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# Quantifying Litter Decomposition Rates on a Semi-Intensive Green Roof

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# QUANTIFYING LITTER DECOMPOSITION RATES ON A SEMI-INTENSIVE GREEN ROOF

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**Abstract:**

The overall goal of this study was to provide a measure of the decomposition rate constant on a semi-intensive green roof located in Fayetteville, Arkansas. The specific approach chosen was the use of the Tea Bag Index (TBI), a standardized plant litter decomposition test. There was some heterogeneity observed on site and the locations of samples tested were chosen based on this. Additional laboratory tests were conducted in order to determine whether there would be a large impact of temperature on decomposition or if it would be outweighed by other factors. The temperatures compared were 5°C, 20°C, and 30°C. Decomposition data collected in the laboratory test fit to some extent with the exponential decay model suggested ($0.9 > P > 0.85$). This data suggests a comparable rate of decomposition to other biomes which had been previously studied, which was further supported by samples tested on site. The decomposition rate constants calculated from data collected on site were slightly less than those seen in grasslands or healthy forests but higher than those seen in more arid environments. The stabilization factor was more similar to those seen in sandy soils, which fits with the composition of the engineered soil used. The main recommendation for future research is replication during the summer months, which could confirm the influence of season or temperature on decomposition while further assessing soil health at the site.

## Introduction:

Green roofs contain engineered soils that are designed in accordance with specific standards, most of which are provided in the Whole Building Design Guide (WBDG) Document 073363. These initial standards include biological content, drainage rate, and nutrient characteristics which all aim to produce a healthy soil which is capable of supporting the goals of the green roof (Dvorak, 2011). If a green roof is to continue to have the success it did soon after installation, these standards must be maintained.

Maintaining the health of the soil present on a site is fundamentally dependent on its bacteria, fungi, and other microbes that shape the soil's ability to enable healthy nutrient cycling (NRCS, 2019). The decomposition rate of the soil is the ability to break down plant matter as it is added, which can help keep the biological content and nutrient characteristics consistent. Breaking down plant matter also prevents it from blocking off drainage of water through the soil. Having the decomposition rate of a soil measured in a laboratory can be highly expensive, so a less costly alternative was used to measure the amount of decomposition present in the soil layer on the Hillside Green Roof.

Previous studies have disagreed on the relationship between the temperature at which decomposition occurs and the rate of that decomposition. Older studies have suggested a doubling of decomposition with every 10 °C increase in temperature based on modeling (Friedlingstein et al., 2006), while more recent studies suggest this relationship can be outweighed by the microbial communities present at specific sites and only exists above a threshold moisture content (Djukic et al., 2018).

There are visible spatial heterogeneities that add to the complexity which exists within this soil. The main component of this is the soil horizon effect, which describes the tendency of a natural soil to exhibit distinct layers separated vertically, with the highest density of organic material typically occurring in the uppermost layer and a layer dense in minerals forming under that (NRCS, 2010). Despite potential efforts to homogenize the soil present on this roof, this distribution will naturally occur as

plants deposit organic matter at the surface when they die. This was most visible through the large amount of roots present through the top inch of the soil.

To approximate the amount of decomposition occurring in the soil, and established method, the Tea Bag Index (TBI) is selected. While this method has been used in other soil systems, it has not been tested in the context of a green roof. The TBI relies on the placement of standardized tea bags in soil over a set period of time to provide a measurement of the amount of decomposition which occurred over that duration (Keuskamp et al., 2013). This data can be used to calculate the decomposition rate constant for the tea in the specific soil, and also allows comparison across different sites to create a comparative network. The relatively small investment compared to other methods of monitoring the decomposition rate of soils makes this approach more accessible to both smaller installations and individuals.

The overall goal of this study is to establish the decomposition rate constant of tea litter in the soil present on this green roof to test whether it fits into an expected range for the soil type on site and climate conditions in the region. Two distinct experiments were conducted to determine key metabolic parameters of the soil. First, an on-site tea litter experiment was designed with the goal of testing for a correlation between decomposition rate and either the depth in the soil profile or location horizontally along the elevation gradient of the roof. Second, laboratory incubations were used to test the effects of temperature variations on litter decomposition.

**Literature Review Summary:**

Maintaining the ability of a green roof to support hydrological functions through water storage, plant growth and ecology, and species diversity is dependent on proper execution of the design process and upkeep afterward (FLL, 2018). None of these goals can be met unless there is healthy soil in place which can adequately store water and serve as growth media for plants (NRCS, 2019). Previous litter decomposition studies were subject to unreliability resulting from significant variability in the litter used (Djukic et al., 2018), while more stable testing methods are prohibitively costly due to the laboratory technology and experience needed (McDaniel, 2017).

The Tea Bag Index (TBI) has been found to provide more comparable data than previous strategies (Djukic et al, 2018) and the materials needed are both significantly cheaper than previous testing methods and available globally through online vendors (Keuskamp et al, 2013). No green roof studies currently exist in the TBI database, but the specifics of the method are well-suited to use with the thin soil layer present. There have also been less green roof studies conducted in the region where this study was completed compared to other regions (Dvorak & Volder, 2010). Those which were found locally focused on factors other than decomposition or other soil parameters, though they did provide a great deal of other information on the functioning of green roofs in the region (Toland et al., 2011 & Toland et al., 2014). What follows is a description of specific, helpful articles influencing this study.

**Detailed Article Review:**

The FLL Green Roof Guidelines were developed in Germany with the initial public release in 1999 and updates following in 2002, 2008, 2016, and this most recent edition in 2018. These remain one of the most influential sets of design criteria in Europe and have served as the basis of national regulations in multiple countries. They state the possibilities for green roofs to provide ecological, functional, and aesthetic benefits to the areas where they are installed (FLL, 2018). A relevant section to

this study is the “turf substrate requirement profiles,” which are identified as an essential component to the functioning of the roof, specifically supporting the flora and fauna that depend on the roof.

The United States Environmental Protection Agency states that for design aspects of green roofs the FLL guidelines developed in Germany can be followed to reliably design high-quality green roofs until more specific design protocols are put into place in the United States (Philippi, 2017). It also recommends following recommendations from the American Standard Testing Methods (ASTM) to establish the required architectural standards in the construction of green roofs. It does caution that, “The words of the English translation may sometimes be quite awkward for American readers, but nevertheless it can be a very helpful tool for many purposes” (Philippi, 2017).

In designing substrate for a green roof, the use of high levels of organic matter may lead to problems such as soil shrinkage due to decomposition and seepage of nutrients (Rowe & Rugh, 2006). In order to find the best mixture, substrate compositions were tested with varying levels of heat-expanded slate and the remainder of the substrate made up of a blend of sand, Michigan peat, and aged compost. The result was that around 80% expanded slate can support optimal water storage while still providing for plant growth. It recommends deeper substrates to increase tolerance to frost as well as water-holding capacity and states the necessity of a small amount of fertilization to sustain growth, with 50 $g \cdot m^{-2}$ per year of Nutricote 13N–5.7P–10.8K Type 180 controlled-release fertilizer proving sufficient. Based on this recommended lower rate of inclusion of organic material, I would expect a correspondingly reduced level of organic activity and decomposition relative to more detritus-heavy soils.

A literature review of previous studies conducted in 2010 contrasted the regional density of previous green roof studies in the S. Central Great Lakes ecoregion and along the Atlantic Coast with a relative lack of studies conducted in any other areas of the United States (Dvorak & Volder, 2010). The importance of studies conducted directly on a green roof is highlighted in the review; problems with

simulations used in research sites were stressed, specifically due to the increased prevalence of wind and sun stressors. This review suggests a need for studies conducted on functioning green roofs in temperate regions, among others. The only study recorded on a temperate area of North America in this meta-study was conducted in Mexico City, which falls in a temperate sierra, and the main recommendations of that study were limited to the hardiness of sedum varieties to the seasonal drought conditions which occur in the area (Müller Garcia, 2005).

An additional study was conducted on mock green roofs in the Ozark Highlands ecoregion from 2008 through 2009, with the main goal of evaluating "species survival under local environmental conditions," with treatments varying by particle size and the amount of fertilization provided (Toland et al., 2014). The main findings were that fine media with added compost supported the highest amount of survival. This study by Toland et al. (2014) was also conducted in Fayetteville, Arkansas, the location of the green roof currently being researched.

Another study has been conducted on the stormwater runoff collected from three green roofs in Fayetteville, Arkansas, with the aim of comparing the "nutrient concentrations in runoff water from conventional roofs, green roofs, and urban streams, focusing on the impacts of compost addition" (Toland et al., 2011). This study concluded that the compost which was added to the green roofs is adding nutrients to the runoff water. Toland et al. included that the compost used on the roofs in this study was a mushroom compost and "was mixed with the media at the industry standard of 15% by volume". This is the same type of compost used on the green roof researched here, which was confirmed by Mr. Jay Huneycutt with the Facilities Management staff at the University of Arkansas.

One article discussing methods for measuring the factors contributing to soil health made the recommendation that a decomposition test should be developed which is broadly applicable across soil types, sensitive to management practices, and inclusive of the physical, chemical, and biological influences on soil health (McDaniel, 2017). It would be ideal if this test were cheaper than current tests,

which are stated to cost up to $150 per sample. McDaniel mentions the use of tea decomposition is mentioned as showing potential, but needing additional research before being confirmed as a viable alternative to established laboratory methods.

One study analyzing the possibility of using bags of shredded leaves to measure plant litter decomposition strongly recommended setting up a standard method which could be compared across ecosystems (Fritz et al., 2011). It mentioned that using leaves from trees which are common in the region where a study is conducted can provide useful information but is not sufficiently standard to allow detailed comparisons. The standardized organic material, reasonable price point, and global availability of Lipton tea bags makes them a promising candidate for this comparison. This does not mean that they would replace the use of native plant litter, which account for local variability, but they could be used simultaneously to help with comparison across sites.

A meta-analysis comparing the use of the Tea Bag Index to other types of litter decomposition studies found that the makeup of the plant matter in tea bags was very consistent, with the Green Tea used having a much higher cellulose content and the Rooibos containing much more lignin (Djukic et al., 2018). It concluded that this differences in the makeup of the plant matter was the largest factor affecting decomposition rates, stating that, “65% of the variation in the remaining litter mass was related to tea type while 13% was related to biome” (Djukic et al., 2018). The specific factors used in modeling were temperature, precipitation, and tea type considered. This study recommends additional long-term studies to quantify the relative importance of different climactic drivers of decomposition. This study lists hundreds of sites globally from numerous authors who have applied this methodology, but none of them were conducted on a green roof. The study also recommends additional testing to confirm the exact effects of seasonal differences on decomposition rates, and suggests that insufficient precipitation can prevent a dependence of decomposition rate on temperature.

