Tannin Leaching in the Macatawa Watershed

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Introduction to Tannins

Tannins are complex organic molecules that have very unique chemical reactivities and biological activities (Hagerman, 2002). Their molecular masses can range between 500 and 3000 g/mol. Tannins are part of the phenolic family of plant derived compounds and have a historical use in "tanning" leather. Their name comes from the Celtic word for 'oak 'which was the historical source for tannins used in leather making. Many carboxyl and hydroxyl groups found in tannins readily bond and precipitate proteins, starches, cellulose, minerals, and other macromolecules. Tannins will chelate metal ions and readily scavenge radicals as well.

As far as their biological functions go, tannins perform defensive functions within plants in that they prevent animal and insect browsing through their astringency properties, as well as having strong anti-microbial and anti-viral effects (Cannas, 2001). Tannins are also secondary plant compounds in that they don't appear to play a function in the primary functions of cells (i.e. metabolism). Tannins are biological anti-oxidants and they also have the ability to lower both carbohydrate and protein digestibility.

Relevance to the Macatawa Watershed

There are many ways that tannins are relevant to the Macatawa Watershed as well as human activities that surround the watershed as well. Anti-microbial properties that tannins possess can depress the decomposition of organic material in the water column. Also, the astringency properties are known to depress food intake in livestock because they reduce the palatability of drinking water and unless filtered, tannins impart the same astringent taste to drinking water.

There are many trees in the local watershed that contain tannins in relatively high amounts, including oaks, maples, birches, pines, and willows (Cannas, 2001). The tannins in these plants are found in all parts of the trees, but the tannins in the leaves have the most direct annual interaction with the environment as a consequence of the fall leaf drop. Because tannins easily dissolve in water, leaves that fall into a stream in a watershed would conceivably have their tannins leached from them.

The Working Hypothesis

There are two main questions that we hoped to answer with our research. First, what is the timeframe for the interaction between the leaves from the fall leaf drop and the watershed? And secondly, at what rate do the leaves in a body of water lose their tannins to the surrounding water? Our working hypothesis that we started out with was: Leaves submerged in a body of water will lose their tannins at a rapid rate due to their high water solubility.

Methodology

There are many methods are available to test for tannins. The two main ones are the HPLC methods and the functional group methods. The functional group methods include the Prussian Blue method, the Acid Butanol method, and the Vanillin method. The HPLC method is long, complicated, expensive, but very accurate. The Functional Group methods are quicker, cheaper, but do give up some quantitative accuracy. Because we planned to look at the relative change in tannin levels over a period of time as opposed to looking at specific quantitative values at a specific point, we chose to use the

Vanillin method, specifically the Vanillin method that was proposed by Price, Van Scoyoc, and Butler to test the tannin levels in leaves. This method was optimized to provide better results than what you normally may get.

Our plan of action was to use polyester mesh fabric that was sewn into two bags using fishing line as thread (figure 2). Mesh fabric was used in order to allow tannins to easily be removed from the bags once they were leached out of the leaves. Yellowing leaves from a single sugar maple tree were collected and placed into the bags. Two locations along Pine Creek were chosen as placement sites because of their easy accessibility and distance apart.

