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DATE: February 8, 2023

TO: Edward A. Blazel, Assembly Chief Clerk
Michael J. Queensland, Senate Chief Clerk

FROM: Dr. Robert Michitsch, Professor, Soil and Waste Resources, College of Natural Resources
University of Wisconsin-Stevens Point

RE: 2021 Wisconsin Act 67 Report Submission

The attached document reflects the findings of a septage characterization study and required by 2021 Wisconsin Act 67. The study was conducted by the Department of Soil and Waste Resources of the College of Natural Resources of the University of Wisconsin-Stevens Point and funded by the Wisconsin Department of Safety and Professional Services. Wisconsin Act 67 states that this report *shall submit the report to the appropriate standing committees of the legislature in the manner provided under s. 13.172 (3).* I request that you share this submitted report with the appropriate committees.

If you have any questions regarding this information, please contact either Deej Lundgren (dlundgren@uwsa.edu) or Kathy Divine (kdivine@uwsa.edu).

Sincerely,

Dr. Robert Michitsch

cc: Senator Andre Jacque
Representative Scott Krug
Secretary-designee Dan Hereth, Departmennt of Safety and Professional Services
Deej Lundgren, UW System
Kathy Divine, UW System
Rob Manzke, UW-Stevens Point

Determining Proper Nutrient and Heavy Metal Contents in WI Septage to Manage Current WI Regulations

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2021 Wisconsin Act 67

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LIST OF ABBREVIATIONS

NR 113: Chapter NR 113, Wis. Adm. Code

ANOVA: Analysis of Variance

CNR: UWSP College of Natural Resources

EC: Electrical Conductivity

EMARL: Environmetal Microbial Analysis and Research Laboratory

EPA: United States Environmental Protection Agency

GI1: Growth Index 1

GI2: Growth Index 2

HSD: Honestly Significant Difference

IRB: Institutional Review Board

POWTS: Private Onsite Wastewater Treatment System

UWSP: University of Wisconsin-Stevens Point

WDNR: Wisconsin Department of Natural Resources

WDSPS: Wisconsin Department of Safety and Professional Services

WLWCA: Wisconsin Liquid Waste Carriers Association

1.0 INTRODUCTION

Private onsite wastewater treatment systems (POWTS) generate the majority of septage that requires disposal in Wisconsin. Septage is the wastewater and solids contents of septic tanks and holding tanks. Septage also includes wastes generated from dosing chambers, grease interceptors, seepage beds, seepage pits, seepage trenches, privies or portable restrooms.

In 1992, the United States Environmental Protection Agency (EPA) set standards for calculating nitrogen (N) content in septage from a limited number of samples (EPA, 1984, 1994). This formula assumes 100 lbs of available N per acre when septage is applied to fields at a rate of approximately 39,000 gallons per acre. Septage also contributes other nutrients, heavy metals and fecal pathogens (Ebeling and Rwatambuga, 2011). The application of other organic and synthetic amendments to land also contribute additional nutrients, heavy metals and potentially fecal pathogens (eg manure sources) to the landscape. Since the introduction of these regulations, numerous efforts have resulted in lower exogenous nutrients in septage. Recent pilot studies have shown that N and phosphorus (P) levels in WI septage samples are below the 1992 thresholds; however, these results are preliminary and not well researched. This study focused on the waste being generated from POWTS septic tanks.

Household POWTS are designed to store and/or treat domestic wastewater. In October 2017, all septic systems in Wisconsin were to have been identified and documented. At the beginning of 2022, there were 778,451 installed septic systems in Wisconsin; 13,923 were installed in 2020 and 14,712 were installed in 2021 (personal communication, B. Johnson, WDSPS, February 2023).

When a POWTS is used for onsite wastewater treatment, the POWTS typically incorporates two separate components:

- An aerobic treatment tank where wastewater is treated physically by settling and flotation, biologically with anaerobic treatment, and limited chemical processes. The solids located at the bottom of the tank is referred to as sludge, while the floatable materials include greases, oils, and scum. When the tank is emptied, the sludge, scum, and other wastewaters in the tank are referred to as septage or septic tank waste.
- Aerobic treatment processes are used to treat the liquid (aka effluent) leaving the septic tank as it passes through the a natural or engineered soil profile.

