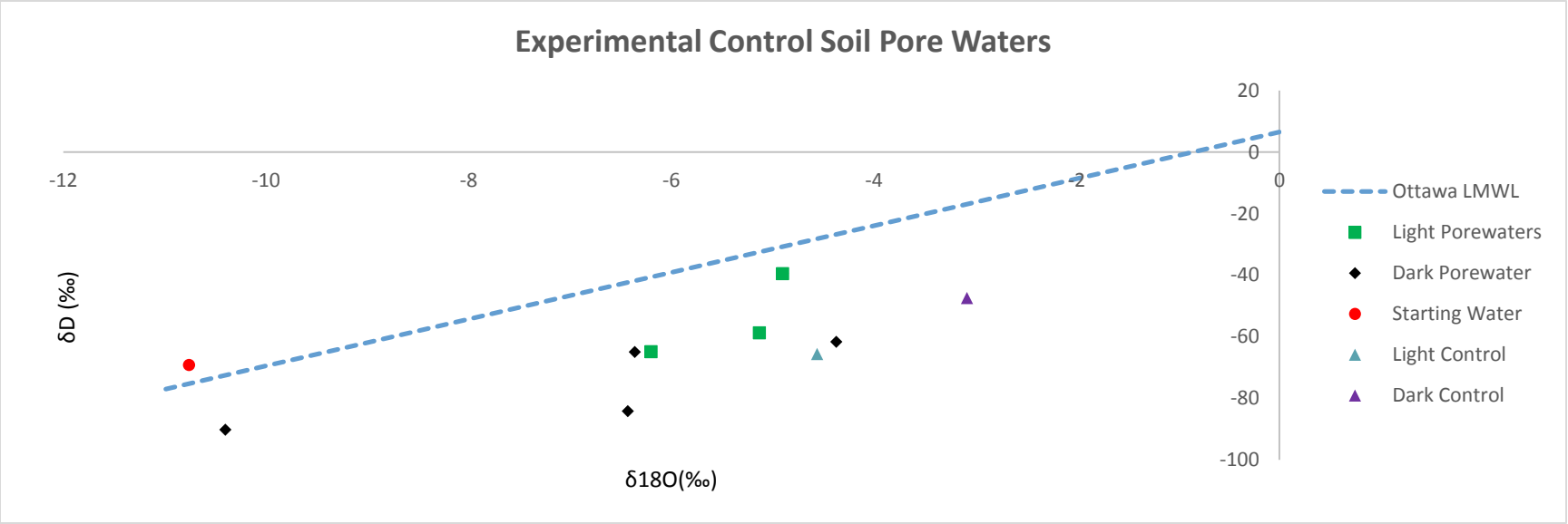


Figure 19: δD and $\delta^{18}O$ of soil pore waters in the Experimental Control run, plotted with the Ottawa LMWL described in Equation [6]. This experiment included two separate trials: potted plants in light, and potted plants in dark. The Light and Dark Controls are samples that were removed from the growth chamber during the experiment, though subjected to the same lighting conditions (Environmental Controls). Note that the Starting Water isotope measurements for the Dark Experiment were unavailable, therefore the data in this figure is compared to that of the Light Experiment Starting Water.

The Experimental Control plants were grown two to a pot. Some plants began bolting, growing a tall stem and flowering at the top. Within the soil, fine root hairs from each plant perforated the medium and appeared to interconnect. The roots consistently developed to the bottom of the pots in all samples. The stems and leaves showed no enrichment over Environment Controls in both the Light (**Figure 20**) and Dark (**Figure 21**) experiments, though both experiments did show progressive enrichment in both isotopes over the Starting Water. The leaves were more enriched in deuterium and ^{18}O versus the stems, which in turn, were more enriched than the soil pore waters. The range of δD and $\delta^{18}O$ values for stems and leaves in the Light Experiments are summarized in **Table 5** and

Table 6. Those of the Dark Experiments are presented in **Table 7** and **Table 8**.

Table 5: Range of δD (‰) in stems and leaves in the Light Experimental Control

Environmental Control (‰)		**Experimental Control δD (‰)**		
Stems	**Leaves**		**Stems**	**Leaves**
-21.1	15.1	Most Enriched	-33.6	8.6
		Most Depleted	-43.3	-23.4
		Average	**-30.26**	**-8.3**

Table 6: Range of **$\delta^{18}O$(‰)** in stems and leaves in the Light Experimental Control

Environmental Control (‰)		**Experimental Control $\delta^{18}O$(‰)**		
Stems	**Leaves**		**Stems**	**Leaves**
0.08	18	Most Enriched	0.87	8.77
		Most Depleted	-1.96	6.61
		Average	**-0.52**	**9.31**

Table 7: Range of δD (‰) in stems and leaves in the Dark Experimental Control

Environmental Control (‰)		**Experimental Control δD (‰)**		
Stems	**Leaves**		**Stems**	**Leaves**
-5.2	2.8	Most Enriched	-5.2	23.5
		Most Depleted	-51.8	-13.5
		Average	**-44.1**	**-2**

Table 8: Range of **$\delta^{18}O$(‰)** in stems and leaves in the Dark Experimental Control

Environmental Control (‰)		**Experimental Control $\delta^{18}O$(‰)**		
Stems	**Leaves**		**Stems**	**Leaves**
4.57	16.96	Most Enriched	3.81	20.16
		Most Depleted	-3.44	6.26
		Average	**-1.13**	**10.37**

The oxygen and hydrogen isotopes bound to the organic fraction of the plant material demonstrate that leaves were more enriched than stems in both δD and $\delta^{18}O$ (**Figure 22**). Consistent across Light and Dark was a depletion in deuterium, and an enrichment in ^{18}O relative to the tissue waters in **Figure 20** and **Figure 21**.

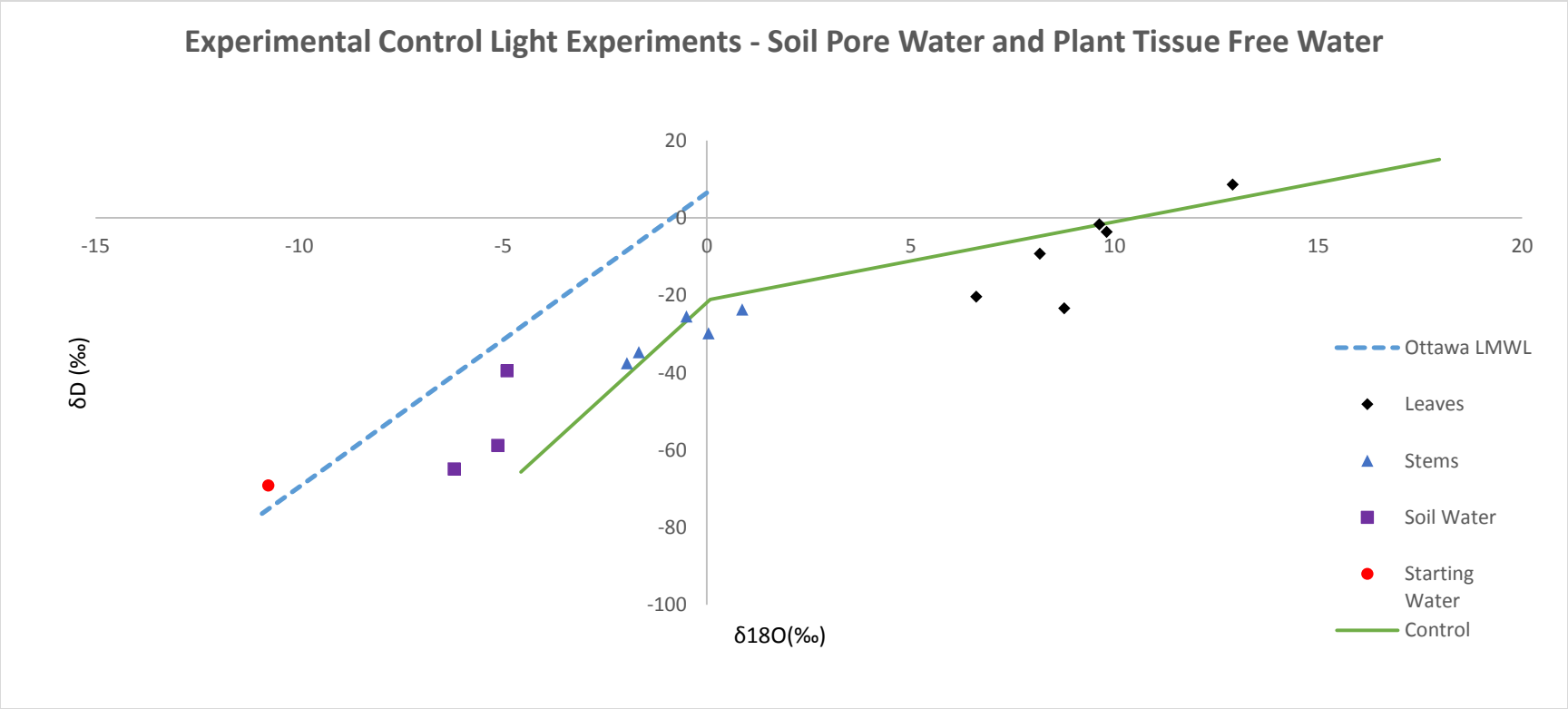


Figure 20: δD and $\delta^{18}O$ of soil pore waters and stem and leaf tissue free waters in the Light Experimental Control run, plotted with the Ottawa LMWL described in Equation [6]. The results are plotted with the soil, stem, and leaf measurements of the Environmental Control. Note that the Experimental Control results plot along the Environmental Control line (green).

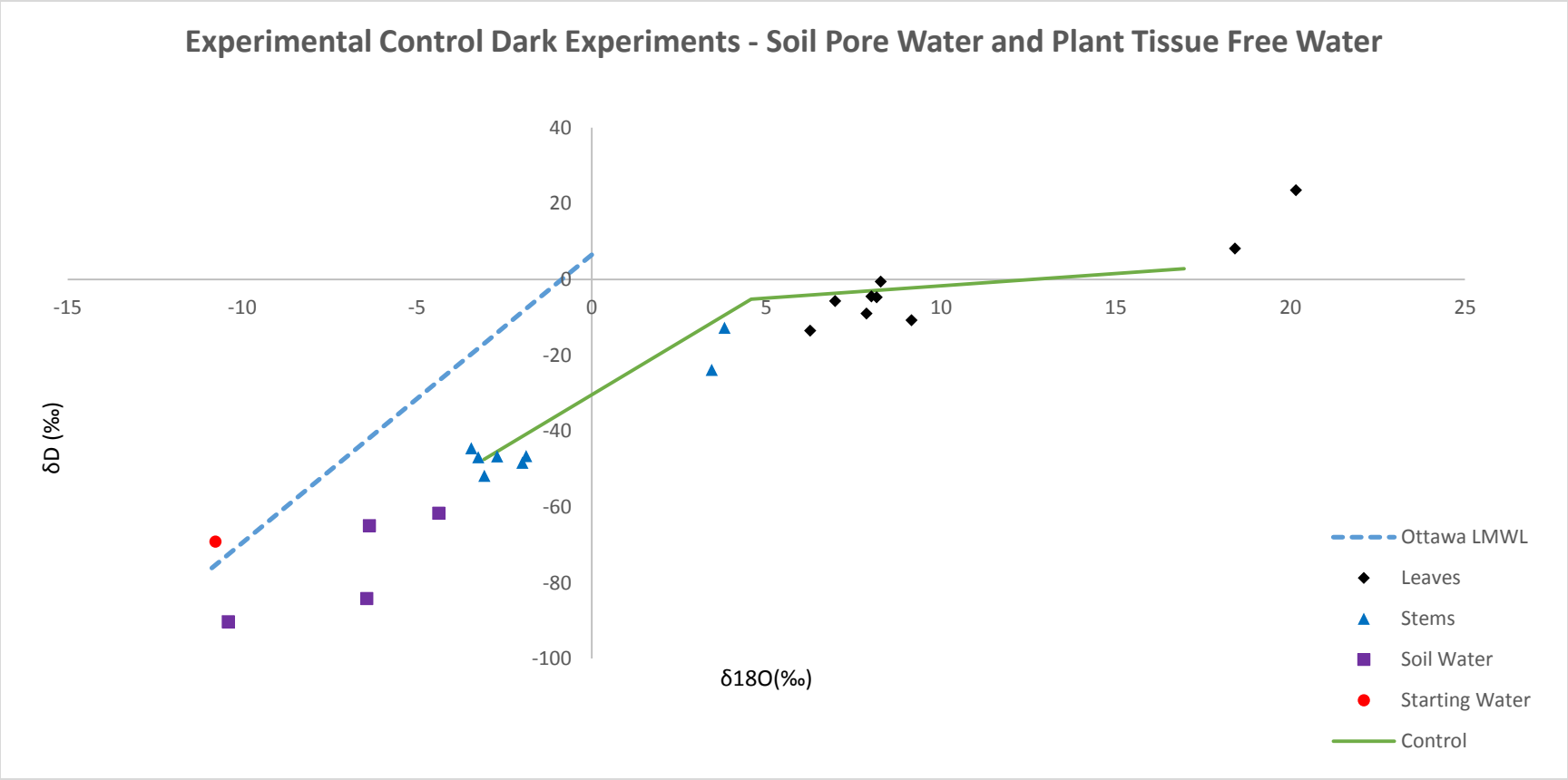


Figure 21: δD and $\delta^{18}O$ of soil pore waters and stem and leaf tissue free waters in the Dark Experimental Control run, plotted with the Ottawa LMWL described in Equation [6]. The results are plotted with the soil, stem, and leaf measurements of the Environmental Control. Note that the Experimental Control results plot along the Environmental Control line (green). Recall that there is no Starting Water data for the Dark Experimental Control experiment, therefore, that of the Light Experimental Control is plotted.

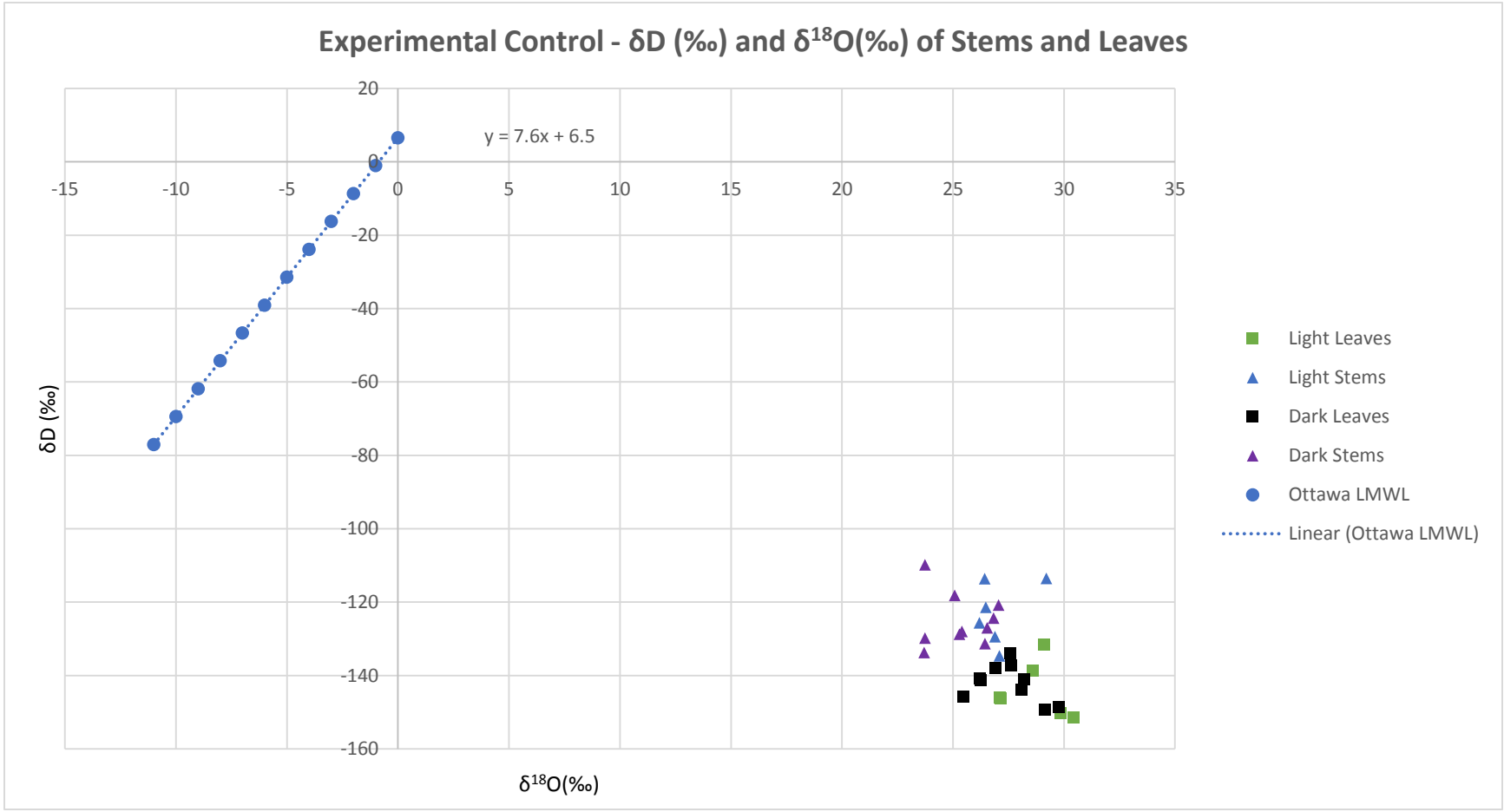


Figure 22: δD (‰) and $\delta^{18}O$(‰) of the stem and leaf organics in the Light and Dark Experimental Control trials, plotted with the Ottawa LMWL described in Equation [6]. Note that the stems and leaves are depleted in deuterium, but enriched in oxygen. There was no observable difference between the Environmental Controls and the samples presented here.

11.2.2 Trial 1 – 24 hours in light and 24 hours in dark

The potted arugula plants, grown four to a pot, displayed some leafy growth but did not gain much height. Some plants in Trial 1 began bolting, growing a tall stem and flowering at the top. Within the soil, fine root hairs from each plant perforated the medium and appeared to interconnect with each other plant. The roots consistently developed to the bottom of the pots, thereby providing access to water throughout the entire soil volume.

As in the Experimental Control, the majority of samples produced sufficient water volume for isotope analysis (requiring only 0.2 mL of water). The same applies to the isotopic measurement of the plant organics, having low sample mass after ball milling, but enough for measurement (a minimum 0.3 to 0.4 mg).

The soil pore water data in Trial 1 is summarized for Light and Dark in **Table 9**. Soil pore waters in the Trial 1 Light Experiments demonstrated deuterium enrichment over the Environmental Control soil, as well as the Trial 1 Light Starting Water used to water the plants(**Figure 23**).The Starting Water had a δD of -66.9‰ and a $\delta^{18}O$ of -7.5‰. The Trial 1 Environmental Control soils actually became isotopically lighter, depleting by -21.1‰ with respect to deuterium, and by -0.72‰ in $\delta^{18}O$. Extracted soil pore waters had δD values ranging from -39.6‰ to -64.2‰ (**Table 10**). This accounts for δD enrichments of +48.4‰ to +23.8‰ over the Environmental Control soil, and enrichments of +2.7‰ to +27.3‰ over the Starting Waters. Changes in pore water $\delta^{18}O$ were relatively minor between exposed plants, the Environmental Control, and Starting Water (**Table 10**).

The Trial 1 Dark Experiments showed less deuterium enrichment versus the Trial 1 Light Experiments, as the Starting Water had a δD of -68.2‰, while the soil pore water δD values ranged from -62.6‰ to -71.7‰, a deviation of only +5.6‰ to -3.8‰ (**Figure 23**). Analysis of pore water $\delta^{18}O$ showed

a maximum deviation of +1.5‰ from the Starting Water, similar to the Trial 1 Light Experiments. Further, the Dark Environmental Control soil selected against deuterium from the Starting Water, with a δD value of -76.9‰ (**Table 11**). $\delta^{18}O$ for the Dark Environmental Control Soil was -6.41‰ while the exposed soils had $\delta^{18}O$ values between -7.07‰ to -5.84‰ (average -6.5‰). Maximum and minimum deuterium enrichment of the exposed soils over the control was +5.2‰ and +14.3‰, respectively, with an average deuterium measurement of -67.1‰ (**Table 11**).

Table 9: Deuterium and oxygen-18 measurements of soil pore waters in the Trial 1 Light and Dark experiments

Trial 1 Soil Pore Water							
Starting Water (Light)		**Starting Water (Dark)**		**Light**		**Dark**	
δD (‰)	$\delta^{18}O$(‰)	δD (‰)	$\delta^{18}O$(‰)	δD (‰)	$\delta^{18}O$(‰)	δD (‰)	$\delta^{18}O$(‰)
-66.9	-7.5	-68.2	-7.34	-39.6	-6.3	-69.9	-7.07
				-47.1	-6.68	-62.6	-5.84
				-51.5	-7.28	-71.7	-6.81
				-64.1	-8.84	-64.3	-6.1
				-64.2	-8.92		
Environmental Control Soil				-88	-8.22	-64.3	-6.1

Table 10: Maximum and minimum δD and $\delta^{18}O$ enrichments over Environmental Controls and Starting Water, Trial 1 Light soil pore waters

Trial 1 Light Enrichment	**Minimum δD (‰)**	**Maximum δD (‰)**	**Minimum $\delta^{18}O$ (‰)**	**Maximum $\delta^{18}O$ (‰)**
Over Environmental Control	+23.8	+48.4	-0.7	+1.92
Over Starting Water	+2.7	+27.3	-1.42	+1.2

Table 11: Maximum and minimum δD and $\delta^{18}O$ enrichments over Environmental Controls and Starting Water, Trial 1 Dark soil pore waters

Trial 1 Dark Enrichment	Minimum δD (‰)	Maximum δD (‰)	Minimum $\delta^{18}O$ (‰)	Maximum $\delta^{18}O$ (‰)
Over Environmental Control	+5.2	+14.3	-0.67	+0.57
Over Starting Water	-3.8	+5.6	+0.27	+1.5

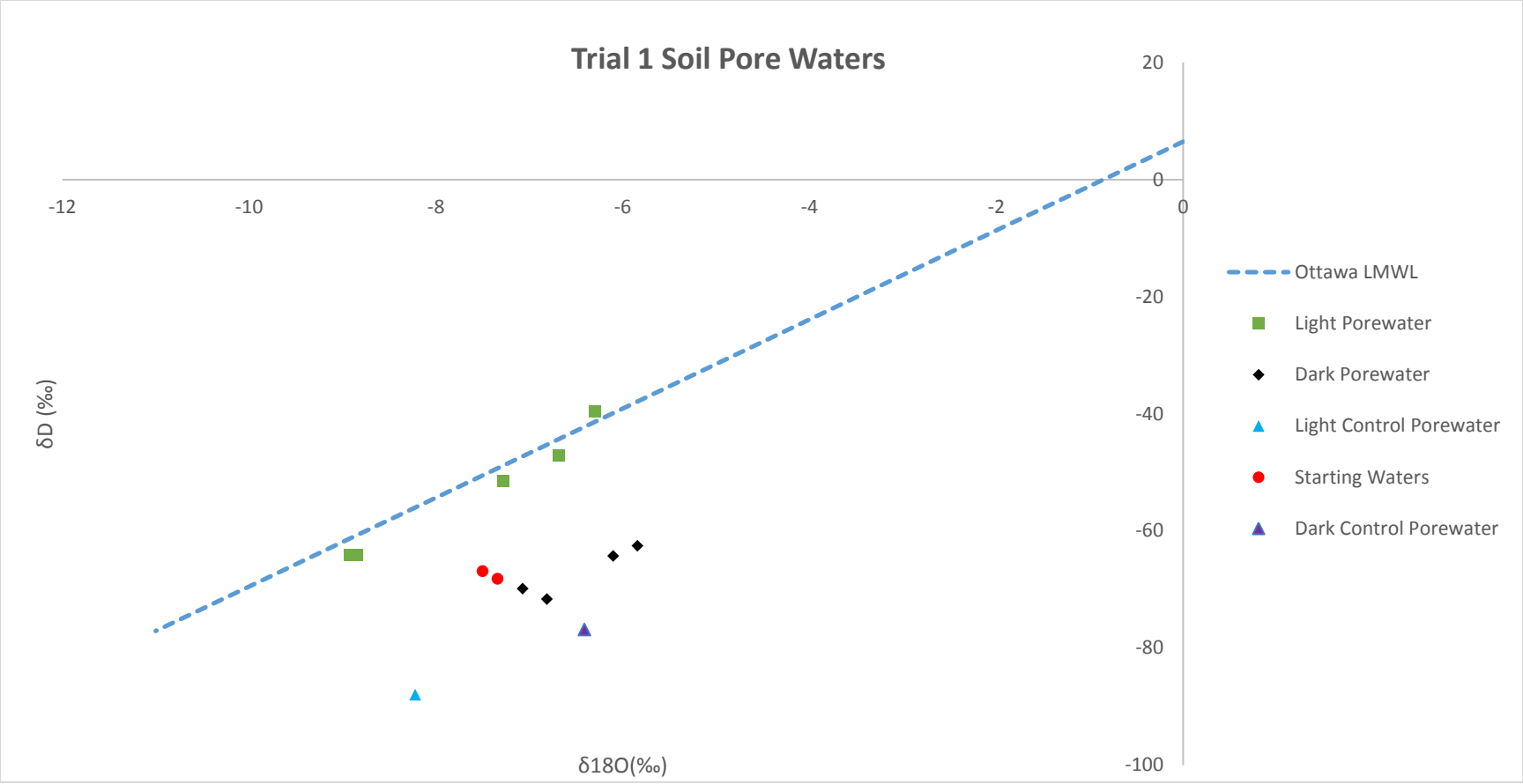


Figure 23: δD and $\delta^{18}O$ of soil pore waters in Trial 1, plotted with the Ottawa LMWL described in Equation [6]. This experiment included two separate trials: potted plants in light, and potted plants in dark. The Light and Dark Environmental Controls are samples that were removed from the growth chamber during the experiment, though subjected to the same lighting conditions.

Overall, both Light and Dark Experiments in Trial 1 appeared to show deuterium enrichment over the Trial 1 Environmental Controls in soils, stems, and leaves, though the level of enrichment in the Dark Experiments appeared minor relative to the light. In comparing the separate sample types, stems were enriched in δD and $\delta^{18}O$ over soils, while leaves were enriched over stems and soils, similarly to the Experimental Control.

Stem and leaf plant tissue waters in the Trial 1 Light Experiments showed enrichment over the stems and leaves of the Light Environmental Control (**Figure 24**). Stem tissue water in the Environmental Control had a δD of -49‰ and a $\delta^{18}O$ of -0.03‰. The average δD of the exposed stems was -18.08‰, with a maximally enriched value of -9.5‰ and a minimally enriched value of -23.3‰ (**Table 12**). Stem $\delta^{18}O$ values of the exposed plants ranged from -2.82‰ to 1.43‰, averaging at -0.75‰ (**Table 13**). The exposed leaves showed some deuterium enrichment over the Environmental Control Leaves, with δD values of -7.0‰ to 10.9‰ (average of 0.14‰). Leaf $\delta^{18}O$ ranged from 8.21‰ to 12.25‰, averaging at 10.10‰. The Environmental Control Leaves do have a higher $\delta^{18}O$ (17.57‰) compared with the exposed leaves, whose highest oxygen isotope measurement was 12.25‰.