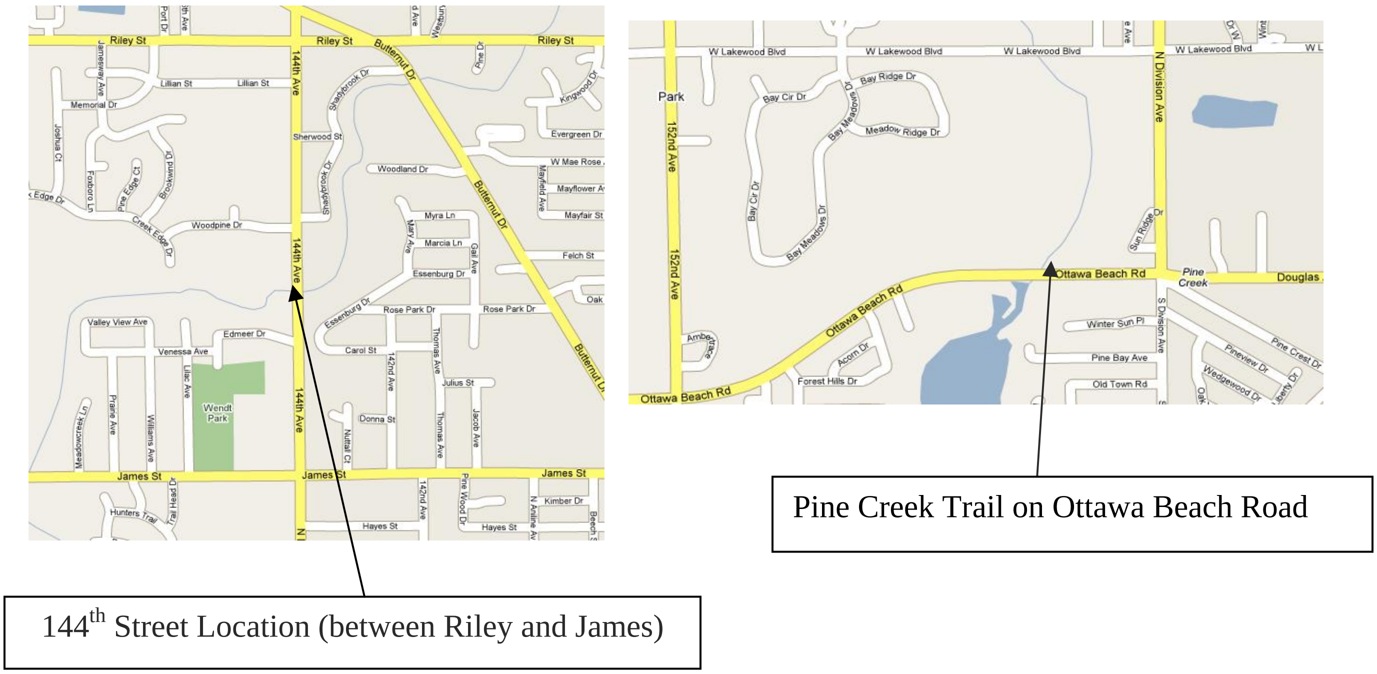

Figure 1: Locations of both leaf bags

Figure 2: Leaf bag example

As shown in figure 1, the two locations chosen were on 144th St. between Riley and James as well as at the Pine Creek Trail head on Ottawa Beach Rd. The leaf bags were placed at each location in areas where they were unlikely to be disturbed or removed from their locations.

Every 7 to 12 days a small sample (approx. 10 leaves) was taken from each leaf bag. The leaves were cleaned of any detritus from the creek by running them under tap water for a short amount of time and were then placed in a vacuum dryer to completely dry them out. These dried leaves were then ground into a fine powder using liquid Nitrogen and a mortar and pestle. 200 micrograms of the powdered leaves were them put into a test tube and 10mL of methanol was added. These test tubes were capped and put in a centrifuge for 20 minutes. Reagent was prepared daily by mixing equal quantities of 1% vanillin in methanol and 8% concentrated HCl in methanol. Once the samples are done centrifuging, they were taken out and 1mL of the aliquot was placed into another vial. The reagent was added to this aliquot 1mL at a time every minute through five minutes (figure 3). The samples were then allowed to sit for 15 minutes in a 30^{o}C water bath in order to allow the reaction to go to completion. A control was made up in the same way that a sample would, but the control itself didn't contain any leaves. The samples were then analyzed by a UV-VIS spectrophotometer (figure 4) in order to find the absorbency of the samples at 500nm.

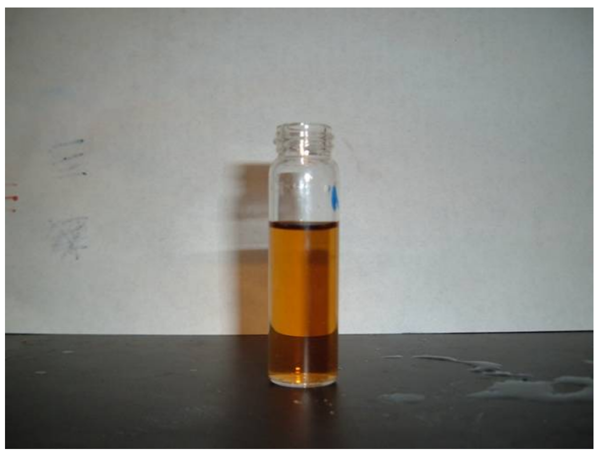

Figure 3: A sample after reacting with Vanillin reagent

Figure 4: UV-VIS Spectrophotometer

Results

Sample Date	Original Sample	10/31/06		11/11/06		11/20/06		11/27/06	
Sample Site	-	144th	Trail Head	144th	Trail Head	144th	Trail Head	144th	Trail Head
Nominal Avg	100	67.5	81.6	40.3	18.7	8.8	5.9	4.5	4.3
STD DEV	5.8	5.8	5.5	4.9	1.0	0.3	0.3	1.4	0.5