Other POWTS only use a storage (eg holding) tank. The solid and/or liquid material removed from the storage tank is termed holding tank septage.

Septage has organic (eg food scraps, fecal matter) and inorganic (eg dish and laundry detergents) waste, and microorganisms that decompose the waste materials (Ebeling and Rwatambuga, 2011). Holding tank waste is removed when the tank is nearly full and prior to overflowing the tank. Septic tank septage must be pumped and removed from septic tanks on a regular basis (typically every 3 years in Wisconsin). It is most often either transported to a public wastewater treatment plant for further treatment, or applied to Wisconsin land as a fertilizer to support plant growth and improve soil nutrient levels, and as a soil amendment adding organic carbon to the soil. Occasionally, septage can be landfilled if properly treated and dewatered.

Chapter NR 113, Wis. Adm. Code (NR 113) regulates septage disposal in Wisconsin and incorporates the requirements from the septage requirements of EPA 40 CFR Part 503 - Standards for the use or disposal of sewage sludge. Currently, domestic septage is exempt from 40 CFR ceiling concentrations for contaminants as the EPA determined that domestic wastewater generated from homes contains low levels of heavy metals. Instead, when septage is land applied it is limited by the N application rates and predicted crop uptake needs. The state standards are N based, allowing a maximum of 39,000 gallons per acre to be applied to low use fields (estimated at 100 lbs of N per acre).

Because of the number of P impaired waters in Wisconsin, efforts are being made to implement P standards through nutrient management plans. Both N and P contribute to eutrophication of water bodies. These efforts mainly focus on manure applications to land; however, wastewater, biosolid, and septage applications to land are also being considered (WDNR, 2017).

High nitrate (NO_3^-) levels in drinking waters commonly provided from groundwater wells can put humans at risk of methemoglobinemia (Blue Baby Syndrome) and possibly cancers when ingested through drinking water or eaten in foods (Kantor, 1997).

The State's standards are currently based on the 1992 EPA standard (EPA 1984, 1994). It is understood that current water-soluble N and P levels are significantly lower than this 1992 data suggests. When applying septic tank waste to land, approximately 1/3 of the N and 2/3 of the P from the wastewater generated in the home is trapped in the solid septage materials. In general, this trapped N may be released (ie mineralized) at approximately 30%, 20%, 10% and 5% over a 4-year period from the solid portion of an organic waste according to regulations; more P (~60%) becomes available in the first year. State and Federal regulations adopt a variable scale for this N/P release depending on location within the US, and in Wisconsin, NR 113 does not specify a mineralization rate. Since the 1992 EPA standard was adopted, there has been a concurrent reduction of P in general-use commercial detergents, and the adoption of the Great Lakes Phosphorus ban from detergents (WDNR, 2015). Allowing higher volume applications of septage to land could add more N for plants and lessen the need for synthetic or other costly soil nutrient amendments, while still adhering to nutrient load goals of the original EPA guidelines based solely on N content; however, higher applications of P or heavy metals may be a concern. This higher volume application could also require much less land to be used for septage applications, thereby potentially decreasing the environmental footprint, road-wear, and transportation costs of the individuals physically applying this septage material to land. As a result, potentially increased profit margins for everyone from farmers and producers to applicators may be realized.

The Wisconsin Liquid Waste Carriers Association (WLWCA) is the premier trade organization for Wisconsin's liquid waste industry (www.wlwca.com/). In 2011, the WLWCA commissioned a study to examine P availability from Wisconsin septage as a means to offset the production of commercial P fertilizers by reusing a 'septage waste' material (Ebeling and Rwatambuga, 2011). It was determined that 2011 septage P levels were lower than septage P levels from 20-30 years ago on which the EPA 1992 standards are based. While the current EPA standard is based on N, very limited septic tank N analyses were performed as part of this 2011 study.