Table 12: Range of δD (‰) in stems and leaves in the Trial 1 Light Experiment

Environmental Control δD(‰)		**Exposed to Deuterium (δD(‰))**		
Stems	Leaves		Stems	Leaves
-49	-2.7	Most Enriched	-9.5	-7
		Most Depleted	-23.3	10.9
		Average	**-18.08**	**0.14**

Table 13: Range of **$\delta^{18}O$(‰)** in stems and leaves in the Trial 1 Light Experiment

Environmental Control δD(‰)		**Trial 1 $\delta^{18}O$(‰)**		
Stems	Leaves		Stems	Leaves
-0.3	17.57	Most Enriched	1.43	12.25
		Most Depleted	-2.82	8.21
		Average	**-0.75**	**10.12**

In the Trial 1 Dark Experiments, the same trends were observed as in the Trial 1 Light Experiments; however, both stems and leaves had a consistently lower enrichment over the Environmental Controls in terms of tissue water (**Figure 25**). The stems had an average δD of -38.3‰ and an average $\delta^{18}O$ of -1.24‰ (**Table 14** and **Table 15**). The δD values of the Dark exposed stems ranged from -43.3‰ to -33.6‰, with a Dark Environmental Control stem value of -38.5‰. $\delta^{18}O$ of the Dark exposed stems was -2.18‰ to 0.49‰, while the Dark Environmental Control stem was slightly more enriched at 1.56‰. Exposed leaves of the Trial 1 Dark Experiments had an average δD of -15.23‰ and an average $\delta^{18}O$ of 22.84‰. The deuterium values of the exposed leaves ranged from -17.3‰ to -13.5‰ versus the Dark Environmental Control leaf, which had a δD value of 0.3‰. The Dark exposed leaves had similar $\delta^{18}O$ values, ranging from 7.31‰ to 7.91‰, while the Dark Environmental Control leaves were considerably more enriched at 17.2‰.

Table 14: Range of δD (‰) in stems and leaves in the Trial 1 Dark Experiment

Environmental Control δD(‰)		**Exposed to Deuterium (δD(‰))**		
Stems	Leaves		Stems	Leaves
-38.5	0.3	Most Enriched	-33.6	-13.5
		Most Depleted	-43.3	-17.3
		Average	**-38.3**	**-15.23**

Table 15: Range of **$\delta^{18}O$(‰)** in stems and leaves in the Trial 1 Dark Experiment

Environmental Control (‰)		**Trial 1 $\delta^{18}O$(‰)**		
Stems	Leaves		Stems	Leaves
1.56	17.2	Most Enriched	1.56	7.91
		Most Depleted	-2.18	7.31
		Average	**-1.03**	**7.61**

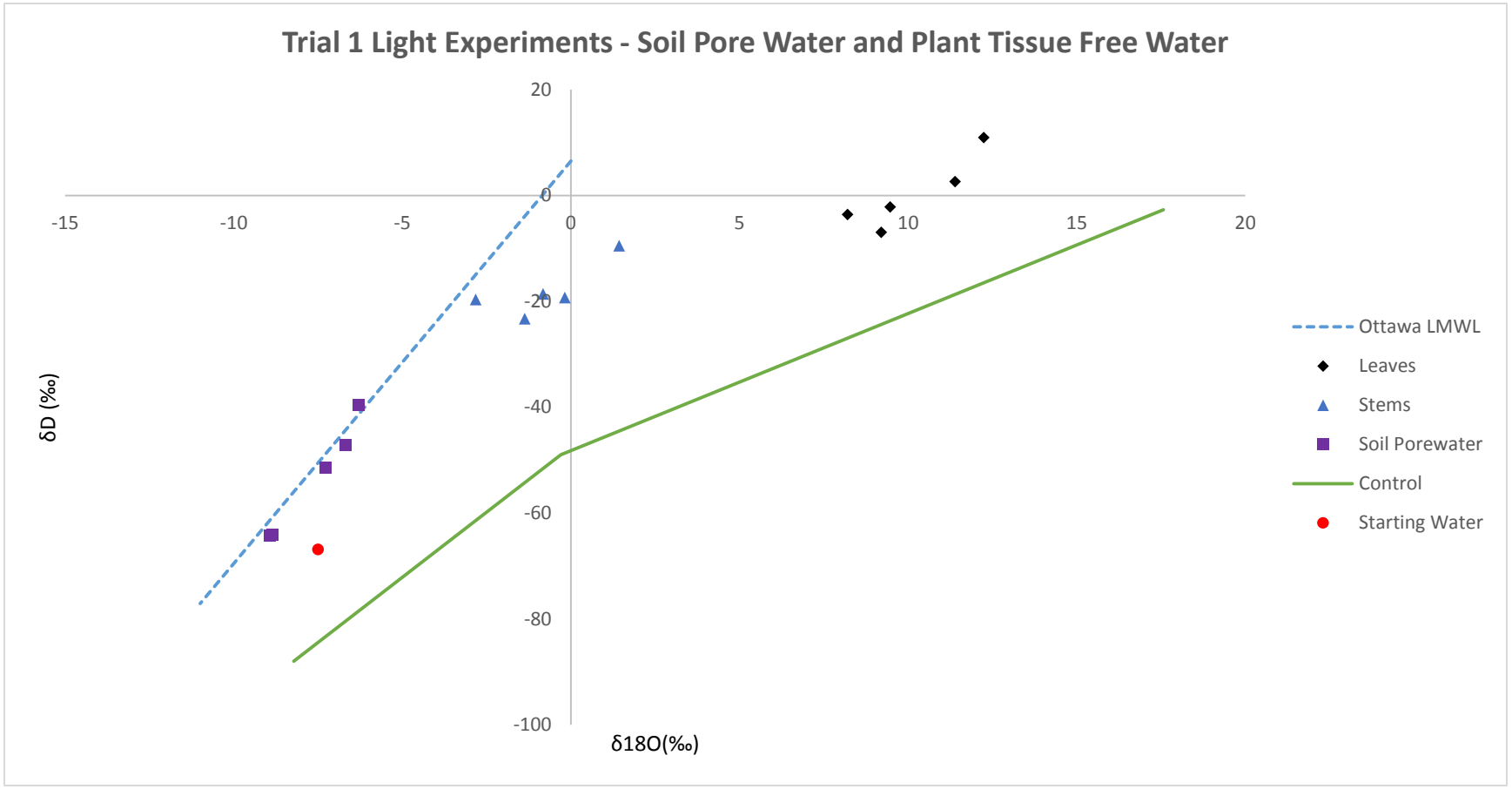


Figure 24: δD and $\delta^{18}O$ of soil pore waters and stem and leaf tissue free waters in the Trial 1 Light Experiment, plotted with the Ottawa LMWL described in Equation [6]. The results are plotted with the soil, stem, and leaf measurements of the Environmental Control (green line). Note that the soils appear to show deuterium enrichment over both the Starting Water and the Environmental Control.

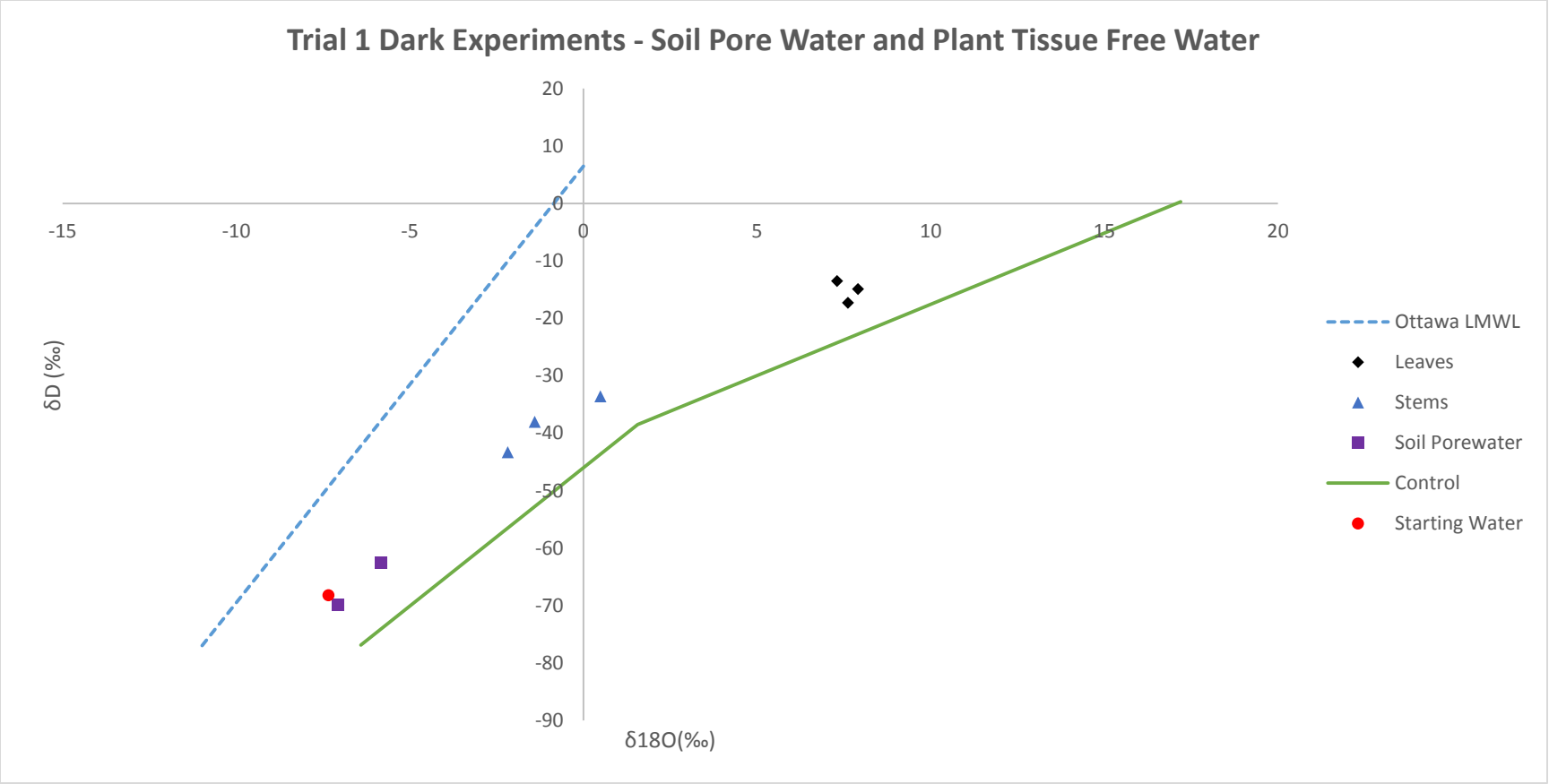


Figure 25: δD and $\delta^{18}O$ of soil pore waters and stem and leaf tissue free waters in the Trial 1 Dark Experiment, plotted with the Ottawa LMWL described in Equation [6]. The results are plotted with the soil, stem, and leaf measurements of the Environmental Control (green line). Note that the exposed soils do not appear to show any deuterium enrichment over the Starting Water.

The results of the Trial 1 organics isotope measurements are presented in **Figure 26**. Measurement of the hydrogen and oxygen isotopes of the Trial 1 plant organics (both Light and Dark Experiments) demonstrated no discernable enrichment between Environmental Controls and exposed plants. On the contrary, exposed plants were slightly depleted in deuterium and oxygen relative to the Environmental Controls. The Light Environmental Control leaves and stems measured a δD value of -143.76‰ and -123.46‰ respectively, while the average measurement of the Light exposed leaves and stems was -154.56‰ and -130.09‰. $\delta^{18}O$ values remained consistent between Environmental Control stems and leaves, showing only minor variations. The Environmental Control leaves had a $\delta^{18}O$ value of 25.58‰, and the exposed leaves averaged 25.93‰. Exposed stems showed an average $\delta^{18}O$ value of 24.46‰ versus the Environmental Control stem value of 24.82‰ (**Table 16**).

Table 16: δD (‰) in stem and leaf tissue in the Trial 1 Light Experiments

Control δD(‰)		**Exposed to Deuterium (δD(‰))**		
Stems	Leaves		Stems	Leaves
-123.46	-143.76	Most Enriched	-128.29	-150.67
		Most Depleted	-131.39	-158.93
		Average	**-130.09**	**-154.56**

The Trial 1 Dark Experiment plant tissue had similar measurements to the Trial 1 Light Experiments, showing some deuterium depletion in the stems and leaves over the Dark Environmental Controls, or otherwise showing little deviation (**Table 17**). The Dark Environmental Control stem had a δD value of -127.39‰ and a $\delta^{18}O$ value of 24.47‰. Dark exposed stems averaged -127.60‰ for δD and 24.51‰ for $\delta^{18}O$. Dark Environmental Control leaves demonstrated a deuterium signature of -145.96‰ and an oxygen-18 value of 26.43‰, which are both similar to the Dark exposed leaf average of -

155.33‰ and 25.32‰ for deuterium and $\delta^{18}O$ respectively, although there is a depletion of -9.37‰ between the Dark Environmental Control leaves and the average of the exposed leaves.

Table 17: δD (‰) in stem and leaf tissue in the Trial 1 Dark Experiments

Control δD(‰)		**Exposed to Deuterium (δD(‰))**		
Stems	Leaves		Stems	Leaves
-127.39	-145.96	Most Enriched	-123.18	-151.36
		Most Depleted	-133.36	-162.04
		Average	**-127.60**	**-155.33**

The data further indicates that the leaves of both Light and Dark Experiments in Trial 1 are consistently more depleted with respect to deuterium in the organic fraction relative to the stems. The average difference between the tissues of the stems and leaves of both Dark and Light Experiments (including Environmental Controls) was -24.44‰ (**Table 18**).

Table 18: Comparison of δD (‰) between stem and leaf plant organic tissue - Trial 1 Light and Dark Experiments

Trial 1 Deuterium Measurements (δD (‰))			
Sample	**Stems (‰)**	**Leaves (‰)**	**Difference (‰)**
PO LC	-123.46	-143.76	**-20.3**
PO L-P1	-131.39	-158.93	**-27.54**
PO L-P2	-130.98	-158.24	**-27.26**
PO L-P3	-129.43	-150.75	**-21.32**
PO L-P4	-130.37	-150.67	**-20.3**
PO L-P5	-128.29	-154.2	**-25.91**
PO DC	-127.39	-145.96	**-18.57**
PO D-P1	-133.36	-162.04	**-28.68**
PO D-P2	-123.18	-151.36	**-28.18**
PO DW	-126.25	-152.58	**-26.33**
		AVERAGE	**-24.439**

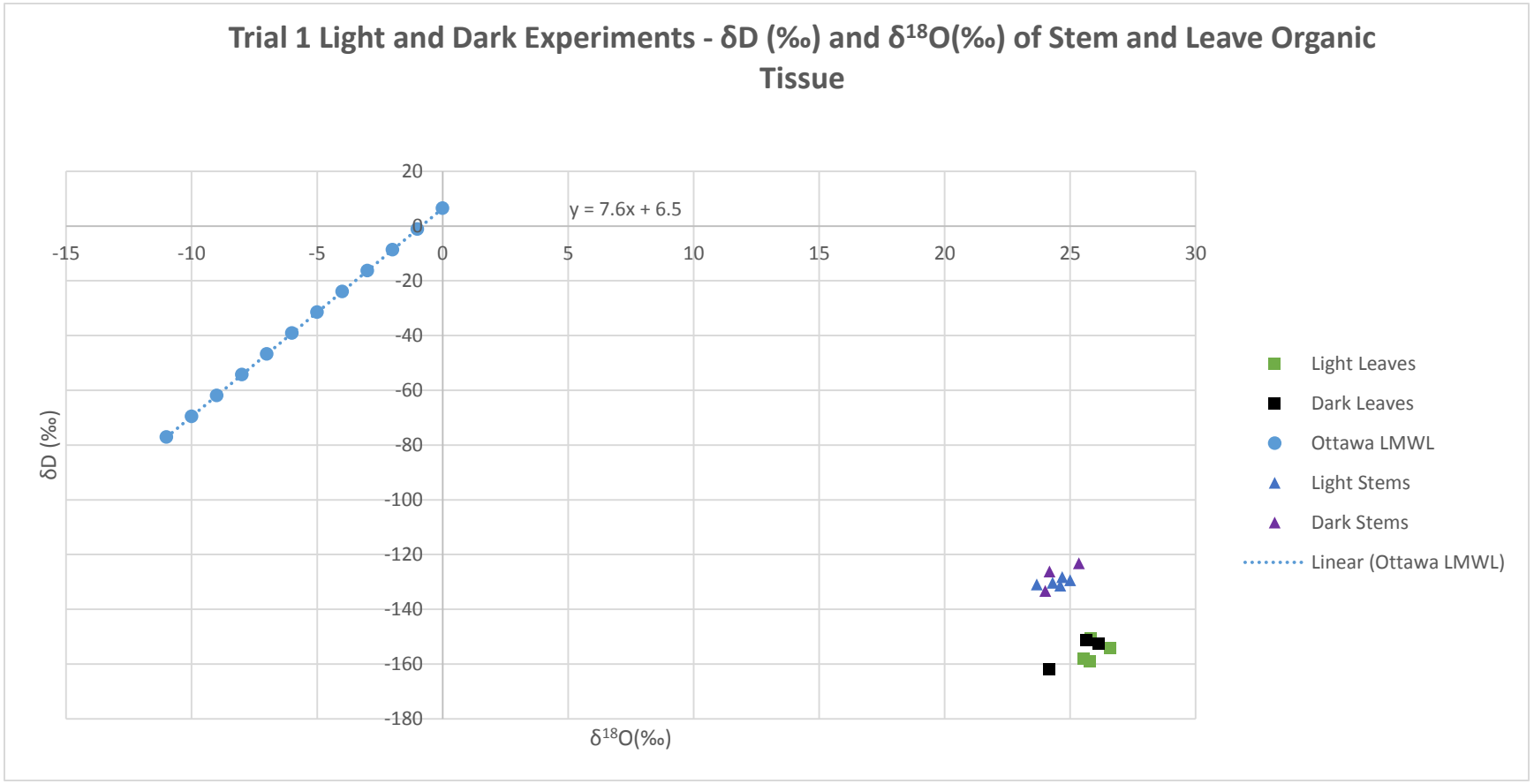


Figure 26: δD (‰) and $\delta^{18}O$(‰) of the stem and leaf organics in the Trial 1 Light and Dark runs, plotted with the Ottawa LMWL described in Equation [6]. Note that the stems and leaves are depleted in deuterium, but enriched in oxygen. There was no observable difference between the Environmental Controls and the samples presented here.

11.2.3 Trial 2 – 36 Hour Light Exposure and Silicone Caulking Soil Isolation Experiment

Air samples taken within the growth chamber during Trial 2 show the HD signal progressively increasing throughout the exposure period (**Figure 27**). The HD signal changes from -41.2‰ at 4:40 PM to -7.00‰ at 6:30 PM the following day, although a baseline measurement in the chamber was not taken prior to exposure. A deuterium peak (90.9‰) in the HD signal occurs at 8:45 AM, though the 90.9‰ peak was during a longer time-interval of approximately 11 hours, versus the other measurements, which were typically five hours, with the exception of the first measurement. The deuterium signals of the air sampler HD water vial are enriched from the Starting Water used in the vials, which has a δD of -82.65‰ (**Table 19**). The deuterium signal of the atmospheric water vapor (HDO) remains relatively unchanged and depleted relative to the HD, and deviates only slightly from the Starting Water signal (

Table **20**). The maximum deviation in HDO (+11.85‰) occurs over the same longer time interval of 10:00 PM to 8:45 AM the following day. Note that the values are not corrected for the amount of air moving through the bubblers in this Trial.

Table 19: δD (‰) and δ18O (‰) isotope deviations from Starting Waters in air sample HD - Trial 2

Trial 2 - Deuterium and Oxygen Isotopes of the Growth Chamber Air (HD)				
Time	**δD (‰)**	**Deviation From Starting Water (δD ‰)**	**$\delta^{18}O$ (‰)**	**Deviation From Starting Water ($\delta^{18}O$ ‰)**
4:40 PM	-41.2	41.45	-9.73	1.16
10:00 PM	-30.3	52.35	-9.74	1.15
8:45 AM	90.9	173.55	-8.89	2
1:30 PM	-8.5	74.15	-9.45	1.44
6:30 PM	-7	75.65	-9.38	1.51
Tap Water	**-82.65**		**-10.89**	

Table 20: δD (‰) and δ18O (‰) deviations from Starting Water in air sample HDO - Trial 2

Deuterium and Oxygen Isotopes of the Growth Chamber Air (HDO)				
Time	**Delta D (‰)**	**Deviation From Starting Water (δD ‰)**	**Delta 18O (‰)**	**Deviation From Starting Water (δ18O ‰)**
4:40 PM	-76.4	6.25	-9.94	0.95
10:00 PM	-77.3	5.35	-9.9	0.99
8:45 AM	-70.8	11.85	-9.49	1.4
1:30 PM	-76.4	6.25	-9.69	1.2
6:30 PM	-76.5	6.15	-10.32	0.57
Tap Water	**-82.65**		**-10.89**	

The $\delta^{18}O$ remains almost unchanged from the Starting Water in both the HD and HDO samples as well, and does not exceed an enrichment of +2‰. The Starting Water showed a $\delta^{18}O$ of -10.89‰, while the greatest deviation (+2.00‰) occurred in the HD sample that peaked in deuterium during the 8:45 AM interval.

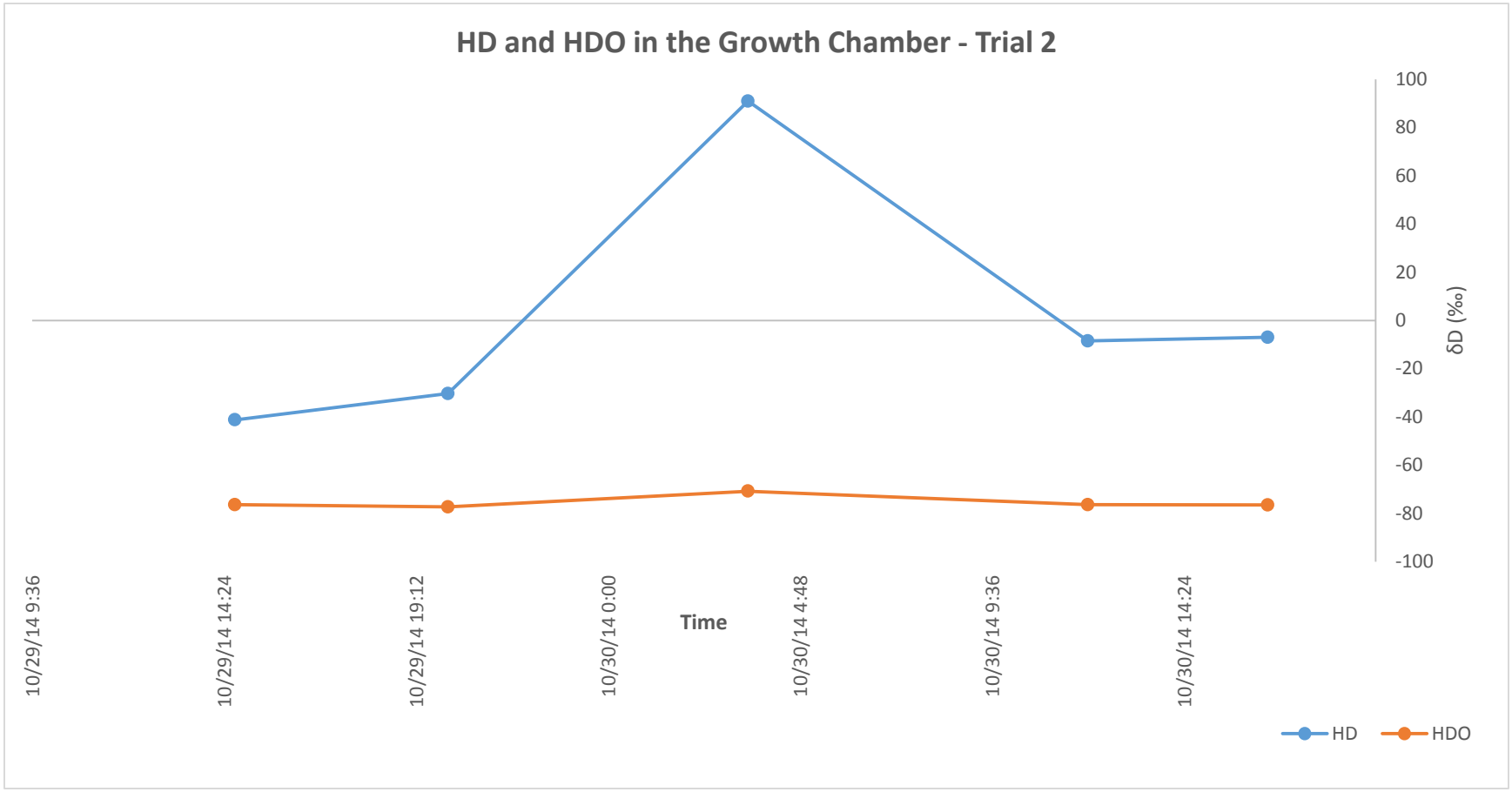


Figure 27: HD and HDO measurements taken with the active air sampler in the growth chamber during Trial 2. The measurements presented are uncorrected for the air volume moving through the bubblers, however the intent is to demonstrate the relative deuterium signatures of the HDO versus HD. The HD peak at 8:45 AM is the result of the longer sample time interval, as more HD is pushed through the bubblers.

The Starting Water used to flush the plant soils had a δD value of -80.9‰ and a $\delta^{18}O$ value of -11.2‰. Soil water samples were taken for isotopic measurement, however, at the time of writing the results remain unavailable.

Generally, the Trial 2 stem and leaf waters show the same trends as observed in the Experimental Control and Trial 1, with leaves being more enriched than the stems, and both being more enriched than the Starting Water with respect to deuterium and oxygen-18 (**Figure 28**). Both the stems and leaves are more enriched in deuterium versus the Environmental Control stems and leaves (**Table 21**). The average deuterium measurement of the exposed stems is -28.6‰, a +27.24‰ enrichment in deuterium over the Environmental Control stems, and a +52.34‰ enrichment over the Starting Water. Similarly, the exposed leaves show a +30.57‰ and +73.67‰ enrichment over the Environmental Control leaves and Starting Water respectively. Oxygen-18 measurements were consistent with the Experimental Control and Trial 1 (**Table 22**).

Table 21: δD(‰)in stem and leaf tissue free water – Trial 2

Environmental Control δD (‰)		**Exposed to Deuterium (δD (‰))**		
Stems	Leaves		Stems	Leaves
-55.8	-37.8	Most Enriched	-28.8	-2.6
		Most Depleted	-32.1	-15.1
		Average	**-28.56**	**-7.23**

Table 22: $\delta^{18}O$(‰)in stem and leaf tissue free water – Trial 2

Environmental Control $\delta^{18}O$ (‰)		**Exposed to Deuterium ($\delta^{18}O$ (‰))**		
Stems	Leaves		Stems	Leaves
-3.60	12.03	Most Enriched	-0.52	9.6
		Most Depleted	-2.29	7.52
		Average	**-1.49**	**8.54**

Shortly after sealing the soil surface with the silicone, the plants began to yellow and wilt. As a consequence, no water could be extracted from those leaves, however there was sufficient quantity in the stems. Two of the stems showed were depleted in deuterium relative to the Environmental Control, though slightly enriched versus the Starting Water, having δD values of -55.2‰, -63.9‰, and -73.0‰. Oxygen-18 measurements for the silicone test stems were -2.52‰, -2.59‰, and -3.87‰ respectively.