Figure 5: Tannin levels over time in terms of percentage

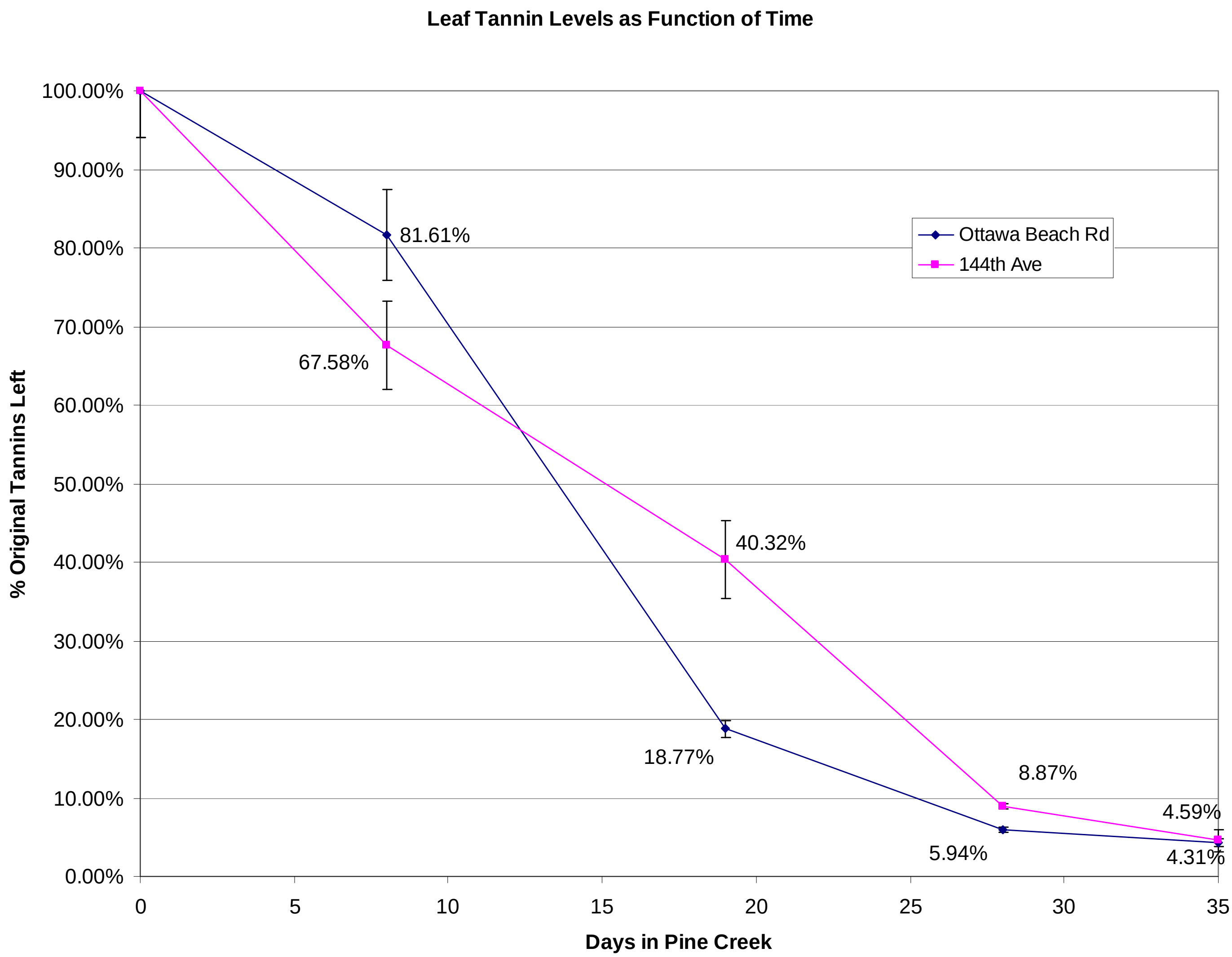

Figure 6: tannin levels shown as a function of the days left in the creek

As shown in figures 5 and 6 above, leaves at both sites showed an immediate drop in tannin levels within the first week of being placed. Also, leaves at both sites lost the vast majority of their tannins within a month of being placed. This is indicated by the drop to less than 5% of original tannin levels after about a month. One other relationship of note is that there appears to be a slight relationship between stream flow rates and the tannin release rate as the flow around the bag placed at the Pine Creek Trail head is significantly faster than the flow at the other bag (the one placed on 144th Ave.). This difference in tannin release rate is especially evident in the data between 8 and 19 days, where the leaves at the Trail Head site lost about 62.84% of their tannin levels while the leaves at the 144th Ave. site lost only 27.26% of their tannins. Also, there was a good grouping of every sample set as the standard deviations were low across the board.

Conclusions

Using the Vanillin method optimized by Price, Van Scoyoc, and Butler, our hypothesis originally stated was supported by the data collected during the experiment in that leaves from both sites showed an immediate drop off in terms of tannin levels immediately after being placed in the streams. The tannin levels within the leaves dropped below measurable levels in approximately one month, which gives us the timetable we were searching for in terms of over how much time leaves lose their tannins. As mentioned previously, a casual correlation was found in terms of tannin release rate and the rate of flow at different parts of the stream. However, we do not have enough data to completely support this correlation.

There are many ideas for future studies that could possibly branch out from the research we've performed. First, more experiments of this type could be run to come up with stronger data supporting our hypothesis. Second, a study of the tannin release rate as a function of flow rate is a promising area of study as, like previously stated, we found a casual correlation between the two variables but we didn't have enough data to completely support it. One way this could be done is to place leaves at at least 4-5 different sites, all with different flow rates, and see what kind of data can be gathered. Third, a detailed study of the tannins adsorbed to the bottom sediments would be necessary to have a more complete picture of the transport of tannins in the watershed. Finally, a quantitative analysis of the tannins dissolved into the water column could provide interesting results, especially as a function of time after the leaf drop and after heavy rain events. Variables that could be looked at here could possibly include how many tannins are washed into the streams via runoff, both through surface runoff as well as through groundwater influxes, as both would increase in heavy rain events.

References

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