This study advocating for the use of the tea bag index in modeling soil decomposition rates expounds on the exact process for completing this study in soil media (Keuskamp et. al, 2013). The availability, relative cheapness, low entry level knowledge required, and inclusion of different influences on soil health all make this an attractive option for monitoring soil health. The study expands on the exact strategy for applying this method, including the number of samples to take, the process for burying tea bags, the length of time to leave samples buried, the temperature to dry samples, and the length of time for drying. There is also a strategy set out for a laboratory test to calculate baseline decomposition rate constants for the soil being studied. The relatively very small size of litter samples and non-invasive placement strategy make this approach especially attractive for applications on the smaller and strictly controlled green roof sites.

**Methods**:

The specific teas needed to complete the Tea Bag Index are the Lipton Indonesian Tea Sencha Tradition, EAN 87 22700 05552 5 or the Lipton Herbal Infusion Rooibos, EAN 87 22700 18843 8. These varieties are sold online from dutchsupermarket.com, who is partnered with the tea bag index and provides a discount to buyers who intend them for that purpose (Tea Bag Index, 2016). This availability makes obtaining the needed materials very simple for those who would like to participate in the project globally. The Sencha Green tea and Rooibos tea used can be seen in Fig. 1.


*Figure 1: Sencha Green Tea (left) and Rooibos Tea (right) used in the Tea Bag Index*

Litter decomposition rates should generally fit with an exponential decay function, seen in Equation 1, until the readily decomposable fraction is exhausted. In this equation, $X_t/X_o$ is the proportion of the original plant mass remaining at time t and k is the decomposition rate constant; k can be calculated by fitting Equation 1 to a plot of t vs. $X_t/X_o$ (Karberg et al., 2008).

$$X_t/X_0 = e^{-kt} \qquad \text{(Equation 1)}$$

Based on a recommendation of having at least ten tea bags of each type on the site from a previous study, it was decided that sixteen bags each of the Green Tea and Rooibos tea would be used (Keuskamp et al., 2013). In an effort to understand the amount which the soil horizon affects the current decomposition rate, the tea bags were separated into four profiles for each tea type, consisting of four bags buried at depths of 1”, 2”, 3”, and 4” to construct a full profile of the 5” of soil in place on the roof. These also match the depths at which the sensors placed to measure the soil volumetric water content, temperature, and electrical conductivity had been placed. The tea bags were placed by first extracting the soil in 3”x3” square sections, as small an area as was feasible, in order to minimize the impact to the site. The soil was replaced in one-inch sections in as close to the same orientation that it was removed to preserve the character of the soil profile as much as possible.

In addition to this vertical differentiation, there may be some additional differences in decomposition rates caused by the slope of the roof, so the four vertical profiles were spaced along this elevation gradient to establish the strength of this correlation. The profiles of Sencha and Rooibos bags were placed at the same location in the North/South direction along the roof slope in order to keep the elevations consistent between tea types. They are spaced by 8” in the East/West direction to reduce direct interaction within soil, and the Sencha profiles were consistently placed to the West. The location of tea bags placed on the green roof can be seen in Fig. 2.


*Figure 2: Placement of Field Samples on Hillside Green Roof: These placements are not to scale, the separations between profiles 1, 2, 3, & 4 are much closer to correct, but the Sencha and Rooibos profiles at each numbered site are only separated by 8 inches and are spaced more here only to clearly suggest the side of the site each tea type was placed toward. For the exact placement and masses of each individual tea bag see Table A1.*

Keeping with recommendations from previous research, the data from the tea bags incubated on the green roof was used to generate both an initial decomposition rate constant, k, and a stabilization factor, S (Keuskamp et al., 2013). These calculations depend on the assumption that this decomposition can be modeled by separating the overall sample mass into a fraction that is quickly broken down, referred to as the labile fraction, and another portion that is much more resistant to decay, termed the recalcitrant fraction. This general relationship is modeled by Equation 2, in which a is the labile fraction, $k_1$ is the decomposition rate constant associated with the labile fraction, the quantity 1 – a is the recalcitrant fraction, $k_2$ is the rate constant for the recalcitrant fraction, and W(t) is the

fractional weight remaining a specific number of days from the start of decomposition (Keuskamp et al., 2013).

$$W(t) = ae^{-k_1 t} + (1-a)e^{-k_2 t} \qquad \text{(Equation 2)}$$

The additional assumption that the recalcitrant fraction will break down by a negligible amount during the early stages of decomposition allows the simplification of Equation 2 to Equation 3. This equation will be used to model the early stage decomposition of Rooibos tea, since it should still be in the early stages of its slower decomposition. The only qualification given on this is that in tropical and extremely biologically active areas the study may need to be shortened somewhat to ensure the Rooibos has not already reached its recalcitrant stage of decomposition (Keuskamp et al., 2013).

$$W(t) = ae^{-kt} + (1-a) \qquad \text{(Equation 3)}$$

Since the Sencha tea has been found to consistently reach the end of its early stage decomposition within 60 days within a broad range of climate conditions and ecosystems, the decomposable fraction for Green Tea, $a_g$, can be approximated as the amount which has decomposed by the time the samples placed in the field are collected. The hydrolysable fraction, $H_g$, which could theoretically be broken down in perfect decomposition conditions is known from previous experimentation, we can use this to calculate the stabilization factor, S, which is defined as the environmental shift to a lower actual rate of decomposition of the labile fraction than would be theoretically possible (Keuskamp et al., 2013). The hydrolysable fraction used for Green Tea, $H_g$, is 0.842 and for Rooibos the hydrolysable fraction, $H_r$, is 0.552. This relationship as it is used with Green Tea can be seen in Equation 4.

$$S = 1 - \frac{a_g}{H_g} \qquad \text{(Equation 4)}$$

Due to the stabilization factor being dependent entirely on environmental conditions, it is assumed to be the same across tea types, which was confirmed through additional testing (Keuskamp et al., 2013). As such, once S is calculated from the data obtained with the Sencha Green Tea, it can be used to calculate the readily decomposable fraction of the Rooibos tea, $a_r$, as well. This relation is shown in Equation 5 using $H_r$ and $a_r$. Once $a_r$ is calculated from Equation 5, all quantities except k are known in Equation 3 for Rooibos tea, so k can be calculated by substituting these in and solving the equation.

$$a_r = H_r(1 - S) \qquad \text{(Equation 5)}$$

A separate, lab conducted test was also performed in vitro in order to establish a baseline decomposition rate constant of Sencha Green and Rooibos tea bags under controlled conditions over time, following the established procedure with some slight variations due to available materials, space, and time constraints (Keuskamp et al., 2013). In an effort to understand the impact which temperature has on the biological activity in this specific soil, a set of laboratory tests were conducted in which tea bags were incubated at different temperatures. This test was completed by using soil collected from the green roof and keeping moisture content, light exposure, and air flow constant while manipulating the temperature at which tea bags were incubated.

Three separate trials were conducted at 5°C, 20°C, and 30°C. The least temperature (5°C) is within expected temperatures during winter months, while 20 and 30°C represent a range of summer temperatures. The tea bags incubated at 30°C were maintained at their set temperature by placing them in an oven intended for soil drying but which allowed significantly lower temperature settings. The tea bags maintained at 20°C were simply stored in a room set to that temperature using the thermostat, but were stored in an out-of-use refrigerator to control a large part of the variation which would have otherwise occurred. The remaining tea bags were stored in a refrigerator set to maintain at 5°C. While it

is acknowledged that there will be some variation around the set point for each of these systems, these steps aim to keep that range of temperatures as small as possible.

The tea bags were incubated in chambers enclosed from light and major air flow in partially covered boxes to allow some air to exchange and prevent condensation from forming on the tops of the boxes and then dripping onto the tea bags. They were incubated on top of an inch-thick layer of the engineered soil collected from the site being studied, under this soil layer there was a three-inch-thick layer of saturated sand to keep the soil moist following the established procedure (Keuskamp et al., 2013). These bags were retrieved after 0, 4, 7, 14, and 30 days of incubation.

Following established convention when working with the Tea Bag Index, all samples were dried at 70 °C for not less than 48 hours after the set incubation period was complete (Tea Bag Index, 2016). No recommendations were made for maximum drying time, since over-drying was not a concern. The tea bags were weighed with strings attached, but after removing the labels from these strings. The difference between this final mass after decomposition and the initial mass recorded was used to calculate the percent lost. The mass loss was fit to the simpler exponential decay function (Equation 1) for both tea types in the laboratory trials, while the specimens incubated on the green roof itself were used to calculate both the stabilization factor, S, and the decomposition rate constant, k, as recommended in the study on which this methodology was based (Keuskamp et al., 2013).

The fit of the decomposition data collected in the laboratory tests to the exponential decay relationship shown in Equation 1 were initially tested with the $R^2$ values for the equation and then confirmed as significant by regression analysis conducted on the linearized values. An alpha value of 0.05 was selected for comparison to the P-values calculated from this statistical analysis. This analysis was completed using the Data Analysis Tools provided in Microsoft Excel.

Whether or not there were differences in the decomposition rate constants calculated for the tea bags resulting from either the depths at which tea bags were buried in the soil profile or due to the location of profiles along the slope of the roof were initially tested by completing a Two-Way Analysis of Variance (ANOVA) test. An alpha value of 0.05 was again selected for comparison to the P-values found in this test. If it had been determined that there was a significant difference, least significant difference tests would have been used to determine whether there were differences between the means calculated for individual sites or depths. The ANOVA tests were also completed using the Data Analysis Tools in Microsoft Excel; the LSD analysis was also completed in Excel, but was done manually as it was not included in the tools provided.

The differences in the decomposition rates calculated at different temperature incubations in the lab were tested using Analysis of Covariance (ANCOVA) tests. For these tests an alpha value of 0.05 was selected again. These tests were completed using a template for Microsoft Excel provided by Vassar Stats (Lowry, 2004). These were all completed by the LN transformed data so that the linearized forms could be compared.