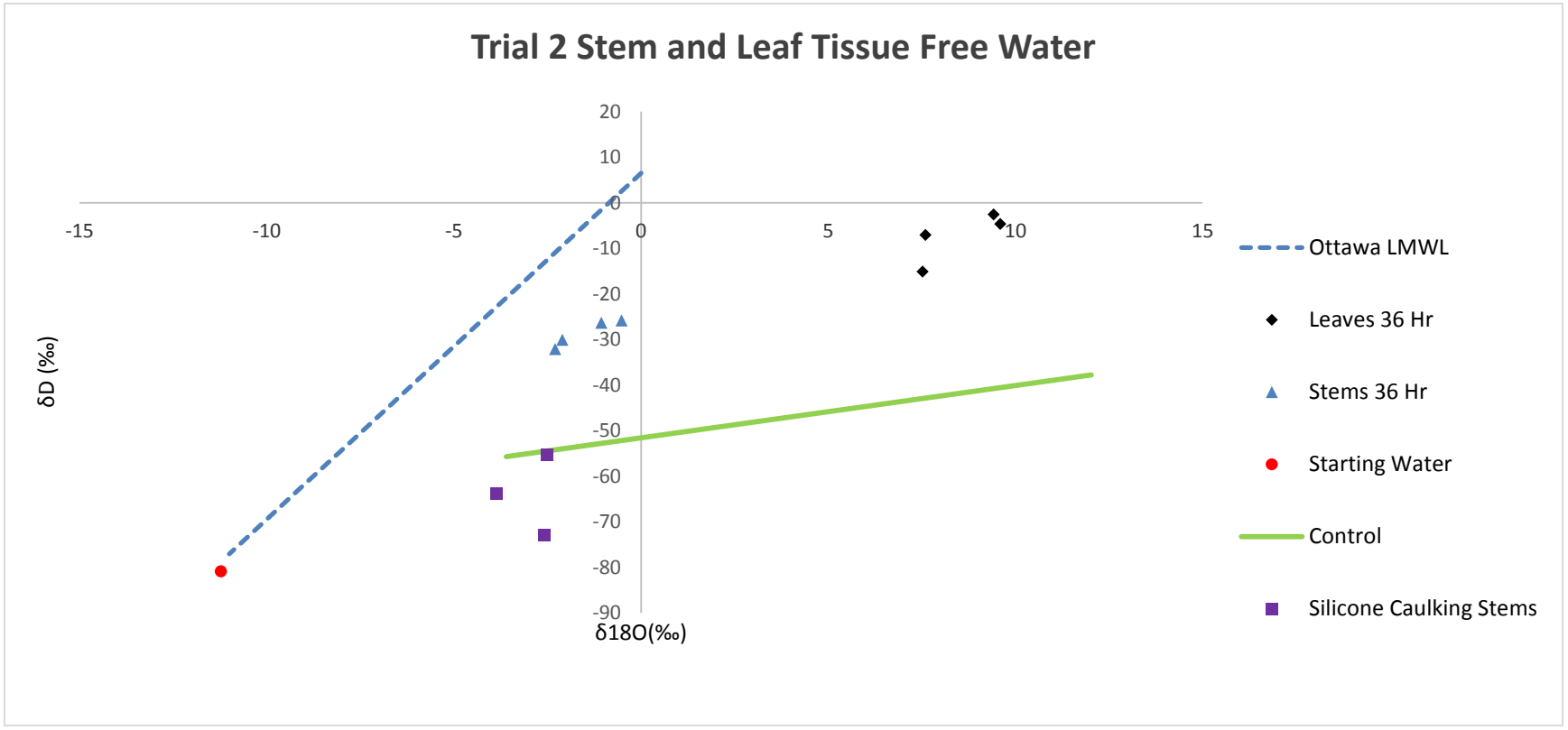


Figure 28: δD and $\delta^{18}O$ of stem and leaf tissue free waters in the Trial 2 run, plotted with the Ottawa LMWL described in Equation [6]. The results are plotted with the stem and leaf measurements of the Environmental Control (green line). Note the stems from the silicone caulking soil isolation samples.

Exposed leaf and stem organics in Trial 2 showed depletion in deuterium from the tissue water, though there is no clear variation from the isotopic signature of the organics of the Environmental Control leaves and stems (**Table 23**). The leaves are more depleted with respect to deuterium than the stems in both the Environmental Control and exposed plants. There was little change in the oxygen-18 signature between the exposed and Environmental Control plants, with an average $\delta^{18}O$ of 26.45‰ for the exposed stems and 28.68‰ for the exposed leaves. The Environmental Control stems measured a $\delta^{18}O$ of 26.25‰ and 26.98‰ in the leaves. The leaves appeared to be more depleted than the stems and slightly enriched in ^{18}O (**Figure 29**). The average isotopic composition of the leaf tissue was -157‰ as opposed to -128‰ in the stems. Interestingly, the leaf organic tissues plot with the stems, which has not been observed in either the Experimental Control or Trial 1.

Table 23: δD(‰) in stem and leaf organic tissue– Trial 2

Environmental Control δD (‰)		**Exposed to (δD (‰))**		
Stems	Leaves		Stems	Leaves
-134.72	-154.77	Most Enriched	-112.4	-152.1
		Most Depleted	-139.5	-161.6
		Average	**-128.2**	**-156.6**

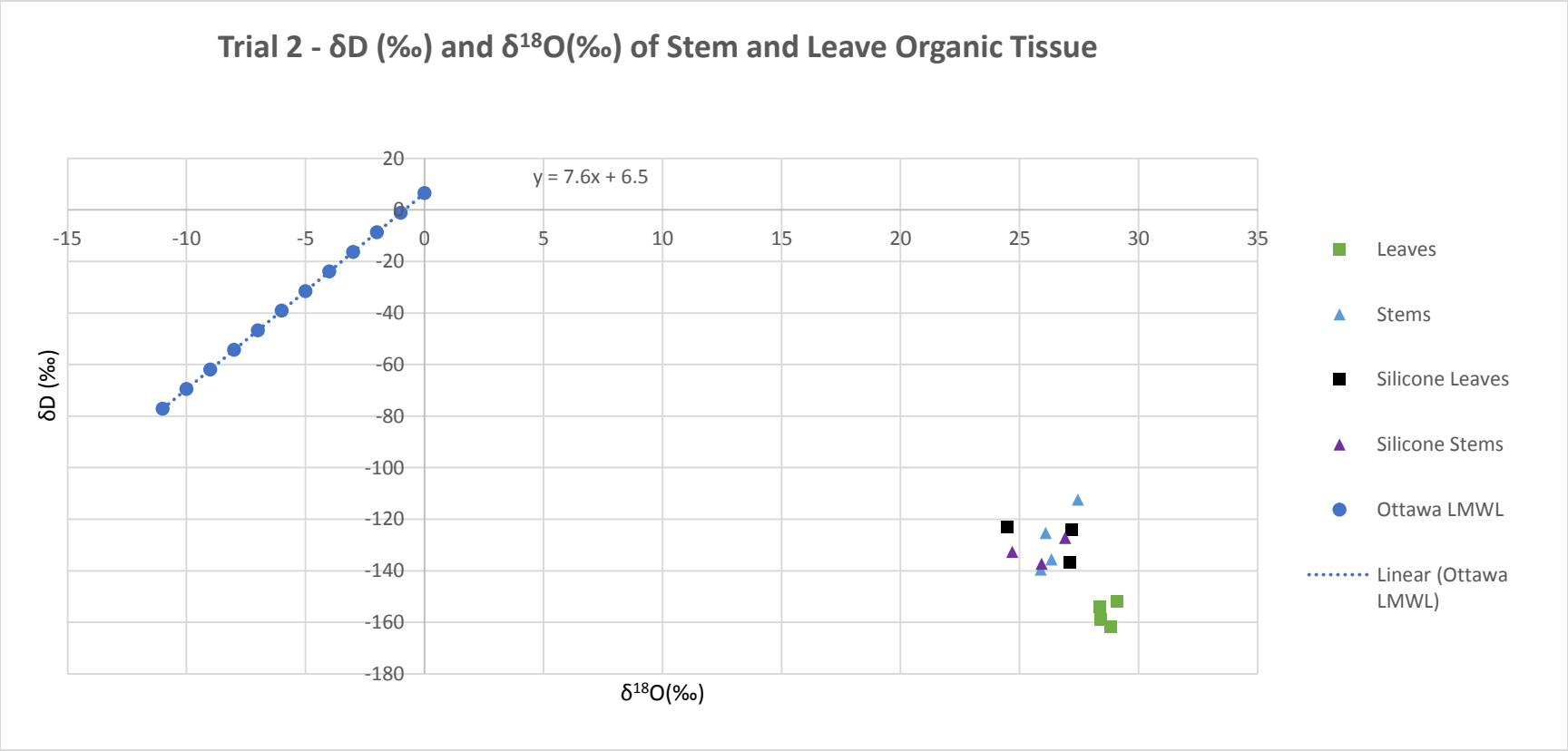


Figure 29: δD (‰) and $\delta^{18}O$(‰) of the stem and leaf organics in Trial 2, plotted with the Ottawa LMWL described in Equation [6]. That the stems and leaves are depleted in deuterium, but enriched in oxygen. There was no observable difference between the Environmental Controls and the samples presented here, however, the silicone test leaves plot with the stems, which is not seen in the other experiments.

11.3 SRBT Field Experiments

The SRBT stack monitors reported a spike in total tritium (HT and HTO combined) emissions at approximately 9:45 AM on the day of exposure. A second major spike, larger than the first, occurred at about 1:45 PM (**Figure 30**). The active air sampler indicated a spike in atmospheric HT to 25 Bq/m^3 over a time interval of 2 hours between 10:20 AM and 12:20 PM (**Figure 31**). The HT signal dropped over the next 3 hour interval to 5 Bq/m^3, never rising above 1 Bq/m^3 throughout the rest of the sample period. The HTO signal remained below HT, measuring at 11 Bq/m^3 from 10:20 AM to 12:20 PM, and spiking in the following time interval to 29 Bq/m^3 (**Figure 32**). Like HT, HTO concentrations fall to below 1 Bq/m^3 for the rest of the sampling period, which includes evening and the night time hours.

Measurements of soil HTO showed no major change throughout the day, and fluctuated between 10 and 11 Bq/L. Control soil samples, remaining in Ottawa, were measured at 4 Bq/L. Conversely, plant leaves and stems responded quickly to the increase in atmospheric tritium concentrations, with the leaves peaking at 533 Bq/L over a 4 hour interval from 8:00 AM until 12:00 PM (**Figure 33**). The leaf tritium concentration remained similar (492 Bq/L) for the 12:00 PM to 4:00 PM interval, before dropping rapidly for the next interval. There was a slight increase in leaf tritium concentration from 12:00 AM to 4:00 AM to 175 Bq/L. Plant stems peaked during the 8:00 AM to 12:00 PM interval, achieving a maximum concentration of 239 Bq/L before dropping rapidly to below 50 Bq/L for the remainder of the sampling period.

Measurement of plant organically bound tritium of the plants brought on site is on-going and will not be presented here.

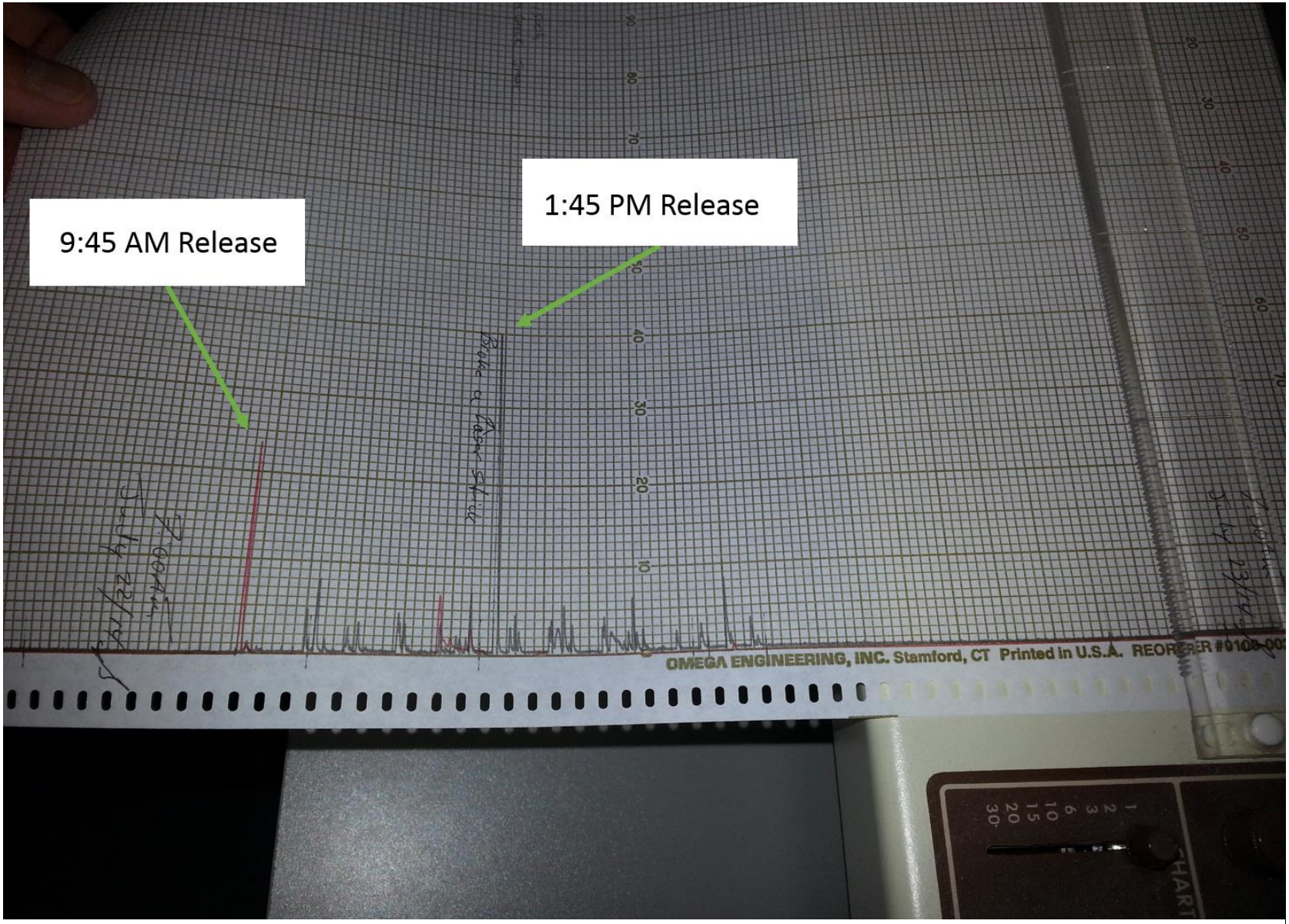


Figure 30: Stack emissions report on July 22, 2015 from the SRBT monitoring system. The first major spike in emissions occurred at 9:45 AM, followed by a second at 1:45 PM. The monitoring system cannot distinguish between HT or HTO, therefore the emissions could represent a combination of the two species.

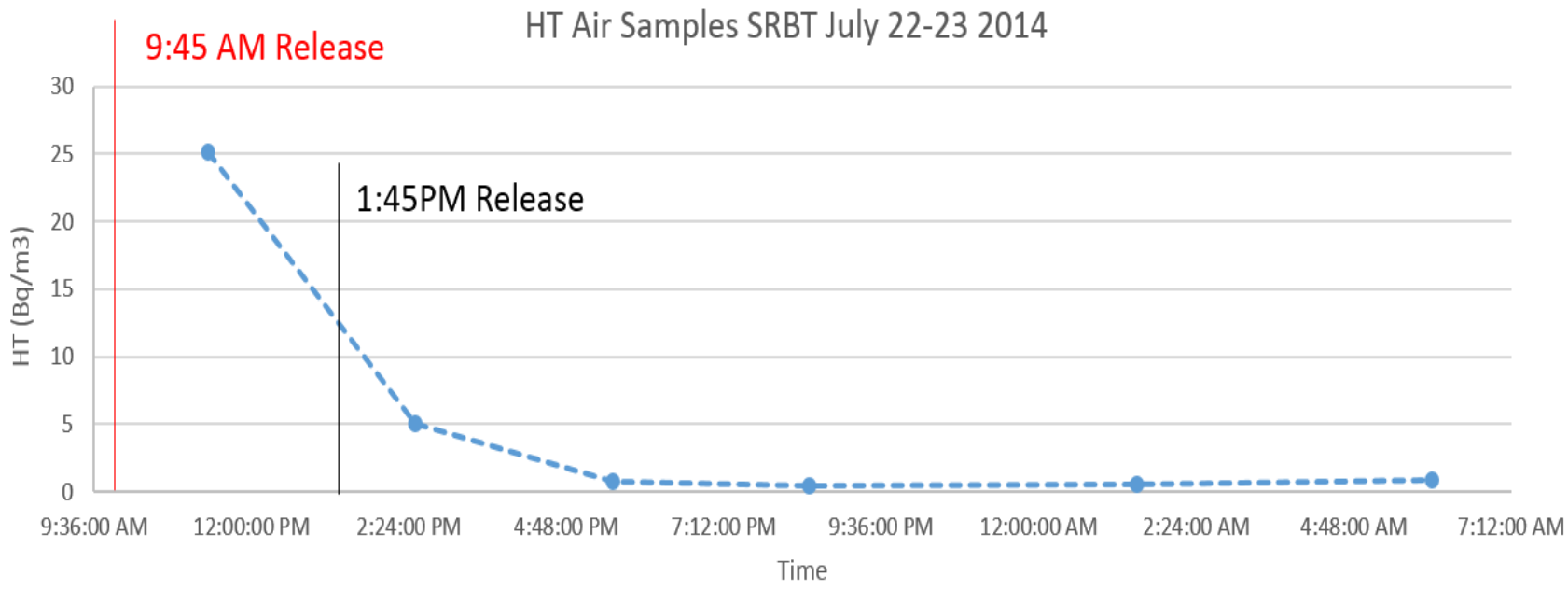


Figure 31: Atmospheric HT concentration from the morning of July 22 to the morning of July 23 at SRBT in Pembroke, Ontario. The measurements have been corrected for the volume of air moved through the bubblers. Note that the HT spikes shortly after the 9:45 AM stack release, but not the second.

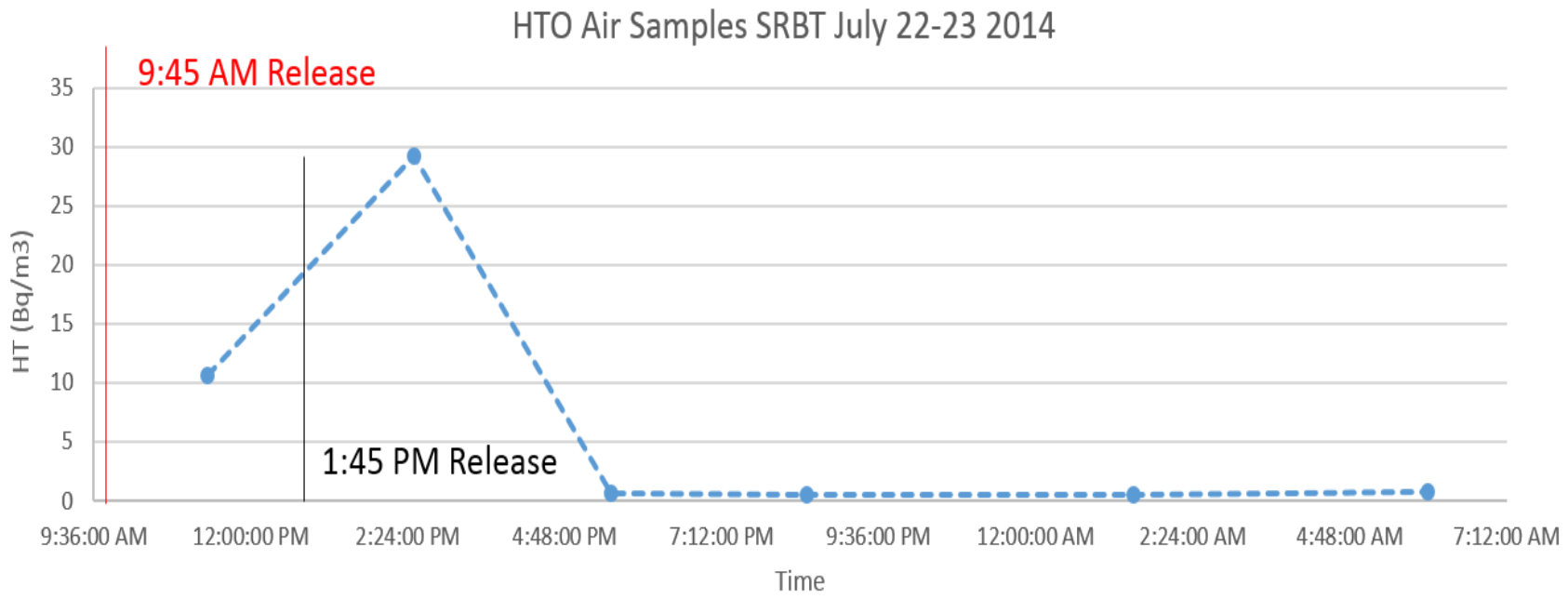


Figure 32: Atmospheric HTO concentration from the morning of July 22 to the morning of July 23 at SRBT in Pembroke, Ontario. The measurements have been corrected for the volume of air moved through the bubblers. Note that the HTO spikes shortly after the 1:45 PM stack release, but not the first.

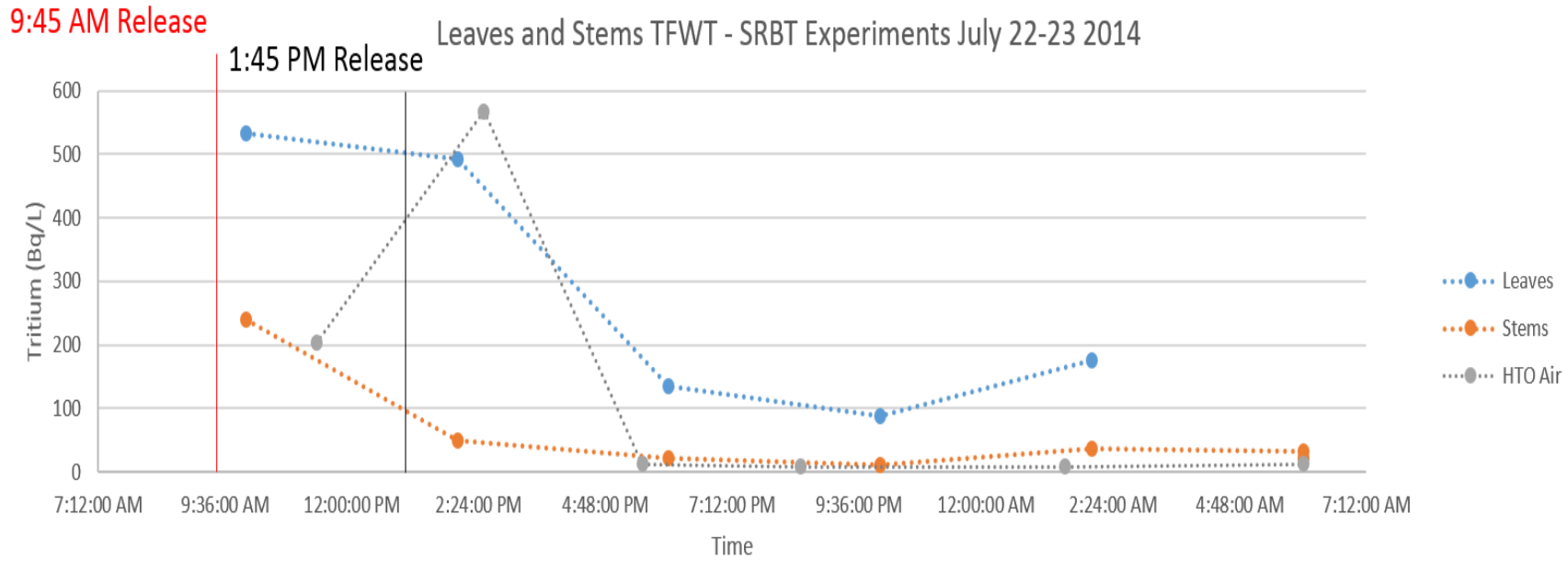


Figure 33: TFW tritium concentrations in the leaves and stems, compared to atmospheric HTO concentrations (adjusted for relative humidity). The leaves and stems peak after the 9:45 AM stack release, steadily dropping, even during the second release that occurred at 1:45 PM**.**

12 Discussion

12.1 SRB Technologies Soil Profiles

Both HT and HTO are released from the SRBT stacks during routine daily operations. Only during rainfall events are the stacks not operating. The spikes in pore water tritium in the upper 10 cm of both cores are likely the combined result of recent dry and wet deposition of HT and HTO emitted from the stacks. HTO is deposited to the soil surface as water vapor, or as liquid water during condensation and precipitation events (Boyer *et al.*, 2009). HT can be converted to HTO following dry deposition by microbial oxidation near the soil surface (McFarlane *et al.*, 1978; Sweet and Murphy, 1981). Once in the soil, some of the HTO can be reemitted to the atmosphere, either through plant uptake and transpiration, or evaporation from the soil surface. Other processes contribute to the attenuation of HTO, including percolation into deeper soil and groundwater, as well as incorporation into organic matter. Following short term exposure, the majority of deposited tritium is reduced by half on the order of one to five days, depending on the soil characteristics and weather conditions (Kirchmann *et al.*, 1971; Korando and Martin, 1971; Belot, 1986). Given that the SRBT stacks regularly emit tritium, the spikes near the core surface likely reflect stack emissions within the past several days.

The decreasing tritium profile to about 22 cm depth seen in both SRBT cores is consistent with previous assessments of tritium in soils (Belot *et al.*, 1996), where the same trend is evident. The decrease could be attributed to poor soil drainage, or dilution with less tritiated pore waters derived from precipitation as suggested by McFarlane *et al.*, (1978). Some light rain did occur on June 16, 2013, 4 days prior to sampling, potentially representing a zero emission rain day, and dilution as rain water mixes with tritiated soil water. This decrease was expected to continue deeper into the profile. However, pore water tritium in both soil cores begins to increase after about 22 cm depth. This reversal

could be a historical signal from higher emissions in the past, as pore waters percolate downwards through the soil.

A comprehensive groundwater study carried out by EcoMetrix Ltd. (2006) estimated the average vertical groundwater velocity in the unsaturated silty-clay overburden to be approximately 0.33 m/year. Average depth to the water table was measured at about 5 m. Therefore, the entirety of the SRBT cores were likely in the unsaturated zone of the soil profile, as the maximum coring depths were 1.2 m. In effect, it would take 3.6 years for tritium to travel from the ground surface to the bottom of each core, and approximately 15 years to reach the water table. A follow-up study by EcoMetrix (2008) analyzed soil waters sampled at various depths from the monitoring wells. Almost all of the EcoMetrix (2008) profiles show the same trend as those of the SRBT cores in this study. Given the length of time required for tritium to migrate downwards from the surface, the increasing pore water tritium in the SRBT cores is likely a historical signal from when emissions were higher than present. Emissions peaked in the year 2000 at 1 700 000 GBq HTO as reported by SRB Technologies. While emissions fell each consecutive year, the low vertical soil water velocities help retain the tritium in the profile. Further, compliance reports produced by SRB Technologies for the CNSC provide tritium concentration measurements from the monitoring wells around the site. The yearly average of groundwater tritium measurements taken at these wells is similar to the SRBT Core measurements (SRBT, 2013).