The UWSP College of Natural Resources (CNR) Environmental Microbial Analysis and Research Laboratory (EMARL) was contracted in 2016 by the WLWCA to conduct a nutrient and heavy metal analysis of 20 septage samples collected around Wisconsin. Due to sampling inconsistencies based on residents sampling their own systems, the results were inconclusive. In Spring 2017, an internal University of Wisconsin-Stevens Point (UWSP) Professional Development Committee grant was funded to analyze 50 septage samples collected from strategic locations around Wisconsin to correlate nutrient and EPA-sanctioned heavy metal levels with storage tank location, size, and household demographics. Results of this pilot study were again highly variable, suggesting the need for a larger and more complete characterization of Wisconsin septic tank waste.

In 2021, Wisconsin Act 67 through the Wisconsin Department of Safety and Professional Services (WDSPS) approved funding for the subsequent collection and analysis of 360 septic system samples from around Wisconsin in order to better assess nutrient and heavy metal levels in septage that may be applied to land. Samples were collected from 36 different counties across the state of Wisconsin to better understand the nutrient and heavy metal contents of Wisconsin septic tank wastes. A greenhouse-based plant-growth trial was also conducted to determine the Carbon:Nitrogen (C:N) content of plant material, along with general plant health and growth measurements, based on three methods of how septage is applied to land in Wisconsin.

2.0 METHODS

2.1 Septage Characterization

2.1.1 Sample Population Identification

To establish the sample population, Wisconsin based septic waste haulers were identified by the WLWCA (personal communication, D. Kons, March 2022). The haulers were contacted, briefed on the project scope, and provided the questionnaire form (Appendix A). Using their base of clientele, the septic haulers identified approximately ten sampling locations in their county that aligned with their usual pumping schedules during the timeframe for visiting their location. This process was repeated for each of the 36 counties sampled across all areas of Wisconsin. The questionnaire form was approved by the UWSP Institutional Review Board (IRB) in order to gather minimal demographic data per household while maintaining anonymity for the personal households.

2.1.2 Sample Collection

The septic samples were collected according to a modified EPA 503 guidance (EPA, 1994). Composite samples were collected from each tank(s) from each individual household septic system. If a system contained multiple tanks, or a single tank with multiple chambers, all tanks/chambers were sampled, and a composite sample was collected. All samples were collected directly and only by UWSP personnel to avoid any perception of bias from State or industry affiliated entities. Samples were collected using a TruCore Sludge Sampler (SIM/TECH Filter, Inc.; Appendix B). The sampler is a 1-1/4" diameter clear polyethylene tube with a cord and a stopper on one end. The open end is inserted into the septic tank and lowered to the tank's terminal depth, and a vertical profile of the tank's contents fills the tube. The cord is then pulled tight thereby capturing the sample. Upon removing the sample(s) from the tank(s), the sample(s) was homogenized in a three-gallon polyethylene container to suspend any solids present within the sample column before transferring into a sample container. One liter of sample was collected. All samples collected were stored on ice and later refrigerated until sample analysis.

2.1.3 Sample Analysis

Septage samples were analyzed by the UWSP EMARL. Septage samples were characterized for physical parameters, nutrients and heavy metals. Physical parameters included: percent total solids, pH and electrical conductivity (EC). pH and EC were measured using a pH and conductivity probe (Oakton, PC 700, pH/mV/Conductivity/°C/°F meter). Nutrient analyses included: extractable nitrate (NO_3) and ammonium (NH_4^+), total N percentage (solid portion), extractable potassium (K), extractable P, and total C. Three extractions were performed during laboratory analyses. A potassium chloride extraction was used to measure extractable NO_3 and NH_4^+. A water extraction was used to measure extractable P. An ammonium acetate extraction was used to determine extractable K. Following the extractions Lachat Technology (SEAL Analytical, AQ300) was used to measure extractable nutrients. Percent total N and percent total C were determined using C/N Analyzer Technology (LECO, CN828). Heavy metal characterizations included metals contained within the EPA heavy metals list and included analysis of aluminum, arsenic, boron, barium, calcium, cadmium, cobalt, chromium, copper, iron, potassium, lithium, magnesium, manganese, molybdenum, sodium, nickel, phosphorus, lead, sulfur, selenium, strontium, and zinc. Heavy metals were analyzed using a microwave digestion followed by Inductively Coupled Plasma Technology (Agilent Technologies, 5110 ICP-OES).