**Results:**

There was a large difference seen between the average amount Green Tea and Rooibos tea that was decomposed in the on-site test; the average mass loss for Rooibos teas was 23.88%, with a standard deviation of 1.95%, and the average mass loss for Green Tea was 51.65%, with a standard deviation of 2.53% (calculations can be seen in Table A1). The resultant decomposition rate constants calculated from the tea bags which were left in the soil on the Hillside green roof showed some variation, with an average of 0.018 $days^{-1}$ for the Rooibos teas and a standard deviation of 0.0045; S had slightly less variation, with an average of 0.387 and a standard deviation of 0.030, as seen in Table 1. The results are visually represented by averages calculated based on both depth and the site where incubation was completed in Fig. 3.

*Table 1:Averaged S and k values from Sencha and Rooibos % Mass Loss Data; Initial and Final Mass Values can be seen in Table A1; Calculations of individual S and k values can be seen in Table A2; t = total time placed, 72 days; Average S = 0.387 with STDEV = 0.0030; Average k = 0.018 with STDEV = 0.0045*

| | Average S | Standard Deviation of S | Average k | Standard Deviation of k |
|---|---|---|---|---|
| 1-Inch Depth, All Sites | 0.372 | 0.04376 | 0.015 | 0.00441 |
| 2-Inch Depth, All Sites | 0.393 | 0.02255 | 0.020 | 0.00621 |
| 3-Inch Depth, All Sites | 0.389 | 0.03412 | 0.019 | 0.00486 |
| 4-Inch Depth, All Sites | 0.392 | 0.02330 | 0.017 | 0.00168 |
| Site 1, All Depths | 0.397 | 0.03480 | 0.021 | 0.00805 |
| Site 2, All Depths | 0.401 | 0.02894 | 0.018 | 0.00145 |
| Site 3, All Depths | 0.390 | 0.01448 | 0.017 | 0.00236 |
| Site 4, All Depths | 0.357 | 0.02580 | 0.015 | 0.00223 |
| All Sites and Depths | 0.387 | 0.03006 | 0.018 | 0.00447 |

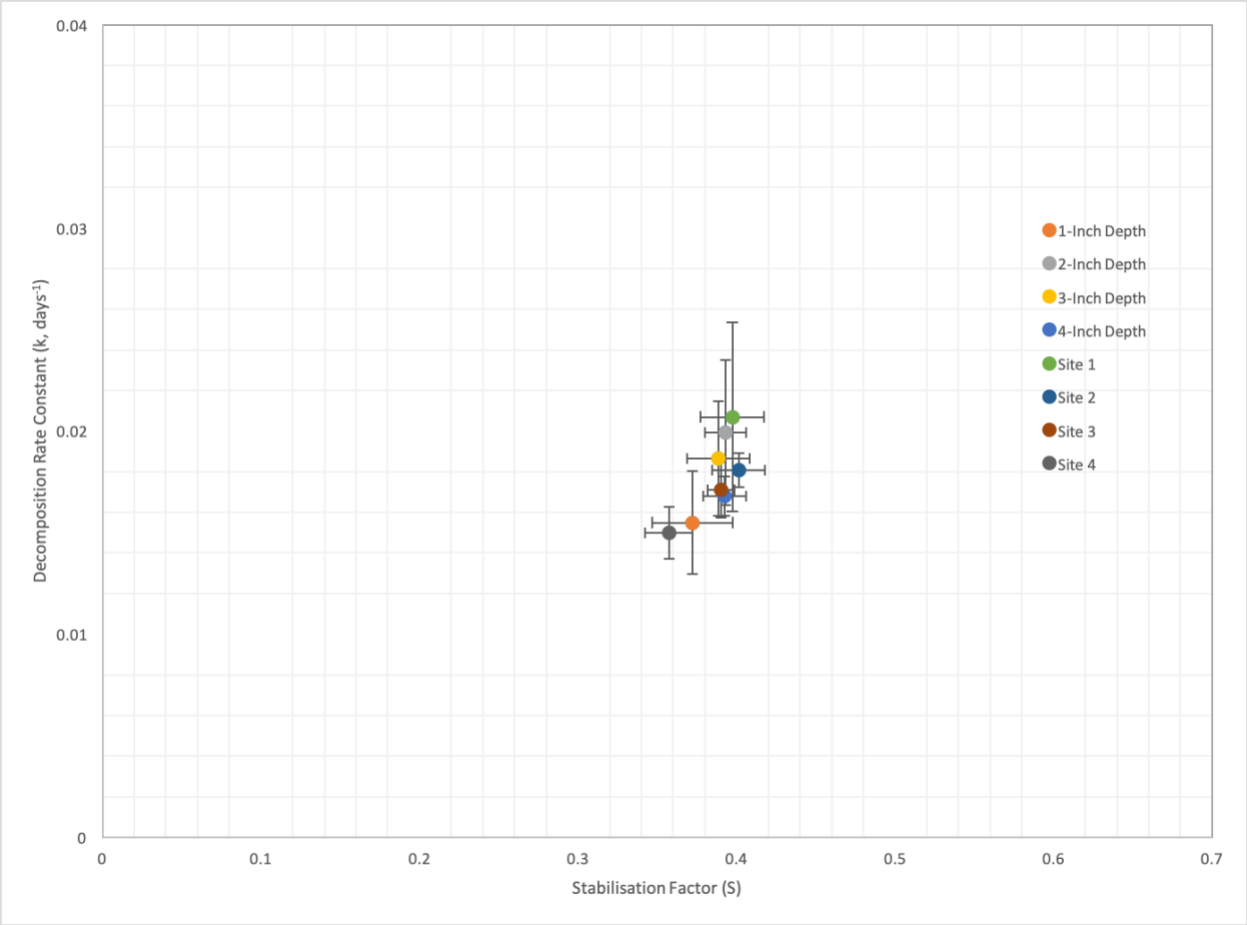


*Figure 3: Stabilization Factor (S) and Decomposition Rate Constant (k) Values Averaged by Site and Depth of Incubation, values these are based are compiled in Table 1, raw data can be seen in Table A1 and the calculations can be seen in Table A2.*

The values for percent mass remaining were found not to be significantly different by either site or depth of incubation, with negligible interaction between the two (ANOVA 2 Factor, $P > 0.05$, Tables A3 and A4).

The data for both tea types at 20 and 30°C did fit the exponential model recommended by Keuskamp et al. (2013) (Sencha at 20°C: $R^2 = 0.891$, $P < 0.05$, Table A5; Sencha at 30°C: $R^2 = 0.890$, $P < 0.05$, Table A5; Rooibos at 20°C: $R^2 = 0.861$, $P < 0.05$, Table A6; Rooibos at 30°C: $R^2 = 0.998$, $P < 0.05$, Table A6). The decomposition data for both tea types failed to fit the exponential model proposed when incubated at 5°C (Sencha at 5°C: $R^2 = 0.367$, $P > 0.05$, Table A5; Rooibos at 5°C: $R^2 = 0.489$, $P > 0.05$, Table A6).

The decomposition rate constants were not found to be different between the 20°C and 30°C incubations for either the Rooibos tea or Sencha Green tea (Sencha: ANCOVA, P = 0.90, Table A7; Rooibos: ANCOVA, P = 0.4415, Table A8). There were significant differences seen between the decomposition rates constants calculated for the 20 and 30°C incubations and the 5°C trial for both tea types (Sencha: ANCOVA, P = 0.003, Table A9; Rooibos: ANCOVA, P = 0.0002, Table A10).

Differences between the rates of decomposition seen in the Sencha Green Tea and Rooibos tea are most apparent in the graph of their relative masses remaining after a natural log transformation, seen in Fig. 4. The decomposition rate constants calculated for the Sencha tea were significantly higher than those for the Rooibos tea at both 20 and 30°C, and still somewhat higher at 5°C, as seen in Fig. 5.

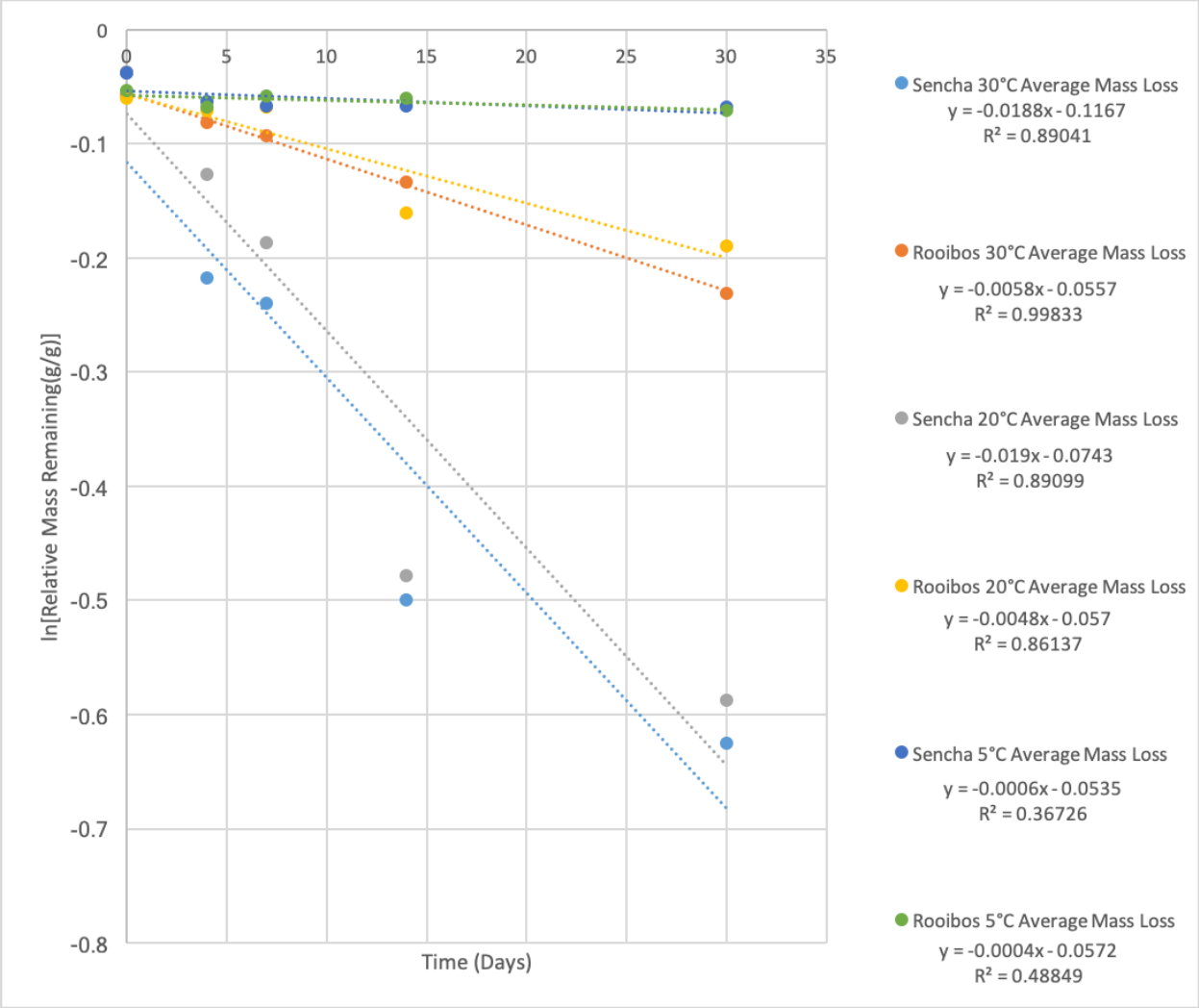


*Figure 4: ln(Relative Mass Remaining) for both Sencha and Rooibos Teas vs Time; a natural log transformation was used to linearize the relative mass remaining with respect to time by viewing it in log space due to the original fit being exponential; raw data can be seen in Table A11*