Organically bound tritium decreases with depth in both cores, which likely reflects the lack of organic material as depth increases, assuming the organic content of SRBT Core 1 can be extrapolated to Core 2. At both core locations, there is vegetation (lawn grass at SRBT Core 1 with topsoil and wild long grass at SRBT Core 2 with no top soil). During photosynthesis, tritium is incorporated into primary photosynthates and eventually into plant cellulose. Plant material is subsequently added to the soil organic detritus thus contributing to the OBT signal found in the soil. As plant detritus becomes scarce

with depth, so too does the OBT concentration. Loss on Ignition analysis was only performed down to 90 cm depth on SRBT Core 1. The limited data does indicate a weakly decreasing correlation of organics with depth (R^2 = 0.34) which supports the fact that OBT is, in this case, associated with decaying plant material near the soil surface. It is expected that OBT levels remain at background deeper into the subsurface. A small portion of tritium could also be being incorporated into microbial biomass at the surface through metabolic processes, and the results would suggest that this microbial influence is not as prominent with depth. Further, while any tritium incorporation into the mineral fraction of sediments is a little explored possibility, this study does not provide any evidence to support or refute the possibility.

12.2 Deuterium Exposure Experiments

The effects of exposing soils and plants to D_2 gas in the growth chamber are difficult to differentiate from normal evapotranspiration. Firstly, the actual presence of D_2 gas had to be confirmed in the chamber atmosphere. The challenge in this respect was in attempting to reproduce similar HT concentrations found in the air around SRBT using deuterium in the growth chamber. The growth chamber is designed to constantly move air, generating a residence time of approximately five minutes. Therefore, the D_2 gas had to be delivered at a rate that could simultaneously withstand the air flux while achieving desirable concentrations that were both above instrumental detection limits, and similar to HT measurements in the field. To this end, the D_2 was delivered by diffusion through 26.9 cm^2 of PVC tubing at an approximate rate of 0.257 cc/hour. **Figure 27** shows that in Trial 2, air sampler measurements indicate that HD gas was elevated while HDO remained relatively unchanged from the Starting Waters. The measured concentrations are a function of time, as the longer the sample time interval, the more air is bubbled through the vials. For example, the HD 8:45 AM sample shows the greatest HD enrichment of 90.9‰, while the other five hour interval samples show a modest increase over time, beginning at -

41.2‰ at 4:40 PM and reaching -7‰ at 6:30 PM the following day. HDO measurements ranged from -77.3‰ to -76.4‰ during the five hour intervals, only reaching -70.8‰ during the approximate nine hour interval from 10:00 PM to 8:45 AM the next morning. The atmospheric HDO concentration varied only slightly from the starting water ∂D value of -81.7‰.This finding, that HD gas was present in the chamber throughout the experiments, is supported further by preliminary soil tests where deuterium was introduced to the chamber atmosphere, though at a faster rate, and without air sampler measurements. Water used to moisten the soils prior to exposure had a deuterium signature of -76.5‰. After 48 hours of exposure, the deuterium signature of the soil pore waters ranged from 92‰ to 181‰, a minimum of +168.52‰ increase over the Starting Water (δD = -76.52‰) with no change in $\delta^{18}O$. Air sampler measurements registered HD values of 1890.6‰ (uncorrected for air volume moved through the sampler). This is opposed to a -61.8‰ (uncorrected) HD measurement in a non-exposure test.

Reducing the diffusion rate of deuterium ensured that these values were not seen in the subsequent experiments, and were intended to be more representative of the SRBT tritium conditions of approximately 5 Bq/m^3. Unfortunately, the air sampler was not available for each plant experiment. Therefore, the results of the preliminary soil experiments, as well as the 24-hour air sampling had to be extrapolated to the other experiments (Experimental Control and Trial 1).

The next challenge was in determining if there was deuterium enrichment in the soils and plants above that which was expected from evaporation. Each Trial showed the same pattern of evaporative enrichment from the source water, to soils, stems, and leaves, progressively enriching in both deuterium and ^{18}O from soil to leaf. This general trend alone is from pure evaporative enrichment, although the literature would suggest that stems should have the same signature as the soil water. Leaves on the other hand, are the site of evaporation and as expected, had the greatest enrichment. Back-diffusion and translocation, could be responsible for the unexpected stem isotopic values, as enriched leaf water

mixes with soil water in the stems. In an attempt to differentiate evaporative versus atmosphere enrichment, each trial incorporated both an Environmental and Experimental Control. The Environmental Controls were potted plants that were grown in the chamber, but were removed during the D_2 exposure, and sampled in the same fashion. This was to account for changes in humidity and temperature, though the lighting conditions remained the same (e.g. 24 hours of light or 24 hours of dark). The Experimental Controls were a large set of potted plants that were sampled from the growth chamber, with the deuterium gas dispenser closed. This large sample set was designed to observe changes resulting from increased D_2 gas in the growth chamber atmosphere.

It was anticipated that any deuterium enrichment in the tissue water would translate into the plant organics following photosynthesis. The organics signature of the Experimental Control set was compared with that of (Trial 1 and 2). Recall however, that photosynthesis selects against deuterium, causing primary photosynthates to be enriched with respect to the lighter protium. In essence, a plant enriched by atmospheric HD and/or soil HDO (derived from said HD) should show clearly elevated deuterium signatures in both tissue water and organics versus the controls, while changes in ^{18}O are negligible.

Only with the confirmation of atmospheric HD, deuterium enriched tissue free water and organics, and zero change in the oxygen isotope signature can it be concluded that the exposure experiments were successful.

12.2.1 Experimental Control Trial - 24 hours in light and 24 hours in dark

The Experimental Control Trial was used to evaluate soil and plant isotopic signatures in the growth chamber in the absence of deuterium gas, with the dispenser present and regulator closed. In determining whether or not enrichment in the soils was the result of evaporation, or supplemented by D_2 in the atmosphere, the results were compared with expected isotopic values. The growth chamber

was considered an open system, as air was constantly moved out of the chamber. Equation [7] was used to calculate the expected isotopic signatures of the soil water, using fractionation factors or 1.084 and 1.0098 for hydrogen and oxygen respectively at 20°C. (Majoube, 1971).

[7]

$$\delta = \delta_o - 1000f^{(\alpha-1)} + 1000$$

Where δ equals the isotopic signature in of interest (ex: δD), δ_O is the initial signature of the starting water, f is the fraction of initial water remaining, and α represents the fractionation factor. **Figure 34** and **Figure 35** show the expected deuterium enrichments resulting from Raleigh fractionation, an approximation of evaporative enrichment at the soil surface. No deuterium enrichment above these expected values was observed. Indeed, the soil pore water appears depleted from the Raleigh curve in both Light and Dark experiments. However, with the exception of one soil sample (-39.5‰), the Light soils samples deviated only slightly from the Starting Waters of 68.1‰. It must be noted however, that the water contents of the potted soils were not available for the Experiment Controls, therefore the Light and Dark respective water content averages from Trial 1 were used to estimate water content.

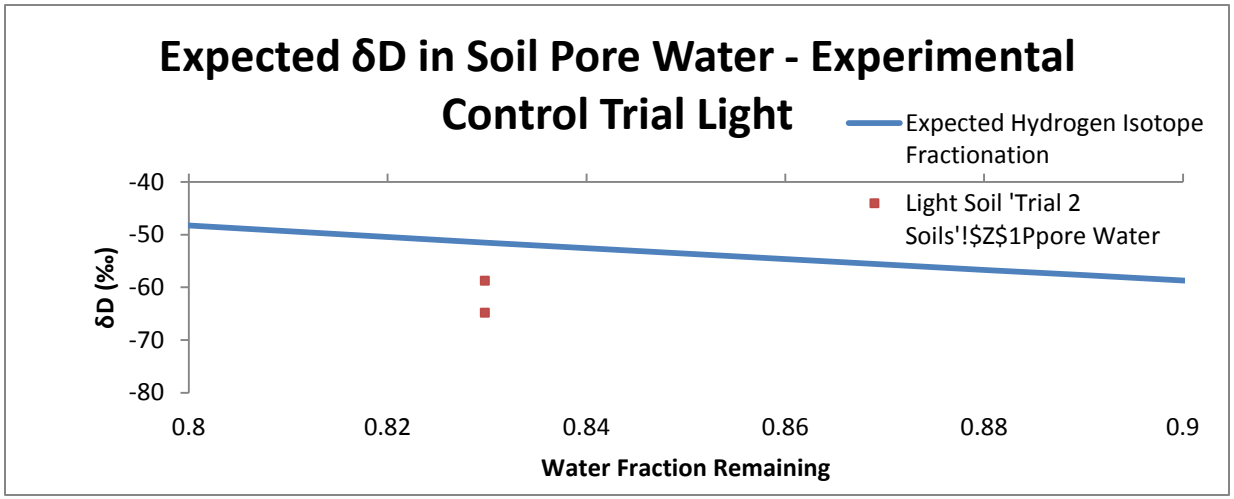


Figure 34: Soil pore water deuterium plotted against the Raleigh fractionation curve in the Light experiments. Water contents were estimated using the available measurements from Trial 1.

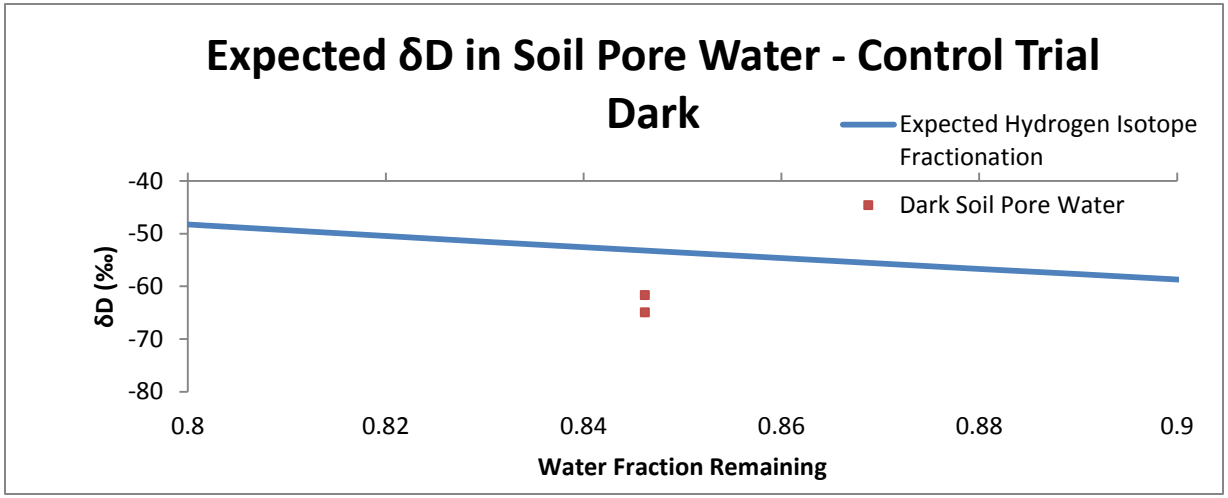


Figure 35: Soil pore water deuterium plotted against the Raleigh fractionation curve in the Dark experiments. Water contents were estimated using the available measurements from Trial 1.

Interestingly, some deuterium measurements in the Experimental Control Dark Experiments were actually isotopically lighter than the Starting Water (δD = -90.3‰, and δD = -84.2‰). These values oppose the expected evaporative enrichment. This could represent back diffusion of isotopically lighter air moisture into the soil, induced by vapour pressure gradients at the soil boundary layer resulting from the reduced heat of night conditions. More likely, it is the result of not having a measurement for the Starting Water in the Experimental Control Dark experiment.

The $\delta^{18}O$ measurements, when compared to Raleigh expectations, are mostly enriched in both Light and Dark Experimental Controls (with the exception of one sample showing depletion). These enrichments were small however, with a maximum enrichment of +4.6‰. Despite the comparison with the Raleigh curves, both deuterium and oxygen are enriched over the Starting Waters, likely the results of enrichment, with the process being more evident, in the Light Experiment, as expected.

The plant tissue water results, presented in **Figure 20** and **Figure 21** show that the evolution of the water isotopic signal from the Starting Water as it moves from soil to stem to leaf, becoming progressively more enriched in both isotopes as it moves through the transpiration stream. In both Light and Dark, the growth chamber plants appear to fall along the Environmental Control line, derived from plants that were removed from the growth chamber. These observations suggest that dramatically changing the environment has no discernible effect on both the nature and magnitude of the isotopic shift. Granted, only one plant was removed per Light and Dark Experiment.

As mentioned previously, the tissue free stem water should be isotopically identical to the soil, as there is no fractionation of the soil water until it reaches the leaf. The Péclet Effect, or the back diffusion of isotopically enriched water, is believed to be limited to the leaf surface. Regardless, leaf water is major component of phloem sap, which is transported opposite to the transpiration stream,

thus bringing enriched water back to the stem. As the stems were analyzed for bulk tissue water, there is no distinction between the contents of the xylem or phloem. Therefore, both water streams could be influencing the isotopic composition of the stems.

Plant organics results presented in **Figure 22**. As expected, deuterium is heavily depleted, and oxygen continued with the trajectory of enrichment seen in the tissue free waters. It is known that the only oxygen fractionation occurring in plants that is related to autotrophic or heterotrophic metabolism is water/carbonyl reactions (producing a shift between +25‰ and +30‰) (Sternberg 1989, Yakir and DeNiro, 1990, Barbour *et al.*, 2000). The average $\delta^{18}O$ of the leaf tissue water in the Light Experiments is 9.3‰. The bulk leaf organics in the Light average 28.5‰, just 6‰ shy of the lower limit. Average stem tissues were 27.1‰, shifting +26.6‰ from the average stem tissue water value of -0.5‰. Both measurements are reasonably close to the expected oxygen shift. It must be noted that fractionations associated with biochemical reactions throughout the plant can alter this signal, which could account for the slight deviation from the expected range (Barbour *et al.*, 2000). There was no major difference in the $\delta^{18}O$ of leaves and stems in the Dark Experimental Control Experiments from the Light, as the primary photosynthates used for organics production were formed from the same water during photosynthesis. Therefore, the oxygen isotopes of the plant organics were as expected.

The isotopic fractionation associated with deuterium in plant organics changes depending on the process involved. Autotrophic metabolism, or photosynthesis, selects against deuterium, depleting the isotope by -120‰ to -171‰ (Yakir and DeNiro, 1990). Conversely, heterotrophic metabolism enriches deuterium by a factor of +144‰ to +166‰ (Yakir and DeNiro, 1990; Luo and Sternberg, 1992). The deuterium measurements shown in **Figure 22** indicate that in the Light Experimental Control trials, the leaf organics were depleted by -137.2‰ on average when compared to the leaf tissue free water. The Light stems were depleted by -114.2‰ relative to the average Light leaf waters (-8.3‰) which

would be used to produce the sugars translocated for stem growth. The leaf organics are within the autotrophic range while the stems are not (though only slightly). It's possible that translocated sugars used to build stem tissue underwent some heterotrophic metabolism however, the measured values are nowhere near the expected range produced by secondary metabolism. The expected fractionation factors were derived from studies of tree ring cellulose exclusively, not including other organic molecules like lipids and proteins that would remain following tissue free water extraction. As these samples were measured in bulk, it is possible the cellulose signature was mixing with other biomolecules. Plant lipids for example, are typically depleted in deuterium by as much as 150‰ to 300‰ (Estep and Hoering, 1980; Hoefs, 2009). The Dark Experiments showed similar results. The Environmental Controls were indistinguishable from the Experimental plants, suggesting that in this case, environmental conditions had little effect on the isotopic signature of the plant organics. This is expected for oxygen, as the isotopic compositions of the plant organics are dictated by the water (Hoefs, 2009). Ultimately, the results of the Experimental Control set a baseline for which Trial 1 and Trial 2 can be compared.

12.2.2 Trial 1 – 24 hours in light and 24 hours in dark

From the growth chamber air sampling events, and confirmation of D_2 to HDO soil conversion, it is assumed that D_2 was present in the chamber atmosphere at above normal concentrations throughout Trial 1.

Figure 36 and **Figure 37** plot the predicted curve given the Starting Water signature in Trial 1 for both Light and Dark Experiments. The soil water content measured during the pore water extractions was used to estimate the remaining water fraction. It was assumed that watering the soils before the experiment saturated the soils. Two data points show greater than expected deuterium enrichment (-39.6‰ and -47.1‰), while the other two data points show less (-51.5‰ and -64.1‰) for the Trial 1

Light Experiment. The $\delta^{18}O$ of these samples all fall below the expected values by showing greater than predicted depletion in deuterium. The Dark Experiments however, both fall below the Raleigh line. Using the Raleigh equation on soils alone does not provide enough evidence to suggest that there was active deuterium oxidation in the soil. However, given that the oxygen measurements are all less than expected, the possibility remains for the Light Experiments.

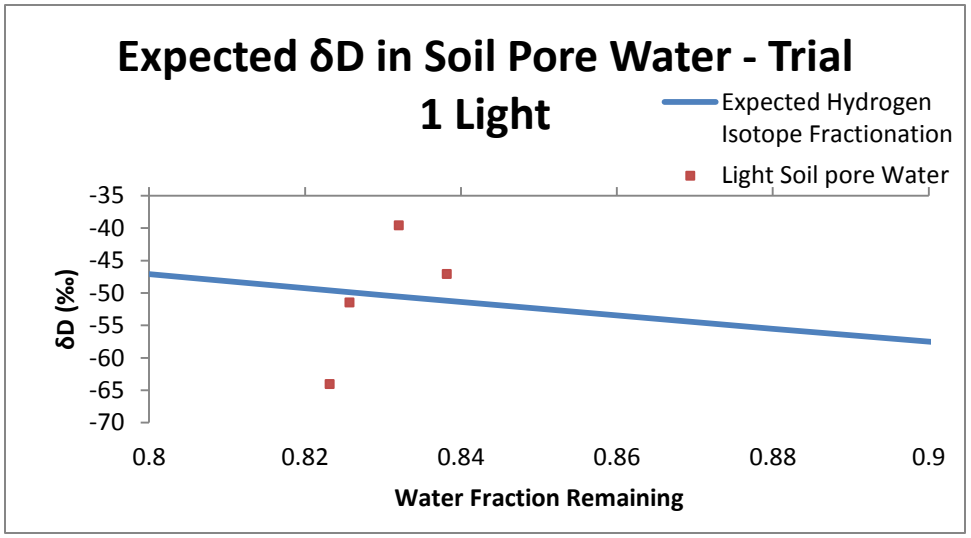


Figure 36: Soil pore water deuterium plotted against the Raleigh fractionation curve in the Trial 1 Light Experiments.

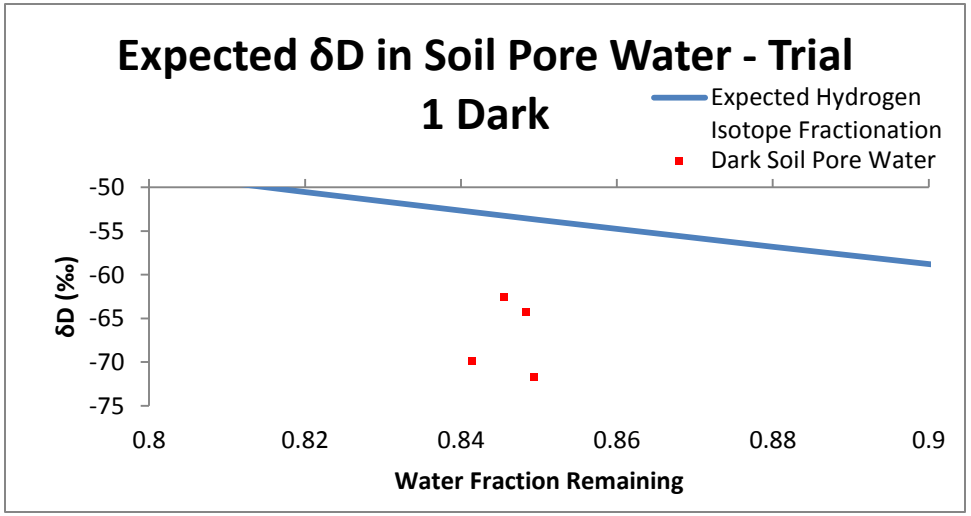


Figure 37: Soil pore water deuterium plotted against the Raleigh fractionation curve in the Trial 1 Dark Experiments.

The soil pore waters were enriched versus the Environmental Controls, with an average enrichment of 34.7‰ in the Light Experiments, and 9.8‰ in the Dark Experiments. The presumably enriched soil waters in Trial 1 are the result of three possibilities; the production of HDO through D_2 oxidation, evaporative enrichment, or a combination of the two processes. The ubiquity of hydrogen gas oxidation mediated by hydrogenase enzymes in the soil suggests that oxidation is at work, assuming the depositional velocity is such that the D_2 gas reaches the soil. However, temperatures in the growth chamber during the day were higher than the Environmental Controls, possibly leading to a greater evaporation rate in the chamber. Further complications in this interpretation arise from the assumption of isotopic uniformity in the soil pore water volume. The whole soil volume was mixed prior to sampling for the extraction process. HD penetration depth within the soil is presumed to be limited. As a result, the HDO signal in the soils could be lost in the sample preparation process, as most of the deuterium remains near the surface. Further, the plant roots, thin and heavily branched, appeared to cover the majority of the soil volume, suggesting that water was being drawn by the plant from the total soil volume, which could impact the plant tissue water depending on where the water was absorbed. Segmenting the soil samples into individual depth intervals would help in delineating the HDO concentrations with depth in the future.

The soil pore water analyses were based on the difference between Starting Waters used to saturate the soils, and the resulting bulk isotopic measurements of the soil following exposure. In Trial 1 (and all other Trials), evaporative enrichment was expected and observed, hence adding to the difficulty in differentiating elevated deuterium measurements resulting from D_2 additions versus evaporation. The Raleigh curves used to evaluate this do not take into account the effects of humidity at the soil surface that could have the biggest impact in dictating evaporative enrichment, as a laminar flow layer above the soil surface could saturate, and limit evaporation.

Figure 24 and **Figure 25** show the results of the Trial 1 Light and Dark Experiments respectively. The stems showed an average deuterium signature of -18.08‰ in the Light Experiments, and -38.3‰ in the Dark Experiments. This amounts to an averaged 30.92‰ and 0.2‰ respective enrichment over the Environmental Control stems. The isotopic signature of stem tissue water should represent the source water (that being the soil pore water subject to root absorption). However, once again, bulk stem tissue water analysis could include back-diffusion or translocation of enriched leaf water, thereby altering the pure xylem (soil) signal. Leaf water, along with dissolved carbohydrates and other biomolecules, is translocated throughout the plant via phloem sap (Farquahar and Lloyd, 1993). In effect, bulk stem water represents a combination of both xylem and phloem sap waters. As expected, the Light Experiments showed a greater enrichment over the Dark, given open stomata and increased temperatures allowing for and inducing transpiration.

Leaf tissue water is enriched in both isotopes when compared to soil and xylem water in the Light and Dark Experiments. Essentially, the same trends, and indeed, the same processes observed in the Experimental Control are observed in Trial 1. However, in the Trial 1 Light Experiments, the soils, stems, and leaves all show enrichment over the Environmental Control, as well as the Starting Waters. This suggests that the D_2 gas being released into the atmosphere registering in the plant tissue waters, via foliar or soil exposure. Previous experiments have shown that it takes approximately three hours for the leaf to reach isotopic steady state with the Source Water, which the experimental timeframe allowed for (Wang and Yakir, 1995; Barbour *et al.*, 2000).

By plotting Trial 1 against the Experimental Control, and conceptualizing the soil-stem-plant into one continuous system (ignoring the nature of the stem signature), Trial 1 appears enriched over the Experimental Control in the Light runs (**Figure 38**). While the trends are the same, the initial conditions for the magnitude are not. Consider the Starting Water for Trial 1 (δD = -66.9‰; $\delta^{18}O$ = -7.5‰). The

presence of D_2 gas in the chamber led to deuterium enrichment in the soils, without the purely evaporative changes in $\delta^{18}O$ seen in the Experimental Control soils. The Trial 1 trend line shows an 8‰ deuterium enrichment over the Experimental Control trend line (though admittedly, an 8‰ enrichment is fairly low given the range in δD seen in plants). Taken together with the perceived enrichment over the Environmental Controls, this analysis suggests that while evaporative enrichment was perhaps the main driver of isotopic change in the system, D_2 gas was being oxidized either in the soil or at the leaf, leading to deuterium enrichments in the stems, though to a lesser extent in the leaves versus the Experimental Control.

When this same comparison is made for the Trial 1 Dark Experiments, there is no enrichment over the Experimental Control (**Figure 39**). Even though deuterium gas was present in the Trial 1 Dark Experiments, it did not register in the tissue waters of the plant. The soils themselves appeared to become depleted with respect to the Starting Water. When considering the Dark Environmental Control, the dark tissue waters were similar to the light in that there was an enrichment in deuterium, however to a much smaller extent. Stomatal pores are typically closed at night, thereby reducing water influx to the plant, either by cutting off the transpiration stream from the soils, or by preventing water vapour diffusion into the leaf. There is no evidence to suggest that soil hydrogen oxidation is light dependent, so if deuterium was elevated in the atmosphere, it should register in the soil pore water signal. While all soil data points are slightly enriched when compared to the Environmental Control, only two are actually enriched versus the Starting Water in the Trial 1 Dark experiments, and even those are more indicative of evaporative enrichment versus oxidation. In either case, the Trial 1 Dark Experiments show no definitive deuterium enrichment in either compartment when both controls are taken into consideration.