2.1.4 Statistical Analysis

The characterization data was analyzed using analysis of variance (ANOVA) and correlation techniques to understand potentially significant trends in the data collected. These calculations are necessary in order to determine if any possible relationship exists between the data collected. Statistical processes, correlation analysis, and calculations were performed using R Studio (RStudio 2021.09.0). R Studio is an integrated development environment for R, a commonly used programming language for statistical computing and graphics. Geometric means, which are used to lessen the effect of extreme outliers in a dataset, were computed along with traditional mean values.

2.2 Greenhouse Comparison of Land Application Methods

2.2.1 Greenhouse Methodology

A greenhouse trial was conducted to compare three different land application methods of septage and two different application rates based on total N content. The trial was conducted in the greenhouse of the Trainer Natural Resources Building on the University of Wisconsin-Stevens Point campus. This equated to 42 pots (six replicates per seven treatments) prepared for the greenhouse land application method and nutrient yield comparison. A tabulated summary of the application treatments is listed in Table 2.2.

Table 2.2. Greenhouse Experiment Treatment Applications

Treatment Number	**Septage Application Method**	**Augmented with NH_4^+-NO_3^-** (Y/N)	**Septage Application Rate** (gallons / acre)	**Nitrogen Application Rate** (lbs N / acre)
1	Surface Application	N	39,000	28.2
2	Surface Application + Incorporation	N	39,000	28.2
3	Injection	N	39,000	28.2
4	Surface Application	Y	39,000	120
5	Surface Application + Incorporation	Y	39,000	120
6	Injection	Y	39,000	120
7	Control	N	0	0

Surface application consisted of pouring the sample volume at each rate over the pots and letting it settle into the pot. Surface application and incorporation consisted of similarly surface applying the septage followed by gently hand-incorporating the volume into the top 2-4 inches of the soil. Lastly an injection method included the use of a traditional meat baster to penetrate the soil surface to an approximate depth of 4” and inject the septage solution directly into the soil. Following the application methods, the soils were allowed to acclimate in the greenhouse prior to planting for 48 hours. When septic waste is strictly surface applied it must be lime treated with either quick lime or hydrated lime to a pH of 12 to neutralize any pathogens per EPA requirements. Prior to our strictly surface application treatment, a subset of our septage sample was limed to a pH of 12 for 30 minutes according to NR 113.

Eight-inch diameter pots were filled to the same volume of soil. The soil used was a Wyocena D (Ap horizon) obtained from central Wisconsin that was available for use at UWSP. The soil was sieved to 3/8” to ensure a consistent grade and remove any large gravel and stones. The septage sample collected for the greenhouse application was a composite of three random septic systems collected during the septage characterization sample collection period in 2022. Three individual systems were bulk sampled (5 gal) then homogenized to a single composite sample. The sample was analyzed for extractable NO_3 , extractable NH_4^+, and total N. An application rate of 39,000 gallons per acre was selected as a direct comparison to the values listed in NR 113. NR 113 estimates that 39,000 gallons of septage applied over an acre of land yields 100 lbs of N. This application rate is the maximum loading rate for low use fields in Wisconsin. It was determined that 740 mL of septage per pot was required to meet our 39,000 gal/acre application rate. Based on sample analysis the application rate of 39,000 gal/acre was calculated to yield 28.2 lbs of total N. Total N is the sum of organic N from the solid portion, and extractable NH_4^+ and extractable NO_3 from the liquid portion. The second application rate is based on conventional crop requirements. To achieve this, the same volume of septage was applied; however, additional augmentation with synthetic NH_4^+-NO_3 was added to raise the N content and meet more traditional crop needs. Prior to the application of septage 0.35 g of NH_4^+-NO_3 was added to augment the septic tank waste and raise the N content of the application to yield 120 lbs of N given a 39,000 gal/acre rate per average crop need. The augmented surface application treatment was limed following the augmentation of NH_4^+-NO_3. Pots were arranged in complete randomized design within the greenhouse area and re-randomized weekly.