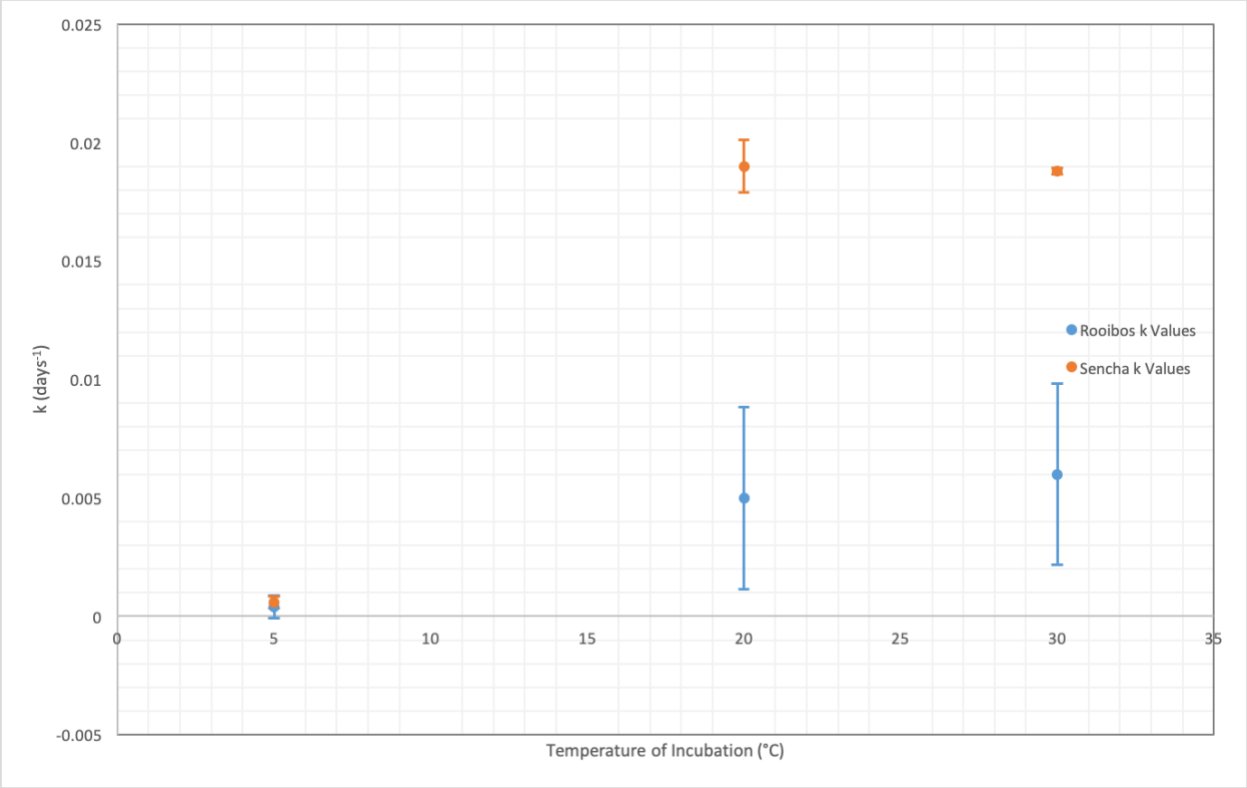


*Figure 5: Decomposition Rate Constants for Sencha and Rooibos Teas with Standard Errors from LN Transformed Linear Regression, Decomposition Rate Constants can be seen as the Slope of the Linearized Decompositions in Figure 4*

**Discussion:**

There is a large set of studies which have contributed data to the TBI, but as of yet one has not been done on a green roof. This would contribute to studying decomposition on green roofs by providing a greater level of comparability across sites. The specific conditions which exist on a green roof make it an especially interesting site of study, including the lack of percolation to underlying soil layers, the engineered nature of the soils, and the fact that the biomass in the soils is falsely included and often single-source. This could provide valuable data for both the scientific community in general and the University of Arkansas, as it could suggest whether the green roof is functioning optimally.

It was hoped that the decomposition rate constant on this site would be comparable to that for a semi-arid, well-fertilized, and sandy soil. This is due to the fact that the structure of the soil is designed to be similar to sand in order to provide increased infiltration and water storage capacity, but compost is included to allow sufficient nutrients to support plant growth. The stabilization factors calculated are closest to those reported values for sandy, desert soils and significantly higher than most forests or grassland as shown by comparison the values reported by Keuskamp et al. (2013). Overall, the decomposition rate constant calculated was higher than those for a wet forest, a pasture, all types of peat, and a sandy desert, but lower than those for all types of grassland and all forests except the extremely wet sample (Keuskamp et al., 2013). These confirmed the original idea of what these should be based on the soil composition described.

The lack of a difference seen in decomposition due to either depth of incubation or the site where tea bags were placed helped answer two major questions set out in this study, whether there would be variation due to depth in the soil profile caused by the soil horizon and whether there would be variation caused by the slope of the roof. The lack of differences in decomposition as depth changed suggests that there is not a significant effect caused by the soil horizon on decomposition. The lack of

differences in decomposition between sites at different heights along the elevation profile suggests that there is not a significant effect caused by the roof slope on decomposition.

One possible correlation recommended in the literature was an approximate doubling of soil respiration rate with every 10°C increase in temperature up to approximately 35°C (NRCS, 2014). However, these results much more closely resemble the recommendation that temperature alone cannot be strongly correlated to decomposition rate of the leaf litter present in tea unless a specific moisture content threshold is met (Djukic et al., 2018). It is possible that due to the tea bags being placed on top of the soil layer instead of being buried they dried enough to inhibit decomposition in the 20°C and 30°C laboratory treatments.

While Keuskamp et al. (2013) did find some variation with temperature in a laboratory test they conducted at both 15°C and 25°C on temperate forest soil, they acknowledged that this relationship did not hold up when comparing a higher temperature set of Icelandic soil to a lower temperature in a close proximity due to the presence of geothermal vents warming the ground at one test site (Keuskamp et al., 2013). It warrants further research to determine the exact nature of the dependence of decomposition on temperature on a green roof, or whether seasonal factors such as precipitation play a larger role.

Tea bags incubated at 5°C had too little decomposition occur to provide a significant fit of the data to the exponential decay model. This temperature was at the extreme low end of the range reported in the study by Djukic et al. (2018), but there was a significantly lower rate of decomposition seen here than in the studies compiled. It is also important to note that the studies conducted at similar temperatures were still representative of summer conditions in the area where they occurred.

The Green tea decomposing much more quickly than the Rooibos tea fits with the trend described for these tea types by both Djukic et al. (2018) and Keuskamp et al. (2013), that the Green tea

has a significantly higher labile fraction while the Rooibos tea has a higher recalcitrant fraction. The reason given for this was the significantly higher cellulose content in Green Tea causing it to be significantly more prone to early decomposition, while the high lignin content of Rooibos leads to lower early stage decomposition (Keuskamp et al., 2018).

**Future Opportunities:**

There are several general improvements that could be made to the experimental setup used in this study if it were repeated in the future; some of the issues encountered were not seen mentioned in any of the literature reviewed in preparation, but would lead to less variability in future studies. Some issues were due to practical concerns of funding, space, and time constraints under which this project was completed.

The previous research by Keuskamp et al. (2013) that the laboratory setup was based on stated that the soil was kept moist by placing it over an underlying saturated sand layer, and that approach was taken here as well. While this would work fine in theory, for the longer trials additional water had to be added to underlying sand, which would have caused variation in the moisture content of both the soil and tea bags being studied. The fact that the tea bags were incubated on top of the soil could also have led to them drying significantly. If this variation in moisture content leads to variation in the decomposition rates of the teas, there will be no way to separate this from the effects of the independent variables being measured, time and temperature of decomposition. In future studies it would be possible to rectify this problem by utilizing a soil moisture sensor in conjunction with a small subsurface irrigation system to maintain a narrow range of moisture content and conducting additional tests where the tea bags are buried in a deeper soil layer. This variability in moisture content could also be remedied less exactly but significantly more cost-effectively by using one of the glass drip globes available for watering potted plants.

The temperatures maintained in the laboratory trials in this study have a certain level of variability due to the systems used to maintain them. If researchers conducting a similar study in the future have access to structures which allow temperature control with less variation, those would be preferred. The oven used in this study was observed to vary to across a 4 °C range (28-32 °C), the refrigerator was more consistent, with no variations more than 1 °C seen (4-6 °C), and the exact range of the room temperature setup was not confirmed. These were the least variable control methodologies available, but there is a large opportunity for improvement in reducing these ranges.

In addition to these temperature fluctuations, these incubations were started almost immediately after soils were collected from the field in late Winter/ early Spring conditions. While this was kept consistent across samples (with the exception of the 14-day samples, which were started later due to a scheduling issue) and treatments, this could be the reason for a large part of the variation seen from the models previously proposed. In the future, if a similar analysis is to be conducted during cold-weather conditions, it would be helpful to see the effects of allowing each soil which will be used for incubation to acclimate to the temperature at which the incubation will be conducted. One article explored the effects of the plant community present on a site on the amount of time it takes the soils to acclimate to changing climate conditions and found that soils with predominantly grass cover can take significantly longer to acclimate to new conditions (Cable et al., 2013). The time for this to occur was generally found to be between 1 and 2 weeks, with grass dominant communities, like the one present on this green roof, falling at the upper end of this range.