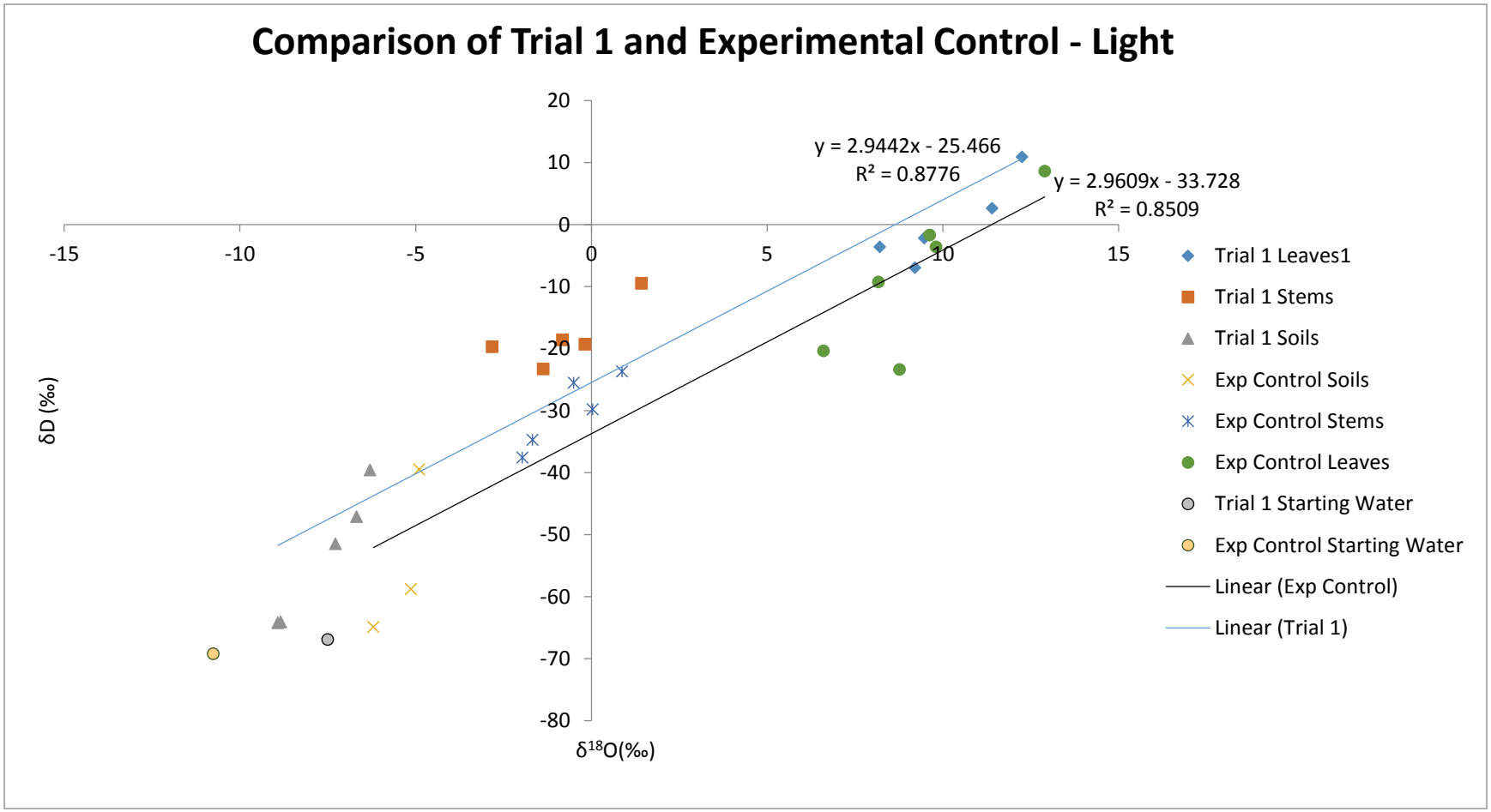


Figure 38: Trial 1 and the Experimental Control Light runs compared to each other, and viewed as one continuous system from soil water to leaf. The Trial 1 trend line produces a deuterium enrichment of 8‰ over the Experimental Control.

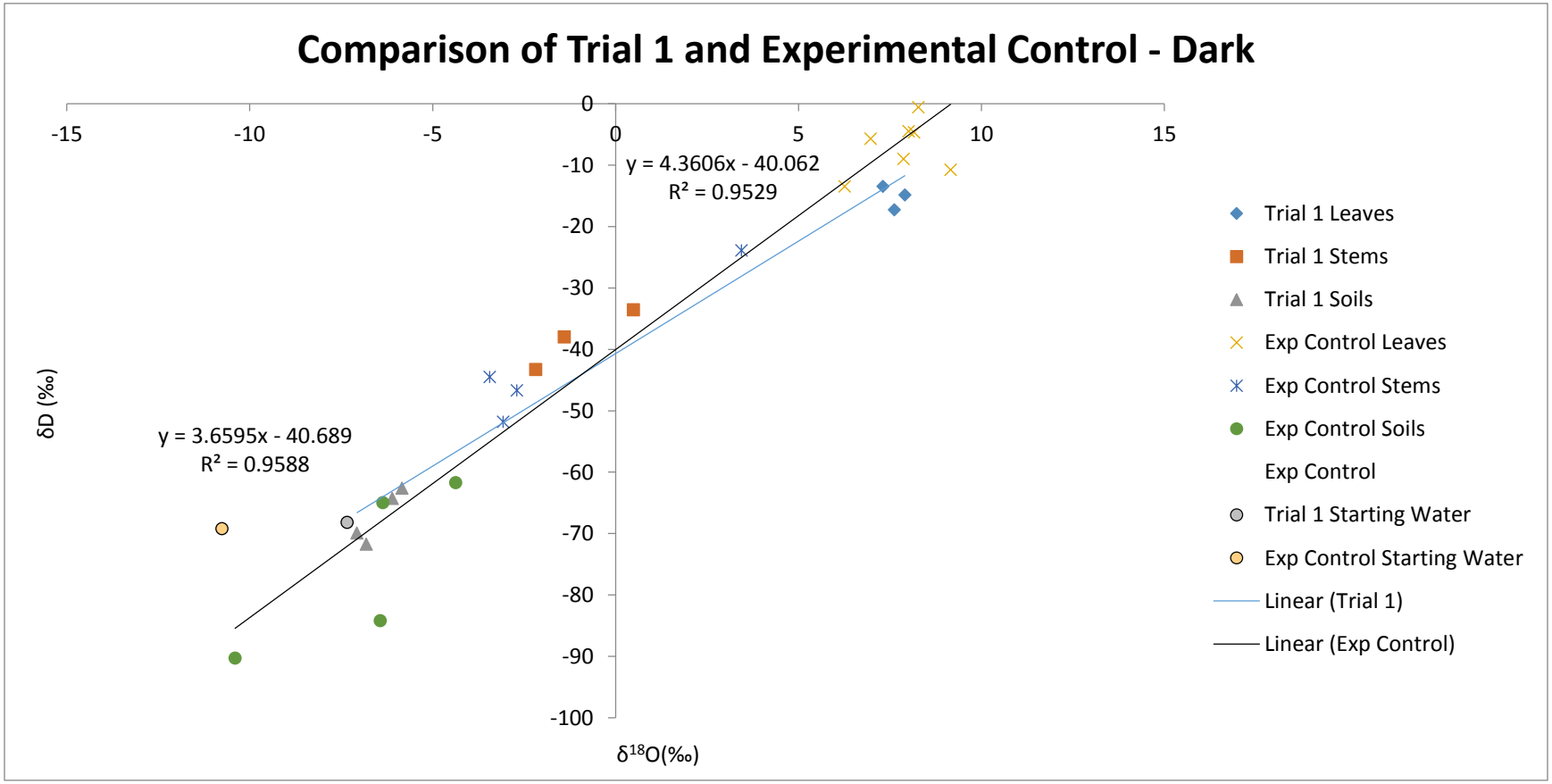


Figure 39: Trial 1 and the Experimental Control Dark runs compared to each other, and viewed as one continuous system from soil water to leaf. There is no deuterium enrichment of one trial over the other.

The isotopic measurements of stem and leaf organics in the Trial 1 Light and Dark Experiments are presented in **Figure 26**. The same trends are observed here as in the Experimental Controls, and there is once again no discernible difference when considering the Environmental Control. The average measurement of light leaf deuterium was -154.6‰ versus the control value of -145.5‰. When considering the average leaf tissue water (δD = 0.14), there was a depletion of -154.5‰ from leaf water to organics, which is within the predicted autotrophic range. The leaf tissue water oxygen isotope measurements averaged 25.9‰ versus 28.5‰ in the Experimental Control, producing a shift from the Trial 1 leaf tissue water of +15.8‰ (which is not entirely consistent with the water/carbonyl fractionation). From this data, it appears that the D_2 signal did not translate into the leaf organics. Given that even the tissue waters only showed an enrichment of 8‰, the D_2 concentration in the atmosphere was likely too low throughout the Trial 1 Light Experiment. Further, if there was enrichment, the signal could have been masked by the bulk analysis.

The stems of the Light Experiment measured a δD = -130.1‰ and a $\delta^{18}O$ = 24.5‰. When comparing to the leaf tissue water, there was a shift of -112.02‰ in deuterium and +25.3‰ in oxygen-18. The oxygen is within the expected fractionation range while once again, the deuterium shows only a slight enrichment from the leaf tissue waters, when compared to the anticipated heterotrophic fractionation of +144 to +166‰. These results are consistent with those observed in the Experimental and Environmental Controls, and there was no remarkable change between Light and Dark.

Based on the results from the Experimental Control and Trial 1, and the initial growth chamber soil tests, it is likely that while D_2 gas was present in the chamber, the standing concentrations were too low to generate a definitive enrichment. With that said, the initial soil tests indicated that at sufficient levels, D_2 gas can lead to large enrichments in the soil pore water. In these experiments, the plants were exposed to D_2 gas, not HDO. And if the conclusions regarding the 8‰ enrichment over the Experimental

Control are sound, the question becomes what mechanism is responsible with regards to the plants? The conventional hypothesis is that conversion occurs in the soil, however the foliar pathway is just as likely, assuming D_2 is oxidized.

12.2.3 Trial 2 – 36 Hour Light Exposure and Silicone Caulking Soil Isolation Experiment

Trial 2 consisted of two sample types: a silicone caulking group, and a 'normal' plant group. Both were exposed to 36 hours of light in the chamber, with active deuterium exposure and the air sampler running. See **Figure 27** for the air sample results. The silicone caulking was used to isolate the soil from the atmosphere in an attempt to monitor strictly foliar deuterium uptake. It must be noted that the silicone caulking used to isolate the soil may have had a degree of toxicity to it that led to the yellowing and wilting of the arugula samples. Sufficient quantities of water could only be extracted from the stems, characterized by an evaporative enrichment trend as shown in (**Figure 28**). There is no enrichment in deuterium above the Environmental Control in this case, indicating that these stems were not influenced by the deuterium present in the chamber air. Soil data is unavailable for these samples, including the Environmental Control. Also shown in **Figure 28** are the normal group plants that demonstrate deuterium enhanced evaporative enrichment above the Environmental Control. This comparison experiment is critical in that it could be showing clear evidence that the D_2 is registering in the plant tissue free waters, versus the silicone group and the Environmental Control. That being said, for 36 hours, the soil waters of the normal group were influenced by evaporation, which could have shifted the isotopic signature above the Starting Waters. This would have set the stem and leaf tissue waters on a trajectory leading them to their current positions above the Environmental Control. The Environmental Control itself was exposed to light for the same period, however the change in relative humidity and temperature could have led to a different evaporation rate. Without the soil data, it is near impossible to draw a confident conclusion. That being said, this experiment had confirmed

atmospheric D_2 in the chamber based on the active air sampler results. Given that the initial soil experiments, and the Trial 1 Light Experiments show soil pore water deuterium enrichment, it is plausible that the stem and leaf tissue water were actually enriched.

One of the main objectives of exposing the plants to light for an extended period was to observe the transfer of enriched photosynthates into plant organics. However, deuterium enriched plant organics were not observed, at least in the leaves (**Figure 29**). The results were generally consistent with those observed in the Experimental Control and Trial 1, including the oxygen measurements. The normal group leaf and stem deuterium measured -156.6‰ and -128.2‰ respectively. This amounted to a -149.1‰ depletion in the leaves, and a -99.6‰ depletion in the stems from the leaf tissue water. The deuterium shift in the stems was the greatest of the three experiments, though it still remains outside of the expected range for heterotrophic metabolism. Given the results of the other trials, this could be evidence for deuterium enhanced organics in the stems, though the uncertainties regarding this experiment tend towards the side of caution. Interestingly, the silicone leaves plotted with the stems, something not observed in the other experiments. As the leaves wilted and yellowed, photosynthesis likely ceased, along with the transpiration stream or any foliar water uptake. If the Experimental Control and Trial 1 are considered, the leaf organic tissue isotope signatures could be a combination of the carbohydrates produced during photosynthesis, and the existing plant tissue. When no new heavily depleted carbohydrates are produced, only the existing and comparatively enriched organics are measured.

12.3 SRBT Field Experiments

SRB Technologies monitors their stack emissions in real-time, however these particular measurements do not differentiate between HTO and HT. Use of the portable active air sampler provides this distinction by quantifying HTO in the first bubbler exchange, and catalyzing HT conversion

to HTO in the second bubbler. It should be noted however, that the portable sampler only measures air concentrations in the immediate vicinity of its location. For example, emission signals from the stacks could be missed if the sampler is not placed within the plume, which is often unpredictable. This is likely what occurred with the sampler measurements on the exposure day. The spike at 9:45 AM was captured with the sampler while the second plume at approximately 2:45 PM was missed. The second spike was the result of a broken vial that released HT almost exclusively, through the stack (SRBT, personal communication). Instead of registering a spike in HT concentrations, there is an increase in HTO. The 9:45 AM emissions could have been the result of an early morning purge, and was derived from the shorter of the two stacks (SRBT, personal communication). Based on the portable active air sampler results, it must have been dominantly HT. Wind measurements taken from the SRBT weather station put the wind direction at 275 degrees from north, or a westward direction during the morning stack purge. Conversely, at 2:45 PM during the second emission spike, the wind travelled in a northern direction (344 degrees from North). Therefore, it is possible that the second plume was missed by the portable air sampler. This suggests that the HTO peak occurring shortly before the HT release at 2:45 PM was the result of HTO evaporating from the natural soil over the course of the day and being captured over the sampler time interval.

During the 9:00 AM to 12:00 PM interval, the tritium level in both leaves and stems is elevated, with the leaves being greater than the latter. Tritium concentrations in the potted plant soil remain low for the same interval, suggesting that the dominant pathway for tritium contamination is through the leaves, and not the soil and roots, in this instance. Further, this time interval represents the highest tritium concentrations throughout the sampling period, as well as greater atmospheric HT concentrations relative to HTO, and therefore there must be a mechanism responsible for HT conversion within the leaf or at the leaf's surface to explain the elevated tritium.

The low solubility of HT in water means that HT gas is not readily absorbed by plants (Belot, 1986). It must be converted to HTO before uptake by vegetation. The potted arugula plants brought on site to SRBT were watered with low tritium water prior to being brought on site for the sampling period. During exposure, the maximum HTO concentration in potted soil was 12 Bq/L, with only a slight increase of 3 Bq/L from the Starting Water tritium concentration of 8 Bq/L. These measurements are far below tritium concentrations in their respective plant leaves or stems. HT conversion to HTO in the soil must have been minimal, thereby making a reduced contribution to plant tritium levels through the transpiration pathway.

The lack of potted plant soil HTO could be attributed to the packing of the soil. The deposition velocity of HT to soil is 10-100 times lower than HTO (HT: 0.01-0.00001m/s) and is dependent on meteorological conditions, as well as soil properties (Murphy 1993, Koarashi *et al.*, 2001). Further, HT conversion to HTO depends on the hydrogenase activity of the soil. The potted plant soils were well packed and consolidated after repeated watering during growth off-site in the growth chamber. This could have limited the available pore space for HT penetration and conversion. Indeed, when compared with loose soils brought to SRBT during the same sampling period, soil HTO ranges from 42-136 Bq/L versus the potted plant soils, peaking at 1:00 PM prior to the second release.

The purpose of exposing plants to deuterium gas in a growth chamber was to examine how deuterium behaves in the plant following atmospheric exposure in controlled conditions, and then attempt to apply those results to what has been observed on site at SRB Technologies and in other tritium plant studies. The general deuterium results are consistent across experiments, with tissue free water in leaves enriched in both deuterium and oxygen-18 over the stems, and the stems over the soils. This trend extends to the tritium field exposure experiments, suggesting that the same processes acting on deuterium could be acting on tritium as well.

All of this was in an attempt to explain the high OBT:HTO ratios seen in previous studies at SRBT (Mihok *et al.*, 2016). This work observed that soils and plants watered with rain or low tritium waters had higher than expected OBT/HTO ratios (9.0 – 13.5, and a mean ratio of 8.3 in native grasses). Further, these values occurred at times when the tritium source was mainly atmospheric. The unique quality at SRBT is the high abundance of atmospheric HT. Therefore, it is possible that HT, and in particular, atmospheric HT, is playing a greater than expected role in OBT formation.

The results of the field exposure indicate that HT contributed directly to the HTO concentration in the plants, as HTO remained low both in the atmosphere and in the potted soils, even though the leaves and stems showed elevated tritium concentrations in the tissue free water. The issue then is identifying a mechanism that could contribute to HT conversion to HTO within the plant itself. Studies by Ichimasa *et al.* (1999) have demonstrated that some plants have the capacity to convert HT to HTO, though similarly, the method remains elusive. The current hypothesis suggests that hydrogen gas conversion is facilitated by hydrogenase enzymes associated with microbial activity (or derived from lysed cells) in soil media. Hydrogenase activity has yet to be identified in plants. The presence of microbes at the leaf surface however, has been confirmed (Ichimasa *et al.*, 1999; Lindow and Brandl, 2003). A potential mechanism for HT conversion could be by microbial mediation or abiontic hydrogenase enzymes at the leaf surface, with HTO subsequently diffusing through the stomata and into the leaf, in close proximity to the photosynthetic sites, thus contributing to the observed OBT/HTO ratios. At the leaf and air interface, and depending on air flow, there is a laminar boundary flow layer where air velocity slows, that plays a critical role in trace gas flux into and out of the leaf (Schuepp, 1993). Conversion of HT to HTO within this layer could concentrate the tritium concentration.

The phyllosphere is the microhabitat within the leaf boundary layer that supports microbial development. Although, research on microbial diversity and the specifics of their contribution to the

plant are scattered and limited. Microbial success is dependent on several factors, including but not limited to moisture at the leaf surface, as well nutrient availability. As such, microbial abundance has been shown to increase in cool, rainy seasons as opposed to the hot and dry (Lindow and Brandl, 2003). There is no known mechanism, enzymatic or otherwise, within the plant structure that has the capacity to convert hydrogen gas (or HT) to water or HTO. However, the presence of HT in the atmosphere, as well as the lack of HTO in the air and the soil during the early hours of exposure, suggest that this conversion is happening, and that it is not originating from the potted soils. The question is both how, and where this conversion is occurring. Assuming the conditions are adequate for microbial activity (sufficient moisture content, nutrient availability, large enough boundary layer thickness) it is proposed that microbial conversion of HT to HTO is occurring at the leaf surface, with HTO diffusing into the leaf, where the boundary layer serves to concentrate tritiated water. This HTO subsequently diffuses through the stomata and into the leaf where it is free to participate in photosynthesis or be transported to other locations in the plant where it can participate in other biochemical reactions, and is subject to the same isotopic shifts observed with deuterium.

13 **Conclusions**

This study sought to elucidate the mechanisms of HT transfer from the atmosphere to plants in the form of HTO. Tritium measurements in plants, taken at a tritium Beta-light facility in Pembroke, Ontario had previously shown unexplained elevated OBT:HTO ratios that did not fit current regulatory models of environmental tritium dynamics. An extensive long-term garden experiment at SRB Technologies in Pembroke found OBT:HTO ratios of 9.0 to 13.5, while the source of tritium was mainly atmospheric (Mihok *et al*., 2016). However, this particular site is subject to chronic, yet sporadic atmospheric tritium releases (HT and HTO), which complicates identifying both the tritium species and

pathway influencing OBT production over extended time periods. Of further consequence is the loss of observable effects of ever changing environmental influence on the overall plant tritium concentration.

To address these issues, potted arugula plants were brought to the SRBT site for a short, 24 hour exposure. Plants (leaves and stems, including roots) were sampled in regular intervals during the exposure experiment, and supplemented with atmospheric tritium measurements of HT and HTO. The air sampler measurements were then compared with stack emissions records from the SRBT facility. While these experiments could account for any immediate plant responses to atmospheric tritium, environmental factors remained uncontrolled.

To this end, deuterium was used as an analogue in laboratory experiments designed to control the dynamic nature of field conditions that could ultimately effect tritium incorporation into plant biomass (temperature, light, and humidity). These experiments served a secondary purpose to test the application of deuterium as an alternative to tritium in laboratory experiments, an often tedious exercise on account of permitting and cost. Soils were sampled as part of the potted plants, and deuterium measurements of the growth chamber atmosphere taken as well, though infrequently.

The final component of this study included pore water and organically bound tritium measurements with depth throughout two soil profiles at SRB Technologies. As soils are the figurative roots of plant water and nutrient uptake, characterizing tritium migration in the subsurface of a chronic exposure site could help elucidate some of the long term influence soils have on plant tritium concentrations. Quantifying HTO and OBT with depth in the SRBT soil profiles would contribute to understanding tritium mobility following chronic atmospheric exposure of HT and HTO, knowledge that is currently missing in the literature.

As expected, OBT generally decreased with depth in the SRBT cores while HTO increased towards the groundwater table (after a brief decrease near the surface). This reflects historic HTO inputs

to groundwater from drainage at the site. Previous practices at SRBT led to high tritium water entering the subsurface, which is believed to be responsible for elevated tritium concentrations in groundwater. The HTO results presented in this study are consistent with previous consultant reports, and given the hydraulic conductivity of the clay overburden, historic HTO inputs could explain the increase. Conversely, the decrease in OBT reflects the lack of organic content in the clay rich sediments with depth. Near the surface, lawn grass and plant detritus is abundant, accounting for higher OBT contents. However once the effects of surface input are limited, OBT concentrations drop. There is no evidence in these results to indicate abundant microbial OBT generation, or concentration in any particular mineral fraction.

The deuterium growth chamber experiments yielded results that were challenging to link to observations of tritium behavior in the field. While it was noted that deuterium gas does oxidize in the soils following atmospheric exposure in the growth chamber, the deuterium enrichment in the plant tissue waters was too low to confidently identify a dominant mechanism or pathway for deuterium enrichment in the plants. Across experiments, a steady evaporative enrichment was observed from the source and soil water, to stems, and then to leaves. Interestingly, stems are expected to maintain the same isotopic signature as the soil water, however this was not observed. The enriched stem waters were likely the result of bulk stem analysis not differentiating the source water in the xylem from the enriched waters of the phloem sap. This should be a consideration in future studies involving deuterium or tritium source studies in plants.

The leaves, as the site of evaporation, behaved expectantly, though any direct influence of deuterium in the atmosphere could not be observed within individual experiments. Only by comparing the Experimental Control and Environmental Controls with the deuterium exposed plants, could any enrichment be observed. That being said, the observed enrichment was so small in these experiments

that extrapolating to plant tritium dynamics is challenging. Future studies should consider using greater atmospheric D_2 gas concentrations.

While the deuterium depletions associated with photosynthesis were observed, there was no evidence of deuterium enrichment in the plant organic tissue, nor even the expected deuterium enrichment resulting from heterotrophic metabolism. Had the secondary metabolic isotopic shifts been observed, it may have been possible to calculate the expected, unaltered change based on the literature, and then compare with the hopefully enriched results. However the observation that the plant organics became more depleted relative to the tissue waters, while demonstrating the expected enrichment in oxygen, warrants further exploration. Perhaps the contribution of heterotrophic metabolism is so minimal when compared with photosynthesis that it has little impact on the organic deuterium (or tritium) signature. This would eliminate a potential pathway for elevated OBT:HTO ratios.

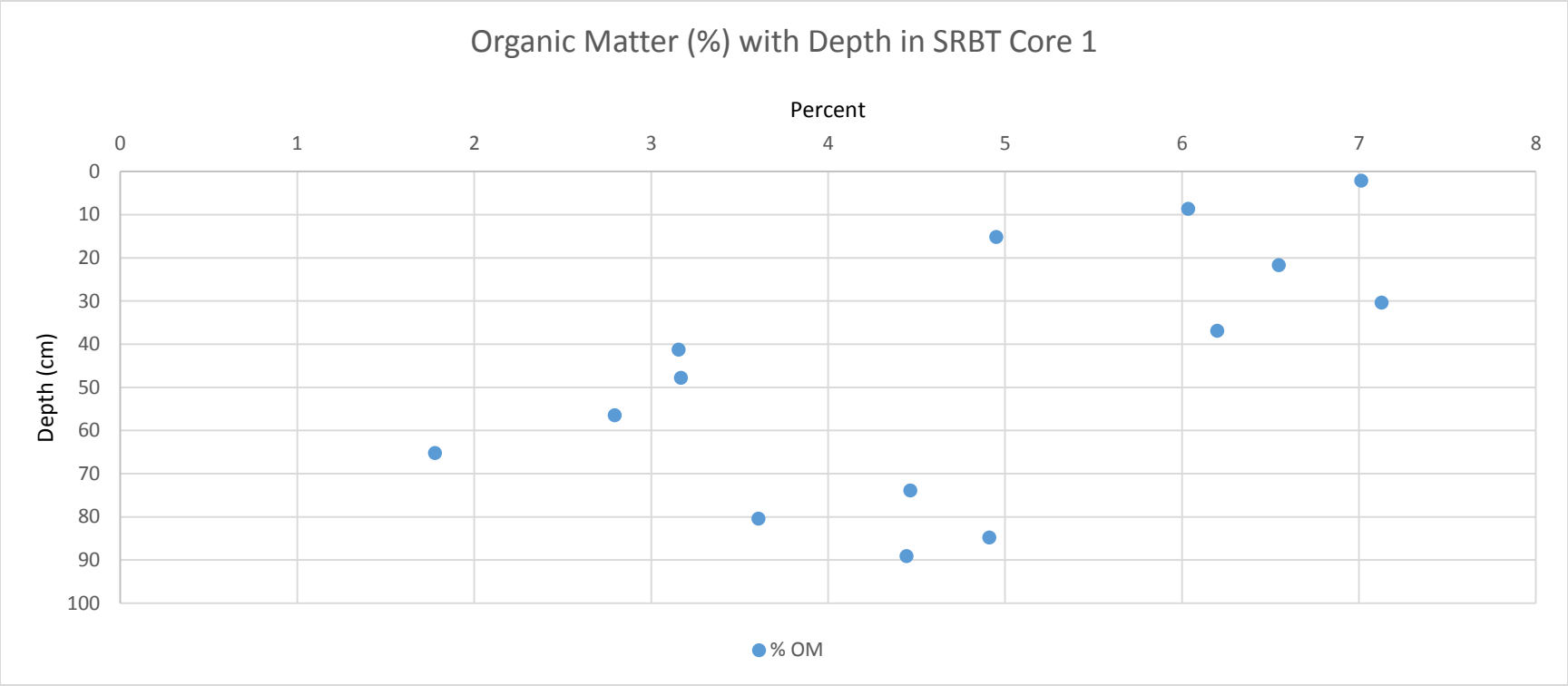
The SRBT field exposures were more enlightening, though the only major similarity to the deuterium experiments was greater tritium signatures in the leaves, versus the stems, and subsequently versus the soil water. Elevated TFWT in the leaves and stems was observed during atmospheric HT peaks which exceeded HTO in the air and soil. Soil pore water tritium was near background, limiting evidence of HT to HTO conversion and subsequent absorption by the plant roots. The results, when considered together, suggest that there is some HT oxidation mechanism occurring at the leaf surface, which ultimately leads to elevated TFWT, and perhaps greater OBT:HTO ratios. This suggests that, similar to soils, there is a hydrogenase-active microbial community on the leaf surface that mediates hydrogen, and tritium, gas oxidation. Increasing tritium concentrations in the leaf boundary could result in tritium diffusion via the stomata and into the leaf tissue free water pool. If this is the case, HTO concentrations would be higher than expected in the leaves, which could result in increased production of tritium labeled sugars, ready for transport and incorporation into plant biomass.