The chosen plant for this experiment was a snap-bush bean (*Phaseolus vulgaris* v. Rocdor). This was selected for its ability to germinate quickly within 7-10 days. Five seeds were planted into each pot equidistantly. Seeds were planted ~1.0 cm below the soil surface and given 2 weeks to germinate, then allowed to grow for 6 weeks to maturation. Any pots that did not show growth in all 5 seeds were replanted following weeks 2 and 4 to ensure enough growth and biomass for sample analyses, and the replanted seeds were allowed to follow the same growing schedule. Seeds that germinated at different time intervals were flagged and recorded in order to allow all replanted seeds to have 6 weeks of growth. Plants were watered with ~200 mL of reverse osmosis water 2-3 times weekly to maintain field capacity in the pots. SPAD readings, which are a measurement of chlorophyll content that relates to N content, were recorded halfway throughout and at the end of the growing period of the plants.

After 6 weeks plants were harvested 1 centimeter above the soil surface and placed into paper bags for drying at 65°C for 3 days. Fresh weight and dry weight measurements were obtained. Plant height, maximum leaf width, 2-3 internodal length, and stem diameter were recorded at the time of harvest. The dry mass and fruiting bodies (ie beans) were separated and analyzed for C:N content.

Dry plant mass was analyzed for total nutrient characterization. Plant samples were analyzed similarly to methods and instruments used for the septage samples.

2.2.2 Statistical Analysis:

The plant growth data was similarly analyzed using an ANOVA to understand potentially significant trends (see Section 2.1.4). A Tukey Honestly Significant Difference (HSD) test was used to identify significant differences. Statical processes and calculations were performed using R Studio (RStudio 2021.09.0).

3.0 RESULTS AND DISCUSSION

3.1 Septage Characterization

3.1.1 Demographic Data

Of the questions asked on the "Septage Sample Information Sheet" (Appendix A), various response frequencies were recorded; 360 responses were not recorded for every single question. On average, a response rate of 90% was recorded per question. Private homeowners and our WLWCA hauler contacts were relied upon to provide this information. The summary statistics from numerical responses were tabulated and are provided in Table 3-1a and 3-1b.

Table 3-1a: Summary of Numerical Demographic Data

Demographic Parameter	Sample Size	Minimum	Median	Mean	Max	SD
Septic System Age	292	1	21	24.27	76	14.71
Last Pumped	354	0	35	29	108	11.64
Pumping Cycle	355	0.5	3	2.84	6	0.68
Number of Residents	346	1	2	2.63	11	1.25
Bedrooms in Home	351	1	3	3.13	9	0.83
Tank Capacity	325	100	1000	1284	4000	455.03

Table 3-1b: Summary of Categorical Demographic Data

Demographic Parameter	Sample Size	Present	Not Present	NA	Full Time	Seasonal
Water Filter (Y/N)	277	57	220	83	--	--
Water Softener (Y/N)	277	67	210	83	--	--
Full-Time vs. Seasonal	360	--	--	0	337	23

3.1.1a Household Size and Occupancy

Three parameters were recorded to determine household size and general occupancy: the number of residents, the number of bedrooms in each household, and whether a residence was occupied full time or seasonally. Out of 346 households the average number of residents was 2.6 with a maximum of 11. Out of 351 households the average number of bedrooms was 3.1 with a maximum of 9. Of the 360 household systems sampled 337 households were full time residences, while 23 were seasonal residences.

3.1.1b Septic System Information

Four parameters were recorded to gather general septic system information. The parameters included: septic system age, septic tank volume, the septic tank pumping cycle, and when the septic tank was last pumped. The average age of a sampled septic system was 24.3 years with a maximum of 76 years and a standard deviation of 14.8 years. Of the systems sampled the tank volume varied. The smallest septic tank was 100 gallons and the largest was 4000 gallons. Average septic tank volume was 1284 gallons. Septic tanks require pumping at various intervals (typically every 3 years) based on filled volume to maintain proper function and ensure system longevity. Of the systems sampled the average pumping cycle is once every 2.9 years with systems formerly pumped on average 29 months prior to sample collection.