It would also be very helpful to see the results of a similar study conducted on the green roof over the summer months. Much of the speculation included with the discussion of the data collected could be resolved if this data were available. The exact relationships between temperature, season, and decomposition rates are complicated, and one test conducted from early to late spring is insufficient to

draw many strong conclusions on what exactly these are. The literature is also inconclusive and disagreement still exists on this matter, which further research could help resolve.

One additional unexpected problem experienced was the growth of mold on a portion the samples incubated at both 20 and 30 degrees C in the lab, which can be seen in Fig. 15. This could potentially be due to cross-contamination from other agricultural materials handled in the same laboratory, as wholly sanitary conditions were not established. No mention of this was seen in previous studies conducted using these tea bags, and it is unknown whether this could jeopardize the value of the data collected. This does raise concerns related to maintaining sanitary conditions in laboratories in which this sort of study is being conducted. From the point at which this was first observed, samples were only handled while wearing nitrile gloves and were thoroughly dried before being manipulated beyond being moved to the drying oven.

The consistency of plant litter and methods in the TBI allow it to serve as the basis for comparison across either a diversity of sites or at one site over time (Tea Bag Index, 2016). If additional decomposition data is taken by replicating this study on this site in the future, it could be compared to the values obtained to establish what trends may be occurring under current management strategies. If the soil ecosystem exhibits significantly decreased capacity to decompose plant litter in the future, it could suggest that management strategies are leading to a deterioration of that community, while an increase in decomposition capacity would be associated with a soil ecosystem becoming more robust.

In addition to providing an indicator to see how soil health is changing over time due to current processes, if specific strategies were undertaken to address the causes of problems in the soil, a replication of this study could be conducted afterward to help see if those mitigation strategies had been effective. To provide a specific example, a recent study has recommended that inoculating green roof soils with additional microbes which function as foundation species can lead to specific benefits

including increased biodiversity at higher trophic levels, overall remediation of soil food webs, and resultant increases in plant growth (Rumble & Gange, 2017). If such an application were attempted on the Hillside green roof, the tea bag index could be used after that application to calculate new decomposition rate constants and determine whether there was an increase, a decrease, or no change to the decomposition rate constant of the soil as a result of the treatment.

**Acknowledgements:**

I would not have been able to complete this thesis without Dr. Benjamin Runkle. As my thesis advisor, he continually kept me on track and re-oriented me in meaningful directions; I truly appreciate the time and effort spent on review, revision, and a few necessary wake-up calls.

I would also like to thank the faculty at the University of Arkansas, including Dr. Kusum Naithani, who introduced me to this study and who will be a member of my thesis committee; Dr. Brian Haggard, who I have worked with closely in my senior design project and has also agreed to serve on my thesis committee; and Dr. G. Scott Osborn, who allowed me to use both his laboratory space and equipment.

The staff in facilities management were crucial in allowing access to the Hillside green roof and extensive information about the site, of whom I worked most closely with Mr. Jay Huneycutt. In the Biological Engineering laboratories, I wanted to thank Mr. Leland Schrader and Mr. Julian Abram. The staff of the Honors College helped me greatly with advice and support throughout my time at the U of A.

I have worked concurrently on a research team studying the Hillside green roof with Madeline Oxner and Zachary Wofford, which was made possible by an Honors College Research Team Grant. Kanaan Hardaway and Eleanor Henson are set to continue related research next year. Each member of this team has provided me with valuable insight and assistance.

I don't know if I would have made it past freshman year without the support and understanding of my friends and family. There are too many people who have provided me with support when I needed it to list here. Instead of listing those who have supported me and will see me graduate, I want to remember two of those whose examples I have relied on and who I will always remember. Thank you Jessamy Samuels, my friend, you helped show me what was worth caring about. Thank you Alton McCartney, my grandfather, you showed me who I should strive to be to those I care about.

## Appendix:

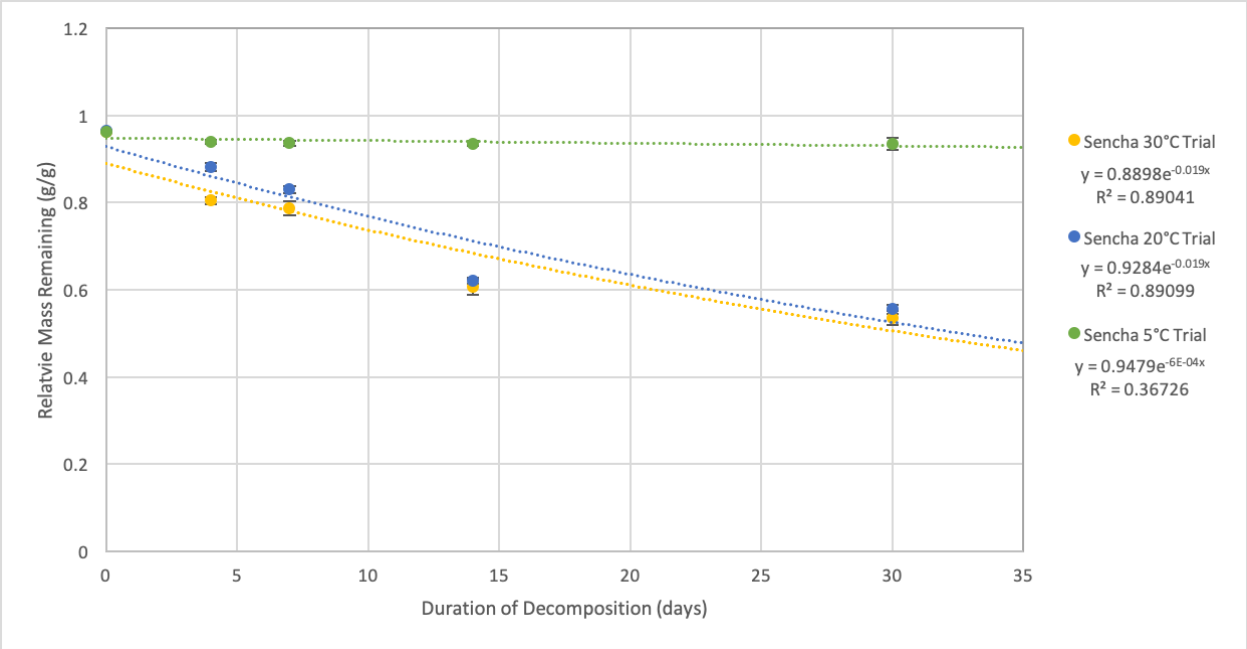


*Figure A1: Lab Trial Sencha Green Tea Relative Mass Remaining Results Fit to Exponential Decay Model Described in Equation 1*

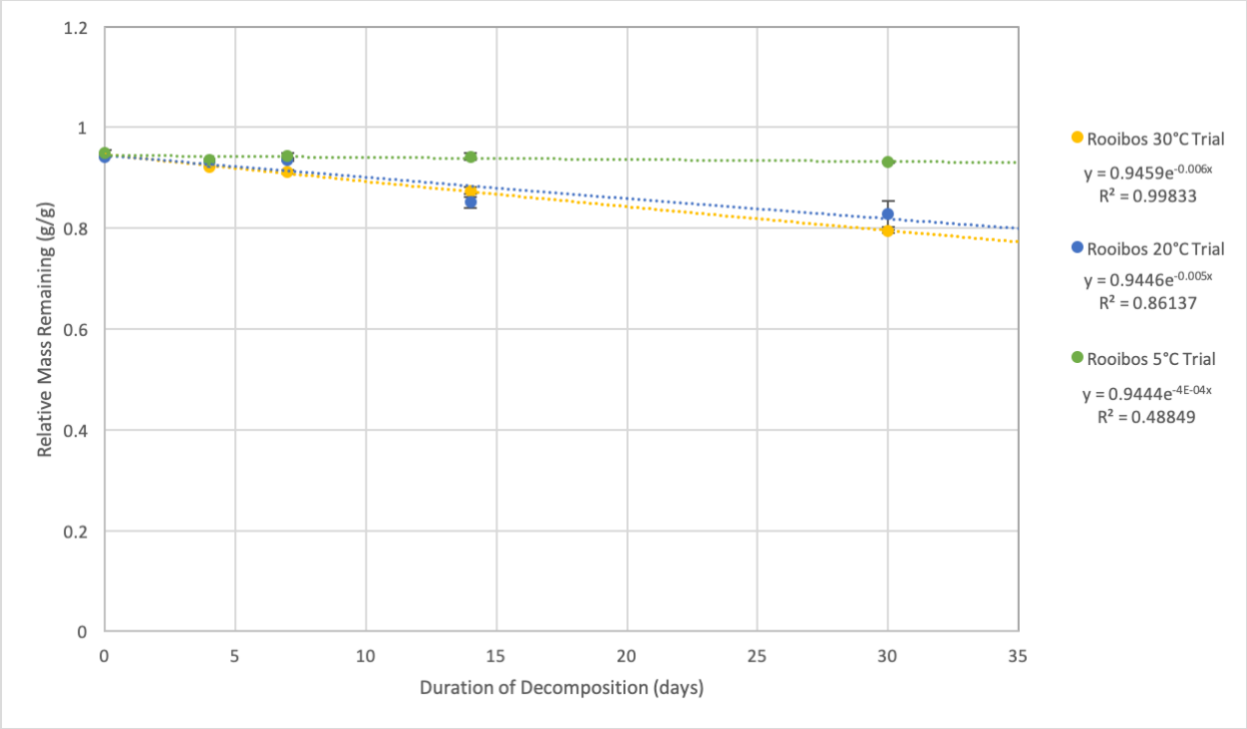


*Figure A2: Lab Trial Rooibos Tea Relative Mass Remaining Results fit to Exponential Decay Model Described in Equation 1*