14 Future Research

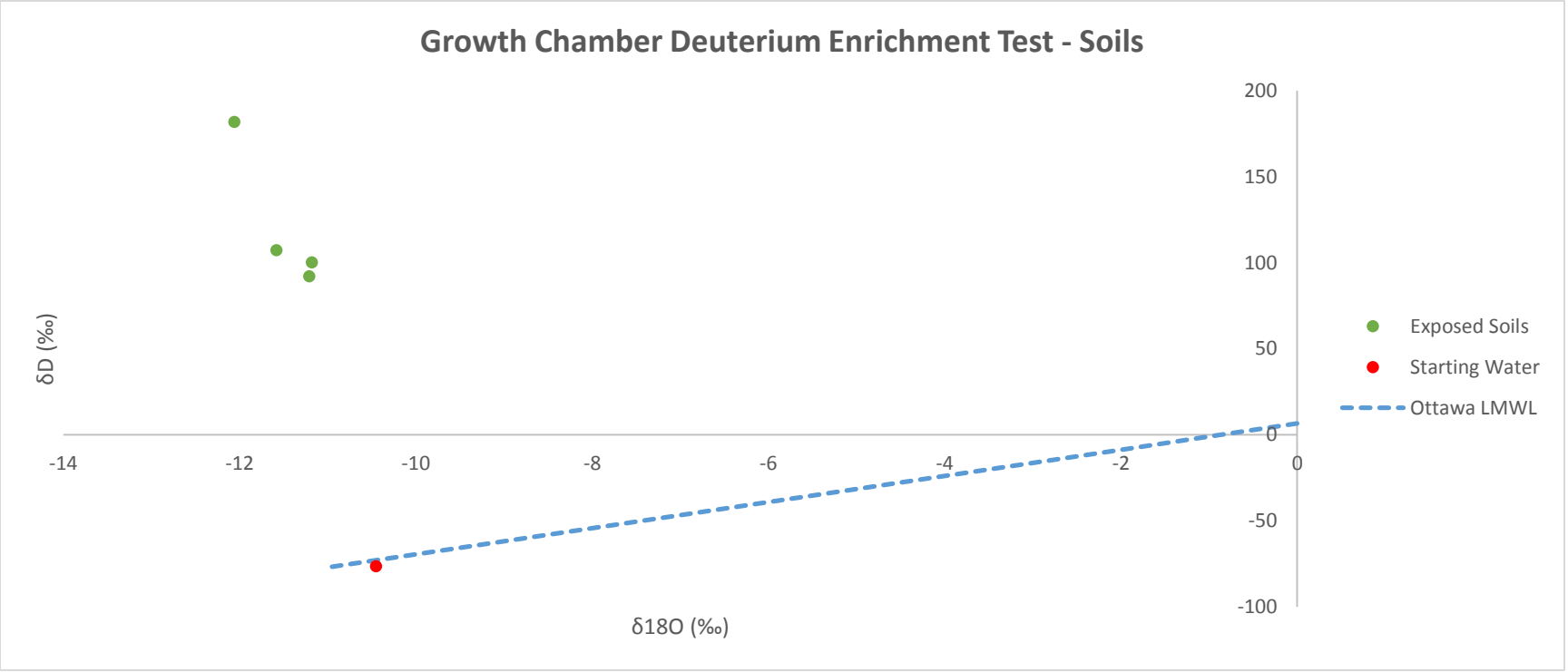
Although the deuterium exposure experiments did not produce ideal results, they highlighted some of the challenges in replicating tritium exposure in a controlled environment. Most notably, managing the D_2 concentration in the chamber air was difficult with respect to getting an enrichment signal in the soils and plants that was unquestionable. Any experiments of this nature should not hesitate to increase the ambient D_2 concentrations.

The seemingly pure foliar conversion of HT to HTO, when there was little HTO present in the atmosphere or plant soils is intriguing. While there was no evidence of phyllosphere activity presented in this study, the possibility of microbially mediated HT-HTO conversion within the leaf boundary layer should not be overlooked. This hypothesis could be explored further by confirming the presence of a microbial community on the leaf, and repeating the SRBT field exposures with natural and sterilized

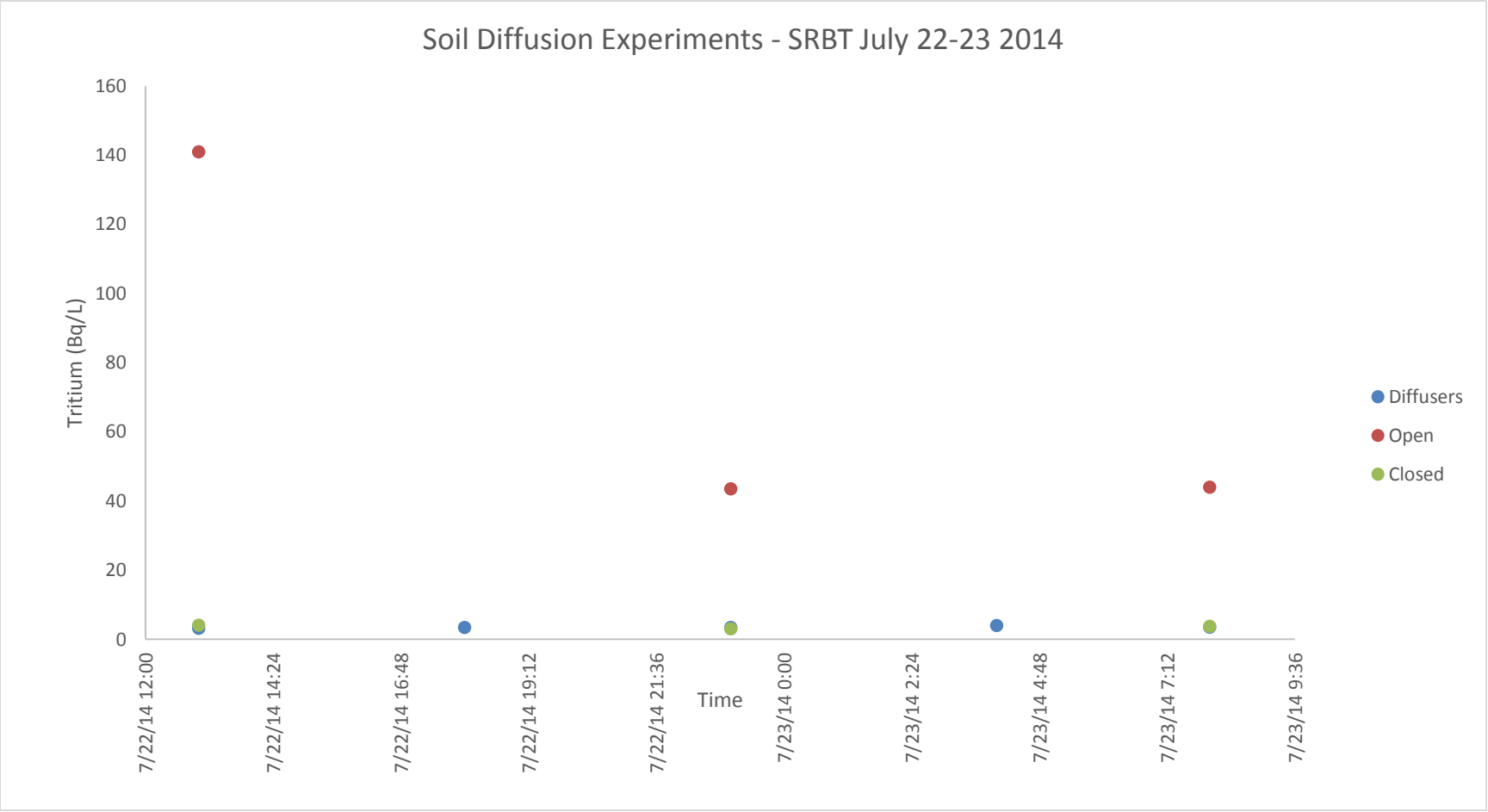
Appendix A



Appendix-A 1: Results of Loss on Ignition analysis of SRBT Core 1, to a depth of approximately 90 cm. Organic Matter generally decreases with depth, though there appears to be a slight spike around 80-90 cm.



Appendix-A 2: Results of an initial deuterium enrichment test in the growth chamber. Soils were exposed to deuterium gas diffusing into the chamber atmosphere using the D_2 lecture bottle diffuser. HD measured 1886.5‰ during the exposure, while HDO measured -60.9‰ (uncorrected for air volume moved through the bubblers).



Appendix-A 3: Soil diffusion experiments carried out at SRB Technologies on July 22-23 2014. Sets of soils in Isojars, each composed of one open container, one sealed container, and one sealed with a diffuser, were sampled throughout a 24 hour period. Elevated pore water tritium was only observed in the open Isojars, and peaked at 1:00 PM, after the 9:45 AM release and before the second 1:45 PM release. This peak coincides with the atmospheric HTO spike observed with the active air sampler.

Appendix B

Pembroke ON SRB Technologies Auger Core # 1 - Pore water Tritium Extraction									
Corrected Depth (cm)	Vial Weight (g)	Vial + Soil (g)	Soil Pre Extraction (g)	Tin Plate (g)	Soil + Tin Post-Extraction (g)	Soil + Tin Dry (g)	Dry Soil (g)	Pore Water Content (g)	Pore Water %
2.17	182.75	349.09	166.34	3.08	152.7	140.98	137.9	28.44	0.17
8.70	194.81	397.73	202.92	3.22	184.29	177.83	174.61	28.31	0.14
15.22	198.02	419.54	221.52	3.08	206.93	188.38	185.3	36.22	0.16
21.75	179.42	355.57	176.15	3.03	162.16	147.88	144.85	31.3	0.18
30.44	198.03	402.31	204.28	3.38	190.47	168.86	165.48	38.8	0.19
36.97	182.77	367.89	185.12	2.99	169.07	154.7	151.71	33.41	0.18
41.32	194.84	328.53	133.69	2.52	118.59	112.69	110.17	23.52	0.18
47.84	179.44	379.39	199.95	3	185.43	167.75	164.75	35.2	0.18
56.54	160.17	389.05	228.88	3.34	212.38	194.14	190.8	38.08	0.17
65.24	212.94	402	189.06	2.57	172.66	161.25	158.68	30.38	0.16
73.93	197.98	435.31	237.33	3.29	222.38	199.5	196.21	41.12	0.17
80.46	159.53	358.26	198.73	2.63	179.31	162.31	159.68	39.05	0.20
84.81	160.14	302.59	142.45	2.25	125.21	115.88	113.63	28.82	0.20
89.15	212.89	388.84	200.95	2.7	159.1	140.62	137.92	63.03	0.31
93.50	197.95	377.65	179.7	2.89	162.62	138.98	136.09	43.61	0.24
100.03	159.31	337.76	178.45	2.95	156.56	138.64	135.69	42.76	0.24
106.55	160.13	344.21	184.08	2.34	167.87	139.44	137.1	46.98	0.26
110.90	212.89	357.23	144.34	2.33	126.79	108.39	106.06	38.28	0.27
115.25	159.2	289.16	129.96	2.68	114.25	97.57	94.89	35.07	0.27

Appendix B 1: Pembroke ON SRB Technologies Auger Core # 1 - Pore water Tritium Extraction

Pembroke ON SRB Technologies Auger Core # 1 - OBT												
Mid-Point Depth (cm)	Trap Weight (Pre) (g)	Weight of Trap (Post) (g)	Water Extracted (g)	Post Trap + H3 Free H2O (g)	Weight of LSV (g)	H2O in LSV (g)	H2O Added (g)	Weight of Boat (g)	Weight of Sample (g)	Weight of Sample and Boat (Pre) (g)	Weight of Sample and Boat (Post)	Sample Combusted (g)
2.17	185.53	186.86	1.33	195.87	7.8	9.97	8.64	28.16	29.95	58.11	-	-
	207.75	207.91	0.16	217.93	7.76	10.04	9.88					
8.70	199.51	200.86	1.35	209.64	7.89	9.98	8.63	28.16	29.97	58.13	56.33	1.80
	194.12	194.19	0.07	204.25	7.93	10.03	9.96					
15.22	199.07	200.44	1.37	209.12	7.89	9.99	8.62	28.16	30.01	58.17	56.14	2.03
	194.55	194.66	0.11	204.6	7.88	9.95	9.84					
21.75	185.83	187.24	1.41	195.86	7.78	9.9	8.49	28.16	30.00	58.16	56.14	2.02
	207.76	207.92	0.16	217.94	7.85	10.02	9.86					
30.44	186.28	187.6	1.32	196.41	7.79	10.05	8.73	28.15	30.00	58.15	55.73	2.42
	207.32	207.47	0.15	217.36	7.87	9.97	9.82					
36.97	199.09	200.37	1.28	209.21	7.82	10	8.72	28.17	29.96	58.13	55.95	2.18
	194.57	194.66	0.09	204.6	7.86	9.9	9.81					
41.32	199.52	200.77	1.25	209.55	7.81	9.97	8.72	28.15	29.98	58.13	56.28	1.85
	194.13	194.22	0.09	204.2	7.79	9.97	9.88					
47.84	199.07	200.13	1.06	209.13	7.79	9.96	8.9	28.15	30.05	58.20	56.5	1.70
	194.36	194.47	0.11	204.52	7.88	10.02	9.91					
56.54	199.07	200.09	1.02	209.12	7.81	9.97	8.95	28.16	30.00	58.16	-	-
	194.32	194.43	0.11	204.32	7.85	9.85	9.74					
65.24	185.83	186.62	0.79	195.94	7.85	10	9.21	28.14	30.78	58.92	57.59	1.33
	207.56	207.77	0.21	217.62	7.91	9.94	9.73					
73.93	186.28	187.12	0.84	196.69	7.88	10.32	9.48	28.15	30.07	58.22	57.45	0.77

	207.12	207.29	0.17	217.14	7.87	9.91	9.74					
80.46	185.82	187.11	1.29	195.89	7.89	9.96	8.67	28.14	30.01	58.15	56.75	1.40
	207.55	207.61	0.06	217.64	7.9	9.98	9.92					
84.81	186.31	187.71	1.4	196.55	7.82	10.14	8.74	28.15	30.04	58.19	-	-
	207.12	207.16	0.04	217.29	7.92	10.07	10.03					
89.15	199.05	200.3	1.25	209.08	7.9	9.91	8.66	27.84	29.96	57.80	56.18	1.62
	194.36	194.43	0.07	204.44	7.87	10.03	9.96					
93.5	186.29	187.74	1.45	196.14	7.87	9.69	8.24	27.81	30.08	57.89	56.14	1.75
	207.12	207.22	0.1	217.4	7.82	10.17	10.07					
100.03	199.09	200.42	1.33	209.34	7.93	10.07	8.74	27.82	30.00	57.82	56.51	1.31
	194.34	194.62	0.28	204.44	7.86	9.99	9.71					
106.55	185.85	186.59	0.74	195.96	7.95	10.03	9.29	27.81	30.04	57.85	56.66	1.19
	207.57	207.65	0.08	218.01	7.9	10.37	10.29					
110.9	186.29	187.26	0.97	196.48	7.91	10.09	9.12	27.79	30.07	57.86	56.47	1.39
	207.12	207.21	0.09	217.24	7.93	9.99	9.9					
115.25	199.54	200.48	0.94	209.61	7.9	9.93	8.99	27.8	30.03	57.83	56.58	1.25
	193.89	194.02	0.13	204.05	7.84	10.02	9.89					

Appendix B 2: Pembroke ON SRB Technologies Auger Core # 1 - OBT

Pembroke ON SRB Auger Core #1 - Tritium Analysis Results - Pore water			
Standard	**Corrected Depth (cm)**	**Field Tritium (Bq/L)**	**Field Tritium Error (Bq/L)**
2	2.17	614.9591	13.34373
2	8.70	391.1909	10.19282
2	15.22	380.776	10.11326
2	21.75	373.8589	10.0148
2	30.44	402.8807	10.44966
2	36.97	408.3082	10.53454
2	41.32	450.2965	11.15006
2	47.84	472.0561	11.46417
2	56.54	510.3861	12.00761
2	65.24	646.8846	13.86296
2	73.93	881.8765	16.78352
2	80.46	840.1049	16.36595
2	84.81	885.9192	16.94179
2	89.15	976.9232	18.06911
2	93.50	1067.641	19.17706
2	100.03	1240.419	21.25113
2	106.55	1358.307	22.64365
2	110.90	1508.206	24.39798
2	115.25	1540.992	24.92027

Appendix B 3: Pembroke ON SRB Auger Core #1 - Tritium Analysis Results - Pore water

Pembroke ON SRB Auger Core #1 - Tritium Analysis Results – Pore water			
Standard	**Corrected Depth (cm)**	**Field Tritium (Bq/L)**	**Field Tritium Error (Bq/L)**
5	2.17	580.2978	57.12829
5	8.70	368.9493	36.70399
5	15.22	329.0951	31.79494
5	21.75	278.4955	25.80976
5	30.44	299.6758	27.68591
5	36.97	303.9475	28.07516
5	41.32	335.4343	30.90091
5	47.84	351.7873	32.36276
5	56.54	380.2054	34.89888
5	65.24	481.1789	43.89178
5	73.93	658.8396	59.78495
5	80.46	625.9757	56.86111
5	84.81	659.5303	59.83867
5	89.15	727.2277	65.88527
5	93.50	795.1564	71.97006
5	100.03	923.2742	83.39414
5	106.55	1012.568	91.4328
5	110.90	1123.643	101.3317
5	115.25	1172.596	107.2834

Appendix B 4: Pembroke ON SRB Auger Core #1 - Tritium Analysis Results – Pore water.

Pembroke ON SRB Auger Core #1 - Tritium Analysis Results - OBT					
Standard	**Mid Point Depth (cm)**	**Field Tritium (Bq/L)**	**Field Tritium Error (Bq/L)**	**Field Tritium (Bq/Kg)**	**Field Tritium Error (Bq/Kg)**
2	2.17	5172.673131	14.59348233	190.4308995	0.537256056
2		2992.037132	3.669227821		
2	8.70	949.5462325	5.77366475	36.80504891	0.223791119
2		387.7216839	1.74882376		
2	15.22	470.5234294	4.18121722	17.96794055	0.159668696
2		375.8177192	1.838880046		
2	21.75	485.0499463	4.310248178	18.74649891	0.166585036
2		282.7114642	1.862051697		
2	30.44	567.4715922	4.461733475	20.22630096	0.159028866
2		458.7829887	2.001801624		
2	36.97	412.0459293	3.814980291	14.42695428	0.133573814
2		193.370883	1.671030577		
2	41.32	275.1459738	3.204683886	9.453790478	0.110110316
2		305.9726048	1.736404131		
2	47.84	209.6100734	2.730849651	6.092247716	0.079371245
2		177.658743	1.684887024		
2	56.54	222.0658231	2.747236029	6.294064172	0.077865561
2		245.094544	1.735394807		
2	65.24	252.9244265	2.63300793	5.448435174	0.056719603
2		200.2597553	1.828841562		
2	73.93	146.0967185	2.252875903	3.374075666	0.052029736
2		146.1326301	1.719391313		
2	80.46	85.78497904	2.21380065	2.962934533	0.076462645
2		230.3234465	1.136385599		
2	84.81	121.9100089	2.49183696	4.53208476	0.092635678
2		149.5260501	0.020120888		
2	89.15	98.14980664	2.325309681	2.810586075	0.066586815
2		109.9032587	1.148220973		

2	93.5	114.61836	2.550964901	4.184296947	0.093126395
2		121.9877789	0.684139615		
2	100.03	143.2671865	2.629912944	4.829569371	0.088654962
2		219.6027326	1.992170595		
2	106.55	147.5487905	2.249045839	2.707062572	0.041263014
2		128.8645721	1.089802411		
2	110.9	118.6446604	2.274648956	2.812233811	0.053915993
2		62.9244811	1.128168622		
2	115.25	80.55337525	2.074027585	1.841056038	0.047402123
2		44.30640747	0.029316782		

Appendix B 5: Pembroke ON SRB Auger Core #1 - Tritium Analysis Results – OBT.

Pembroke ON SRB Auger Core #1 -Tritium Analysis Results – OBT Extractions					
Standard	**Mid Point Depth (cm)**	**Field Tritium (Bq/L)**	**Field Tritium Error (Bq/L)**	**Field Tritium (Bq/Kg)**	**Field Tritium Error (Bq/Kg)**
5	2.17	3873.23921	47.59418825	142.5925065	1.752170271
5		2234.707218	4.205472458		
5	8.70	711.3683944	9.652386409	26.80427519	0.363700754
5		301.9984202	1.327675114		
5	15.22	351.2451365	5.324294814	12.98926432	0.196895744
5		284.0376754	1.404371567		
5	21.75	363.6966777	5.660694784	14.08645194	0.219246174
5		214.7013015	1.427859258		
5	30.44	424.4881581	6.007983694	15.57000605	0.220369734
5		349.6442172	1.578411264		
5	36.97	313.5826562	4.638610316	11.07614656	0.163841739
5		147.9037751	1.278815254		
5	41.32	208.2823369	3.380194812	6.927258263	0.112421835
5		233.4948285	1.338485347		
5	47.84	160.1084504	2.589892039	4.277143095	0.069186472
5		134.8859549	1.293944771		
5	56.54	167.9514021	2.596014433	4.252980582	0.065738058
5		185.3030827	1.338148203		
5	65.24	192.3714655	2.427909249	3.605038219	0.045498981
5		148.7284395	1.420639467		
5	73.93	110.4899141	1.896039752	3.086515723	0.052965527
5		112.0070956	1.32318129		
5	80.46	64.90211043	1.845634311		
5		145.7279726	0.874885394		
5	84.81	92.80393273	2.220642523	4.325083416	0.103491995
5		121.2415613	0.056161414		
5	89.15	73.32292285	1.577476102	3.05920072	0.065815925
5		83.93615604	0.839192024		

5	93.5	85.71158526	2.082818719	4.131708731	0.100401833
5		96.71457618	0.581487594		
5	100.03	106.4238317	2.153148592	4.718123208	0.095456254
5		166.7458701	1.555049429		
5	106.55	110.9860666	1.838815495	2.734010961	0.045297053
5		99.42025448	0.89127916		
5	110.9	88.5490114	1.867225365	2.856419723	0.060233076
5		46.32394297	0.941821588		
5	115.25	59.97864001	1.629814973	1.877453267	0.051016519
5		38.59213862	0.116066177		

Appendix B 6: Pembroke ON SRB Auger Core #1 -Tritium Analysis Results – OBT Extractions.

Pembroke ON SRB Auger Core #1 Loss on Ignition					
Midpoint Depth	**Crucible Weight**	**Sample Pre**	**Crucible + Sample Post**	**Sample Post**	**% OM**
2.17	29.08	4.99	33.72	4.64	7.01
8.70	37.21	4.97	41.88	4.67	6.04
15.22	31.63	5.05	36.43	4.8	4.95
21.75	29.2	5.04	33.91	4.71	6.55
30.44	32.1	5.05	36.79	4.69	7.13
36.97	29.1	5	33.79	4.69	6.20
41.32	37.18	5.07	42.09	4.91	3.16
47.84	32.08	5.05	36.97	4.89	3.17
56.54	29.1	5.01	33.97	4.87	2.79
65.24	31.64	5.06	36.61	4.97	1.78
73.93	18.83	5.15	23.75	4.92	4.47
80.46	18.77	4.99	23.58	4.81	3.61
84.81	29.17	5.09	34.01	4.84	4.91
89.15	32.09	4.95	36.82	4.73	4.44

Appendix B 7: Pembroke ON SRB Auger Core #1 - Loss on Ignition.

Pembroke ON SRB Technologies Auger Core # 2 - Pore water Tritium Extractions									
Mid Point Depth (cm)	**Vial Weight (g)**	**Vial + Soil (g)**	**Soil Pre Extraction (g)**	**Tin Plate (g)**	**Soil + Tin Post-Extraction (g)**	**Soil + Tin Dry (g)**	**Dry Soil (g)**	**Pore Water Content (g)**	**Pore Water %**
5.5	182.13	363.83	181.7	3.76	162.92	147.75	143.99	37.71	0.21
15	194.8	393.29	198.49	3.42	170.52	157.59	154.17	44.32	0.22
22.5	179.4	380.2	200.8	2.81	179.36	150.49	147.68	53.12	0.26
30	182.73	399.2	216.47	3.51	190.6	164.91	161.4	55.07	0.25
37	159.03	396.76	237.73	3.81	211.15	176.97	173.16	64.57	0.27
45	194.8	376.7	181.9	3.22	162.45	137.87	134.65	47.25	0.26
53.5	179.4	349.55	170.15	3.38	148.9	129.76	126.38	43.77	0.26
59	182.75	356.71	173.96	2.98	152.59	131.2	128.22	45.74	0.26
63	159.05	321.11	162.06	3.46	142.52	124.75	121.29	40.77	0.25
69.5	194.81	346.1	151.29	3.2	133.63	116.17	112.97	38.32	0.25
75	179.42	300.23	120.81	3.37	103.81	94.03	90.66	30.15	0.25
79.5	182.74	339.72	156.98	2.51	138.72	120.71	118.2	38.78	0.25
86	159.05	341.14	182.09	3.34	161.36	140.16	136.82	45.27	0.25
92	194.79	371.25	176.46	2.9	159.05	136.97	134.07	42.39	0.24
97.5	179.4	338.38	158.98	2.46	138.97	123.73	121.27	37.71	0.24
101.5	182.76	316.98	134.22	3.45	115.62	104.76	101.31	32.91	0.25
107	159.06	323.84	164.78	3.55	145.78	127.67	124.12	40.66	0.25
113	194.83	352.62	157.79	3.6	142.53	122.69	119.09	38.7	0.25
118	179.43	313.24	133.81	3.79	122.57	103.64	99.85	33.96	0.25

Appendix B 8: Pembroke ON SRB Technologies Auger Core # 2 - Pore water Tritium Extractions.