3.1.1c Water Treatment

Two parameters were collected to determine the presence of additional water treatment at the household. The presence of a water filtration system, such as an iron filter or a water softener, may alter the contents of the waste due to the prior treatment of household water and waste discharges from such systems. Out of 277 responses for this question, 57 households indicated use of a water filter and 67 households indicated the use of a water softener.

3.1.2 Physical Parameters

Three physical parameters were measured in the septic tank waste: electrical conductivity (EC), pH, and total percent solids. Physical parameters can provide useful information regarding the physical nature of a substance and can be indicative of chemical relationships and compositions. The summary statistics for each physical parameter is provided in Table 3-1c.

Table 3-1c: Summary Statistics of Measured Physical Parameters

Parameter	Sample Size	Minimum	Median	Mean	Max	SD
pH	360	5.00	6.51	6.45	7.15	0.34
Electrical Conductivity (mS/cm)	360	0.13	2.33	3.66	22.50	3.26
Percent Solids (%)	352	0.01	0.57	0.72	7.97	0.79

Notes:

1.) Eight samples collected lacked enough volume to determine percent solids content.

3.1.2a Electrical Conductivity

Electrical conductivity measures the amount of electrical current a material can carry and can indicate the presence of ions/salts within a material. This is primarily a measurement of salt content. As measured in 360 samples, the average EC was 3.66 (mS/cm), minimum of 0.13 (mS/cm), and a maximum of 22.5 (mS/cm). The standard deviation was recorded at 3.26 (mS/cm). The geometric mean was calculated at 2.71 (mS/cm). It is anticipated that EC measurements may be higher in a septic tank due to the concentration of nutrients or discharge from filtration devices. Most growing plants (according to general standards) can tolerate 2-4 mS/cm for land application before becoming problematic.

3.1.2b pH

pH plays a vital role in the availability of nutrients and metals in various chemical forms present in a substance. Heavy metals are increasingly available at pH's of <5 or >8. Nitrogen, a vital plant nutrient, is most plant available at pH's of 6-8 but can be lost as ammonia (a gas) when the pH reaches 8.2 or above. Of 360 samples analyzed the average sample pH was 6.44, a minimum of 5.00 and a maximum of 7.15, with a standard deviation of 0.34. The geometric mean was calculated at 6.44.

3.1.2c Percent Solids

Percent solids is the measure of solid materials contained in a mixed medium and is quantified as a percentage. Higher solids content can impact septic systems longevity and impact the nutrient content of septic waste. Solids appear in septic systems through human waste, food scraps, and other refuse discarded down household drains. This waste is actively broken down through biological processes within the systems themselves. Of 352 samples measured, the average solids percentage was 0.75%, a minimum of 0.01%, and a maximum of 7.97%. The standard deviation among samples was 0.79%. The geometric mean was calculated at 0.50%.

3.1.3 Nitrogen

Nitrogen is a nutrient found in septic waste that is vital to plant growth. Due to the impact of N on surface water, groundwater, and human health, the EPA has imposed regulations and guidance on the maximum loading rates of N applied to land from septage waste (EPA, 1994). In addition, the State of Wisconsin has its own regulation around the practice of land applying septage (NR 113). This State legislation is also N-based and incorporates the federal standard.

Total N is the measure of all present forms of N within a sample, considering both the organic and inorganic fractions of N that may be present. When nitrogen is applied it is estimated that 30% of the N from the solid organic fraction becomes available in the first growing season year. This figure can then be applied to a rate to calculate how much N could be applied at a given rate of septage applied to land. A low use field is limited to 39,000 gallons per acre. It is stated that this agronomic rate will yield a N addition of 100 lbs per acre (EPA, 1994).