*Table A1: Field-Tested Tea Bag Data; Placed 02/05/2019 from 5-6 P.M.; retrieved 04/18/2019 from 4-5 P.M.; total incubation time of 72 days, short of the recommended 90 days but over the minimum of 60 days suggested*

| Depth (in) | Profile (1-4) | Rooibos Tea Initial Mass (g) | Green Tea Initial Mass (g) | Rooibos Tea Final Mass (g) | Green Tea Final Mass (g) | % Initial Mass Remaining, Rooibos | % Initial Mass Remaining, Sencha |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 2.2866 | 2.1077 | 1.8381 | 0.9615 | 0.8039 | 0.4562 |
| 1 | 2 | 2.3056 | 2.0931 | 1.7501 | 1.0774 | 0.7591 | 0.5147 |
| 1 | 3 | 2.2522 | 2.0699 | 1.6871 | 1.0041 | 0.7491 | 0.4851 |
| 1 | 4 | 2.2842 | 2.126 | 1.7814 | 0.9126 | 0.7799 | 0.4293 |
| 2 | 1 | 2.3234 | 2.0522 | 1.6583 | 1.0275 | 0.7137 | 0.5007 |
| 2 | 2 | 2.2845 | 1.9933 | 1.7516 | 0.9858 | 0.7667 | 0.4946 |
| 2 | 3 | 2.3186 | 2.0345 | 1.7483 | 1.017 | 0.7540 | 0.4999 |
| 2 | 4 | 2.2952 | 2.0621 | 1.7648 | 0.9502 | 0.7689 | 0.4608 |
| 3 | 1 | 2.2759 | 2.0833 | 1.6812 | 1.0969 | 0.7387 | 0.5265 |
| 3 | 2 | 2.2436 | 2.1439 | 1.6881 | 0.9902 | 0.7524 | 0.4619 |
| 3 | 3 | 2.2857 | 2.0215 | 1.7745 | 0.9516 | 0.7763 | 0.4707 |
| 3 | 4 | 2.2582 | 2.134 | 1.7038 | 1.0272 | 0.7545 | 0.4813 |
| 4 | 1 | 2.2239 | 2.108 | 1.6918 | 1.0261 | 0.7607 | 0.4868 |
| 4 | 2 | 2.312 | 2.092 | 1.7617 | 1.0716 | 0.7620 | 0.5122 |
| 4 | 3 | 2.3636 | 2.0416 | 1.8248 | 1.0015 | 0.7720 | 0.4905 |
| 4 | 4 | 2.325 | 2.1233 | 1.7842 | 0.9859 | 0.7674 | 0.4643 |
| | | | | | Average: | 0.7612 | 0.4835 |
| | | | | | STDEV | 0.0195 | 0.0253 |

*Table A2: Calculation of S and k values from Sencha and Rooibos % Mass Loss Data; Initial and Final Mass Values can be seen in Table A1 in the Appendix; t = total time placed, 72 days; Average S = 0.387 with STDEV = 0.0030; Average k = 0.018 with STDEV = 0.0045*

| | | Hr | 0.552 | Hg | 0.842 | |
|---|---|---|---|---|---|---|
| | | Substituted % Mass Loss, Green Tea | Substituted % Mass Remaining, Rooibos | S=1-(ag/Hg) | ar=Hr(1-s) | k=[ln(((W(t)-(1-ar))/ar)]/-t |
| Depth (in) | Profile (1-4) | ag | W(t) | S | ar | k |
| 1 | 1 | 0.544 | 0.804 | 0.354 | 0.357 | 0.011 |
| 1 | 2 | 0.485 | 0.759 | 0.424 | 0.318 | 0.020 |
| 1 | 3 | 0.515 | 0.749 | 0.388 | 0.338 | 0.019 |
| 1 | 4 | 0.571 | 0.780 | 0.322 | 0.374 | 0.012 |
| 2 | 1 | 0.499 | 0.714 | 0.407 | 0.327 | 0.029 |
| 2 | 2 | 0.505 | 0.767 | 0.400 | 0.331 | 0.017 |
| 2 | 3 | 0.500 | 0.754 | 0.406 | 0.328 | 0.019 |
| 2 | 4 | 0.539 | 0.769 | 0.360 | 0.353 | 0.015 |
| 3 | 1 | 0.473 | 0.739 | 0.438 | 0.310 | 0.026 |
| 3 | 2 | 0.538 | 0.752 | 0.361 | 0.353 | 0.017 |
| 3 | 3 | 0.529 | 0.776 | 0.371 | 0.347 | 0.014 |
| 3 | 4 | 0.519 | 0.754 | 0.384 | 0.340 | 0.018 |
| 4 | 1 | 0.513 | 0.761 | 0.390 | 0.336 | 0.017 |
| 4 | 2 | 0.488 | 0.762 | 0.421 | 0.320 | 0.019 |
| 4 | 3 | 0.509 | 0.772 | 0.395 | 0.334 | 0.016 |
| 4 | 4 | 0.536 | 0.767 | 0.364 | 0.351 | 0.015 |
| | Average | 0.517 | 0.761 | 0.387 | 0.339 | 0.018 |
| | STDEV | 0.0253 | 0.0195 | 0.0301 | 0.0166 | 0.0045 |

*Table A3*: Sencha Tea % Mass Remaining 2-Factor ANOVA Without Replication by Site and Depth

| | 1-inch Depth | 2-inch Depth | 3-inch Depth | 4-Inch Depth |
|---|---|---|---|---|
| Profile 1 | 0.456184 | 0.500682 | 0.526520 | 0.486765 |
| Profile 2 | 0.514739 | 0.494557 | 0.461869 | 0.512237 |
| Profile 3 | 0.485096 | 0.499877 | 0.470740 | 0.490547 |
| Profile 4 | 0.429257 | 0.460792 | 0.481350 | 0.464324 |

Anova: Two-Factor Without Replication

| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Profile 1 | 4 | 1.970152 | 0.492538 | 0.000859 |
| Profile 2 | 4 | 1.983401 | 0.495850 | 0.000594 |
| Profile 3 | 4 | 1.946259 | 0.486565 | 0.000149 |
| Profile 4 | 4 | 1.835723 | 0.458931 | 0.000472 |
| | | | | |
| 1-inch Depth | 4 | 1.885276 | 0.471319 | 0.001358 |
| 2-inch Depth | 4 | 1.955908 | 0.488977 | 0.000360 |
| 3-inch Depth | 4 | 1.940478 | 0.485120 | 0.000825 |
| 4-Inch Depth | 4 | 1.953873 | 0.488468 | 0.000385 |

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Rows | 0.0034 | 3 | 0.001130 | 1.884020 | 0.202736 | 3.862548 |
| Columns | 0.0008 | 3 | 0.000274 | 0.457363 | 0.718701 | 3.862548 |
| Error | 0.0054 | 9 | 0.000600 | | | |
| Total | 0.0096 | 15 | | | | |

*Table A4*: Rooibos Tea % Mass Remaining 2-Factor ANOVA Without Replication by Site and Depth

| | 1-inch Depth | 2-inch Depth | 3-inch Depth | 4-Inch Depth |
|---|---|---|---|---|
| Profile 1 | 0.803857 | 0.713738 | 0.738697 | 0.760736 |
| Profile 2 | 0.759065 | 0.766732 | 0.752407 | 0.761981 |
| Profile 3 | 0.749090 | 0.754033 | 0.776349 | 0.772043 |
| Profile 4 | 0.779879 | 0.768909 | 0.754495 | 0.767398 |

Anova: Two-Factor Without Replication

| *SUMMARY* | *Count* | *Sum* | *Average* | *Variance* |
|---|---|---|---|---|
| Profile 1 | 4 | 3.017028 | 0.754257 | 0.001462 |
| Profile 2 | 4 | 3.040185 | 0.760046 | 0.000036 |
| Profile 3 | 4 | 3.051514 | 0.762878 | 0.000178 |
| Profile 4 | 4 | 3.070681 | 0.767670 | 0.000108 |
| | | | | |
| 1-inch Depth | 4 | 3.091891 | 0.772973 | 0.000588 |
| 2-inch Depth | 4 | 3.003412 | 0.750853 | 0.000655 |
| 3-inch Depth | 4 | 3.021947 | 0.755487 | 0.000243 |
| 4-Inch Depth | 4 | 3.062157 | 0.765539 | 0.000027 |

ANOVA

| *Source of Variation* | *SS* | *df* | *MS* | *F* | *P-value* | *F crit* |
|---|---|---|---|---|---|---|
| Rows | 0.000377 | 3 | 0.000126 | 0.271557 | 0.844438 | 3.862548 |
| Columns | 0.001189 | 3 | 0.000396 | 0.856404 | 0.497826 | 3.862548 |
| Error | 0.004163 | 9 | 0.000463 | | | |
| Total | 0.005729 | 15 | | | | |

*Table A5: Regression Analysis for Sencha Lab Trials, Raw Data Can Be Seen in Table A11*

Regression Statistics for ln Sencha 30°C Trial

| | |
|---|---|
| Multiple R | 0.9436 |
| R Square | 0.8904 |
| Adjusted R Squa | 0.8539 |
| Standard Error | 0.0900 |
| Observations | 5 |

ANOVA

| | df | SS | MS | F | Significance F |
|---|---|---|---|---|---|
| Regression | 1 | 0.1975 | 0.1975 | 24.3735 | 0.0159 |
| Residual | 3 | 0.0243 | 0.0081 | | |
| Total | 4 | 0.2218 | | | |

| | Coefficients | Standard Error | t Stat | P-value | Lower 95% | Upper 95% | Lower 95.0% | Upper 95.0% |
|---|---|---|---|---|---|---|---|---|
| Intercept | -0.1167 | 0.0582 | -2.0065 | 0.1385 | -0.3018 | 0.0684 | -0.3018 | 0.0684 |
| Time (days) | -0.0188 | 0.0038 | -4.9370 | 0.0159 | -0.0310 | -0.0067 | -0.0310 | -0.0067 |

Regression Statistics for Sencha 20°C Trial

| | |
|---|---|
| Multiple R | 0.9439 |
| R Square | 0.8910 |
| Adjusted R Squa | 0.8547 |
| Standard Error | 0.0905 |
| Observations | 5 |

ANOVA

| | df | SS | MS | F | Significance F |
|---|---|---|---|---|---|
| Regression | 1 | 0.2006 | 0.2006 | 24.5216 | 0.0158 |
| Residual | 3 | 0.0245 | 0.0082 | | |
| Total | 4 | 0.2252 | | | |

| | Coefficients | Standard Error | t Stat | P-value | Lower 95% | Upper 95% | Lower 95.0% | Upper 95.0% |
|---|---|---|---|---|---|---|---|---|
| Intercept | -0.0743 | 0.0585 | -1.2705 | 0.2935 | -0.2603 | 0.1118 | -0.2603 | 0.1118 |
| Time (days) | -0.0190 | 0.0038 | -4.9519 | 0.0158 | -0.0312 | -0.0068 | -0.0312 | -0.0068 |