Pembroke ON SRB Technologies Auger Core # 2 - OBT												
Mid Point Depth (cm)	Trap Weight (Pre) (g)	Weight of Trap (Post) (g)	Water Extracted (g)	Post Trap + H3 Free H2O (g)	Weight of LSV (g)	H2O in LSV (g)	H3-Free Added	Weight of Boat (g)	Weight of Sample (g)	Weight of Sample and Boat (Pre) (g)	Weight of Sample and Boat (Post)	Sample Combusted (g)
5.5	199.14	200.49	1.35	209.27	7.87	9.94	8.59	28.83	29.98	58.81	55.5	3.31
	194.54	194.73	0.19	204.83	7.91	9.66	9.47					
15	186.35	187.65	1.3	196.37	7.94	9.9	8.6	28.37	30.12	58.49	55.97	2.52
	207.39	207.67	0.28	217.57	7.79	9.94	9.66					
22.5	199.11	200.66	1.55	209.41	7.9	10.08	8.53	27.86	30.03	57.89	55.92	1.97
	194.6	194.72	0.12	204.66	7.8	9.93	9.81					
30	186.3	187.68	1.38	196.46	7.76	10.01	8.63	28.16	30.15	58.31	56.19	2.12
	207.35	207.51	0.16	217.76	7.9	10.28	10.12					
37	199.1	200.5	1.4	209.2	7.9	9.94	8.54	28.15	30.03	58.18	55.93	2.25
	194.58	194.9	0.32	204.83	7.89	9.96	9.64					
45	199.12	200.61	1.49	209.62	7.85	10.36	8.87	28.16	29.95	58.11	55.57	2.54
	194.6	194.71	0.11	204.61	7.82	9.87	9.76					
53.5	185.9	186.95	1.05	196.14	7.9	10.1	9.05	28.15	30.01	58.16	-	-
	207.85	207.97	0.12	218.28	7.88	10.29	10.17					
59	199.16	200.63	1.47	209.4	7.79	10.14	8.67	28.16	30.06	58.22	56.56	1.66
	194.65	194.75	0.1	204.68	7.94	9.96	9.86					
63	185.89	187.22	1.33	196.02	7.95	10.02	8.69	28.16	30.03	58.19	56.32	1.87
	207.79	207.88	0.09	217.86	7.9	9.95	9.86					
69.5	185.9	187.21	1.31	196.14	7.89	10.07	8.76	28.16	30.06	58.22	56.24	1.98
	207.81	207.92	0.11	217.87	7.82	9.93	9.82					
75	185.93	187.36	1.43	196.07	7.85	9.98	8.55	28.16	30.01	58.17	56.57	1.60
	207.82	207.89	0.07	218.13	7.83	10.2	10.13					
79.5	185.89	186.99	1.1	196.04	7.95	10.05	8.95	28.15	30.05	58.20	-	-

	207.78	208.02	0.24	218.04	7.9	10.15	9.91					
86	186.3	187.51	1.21	196.53	7.9	10.07	8.86	28.15	30.06	58.21	56.26	1.95
	207.34	207.56	0.22	217.45	7.83	9.84	9.62					
92	186.31	187.68	1.37	196.46	7.88	10.03	8.66	28.16	30.05	58.21	54.7	3.51
	207.37	207.43	0.06	217.37	7.78	9.9	9.84					
97.5	185.84	187.16	1.32	186.6	7.92	10.61	9.29	28.16	30.07	58.23	56.68	1.55
	207.8	207.97	0.17	217.93	7.94	9.97	9.8					
101.5	185.86	187.3	1.44	195.86	7.74	9.9	8.46	28.15	29.97	58.12	56.44	1.68
	207.79	207.86	0.07	218.05	7.92	10.16	10.09					
107	185.85	187.1	1.25	195.88	7.86	9.95	8.7	28.17	29.96	58.13	56.4	1.73
	207.8	207.85	0.05	217.98	7.81	10.09	10.04					
113	185.83	187.32	1.49	195.83	7.8	9.88	8.39	28.16	30.00	58.16	56.37	1.79
	207.77	207.9	0.13	218.01	7.88	10.07	9.94					
118	199.5	201.03	1.53	209.59	7.82	10	8.47	28.16	30.00	58.16	56.7	1.46
	194.09	194.17	0.08	204.18	7.92	9.98	9.9					

Appendix B 9: Pembroke ON SRB Technologies Auger Core # 2 – OBT.

Pembroke ON SRB Technologies Auger Core # 2 - Tritium Analysis Results – Pore water			
Standard	**Mid Point Depth (cm)**	**Field Tritium (Bq/L)**	**Field Tritium Error (Bq/L)**
2	5.5	484.7243845	11.5551478
2	15	440.312672	10.91792695
2	22.5	425.6061112	10.7037869
2	30	478.5759146	11.46823645
2	37	560.4673636	12.60851368
2	45	714.658328	14.65823077
2	53.5	873.9131594	16.68071737
2	59	1039.323664	18.71628368
2	63	1126.095865	19.76420659
2	69.5	1202.831746	20.68074315
2	75	1334.797154	22.24198591
2	79.5	1485.13112	23.99526939
2	86	1597.757311	25.29705622
2	92	1693.279003	26.39474091
2	97.5	1779.185523	27.3748484
2	101.5	1800.541212	27.61973125
2	107	1807.303801	27.69600054
2	113	1822.119181	27.8639423
2	118	1842.852427	28.10122321

Appendix B 10: Pembroke ON SRB Technologies Auger Core # 2 - Tritium Analysis Results – Pore water.

Pembroke ON SRB Technologies Auger Core # 2 - Tritium Analysis Results – Pore water			
Standard	**Mid Point Depth (cm)**	**Field Tritium (Bq/L)**	**Field Tritium Error (Bq/L)**
5	5.5	457.7997758	45.31373053
5	15	415.8441088	41.25634468
5	22.5	401.3434238	39.82456011
5	30	451.8102882	44.72249483
5	37	528.6485124	52.12444891
5	45	674.7397986	66.26863386
5	53.5	824.877717	80.77113314
5	59	980.1285303	95.72449816
5	63	1062.322212	103.6840701
5	69.5	1134.509847	110.6448103
5	75	1259.861788	122.7968813
5	79.5	1401.0623	136.4046818
5	86	1507.627371	146.7144928
5	92	1597.017433	155.3177654
5	97.5	1678.784181	163.2479178
5	101.5	1698.465653	165.1282004
5	107	1705.578368	165.8444924
5	113	1719.325335	167.1677905
5	118	1739.102677	169.0921462

Appendix B 11: Pembroke ON SRB Technologies Auger Core # 2 - Tritium Analysis Results – Pore water

Pembroke ON SRB Technologies Auger Core # 2 - Tritium Analysis Results – OBT					
Standard	Mid Point Depth (cm)	Field Tritium (Bq/L)	Field Tritium Error (Bq/L)	Field Tritium (Bq/Kg)	Field Tritium Error (Bq/Kg)
2	5.5	12803.9104	27.16403854	456.9010653	0.969334974
2	5.5	5052.312165	5.085697173		
2	15	1111.764747	6.128655746	37.27026874	0.205454119
2	15	503.2180999	2.400915298		
2	22.5	334.9406034	3.791734732	12.71458354	0.143936947
2	22.5	156.0714042	1.187334469		
2	30	232.5775272	3.136071235	7.937163599	0.107024573
2	30	236.1402211	1.84518041		
2	37	101.2427632	2.407169832	3.437955728	0.081741579
2	37	56.24520899	1.142935007		
2	45	85.56374523	2.313012085	3.151032527	0.08518066
2	45	109.6964097	1.687311137		
2	53.5	63.19852852	2.01627294	1.642391421	0.05239852
2	53.5	167.948759	1.192852168		
2	59	75.11954302	2.242244943	2.707619726	0.080819803
2	59	118.4839694	1.058507786		
2	63	99.40873461	2.359281804	3.295110749	0.078203338
2	63	52.22261517	0.370842732		
2	69.5	73.76642858	2.178428902	2.400457288	0.070888962
2	69.5	74.41693943	1.030938165		
2	75	68.53991118	2.189708847	2.450904616	0.078301349
2	75	12.3128912	-0.29476405		
2	79.5	63.22022941	2.032857384	1.742518622	0.056030987
2	79.5	92.00111145	1.745975983		
2	86	36.43659413	1.884239574	1.102040764	0.056989652
2	86	34.97110654	0.403750444		
2	92	36.98262926	1.923507398	1.281029731	0.066627771

2	92	166.4372322	0.42997079		
2	97.5	33.10971087	1.869909898	1.108681438	0.062614089
2	97.5	32.62643077	0.369326152		
2	101.5	49.46027721	2.046492395	1.79377256	0.074220003
2	101.5	134.8072304	4.64871495		
2	107	68.85846805	2.127101041	2.164027766	0.066848796
2	107	75.00950154	0.584084938		
2	113	45.55126631	1.983696476	1.707502261	0.07435943
2	113	98.81946424	0.553774882		
2	118	41.23314275	1.950903954	1.569192097	0.074244718
2	118	149.7357167	1.085077778		

Appendix B 12: Pembroke ON SRB Technologies Auger Core # 2 - Tritium Analysis Results – OBT

Pembroke ON SRB Technologies Auger Core # 2 - Tritium Analysis Results – OBT					
Standard	**Mid Point Depth (cm)**	**Field Tritium (Bq/L)**	**Field Tritium Error (Bq/L)**	**Field Tritium (Bq/Kg)**	**Field Tritium Error (Bq/Kg)**
5	5.5	8320.853294	119.9229254	296.9254404	4.279388925
5	5.5	3271.429423	7.75173179		
5	15	723.573844	10.97032287	24.25674289	0.367763848
5	15	326.1258286	2.055939637		
5	22.5	217.5422472	4.520982891	8.258058434	0.171619726
5	22.5	99.09074881	0.894462747		
5	30	151.3683245	3.225785567	5.165740514	0.11008625
5	30	153.3253982	1.422481451		
5	37	66.10696789	2.07239033	2.244830365	0.070373289
5	37	34.90675386	0.874105928		
5	45	55.30233776	1.935074332	2.036603992	0.071262451
5	45	69.72467943	1.28100458		
5	53.5	40.97849852	1.596318103	1.064941478	0.041484813
5	53.5	107.4577403	0.897763961		
5	59	48.28471721	1.850357584	1.740381364	0.066694558
5	59	76.81467916	0.807514954		
5	63	71.95507038	1.904342214	2.385101539	0.063123412
5	63	37.46768187	0.280616363		
5	69.5	53.26302447	1.690909113	1.733249362	0.055024422
5	69.5	53.47763285	0.770250091		
5	75	49.73051954	1.709312245	1.778303441	0.061122946
5	75	15.7773531	-0.180141493		
5	79.5	46.16362555	1.541326257	1.272392997	0.042483074
5	79.5	64.80856575	1.267740084		
5	86	26.04947462	1.38757618	0.787877781	0.04196785
5	86	23.77003243	0.249421487		
5	92	27.1905435	1.436304818	0.941844734	0.049751713

5	92	114.478603	0.26899437		
5	97.5	23.77897252	1.375288715	0.796240884	0.046051658
5	97.5	22.36535215	0.18591725		
5	101.5	35.1687312	1.544056885	1.275462018	0.055998208
5	101.5	80.70794075	3.293744964		
5	107	49.54729976	1.634248899	1.557132121	0.051359841
5	107	57.41311953	0.446834898		
5	113	34.29217133	1.558311158	1.285451862	0.05841374
5	113	78.22508119	0.469915244		
5	118	30.97119133	1.52160054	1.178657396	0.057906902
5	118	121.0354961	0.836961608		

Appendix B 13: Pembroke ON SRB Technologies Auger Core # 2 - Tritium Analysis Results – OBT

Russell Ontario Background Site Auger Core - Pore water Tritium Extractions									
Mid Point Depth (cm)	**Vial Weight (g)**	**Vial + Soil (g)**	**Soil Pre Extraction (g)**	**Tin Plate (g)**	**Soil + Tin Post-Extraction (g)**	**Soil + Tin Dry (g)**	**Dry Soil (g)**	**Pore Water Content (g)**	**Pore Water %**
11	197.94	334.52	136.58	1.98	-	99.88	97.9	38.68	0.28
27.25	159.18	289.45	130.27	2.66	120.81	96.51	93.85	36.42	0.28
35.25	182.7	301.96	119.26	2.91	126.28	122.34	119.43	-0.17	0.00
42	160.12	309.04	148.92	2.45	101.8	91.21	88.76	60.16	0.40
53	182.69	411.86	229.17	2.52	206.93	200.42	197.9	31.27	0.14
70	212.88	456.59	243.71	2.53	224.27	212.01	209.48	34.23	0.14

Appendix B 14: Russell Ontario Background Site Auger Core - Pore water Tritium Extractions

Russell Ontario Background Site Auger Core - OBT Extractions													
Depth (cm)	**Trap Weight (Pre) (g)**	**Weight of Trap (Post) (g)**	**Water Extracted (g)**	**Post Trap + H3 Free H2O (g)**	**Weight of LSV (g)**	**H2O in LSV (g)**	**H3-Free Added**	**LSV #**	**Weight of Boat (g)**	**Weight of Sample (g)**	**Weight of Sample and Boat (Pre) (g)**	**Weight of Sample and Boat (Post)**	**Sample Combusted (g)**
11	199.6	201	1.4	209.83	7.91	10.07	8.67	5-18	27.81	29.99	57.80	55.86	1.94
	193.94	194.01	0.07	204.01	7.85	9.97	9.9	5-19					
27.25	185.9	186.89	0.99	196.14	7.94	10.15	9.16	5-20	27.8	29.97	57.77	56.6	1.17
	207.61	207.75	0.14	217.74	7.94	9.99	9.85	5-21					
35.25	199.09	200.21	1.12	209.38	7.89	10.1	8.98	5-22	27.81	29.96	57.77	56.12	1.65
	194.36	194.43	0.07	204.49	7.79	10	9.93	5-23					
42	185.88	187.22	1.34	196.03	7.82	9.95	8.61	5-24	27.81	29.97	57.78	55.63	2.15

	207.59	207.7	0.11	217.98	7.89	10.25	10.14	5-25				
53	186.32	187.1	0.78	196.4	7.91	9.91	9.13	6-1	27.81	29.98	57.79	55.63	2.16
	207.14	207.18	0.04	217.35	7.88	10.08	10.04	6-2				
70	199.56	200.26	0.7	209.73	7.87	10.03	9.33	6-3	27.81	30.06	57.87	?	?
	193.9	193.95	0.05	204.11	7.87	10.01	9.96	6-4				

Appendix B-15: Russell Ontario Background Site Auger Core - OBT Extractions

Russell Ontario Background Site Auger Core - Tritium Analysis Results – Pore water			
Standard	**Mid Point Depth (cm)**	**Field Tritium (Bq/L)**	**Field Tritium Error (Bq/L)**
2	11	1.98074988	1.670475202
2	27.25	1.866017671	1.663496973
2	35.25	3.482087988	1.765778527
2	42	3.311756462	1.757220148
2	53	3.470813683	1.769291091
2	70	2.898614183	1.728316819

Appendix B 16: Russell Ontario Background Site Auger Core - Tritium Analysis Results – Pore water

Russell Ontario Background Site Auger Core - Tritium Analysis Results – Pore water			
Standard	**Mid Point Depth (cm)**	**Field Tritium (Bq/L)**	**Field Tritium Error (Bq/L)**
5	11	1.559614996	1.323513
5	27.25	1.453660418	1.316097
5	35.25	2.690221516	1.409636
5	42	2.610629985	1.404941
5	53	2.727074002	1.416833
5	70	2.304855925	1.379919

Appendix B 17: Russell Ontario Background Site Auger Core - Tritium Analysis Results – Pore water

Russell Ontario Background Site Auger Core - Tritium Analysis Results - OBT					
Standard	**Mid Point Depth (cm)**	**Field Tritium (Bq/L)**	**Field Tritium Error (Bq/L)**	**Field Tritium (Bq/Kg)**	**Field Tritium Error (Bq/Kg)**
2	11	12.74399476	1.092539392	0.426434897	0.036558154
2	11	35.29604	1.68073242		
2	27.25	13.37914934	0.533975794	0.318395424	0.012707493
2	27.25	22.33385589	1.6079982		
2	35.25	7.069759567	0.54786454	0.264666811	0.012207133
2	35.25	189.7940196	0.583711708		
2	42	12.36493172	1.647916389	0.329514131	0.043915466
2	42	79.73314803	0.033219593		
2	53	8.570302705	0.521216305	0.192551615	0.011710326
2	53	459.2245782	0.085095446		
2	70	6.486989207	1.051730717	0.129843871	0.02105149
2	70	74.28850538	0.046874189		

Appendix B 18: Russell Ontario Background Site Auger Core - Tritium Analysis Results - OBT

Russell Ontario Background Site Auger Core - Tritium Analysis Results - OBT					
Standard	**Mid Point Depth (cm)**	**Field Tritium (Bq/L)**	**Field Tritium Error (Bq/L)**	**Field Tritium (Bq/Kg)**	**Field Tritium Error (Bq/Kg)**
5	11	9.588464958	0.821276719	0.320845711	0.027481262
5	11	26.93272849	0.851657172		
5	27.25	10.47978018	0.412336528	0.249396577	0.009812736
5	27.25	17.26782846	0.783733971		
5	35.25	5.304634056	0.408813892	0.198586751	0.015304547
5	35.25	153.9267696	0.486726675		
5	42	10.04511974	1.30899046	0.267693262	0.0348834
5	42	62.07846932	0.02243878		
5	53	6.562694456	0.38585535	0.147446066	0.00866913
5	53	350.8125288	0.07557951		
5	70	4.921182023	0.801829706	0.098502603	0.01604946

5	70	62.00132369	0.084774921	[redacted]

Appendix B 19: Russell Ontario Background Site Auger Core - Tritium Analysis Results - OBT

Sample ID	Delta 2H (VSMOW)	Delta 18O (VSMOW)
GC DC-L	0.3	17.20
GC DC-S	-38.5	1.56
GC D1-L	-13.5	7.31
GC D1-S	-43.3	-2.18
GC D2-L	-17.3	7.62
GC D2-S	-38.0	-1.40
GC D2-S QCD		
GC DW-L	-14.9	7.91
GC DW-S	-33.6	0.49
GC LC-2-L	-2.7	17.57
GC LC-2-S	-49.0	-0.30
GC L-1-L	-3.6	8.21
GC L-1-S	-19.7	-2.82
GC L-2-L	10.9	12.25
GC L-2-S	-9.5	1.43
GC L-3-L	2.6	11.40
GC L-3-L QCD		
GC L-3-S	-23.3	-1.37
GC L-4-L	-2.2	9.47
GC L-4-S	-19.3	-0.18
GC L-5-L	-7.0	9.21
GC L-5-S	-18.6	-0.82
SWL	-66.9	-7.50
SWD	-68.2	-7.34
PW L-1	-39.6	-6.30
PW L-2	-47.1	-6.68
PW L-3	-51.5	-7.28
PW L-4	-64.1	-8.84
PW L-4 QCD		
PW L-5	-64.2	-8.92
PW D-1	-69.9	-7.07
PW D-2	-62.6	-5.84
PW W-1	-71.7	-6.81
PW W-2	-64.3	-6.10
PW LC	-88.0	-8.22
PW DC	-76.9	-6.41

Appendix B 20: Trial 1 Soil, Stem, and Leaf Tissue Free Water Isotope Data

Sample ID	Weight (mg)	Delta 2H x 1000
PO LC-L	0.41	-143.8
PO LC-S	0.46	-123.5
PO L-P1-L	0.45	-158.9
PO L-P1-S	0.46	-131.4
PO L-P2-L	0.43	-158.2
PO L-P2-S	0.42	-131.0
PO L-P3-L	0.44	-150.8
PO L-P3-S	0.43	-129.4
PO L-P4-L	0.44	-150.7
PO L-P4-S	0.46	-130.4
PO L-P5-L	0.41	-154.2
PO L-P5-S	0.42	-128.3
PO DC-L	0.44	-146.0
PO DC-S	0.42	-127.4
PO D-P1-L	0.42	-162.0
PO D-P1-S	0.45	-133.4
PO D-P2-L	0.45	-151.4
PO D-P2-S	0.42	-123.2
PO DW-L	0.43	-152.6
PO DW-S	0.4	-126.3

Appendix B 21: Trial 1 Plant Tissue Deuterium

Sample ID		Delta 18O x 1000	%O
PO LC-L		25.580	47.72
PO LC-S		24.820	47.47
PO L-P1-L		25.800	47.59
PO L-P1-S		24.610	44.38
PO L-P2-L		25.550	48.95
PO L-P2-S		23.670	46.69
PO L-P3-L		25.840	49.08
PO L-P3-S		25.000	45.66
PO L-P4-L		25.840	48.09
PO L-P4-S		24.310	47.03
PO L-P5-L		26.600	48.58
PO L-P5-S		24.690	46.34
PO DC-L		26.430	45.53
PO DC-S		24.470	44.7
PO D-P1-L		24.180	43.88
PO D-P1-S		24.010	45.62
PO D-P2-L		25.650	49.87
PO D-P2-S		25.350	45.95
PO DW-L		26.140	48.83
PO DW-S		24.180	46.15

Appendix B 22: Trial 1 Plant Tissue Oxygen-18

Sample ID		Weight (ug) submit	Weight (mg) entered	%N	%C	%H	%S
PO LC-L		0.000	5.280	0.90	39.21	10.40	0.77
PO LC-S		0.000	5.630	0.65	40.85	10.97	0.43
PO L-P1-L		0.000	5.869	0.81	39.55	11.31	0.43
PO L-P1-S		0.000	5.860	0.67	40.68	11.08	0.45
PO L-P2-L		0.000	4.295	0.76	39.25	9.57	0.99
PO L-P2-S		0.000	5.280	0.71	40.93	10.54	0.60
PO L-P3-L		0.000	4.370	0.80	39.31	9.66	1.02
PO L-P3-S		0.000	4.410	0.68	40.08	9.71	0.55
PO L-P4-L		0.000	5.760	0.85	39.30	11.19	0.89
PO L-P4-S		0.000	4.670	0.68	41.17	9.85	0.66
PO L-P5-L		0.000	6.360	0.77	39.36	11.58	0.79
PO L-P5-S		0.000	4.820	0.70	40.78	10.18	0.68
PO DC-L		0.000	4.420	0.90	38.98	9.69	1.18
PO DC-S		0.000	4.640	0.61	41.51	9.96	0.60
PO D-P1-L		0.000	4.430	0.99	37.88	9.55	1.73
PO D-P1-S		0.000	4.895	0.75	40.90	10.12	0.74
PO D-P2-L		0.000	5.380	0.88	39.41	10.94	1.34
PO D-P2-S		0.000	4.160	0.81	41.75	9.50	0.80
PO DW-L		0.000	6.230	0.96	38.88	11.50	1.31
PO DW-S		0.000	4.980	0.74	42.61	10.47	0.54

Appendix B 23: Trial 1 Plant Stem and Leaf Elemental Ratios

Sample ID	Delta 2H (VSMOW)	Delta 2H Comment	Delta 18O (VSMOW)	Delta 18O Comment	Comment
ST SW	-80.9	0	-11.22	0	0
SP1 S	-55.2	0	-2.52	0	0
BTS 36-L	-37.8	0	12.03	0	0
BTS 36-S	-55.8	0	-3.60	0	0
LP1 36-L	-15.1	0	7.52	0	0
LP1 36-S	-32.1	0	-2.29	0	0
LP2 36-L	-2.6	0	9.42	0	0
LP2 36-S	-26.3	0	-1.06	0	0
LP3 36-L	-7.1	0	7.60	0	0
LP3 36-S	-30.1	0	-2.10	0	0
LP4 36-L	-4.7	0	9.60	0	0
LP4 36-S	-25.8	0	-0.52	0	0
?-1 (ST 1 S?)	-73.0	0	-2.59	0	0
?-2 (ST 2 S?)	-63.9	0	-3.87	0	0

Appendix B 24: Trial 2 Stem and Leaf Tissue Free Water Isotope Data

Sample ID		Weight (mg)	Delta 2H x 1000
ST P1-L		0.45	-136.9
ST P1-S		0.49	-137.3
ST P2-L		0.43	-124.0
ST P2-S		0.42	-132.7
ST P3-L		0.44	-123.0
ST P3-S		0.47	-127.3
BTS-L		0.43	-154.8
BTS-S		0.47	-134.7
LP1 36-L		0.48	-152.1
LP1 36-S		0.44	-112.4
LP2 36-L		0.47	-153.9
LP2 36-S		0.43	-135.6
LP3 36-L		0.48	-159.0
LP3 36-S		0.49	-125.3
LP4 36-L		0.41	-161.6
LP4 36-S		0.43	-139.6

Appendix B 25: Trial 2 Plant Tissue Deuterium

Sample ID	Delta 18O (‰)	%O
ST P1-L	27.100	49.1
ST P1-S	25.930	51.9
ST P2-L	27.210	47.3
ST P2-S	24.700	52.1
ST P3-L	24.500	53.0
ST P3-S	26.920	49.2
BTS-L	26.980	56.5
BTS-S	26.250	51.4
LP1 36-L	29.090	56.9
LP1 36-S	27.450	48.4
LP2 36-L	28.390	55.8
LP2 36-S	26.340	52.2
LP3 36-L	28.430	54.4
LP3 36-S	26.100	52.9
LP4 36-L	28.820	55.1
LP4 36-S	25.890	55.4

Appendix B 26: Trial 2 Plant Tissue Oxygen-18

Sample ID	Delta 2H (VSMOW)	Delta 2H Comment	Delta 18O (VSMOW)
LC L-2E	15.1	0	17.97
LC S-2E	-21.1	0	0.08
DC P3 L-2E	2.8	0	16.96
DC P3 S-2E	-5.2	0	4.57
DC P1-P1 L-2E	23.5	0	20.16
DC P1-P1 S-2E	-12.8	0	3.81
DC P1-P2 L-2E	8.1	0	18.42
D P1-P1 L-2E	-4.7	0	8.16
D P1-P1 S-2E	-44.5	0	-3.44
D P1-P2 L-2E	-0.6	0	8.28
D P1-P2 S-2E	-51.8	0	-3.07
D P2-P1 L-2E	-5.7	0	6.97
D P2-P1 S-2E	-46.7	0	-2.70
D P3-P1 L-2E	-4.5	0	8.01
D P3-P1 S-2E	-23.9	0	3.44
D P3-P2 L-2E	-13.5	0	6.26
D P3-P2 S-2E	-46.9	0	-3.24
D P4-P1 L-2E	-9.0	0	7.87
D P4-P1 S-2E	-46.6	0	-1.87
D P4-P2 L-2E	-10.8	0	9.16
D P4-P2 S-2E	-48.4	0	-1.98
L P2-P2 L-2E	-1.7	0	9.63
L P2-P2 S-2E	-34.7	0	-1.67
L P1-P1 L-2E	-3.6	0	9.81
L P1-P1 S-2E	-25.5	0	-0.50
L P1-P2 L-2E	8.6	0	12.90
L P1-P2 S-2E	-29.8	0	0.04
L P3-P2 L-2E	-9.3	0	8.17
L P3-P2 S-2E	-37.6	0	-1.96
L P2-P1 L-2E	-20.4	0	6.61
L P2-P1 S-2E	-23.7	0	0.87
L P3-P1 S-2E	-23.4	0	8.77
SPW DP4 2E	-90.3	0	-10.40
SPW DC 2	-47.5	0	-3.08
SPW LC 2	-65.7	0	-4.56
SPW DP1 2	-61.7	0	-4.37
D START 2	-69.2	0	-10.76
SPW LP1 2	-39.5	0	-4.90
SPW DP2 S	-65.0	0	-6.36
SPW DP3 2	-84.2	0	-6.43
SPW LP3 2	-64.9	0	-6.20
SPW LP2 2	-58.8	0	-5.13

Appendix B 27: Experimental Control Soil, Stem, and Leaf Tissue Free Water Isotope Data

Sample ID	Weight (mg)	Delta 2H x 1000
LC L-2	0.44	-154.3
LC S-2	0.41	-118.6
DC P3 L-2	0.46	-144.6
DC P31 S-2	0.43	-118.9
DC P32 S-2	0.44	-109.9
DC P1 L-2	0.46	-141.0
DC P1-P2 L-2	0.46	-144.0
DC P1-P1 S-2	0.46	-133.8
D P1-P1 L-2	0.44	-141.3
D P1-P1 S-2	0.44	-129.9
D P1-P2 L-2	0.4	-140.9
D P1-P2 S-2	0.48	-118.2
D P2-P1 L-2	0.42	-145.9
D P2-P1 S-2	0.41	-128.8
D P2-P2 L-2	0.47	-134.0
D P2-P2 S-2	0.41	-128.0
D P3-P1 L-2	0.44	-138.0
D P3-P1 S-2	0.47	-124.4
D P3-P2 L-2	0.43	-137.4
D P3-P2 S-2	0.42	-127.1
D P4-P1 L-2	0.4	-149.4
D P4-P1 S-2	0.42	-131.4
D P4-P2 L-2	0.4	-148.7
D P4-P2 S-2	0.44	-120.9
L P2-P2 L-2	0.43	-146.3
L P2-P2 S-2	0.47	-121.5
L P1-P1 L-2	0.46	-151.4
L P1-P1 S-2	0.42	-134.7
L P1-P2 L-2	0.42	-150.4
L P1-P2 S-2	0.41	-125.7
L P3-P2 L-2	0.43	-138.6
L P3-P2 S-2	0.41	-129.5
L P2-P1 L-2	0.44	-131.7
L P2-P1 S-2	0.43	-113.6
L P3-P1 S-2	0.48	-113.6
L P3-P1 L-2	0.45	-146.1