Regression Statistics for Sencha 5°C Trial

| | |
|---|---|
| Multiple R | 0.6060 |
| R Square | 0.3673 |
| Adjusted R Squa | 0.1563 |
| Standard Error | 0.0116 |
| Observations | 5 |

ANOVA

| | df | SS | MS | F | Significance F |
|---|---|---|---|---|---|
| Regression | 1.0000 | 0.0002 | 0.0002 | 1.7413 | 0.2786 |
| Residual | 3 | 0.0004 | 0.0001 | | |
| Total | 4 | 0.0006 | | | |

| | Coefficients | Standard Error | t Stat | P-value | Lower 95% | Upper 95% | Lower 95.0% | Upper 95.0% |
|---|---|---|---|---|---|---|---|---|
| Intercept | -0.0535 | 0.0075 | -7.1589 | 0.0056 | -0.0773 | -0.0297 | -0.0773 | -0.0297 |
| Time (days) | -0.0006 | 0.0005 | -1.3196 | 0.2786 | -0.0022 | 0.0009 | -0.0022 | 0.0009 |

*Table A6: Regression Analysis for Rooibos Lab Trials, Raw Data can be seen in Table A11*

*Table A7: ANCOVA Analysis of LN Transformed Sencha Decomposition at 20°C and 30°C*

| Time (Days) | % Mass Remaining (30°C) | Time (Days) | % Mass Remaining (20°C) |
|---|---|---|---|
| 0 | 0.963 | 0 | 0.964 |
| 4 | 0.805 | 4 | 0.881 |
| 7 | 0.786 | 7 | 0.830 |
| 14 | 0.607 | 14 | 0.620 |
| 30 | 0.535 | 30 | 0.556 |

## One-Way ANCOVA: Summary Statistics ANCOVA Results (k=2)

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| adjusted means | 0.00 | 1 | 0.00 | 0.49 | 0.5061 |
| adjusted error | 0.03 | 7 | 0.00 | | |
| adjusted total | 0.04 | 8 | | | |

## Test for Homogeneity of Regressions

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| between regressions | 0.00 | 1 | 0.00 | 0.02 | 0.9037 |
| remainder | 0.03 | 7 | 0.00 | | |

| | | CV | DV | |
|---|---|---|---|---|
| | Means | Observed | Observed | Adjusted |
| | 1 | 11.00 | 0.74 | 0.74 |
| | 2 | 11.00 | 0.77 | 0.77 |

*Table A8: ANCOVA Analysis of LN Transformed Rooibos Decomposition at 20°C and 30°C*

| Time (Days) | % Mass Remaining (30°C) | Time (Days) | % Mass Remaining (20°C) |
|---|---|---|---|
| 0 | 0.944 | 0 | 0.942 |
| 4 | 0.922 | 4 | 0.932 |
| 7 | 0.911 | 7 | 0.935 |
| 14 | 0.875 | 14 | 0.852 |
| 30 | 0.794 | 30 | 0.828 |

## One-Way ANCOVA: Summary Statistics ANCOVA Results (k=2)

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| adjusted means | 0.00 | 1 | 0.00 | 0.67 | 0.4415 |
| adjusted error | 0.00 | 7 | 0.00 | | |
| adjusted total | 0.00 | 8 | | | |

## Test for Homogeneity of Regressions

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| between regressions | 0.00 | 1 | 0.00 | 0.71 | 0.4275 |
| remainder | 0.00 | 7 | 0.00 | | |

| | | CV | DV | |
|---|---|---|---|---|
| | Means | Observed | Observed | Adjusted |
| | 1 | 11.00 | 0.89 | 0.89 |
| | 2 | 11.00 | 0.90 | 0.90 |

*Table A9: ANCOVA Analysis of LN Transformed Sencha Decomposition at 5°C, 20°C, and 30°C*

| Time (Days) | % Mass Remaining (30°C) | Time (Days) | % Mass Remaining (20°C) | Time (Days) | % Mass Remaining (5°C) |
|---|---|---|---|---|---|
| 0 | 0.963 | 0 | 0.964 | 0 | 0.962 |
| 4 | 0.805 | 4 | 0.881 | 4 | 0.939 |
| 7 | 0.786 | 7 | 0.830 | 7 | 0.936 |
| 14 | 0.607 | 14 | 0.620 | 14 | 0.935 |
| 30 | 0.535 | 30 | 0.556 | 30 | 0.934 |

## One-Way ANCOVA: Summary Statistics ANCOVA Results (k=2)

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| adjusted means | 0.12 | 2 | 0.06 | 6.78 | 0.0121 |
| adjusted error | 0.10 | 11 | 0.01 | | |
| adjusted total | 0.21 | 13 | | | |

## Test for Homogeneity of Regressions

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| between regressions | 0.06 | 2 | 0.03 | 9.99 | 0.0034 |
| remainder | 0.03 | 11 | 0.00 | | |

| | CV | DV | |
|---|---|---|---|
| Means | Observed | Observed | Adjusted |
| 1 | 11.00 | 0.74 | 0.74 |
| 2 | 11.00 | 0.77 | 0.77 |
| 3 | 11.00 | 0.94 | 0.94 |

*Table A10: ANCOVA Analysis of LN Transformed Rooibos Decomposition at 5°C, 20°C, and 30°C*

| Time (Days) | % Mass Remaining (30°C) | Time (Days) | % Mass Remaining (20°C) | Time (Days) | % Mass Remaining (5°C) |
|---|---|---|---|---|---|
| 0 | 0.944 | 0 | 0.942 | 0 | 0.948 |
| 4 | 0.922 | 4 | 0.932 | 4 | 0.935 |
| 7 | 0.911 | 7 | 0.935 | 7 | 0.943 |
| 14 | 0.875 | 14 | 0.852 | 14 | 0.942 |
| 30 | 0.794 | 30 | 0.828 | 30 | 0.931 |

## One-Way ANCOVA: Summary Statistics ANCOVA Results (k=2)

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| adjusted means | 0.01 | 2 | 0.00 | 4.80 | 0.0317 |
| adjusted error | 0.01 | 11 | 0.00 | | |
| adjusted total | 0.02 | 13 | | | |

## Test for Homogeneity of Regressions

| Source | SS | df | MS | F | P |
|---|---|---|---|---|---|
| between regressions | 0.01 | 2 | 0.00 | 20.94 | 0.0002 |
| remainder | 0.00 | 11 | 0.00 | | |

| | | CV | DV | |
|---|---|---|---|---|
| | Means | Observed | Observed | Adjusted |
| | 1 | 11.00 | 0.89 | 0.89 |
| | 2 | 11.00 | 0.90 | 0.90 |
| | 3 | 11.00 | 0.94 | 0.94 |

*Table A11: Laboratory Incubation Initial and Final Weights and Mass Fractions Remaining (g/g)*

| Treatment | Duration (Days) | Sencha Initial Weight | Rooibos Initial Weight | Sencha Final Weight | Rooibos Final Weight | Mass Remaining (g/g) | Mass Remaining (g/g) |
|---|---|---|---|---|---|---|---|
| Hot | 0 | 2.116 | 2.2267 | 2.0365 | 2.1055 | 0.9624 | 0.9456 |
| Hot | 0 | 2.048 | 2.2791 | 1.9701 | 2.1502 | 0.9620 | 0.9434 |
| Hot | 0 | 2.0692 | 2.3009 | 1.9941 | 2.1713 | 0.9637 | 0.9437 |
| Cold | 0 | 2.0155 | 2.261 | 1.9394 | 2.1255 | 0.9622 | 0.9401 |
| Cold | 0 | 2.0276 | 2.1989 | 1.9505 | 2.1148 | 0.9620 | 0.9618 |
| Cold | 0 | 2.0157 | 2.2485 | 1.9411 | 2.1203 | 0.9630 | 0.9430 |
| Room | 0 | 2.1127 | 2.2452 | 2.0379 | 2.1174 | 0.9646 | 0.9431 |
| Room | 0 | 1.9878 | 2.247 | 1.9146 | 2.1127 | 0.9632 | 0.9402 |
| Room | 0 | 2.0307 | 2.2932 | 1.9567 | 2.1617 | 0.9636 | 0.9427 |
| Hot | 4 | 2.0167 | 2.2953 | 1.6502 | 2.1166 | 0.8183 | 0.9221 |
| Hot | 4 | 2.0698 | 2.2994 | 1.6368 | 2.1177 | 0.7908 | 0.9210 |
| Hot | 4 | 2.0048 | 2.2584 | 1.6149 | 2.0831 | 0.8055 | 0.9224 |
| Cold | 4 | 2.0924 | 2.2483 | 1.9456 | 2.0934 | 0.9298 | 0.9311 |
| Cold | 4 | 2.0248 | 2.228 | 1.9075 | 2.0847 | 0.9421 | 0.9357 |
| Cold | 4 | 2.0445 | 2.1301 | 1.9302 | 1.9959 | 0.9441 | 0.9370 |
| Room | 4 | 2.0644 | 2.2483 | 1.7914 | 2.0975 | 0.8678 | 0.9329 |
| Room | 4 | 2.0833 | 2.1948 | 1.8304 | 2.0392 | 0.8786 | 0.9291 |
| Room | 4 | 2.1568 | 2.2821 | 1.9337 | 2.1299 | 0.8966 | 0.9333 |
| Hot | 7 | 2.0386 | 2.2734 | 1.5417 | 2.0618 | 0.7563 | 0.9069 |
| Hot | 7 | 2.0141 | 2.2724 | 1.6036 | 2.0874 | 0.7962 | 0.9186 |
| Hot | 7 | 2.0232 | 2.3089 | 1.6327 | 2.0984 | 0.8070 | 0.9088 |
| Cold | 7 | 2.0797 | 2.2268 | 1.9501 | 2.0892 | 0.9377 | 0.9382 |
| Cold | 7 | 2.134 | 2.2474 | 2.0126 | 2.109 | 0.9431 | 0.9384 |
| Cold | 7 | 2.0781 | 2.1756 | 1.924 | 2.075 | 0.9258 | 0.9538 |
| Room | 7 | 2.0771 | 2.3121 | 1.6919 | 2.1539 | 0.8145 | 0.9316 |
| Room | 7 | 2.0156 | 2.2163 | 1.674 | 2.076 | 0.8305 | 0.9367 |
| Room | 7 | 2.0782 | 2.2697 | 1.754 | 2.1245 | 0.8440 | 0.9360 |
| Room | 14 | 2.0391 | 2.2451 | 1.2356 | 1.9596 | 0.6060 | 0.8728 |
| Room | 14 | 2.0772 | 2.2429 | 1.3005 | 1.874 | 0.6261 | 0.8355 |
| Room | 14 | 2.0614 | 2.2389 | 1.2945 | 1.8956 | 0.6280 | 0.8467 |
| Cold | 14 | 2.0477 | 1.9138 | 1.8924 | 1.784 | 0.9242 | 0.9322 |
| Cold | 14 | 2.0832 | 2.266 | 1.9487 | 2.1638 | 0.9354 | 0.9549 |
| Cold | 14 | 2.0019 | 2.1616 | 1.8936 | 2.0287 | 0.9459 | 0.9385 |
| Hot | 14 | 2.1466 | 2.2302 | 1.2748 | 1.9561 | 0.5939 | 0.8771 |
| Hot | 14 | 2.0732 | 2.2788 | 1.334 | 1.96682 | 0.6434 | 0.8631 |
| Hot | 14 | 2.1072 | 2.2334 | 1.2285 | 1.974 | 0.5830 | 0.8839 |
| Hot | 30 | 1.966 | 2.296 | 0.9901 | 1.8154 | 0.5036 | 0.7907 |
| Hot | 30 | 2.0324 | 2.2107 | 1.1388 | 1.7483 | 0.5603 | 0.7908 |
| Hot | 30 | 1.9543 | 2.2203 | 1.0585 | 1.7776 | 0.5416 | 0.8006 |
| Cold | 30 | 2.0747 | 2.197 | 1.9476 | 2.0473 | 0.9387 | 0.9319 |
| Cold | 30 | 2.0454 | 2.2142 | 1.8604 | 2.0561 | 0.9096 | 0.9286 |
| Cold | 30 | 2.0973 | 2.2675 | 2.0025 | 2.1169 | 0.9548 | 0.9336 |
| Room | 30 | 2.1451 | 2.2811 | 1.1486 | 1.9605 | 0.5355 | 0.8595 |
| Room | 30 | 2.0026 | 2.2602 | 1.1365 | 1.913 | 0.5675 | 0.8464 |
| Room | 30 | 2.1194 | 2.2683 | 1.1948 | 1.7632 | 0.5637 | 0.7773 |

*Table A12: Laboratory Incubation Averaged Mass Fractions Remaining (g/g) and Standard Errors*

| Duration (Days) | Sencha Hot Average Mass Loss | Rooibos Hot Average Mass Loss | Sencha Room Average Mass Loss | Rooibos Room Average Mass Loss | Sencha Cold Average Mass Loss | Rooibos Cold Average Mass Loss |
|---|---|---|---|---|---|---|
| 0 | 0.9627 | 0.9442 | 0.9638 | 0.9420 | 0.9624 | 0.9483 |
| 4 | 0.8049 | 0.9218 | 0.8810 | 0.9318 | 0.9387 | 0.9346 |
| 7 | 0.7865 | 0.9114 | 0.8297 | 0.9348 | 0.9355 | 0.9435 |
| 14 | 0.6068 | 0.8747 | 0.6200 | 0.8517 | 0.9352 | 0.9419 |
| 30 | 0.5352 | 0.7940 | 0.5556 | 0.8277 | 0.9344 | 0.9313 |
| Duration (Days) | Sencha Hot Average STD Errors | Rooibos Hot Average STD Errors | Sencha Room Average STD Errors | Rooibos Room Average STD Errors | Sencha Cold Average STD Errors | Rooibos Cold Average STD Errors |
| 0 | 0.0005 | 0.0007 | 0.0004 | 0.0009 | 0.0003 | 0.0068 |
| 4 | 0.0079 | 0.0004 | 0.0084 | 0.0013 | 0.0045 | 0.0018 |
| 7 | 0.0154 | 0.0036 | 0.0085 | 0.0016 | 0.0051 | 0.0051 |
| 14 | 0.0186 | 0.0061 | 0.0070 | 0.0111 | 0.0063 | 0.0068 |
| 30 | 0.0167 | 0.0033 | 0.0101 | 0.0255 | 0.0132 | 0.0015 |

*Table A13: Single Factor ANOVA Test Conducted to Test Statistical Differences by Depths Rooibos Placed On Site*

Rooibos % Remaining Analyzed by Depth

| | 1 Inch | 2 Inch | 3 Inch | 4 Inch |
|---|---|---|---|---|
| % Mass Remaining | 0.8039 | 0.7137 | 0.7387 | 0.7607 |
| % Mass Remaining | 0.7591 | 0.7667 | 0.7524 | 0.7620 |
| % Mass Remaining | 0.7491 | 0.7540 | 0.7763 | 0.7720 |
| % Mass Remaining | 0.7799 | 0.7689 | 0.7545 | 0.7674 |

Anova: Single Factor

SUMMARY

| Groups | Count | Sum | Average | Variance |
|---|---|---|---|---|
| 1 Inch | 4 | 3.09189 | 0.77297 | 0.00059 |
| 2 Inch | 4 | 3.00341 | 0.75085 | 0.00066 |
| 3 Inch | 4 | 3.02195 | 0.75549 | 0.00024 |
| 4 Inch | 4 | 3.06216 | 0.76554 | 0.00003 |

ANOVA

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 0.00119 | 3 | 0.00040 | 1.04709 | 0.40725 | 3.49029 |
| Within Groups | 0.00454 | 12 | 0.00038 | | | |
| Total | 0.00573 | 15 | | | | |

*Table A14: Single Factor ANOVA Test Conducted to Test Statistical Differences by Depths Sencha Green Placed On Site*

Sencha % Remaining Analyzed by Depth

| | 1 Inch | 2 Inch | 3 Inch | 4 Inch |
|---|---|---|---|---|
| % Mass Remaining | 0.4562 | 0.5007 | 0.5265 | 0.4868 |
| % Mass Remaining | 0.5147 | 0.4946 | 0.4619 | 0.5122 |
| % Mass Remaining | 0.4851 | 0.4999 | 0.4707 | 0.4905 |
| % Mass Remaining | 0.4293 | 0.4608 | 0.4813 | 0.4643 |

Anova: Single Factor

SUMMARY

| Groups | Count | Sum | Average | Variance |
|---|---|---|---|---|
| 1 Inch | 4 | 1.88528 | 0.47132 | 0.00136 |
| 2 Inch | 4 | 1.95591 | 0.48898 | 0.00036 |
| 3 Inch | 4 | 1.94048 | 0.48512 | 0.00083 |
| 4 Inch | 4 | 1.95387 | 0.48847 | 0.00039 |

ANOVA

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 0.00082 | 3 | 0.00027 | 0.37458 | 0.77294 | 3.49029 |
| Within Groups | 0.00879 | 12 | 0.00073 | | | |
| Total | 0.00961 | 15 | | | | |

*Table A15: Single Factor ANOVA Test Conducted to Test Statistical Differences by Location Rooibos Green Placed On Site*

Rooibos % Remaining Analysed by Site

| | Site 1 | Site 2 | Site 3 | Site 4 |
|---|---|---|---|---|
| % Mass Remaining | 0.8039 | 0.7591 | 0.7491 | 0.7799 |
| % Mass Remaining | 0.7137 | 0.7667 | 0.7540 | 0.7689 |
| % Mass Remaining | 0.7387 | 0.7524 | 0.7763 | 0.7545 |
| % Mass Remaining | 0.7607 | 0.7620 | 0.7720 | 0.7674 |

Anova: Single Factor

SUMMARY

| Groups | Count | Sum | Average | Variance |
|---|---|---|---|---|
| 1 Inch | 4 | 3.01703 | 0.75426 | 0.00146 |
| 2 Inch | 4 | 3.04019 | 0.76005 | 0.00004 |
| 3 Inch | 4 | 3.05151 | 0.76288 | 0.00018 |
| 4 Inch | 4 | 3.07068 | 0.76767 | 0.00011 |

ANOVA

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 0.00038 | 3 | 0.00013 | 0.28167 | 0.83765 | 3.49029 |
| Within Groups | 0.00535 | 12 | 0.00045 | | | |
| Total | 0.00573 | 15 | | | | |

*Table A16: Single Factor ANOVA Test Conducted to Test Statistical Differences by Location Sencha Green Placed On Site*

Sencha % Remaining Analysed by Site

| | Site 1 | Site 2 | Site 3 | Site 4 |
|---|---|---|---|---|
| % Mass Remaining | 0.4562 | 0.5147 | 0.4851 | 0.4293 |
| % Mass Remaining | 0.5007 | 0.4946 | 0.4999 | 0.4608 |
| % Mass Remaining | 0.5265 | 0.4619 | 0.4707 | 0.4813 |
| % Mass Remaining | 0.4868 | 0.5122 | 0.4905 | 0.4643 |

Anova: Single Factor

SUMMARY

| Groups | Count | Sum | Average | Variance |
|---|---|---|---|---|
| 1 Inch | 4 | 1.97015 | 0.49254 | 0.00086 |
| 2 Inch | 4 | 1.98340 | 0.49585 | 0.00059 |
| 3 Inch | 4 | 1.94626 | 0.48656 | 0.00015 |
| 4 Inch | 4 | 1.83572 | 0.45893 | 0.00047 |

ANOVA

| Source of Variation | SS | df | MS | F | P-value | F crit |
|---|---|---|---|---|---|---|
| Between Groups | 0.00339 | 3 | 0.0011 | 2.1797 | 0.1434 | 3.4903 |
| Within Groups | 0.00622 | 12 | 0.0005 | | | |
| Total | 0.00961 | 15 | | | | |


*Figure A3: Mettler Toledo AB104-S Used to Take Weights*

*Figure A4: Profiles of Bags Flagged Down Roof Slope*

*Figure A5: Placement of One Profile Each of Sencha and Rooibos Tea Bags*

*Figure A6: Mold Growth Noted on Tea Bags after Approximately 30 Days of Laboratory Incubation*

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