Appendix B 28: Experimental Control Plant Tissue Deuterium

Sample ID	Delta 18O (‰)	%O
LC L-2	27.500	53.6
LC S-2	23.700	52.0
DC P3 L-2	28.210	51.5
DC P31 S-2	23.600	50.1
DC P32 S-2	23.750	54.5
DC P1 L-2	28.220	48.3
DC P1-P2 L-2	28.110	53.4
DC P1-P1 S-2	23.710	50.9
D P1-P1 L-2	26.250	51.0
D P1-P1 S-2	23.750	50.2
D P1-P2 L-2	26.200	53.4
D P1-P2 S-2	25.080	51.6
D P2-P1 L-2	25.480	52.6
D P2-P1 S-2	25.300	50.6
D P2-P2 L-2	27.590	52.7
D P2-P2 S-2	25.410	50.7
D P3-P1 L-2	26.930	49.4
D P3-P1 S-2	26.840	46.0
D P3-P2 L-2	27.640	48.8
D P3-P2 S-2	26.540	52.1
D P4-P1 L-2	29.130	55.0
D P4-P1 S-2	26.450	50.4
D P4-P2 L-2	29.760	53.8
D P4-P2 S-2	27.060	51.6
L P2-P2 L-2	27.170	53.3
L P2-P2 S-2	26.480	48.8
L P1-P1 L-2	30.450	48.8
L P1-P1 S-2	27.100	51.7
L P1-P2 L-2	29.850	52.1
L P1-P2 S-2	26.200	50.2
L P3-P2 L-2	28.590	50.5
L P3-P2 S-2	26.900	50.5
L P2-P1 L-2	29.100	51.7
L P2-P1 S-2	26.430	54.1
L P3-P1 S-2	27.100	52.0
L P3-P1 L-2	29.210	54.0

Appendix B 29: Experimental Control Plant Tissue Oxygen-18

Sample	Measured Tritium (Bq/L)avg	Measured Tritium Error (Bq/L)	Field Tritium (Bq/L)	Field Tritium Error (Bq/L)	Backround Corrected (Bq/L)
Pembroke Hotel Tap Water	3	1	3	1	
Pembroke Soil H20 Water Start	3	1	3	1	
SRBT 12:20 HT	75	3	77	3	74
SRBT 12:20 HTO	34	2	34	2	31
SRBT 3:30 HT	17	2	17	2	14
SRBT 3:30 HTO	82	3	84	3	81
SRBT 6:30 HT	5	1	5	1	2
SRBT 6:30 HTO	5	1	5	1	2
SRBT 9:30 HT	4	1	4	1	1
SRBT 9:30 HTO	4	1	5	1	1
SRBT 4:30 HT	6	1	6	1	3
SRBT 4:30 HTO	6	1	6	1	3
SRBT JULY 23 8:00AM HT	6	1	6	1	3
SRBT JULY 23 8:00AM HTO	5	1	5	1	2

Appendix B 30: SRBT Field Experiments Air Sampler HT and HTO Data

Sample	Measured Tritium (Bq/L)avg	Measured Tritium Error (Bq/L)	Field Tritium (Bq/L)	Field Tritium Error (Bq/L)
SRBT 12PM LEAVES	518	3	533	3
SRBT 12PM STEMS	233	3	240	3
SRBT 4PM LEAVES	478	2	492	2
SRBT 4PM STEMS	48	1	50	1
SRBT 8PM LEAVES	132	2	136	2
SRBT 8PM STEMS	21	1	22	1
SRBT 12AM LEAVES	86	2	88	2
SRBT 12AM STEMS	11	1	11	1
SRBT 4AM LEAVES	170	1	175	1
SRBT 4AM STEMS	35	1	36	1
SRBT 8:00AM STEM	30	1	31	1
SRBT 8:00AM STEM 2	20	1	21	1

Appendix B 31: SRBT Field Experiments Stem and Leaf Tissue Free Water Tritium

Sample Name	Time	Measured Tritium (Bq/L)avg	Measured Tritium Error (Bq/L)	Field Tritium (Bq/L)	Field Tritium Error (Bq/L)
12 PM Plant Soil	7/22/2014 10:00	11	1	12	2
4 PM Plant Soil	7/22/2014 14:00	10	1	10	2
8 PM Plant Soil	7/22/2014 18:00	10	1	11	2
12 AM Plant Soil	7/22/2014 22:00	10	1	11	2
4 AM Plant Soil	7/23/2014 2:00	12	2	12	2
8 AM Plant Soil	7/23/2014 6:00	11	1	11	2

Appendix B 32: SRBT Field Experiments Potted Soil Pore Water Tritium

Sample	Time	Measured Tritium (Bq/L)avg	Measured Tritium Error (Bq/L)	Field Tritium (Bq/L)	Field Tritium Error (Bq/L)
Control Diffuser		3	1	3	1
Control Closed		4	1	5	1
Control Open		4	1	4	1
1 PM Soil Closed	7/22/14 1:00 PM	4	1	4	1
11 PM Soil Closed	7/22/14 11:00 PM	3	1	3	1
8 AM 23rd Soil Closed	7/23/14 8:00 AM	4	1	4	1
8 AM 23rd Soil Diffuser	7/23/14 8:00 AM	3	1	4	1
4 AM 23rd Soil Diffuser	7/23/14 4:00 AM	4	1	4	1
11 PM Soil Diffuser	7/22/14 11:00 PM	3	1	3	1
1 PM Soil Diffuser	7/22/14 1:00 PM	3	1	3	1
8 AM 23rd Soil Open	7/23/14 8:00 AM	42	2	44	3
11 PM Soil Open	7/22/14 11:00 PM	42	2	44	3
6 PM Soil Diffuser	7/22/14 6:00 PM	3	1	3	1
1 PM Soil Open	7/22/14 1:00 PM	136	4	141	4

Appendix B 33: SRBT Field Experiments Isojar Soil Pore Water Tritium

References

Alvarez, L., Cornog, R., (1939). Helium and Hydrogen of Mass 3. *Physical Review* ,**56**, 6, 613.

Amano, H., Atarashi, M., Noguchi, H., Yokoyama, S., Ichimasa, Y., Ichimasa, M., (1995). Formation of organically bound tritium in plants during the 1994 chronic HT release experiment at Chalk River. *Fusion Technology*. **28**, 803–808.

Amano, H., Garten, C.T., (1991). Uptake of tritium by plants from atmosphere and soil. *Environment International*, **17**, 23-29.

Amano, H., Kasai, A., (1988). The transfer of atmospheric HTO released from nuclear facilities during normal operation. *Journal of Environmental Radioactivity*, **8**, 239-253.

Baglan, N., Alanic, G., Pointurier, F., (2005). Tritium determination at trace level: which strategy to determine accurately HTO and OBT in environmental samples? *Fusion Technology*, **48**, 749–754.

Barbour, M.M., Schurr, U., Henry, B.K., Chin Wong, S., Farquhar, G.D., (2000). Variation in the oxygen isotope ratio of phloem sap sucrose from castor bean. Evidence in support of the Péclet Effect. *Plant Physiology*, **123**, (2), 671-680.

Baumgärtner, F., (2005). Accumulative tritium transfer from water into biosystems. *Fusion Science Technology*, **48**, 787-790.

Baumgärtner, F., Donhaerl, W., (2004). Non-exchangeable organically bound tritium (OBT): its real nature. *Analytical and Bioanalytical Chemistry*, **379**, (2), 204–209

Belot, Y., (1986). Tritium in plants: a review. *Radiation Protection Dosimetry*, **16**, (1-2), 101-105

Belot, Y., Roy, M., Métivier, H., (1996). Le tritium dans l'environment à l'homme. *Institut de Radioprotection et de Sûreté Nucléaire*.

Belovodski, L.F., Gaevoy, V.K., Golubev, A.V., Kosheleva, T.A., (1997). Tritium oxide wash-out by drops. *Journal of Environmental Radioactivity*, **36**, (2-3), 129-139.

Boyer, C., Vichot, L., Fromm, M., Losset, Y., Tatin-Froux, F., Guetat, P., Badot, P.M., (2009). Tritium in plants: A review of current knowledge. *Environmental and Experimental Botany*, **67**, 34-51.

Brown, R.M., (1961). Hydrology of tritium in the Ottawa Valley. *Geochimca et Cosmochimca Acta*, **21**, 199-216.

Canadian Nuclear Safety Commission, (2008). *Standards and guidelines for tritium in drinking water: part of the tritium studies project*. INFO-0766.

Canadian Nuclear Safety Commission, (2010). Compliance Framework Document Associated to the SRB Technologies (Canada) Inc. Nuclear Substance Processing Facility Operating Licence. NSPFOL-13.00/2015 E, E-DOCS-#3572664.

Clark, I., Fritz, P., (1997). Environmental isotopes in hydrogeology. CRC Press, 342 pp.

Clark, I., Wilk, M., Lacelle, D. (2010). Environmental fate of tritium in soil and vegetation: final report. *Canadian Nuclear Safety Commission*, Contract No. 87055-07-0947:R388.1

Constant, P., Chowdhury, S., Hesse L., Conrad R., (2011). Co-localization of atmospheric H_2 oxidation activity and high affinity H_2-oxidizing bacteria in non-axenic soil and sterile soil amended with Streptomyces sp. PCB7. *Soil Biology and Biochemistry*, **43**, 9, 1888-1893.

Craig, H., (1961). Isotopic variations in meteoric waters. *Science*, **133**, 1702-1703.

Dansgaard, W., (1964). Stable isotopes in precipitation. *Tellus*, **16**, 436-468.

Davis, P.A., Galeriu, D.C., Spencer, F.S., Amiro, B.D., (1995). Evolution of HTO concentrations in soil, vegetation and air during an experimental chronic HT release. *Fusion Technology*, **28**, 833-839.

Dawson, T.E., and Ehleringer, J.R., (1993). Isotopic enrichment of water in the woody tissues of plants – implications for plant water source, water uptake, and other studies which use the stable isotopic composition of cellulose. *Geochimica et Cosmochimica Acta*, **57**, (14), 3487-3492.

DeNiro, M.J., Epstein, S., (1979). Relationship between oxygen isotope ratios of terrestrial plant cellulose, carbon dioxide and water. *Science*, **204**, 51-53.

Diabaté, S., Strack, S., (1993). Organically bound tritium. *Health Physics*, **65** (6), 698–712.

Diabaté, S., Strack, S., (1997). Organically bound tritium in wheat after short-term exposure to atmospheric tritium under laboratory conditions. *Journal of Environmental Radioactivity*, **36**, (2-3), 157-175.

Dinner, P.J., Gorman, D.J., Spencer, F.S., (1980). Tritium dynamics in vegetables: experimental results. *Applied Radiation and Isotopes*, **31**, (8), 460.

EcoMetrix Incorporated, (2006). Derived Release Limits (DRLs) for the SRB Pembroke Facility – 2006. Report prepared for SRB Technologies (Canada) Incorporated, *EcoMetrix Incorporated*, Ref. 05-1248.01.

EcoMetrix Incorporated, (2008). Comprehensive Report – Groundwater Studies at the SRB Technologies Facility, Pembroke, ON. *EcoMetrix Incorporated*.

Epstein, S., Yapp, C.J., Hall, J.H., (1976). The determination of the D/H ratio of non-exchangeable hydrogen in cellulose extracted from aquatic and land plants. *Earth Planet Science Letters*, **30**, 241-251.

Eisenbud, M., Bennett, B., Blanco, R., Compere, E., Goldberg, E., Jacobs, D., Koranda, J., Moghisi, A., Rust, J., Soldat, J., Crandell, J., Bailey, C., Reinig, W., Grimes, W., Fearon, T., (1978). Tritium in the environment. *National Council on Radiation Protection Measurements*, NCRP Report 62.

Epstep, M.F., Hoering, T.C., (1980). Biogeochemistry of the stable hydrogen isotopes. *Geochimica et Cosmochimica Acta*, **44**, 1197-1206.

Farquhar G.D., Hubick K.T., Condon A.G., Richards R.A., (1988). Carbon isotope fractionation and plant water-use efficiency. *Stable Isotopes in Ecological Research*. Springer-Verlag, Berlin, 21-40.

Farquhar G.D., Ehleringer J.R., Hubick K.T. (1989). Carbon isotope discrimination and photosynthesis. *Annual Review of Plant Physiology and Plant Molecular Biology*, **40**, 503-537.

Farquhar, G.D., Barbour, M.M., Styles, J.M., Masle, J., Read, J.J., Wong, S.C., Miller, J.M., Fischer, R.A., Sayre, K.D., Henry, B.K., (2000). Oxygen isotope composition of plant matter reveals physiological and genetic effects on plant water use and crop yields. *Proceedings of the National Academy of Sciences*, USA (in press).

Farquhar, G.D., and Cernusak, L.A., (2005). On the isotopic composition of leaf water in the non-steady state. *Functional Plant Biology*, **32**, 293-303.

Farquhar, G.D., Lloyd, J., (1993). Carbon and oxygen isotope effects in the exchange of carbon dioxide between terrestrial plants and the atmosphere. *Stable Isotopes and Plant Carbon-Water Relations*, Academic Press, San Diego; p. 47–70.

Flanagan, L.B., Comstock, J.P., Ehleringer, J.R. (1991). Comparison of modeled and observed environmental influences on the stable oxygen and hydrogen isotope composition of leaf water in *Phaseolus vulgaris* L. *Plant Physiology*, **96**, 588-596.

Fritz, P., Drimmie, R.J., Frape, S.K., O'Shea, O., (1987). The isotopic composition of precipitation and groundwater in Canada. *Isotope Techniques in Water Resources Development*, IAEA Symposium 299, Vienna, 539-550.

Galeriu, D., Davis, P., Raskob,W., Melintescu, A., (2008). Recent progresses in tritium radioecology and dosimetry. *Fusion Technology*, **54**, (1), 237–242.

Galeriu, D., Melintescu, A., Strack, S., Atarashi-Andoh, M., Kim, S.B., (2013). An overview of organically bound tritium experiments in plants. *Journal of Environmental Radioactivity*, **118**, 40-56.

Gascoyne, M., and Kotzer, T., (1995). Isotopic methods in hydrogeology and their application to the Underground Research Laboratory, Manitoba. Report AECL-11370, Atomic Energy of Canada Limited, Pinawa, Manitoba, 100 pp.

Golder Associates Ltd (2003). Renfrew County – Mississippi – Rideau Groundwater Study. Final report prepared for Mississippi Valley Conservation Authority Study Group.

Gonfiantini, R., (1996). On the isotopic composition of precipitation. *Proceedings, International Symposium on Isotope Hydrology*, Vienna.

Gonfiantini, R., Gratziu, S., Tongiorgi E., (1965). Oxygen isotope composition of water in leaves. *Isotopes and Radiation in Soil Plant Nutrition Studies, IAEA Technical Report Series*, **206,** International Atomic Energy Agency: Vienna, 405-410.

Gillespie, J.E., R.E. Wicklund, and B.C., Matthews, (1964). Soil Survey of Renfrew County. Report No. 37 of the Ontario Soil Survey. Prepared Jointly by the Canada Department of Agriculture and the Ontario Department of Agriculture.

Guo, R., Conrad, R., (2008). Extraction and characterization of soil hydrogenases oxidizing atmospheric hydrogen. *Soil Biology and Biochemistry*, **40**, (5), 1149-1154.

Hart, D., (2008). Derived Release Limits Guideline COG Technical Report, *COG* 06-3090-R2-1.

Hisamatsu, S., Katsumata, T., Takizawa, Y., 1998. Tritium concentrations in pine needle, litter and soil samples. *Journal of Radiation Research*, **39**, 129-136.

Hoefs, J., (2009). Stable Isotope Geochemistry. Springer-Verlag, Berlin, 6th edition.

Ichimasa, M., Ichimasa, Y., Yagi, Y., Ko, R., Suzuki, M. and Akita, Y., (1989). Oxidation of atmospheric molecular tritium in plant leaves, lichens and mosses. *Journal of Radiation Research*, **30**, 323–329.

Ichimasa, Y., Ichimasa, M., Jiang, H., Katsuno, K., Noguchi, H., Yokoyama, S., Amano, H. and Atarashi, M., (1995). In vitro determination of HT oxidation activity and tritium concentration in soil and vegetation during the chronic HT release experiment at Chalk River. *Fusion Technology*, **28**, 877–882.

Ichimasa, M., Suzuki, M., Obayashi, H., Sakuma, Y., Ichimasa, Y., (1999). In vitro determination of oxidation of atmospheric tritium gas in vegetation and soil in Ibaraki and Gifu, Japan. *Journal of Radiation Research*, **40**, 243–251.

INTERA Information Technologies (Canada) Limited, (1994). Decommissioning of the Shell Service Station – 340 Mud Lake Road (Boundary Road). Report prepared for Shell Canada Products Limited. INTERA Ref. 94578.

Starz, A., (2015). Developing infrastructure for new nuclear programmes: IAEA services for member states. *International Atomic Energy Association.* Retrieved from: https://www.iaea.org/NuclearPower/Downloadable/News/2015-11-30-nids/Nuclear-Espana-365-Sept2015-Starz.pdf

ITER Organization, (2015). Tritium Breeding. *International Thermonuclear Reactor*. Retrieved from: https://www.iter.org/mach/tritiumbreeding.

Jiménez-Martinez, J., Tamoh K., Candela, L., Elorza, F., Hunkeler, D., (2012). Multiphase transport of tritium in unsaturated porous media – bare and vegetated soils. *Mathematical Geosciences*, **44**, (2), 187-208.

Khdhiri, M., Hesse, L., Elena Popa, M., Quiza, L., Lalonde, I., Meredith, L.K., Röckmann T., Constant, P. (2015). Soil carbon content and relative abundance of high affinity H_2-oxidizing bacteria predict atmospheric H_2 soil uptake activity better than soil microbial composition. *Soil Biology and Biochemistry*, **85**, 1-9.

Kirchmann, R., Van Den Hoek, J., Lafontaine, A., (1971). Transfert et incorporation du tritium dans les constituants de l'herbe et du lait en conditions naturelles. *Health Physics*, **21**, 61–66.

Kim, S.B., Bredlaw, M., Korolevych, V.Y., (2012). HTO and OBT activity concentrations in soil at the historical atmospheric HT release site (Chalk River Laboratories). *Journal of Environmental Radioactivity*, **103**, 34-40.

Kim, S.B., Baglan, N., Davis, P.A., (2013). Current understanding of organically bound tritium (OBT) in the environment. *Journal of Environmental Radioactivity*, **126**, 83-91.

Kim, S.B., Roche, J., (2013). Empirical insights and considerations for the OBT inter-laboratory comparison of environmental samples. *Journal of Environmental Radioactivity*, **122**, 79-85.

Kim, S.B., Stuart, M., (2015). OBT analysis method using polyethylene beads for limited quantities of animal tissue. *Journal of Environmental Radioactivity*, **146**, 51-55.

Koarashi, J., Amano, H., Andoh, M., Lida, T., Koma, T., (2001). A new method for determining HT deposition velocity and dependence of the velocity on environmental factors. *Radiation Protection Dosimetry*, **93,** (3), 237–243.

Koranda, J.J., Martin, J.R., (1971). The movement of tritium in ecological systems. *Tritium Symposium*, Las Vegas, 430–455.

Lapp, A., (2012). Prototype tritium in-air sampler: for HTO and HT. Undergraduate Thesis, *University of Ottawa, Department of Earth Science*.

Leuenberger, M., Borella, S., Stocker, T., Saurer, M., (1998). Stable isotopes in tree rings as climate change stress indicators. *Hochschulverlag AG*, Zurich.

Lindow, S.E., Brandl, M.T., (2003). Microbiology of the phylllosphere. *Applied Environmental Microbiology*, **69**, (4), 1875-1883.

Lucas, L.L., Unterweger, M.P., (2000). Comprehensible review and critical evaluation of the half-life of tritium. *Journal of Research of the National Institute of Standards and Technology*, **105**, (4), 541-549.

Luo, Y., Sternberg, L., (1992). Hydrogen and oxygen isotope fractionation during heterotropic cellulose synthesis. *Journal of Experimental Botany*, **43**, 47–50.

Majoube, M., (1971). Fractionation en oxygène-18 et en deuterium entre l'eau et sa vapeur. *Journal of Chemical Physics*, **197**, 1423-1436.

Marshall, J.D., Brooks, J.R., Lajtha, K., (2008). Sources of variation in the stable isotopic composition of plants. *Stable Isotopes in Ecology and Environmental Science*, Second Edition. Blackwell Publishing.

McFarlane, J., (1978). Tritium accumulation in lettuce fumigated with elemental tritium. *Environmental and Experimental Botany*, **18**, 131-138.

McFarlane, J, Rogers, R., Bradley, D., (1978). Environmental tritium oxidation in surface soil. *Environmental Science and Technology*, **12**, (5), 590-593.

McFarlane, J.C., Beckert, W.F., Brown, K.W., (1979). Tritium in plants. Journal of Environmental Quality, **8**, 269-276.

Mihok, S., Wilk, M., Lapp, A., St-Amant, N., Kwamena, O.A., Clark, I.D. ,(2016). Tritium dynamics in soils and plants grown under three irrigation regimes at a tritium processing facility in Canada. *Journal of Environmental Radioactivity*, **153**, 176-187.

Momoshima, N., Kakiuchi, H., Okai, T., Hisamatsu, S., Maeda, Y., (2000). Tritium in a pine forest ecosystem: relation between fresh pine needles, organic materials on a forest floor and atmospheric. *Journal of Radioanalytical and Nuclear Chemistry*, **243**, 479-482.

Murphy, C.E., (1984). The relationship between tritiated water activities in air, vegetation and soil under steady-state conditions. Health Physics, **47**, 635-639.

Murphy, C.E., (1990). The transport, dispersion and cycling of tritium in the environment. *Westinghouse Savannah River Company*, Aiken, South Carolina, USA.

Murphy C. E., (1993). Tritium cycling and transport in the environment. *Health Physics*, **65**, 683-697.

Noguchi, H., Yokoyama, S., Kinouchi, N., Ichimasa, Y., Ichimasa, M., (1995). Tritium behavior on a cultivated plot in the 1994 chronic HT release experiment at Chalk River. *Fusion Technology*, **28**, 924-929.

Nabors, M.W., (2004). Introduction to Botany. *Benjamin Cummings/Pearson*, 656 pp.

Okada, S., Momoshima, N., (1993). Overview of Tritium: characteristics, sources and problems. *Health Physics*, **65**, 595-608.

Oliphant, M., Harteck, P., Rutherford, E., (1934). Transmutation Effects observed with Heavy Hydrogen. *Nature*, **133,** 3359-3413.

Papke, H., Foerstel, H., (1991). Formation rate of nonexchangeable organically bound tritium from tritiated soilwater. *Health Physics*, **60**, (6), 773–779.

Parkhurst, D.F. (1982). Stereological methods for measuring internal leaf structure variables. *American Journal of Botany*, **69**, 31-39.

Raskob, W., (1995). Assessment of the environmental impact from tritium releases under normal operation conditions and after accidents. *Fusion Science and Technology*, Fifth Tropical Meeting on Tritium Technology in Fission, Fusion, and Isotopic Applications, Belgirate, **28**, 934-939.

Raney, F., Vaadia, Y., (1965). Movement and distribution of THO in tissue water and vapor transpired by shoots of Helianthus and Nicotiana. *Plant Physiology*, **40**, 2, 383-388.

Reece, J.B., Urry, L.A., Cain, M.L., Wasserman, S.A., Minorsky, P.V., Jackson, R.B., Campbell, N.A., (2012). Campbell Biology, 9th edition. *Benjamin Cummings/Pearson*, 1464 pp.

Roden J.S., Ehleringer, J.R., (1999). Observations of hydrogen and oxygen isotopes in leaf water confirm the Craig-Gordon model under wide-ranging environmental conditions. *Plant Physiology*, **120**, (4), 1165-1174.

Rozanski, K., Araguas-Araguas, L., Gonfiantini R., (1993). Isotopic patterns in modern global precipitation. Continental Isotope Indicators of Climate, *American Geophysical Union Monograph*, **78.**

Santrucek, J., Kveton, J., Setlik, J., Bilickova, L., (2007). Spatial variation of deuterium enrichment in bulk water of Snowgum leaves. *Plant Physiology*, **143**, 88-97.

Schuepp, P.H., (1993). Leaf boundary layers. *The New Phytologist*, **125**, (3), 477-507.

Spencer, F.S., Dunstall, T.G., (1986). Molecular tritium conversion in vegetation, litter and soil. *Radiation Protection Dosimetry*, **16**, 89-93.

Sternberg, L., (1989). Oxygen and hydrogen isotope measurements in plant cellulose analysis. *Modern Methods of Plant Analysis*, *Vol. 10*: *Plant Fibers,* Springer-Verlag, Berlin, 89–99.

Strack, S., Diabate, S., Raskob,W., (2005). Organically bound tritium in plants: Insights gained by long-term experience in experimental and modeling research. *Fusion Science Technology*, **48**, 767-770.

Straume, T., (1993). *Tritium risk assessment*. *Health Physics*, **65**, (6), 673-682.

SRB Technologies (Canada) Incorporated, (2012). Annual Compliance and Performance Report.

Sweet, C.W., Murphy, C.E., (1981). Oxidation of molecular tritium by intact soils. *Environmental Science and Technology*, **15,** (12), 1485–1487.

Reece, J.B., Urry, L.A., Cain, M.L., Wasserman, S.A., Minorsky, P.V., Jackson, R.B., Campbell, N.A., (2012). Campbell Biology, 9th edition. *Benjamin Cummings/Pearson*, 1464 pp.

Vichot, L., Boyer, C., Boissieux, T., Losset, Y., Pierrat, D., (2008). Organically bound tritium (OBT) for various plants in the vicinity of a continuous atmospheric tritium release. *Journal of Environmental Radioactivity*, **99**, 1636-1643.

Wang X-F., Yakir, D., (1995). Temporal and spatial variations in the oxygen-18 content of leaf water in different plant species. *Plant, Cell, and Environment*, **18,** 1377-1385.

Wershaw, R.L., Freidman, I., Heller S.L., Frank P.A., (1970). Hydrogen isotopic fractionation of water passing through trees. *Advances in Organic Geochemistry*, Pergamon Press, Oxford, 55-67.

White, J.W.C., Cook E.R., Lawrence, J.R., and Broecker, W.S. (1985). The D/H ratios of sap in trees: implications for water sources and tree ring D/H ratios. *Geochimica et Cosmochimica Acta*, **49,** 237-246.

Williams, D.G. & Ehleringer, J.R., (2000). Intra- and interspecific variation for summer precipitation use in Pinyon-Juniper woodlands. *Ecological Monographs*, **70**, (4), 517-537.

Workman, W.J.B., Kim, S.B., Kotzer, T.G., (2005). Interlaboratory comparison of organically bound tritium measurements in environmental samples. *Fusion Technology*, **48**, (1), 763-766.

Yakir, D., & DeNiro, M.J., (1990). Oxygen and hydrogen isotope fractionation during cellulose metabolism in *Lemma gibba L. Plant Physiology*, **93**, 325-332.

Yakir, D. (1992). Variations in the natural abundance of oxygen-18 and deuterium in plant carbohydrates. *Plant, Cell and Environment*, **15**, 1005-1020.