3.1.3a Nitrogen Fractionations

Septage samples were analyzed for extractable NO_3 and extractable NH_4^+ to capture the inorganic fraction of N, while the N percentage of the solid material captured the total organic portion, which allowed the differentiation between organic and inorganic components. Nitrogen is primarily present in septic systems as NH_4^+ due to the transformations performed by anaerobic microorganisms. Of 241 samples where measurable extractable NO_3^- was detected, the mean was 1.34 mg/L, the minimum was 0.12 mg/L, and the maximum was 11.77 mg/L. The standard deviation for extractable NO_3^- was 1.31 mg/L. The geometric mean of extractable NO_3^- measurements was 0.99 mg/L. Of 360 samples containing measurable amounts of extractable NH_4^+ the mean was 102.79 mg/L, the minimum was 4.27 mg/L, and the maximum was 909.63 mg/L. The standard deviation for extractable NH_4^+ was 86.15 mg/L. The geometric mean of extractable NH_4^+ samples was 83.32 mg/L. The total N percentage was measured in 356 samples. The total N percentage for four samples could not be measured due to an insufficient amount of solids content. The mean percent total N in the solid fraction was 2.71%, the minimum was 0.08%, and the maximum was 27.28%. The standard deviation for total N was 1.70%. The geometric mean of total N was 2.34%.

3.1.3b 39,000 gal/acre Application Rate

Using the values measured above, a total N loading rate was calculated for each sample. A 39,000 gal/acre rate was calculated as a relevant comparison to the State standards. In addition, a lbs N per 1000 gallon calculation was calculated for relevance to industry standard language. Thirty percent of the solid fraction was input into the equation to compensate for first year N availability. A tabulated summary of calculated N values is provided in Table 3-1d. Of the total N values, 60% of the total N content resides in the solid fraction while 40% is present in the extractable form.

Table 3-1d: Comparison of Nitrogen Additions at Different Application Rates

Parameter	**Minimum** (lbs N)	**Median** (lbs N)	**Maximum** (lbs N)	**Mean** (lbs N)	**Geometric Mean** (lbs N)	**Standard Deviation** (lbs N)
Total N Loading Rate (lbs N/39,000 gal septage)	3.61	65.10	956.73	85.32	62.52	96.00
First Year N Availability (lbs N/39,000 gal septage)	2.23	34.56	405.45	43.81	34.95	40.44
Total N Loading Rate (lbs N/1000 gal septage)	0.09	1.67	24.53	2.19	1.60	2.46
First Year N Availability (lbs N/1000 gal)	0.06	0.89	10.40	1.12	0.90	10.37

These values represent the measure of a single septic system. On average, based on the first year of septage application, 39,000 gal/acre will provide approximately 44 lbs N/acre. Of 360 samples analyzed there were 77 that were calculated to exceed 100 pounds of N when land applied if all the N in the sample was considered. When considering that only 30% of the N present in the solid fraction is available in the first year, only 17 values exceed 100 lbs of N. These values contribute to the overall land application of septic waste; however, a septic truck applying waste to land will apply wastes from numerous systems. This dilutes the elevated values measured from single systems resulting in lower nitrogen contributions being applied to land.

3.1.4 Phosphorus and Potassium

Phosphorus and K play vital roles in plant growth as well. Though NR 113 does not currently consider the addition of these nutrients from a regulatory stance, they still play important roles in nutrient management of agricultural landscapes. Excess P is known to negatively impact waterbodies and sensitive ecosystems when overapplied to agricultural land. Excessive erosion from agricultural fields can negatively impact surface water bodies. In eastern Wisconsin, where karst topography is present, additional concerns to groundwater resources are present. Phosphorus and K levels in septic wastes are relatively unknown.

NOTE: When agricultural lands are applied to with septage wastes, the wastes are required to be included into nutrient management plans pursuant to chs. NR 151, NR 243, ATCP 50, NRCS 590, County and local ordinances. If septage is applied on lands only receiving septage and no commercial fertilizer, the accounting of P is not required.

3.1.4a Phosphorus

As measured in 360 samples, mean extractable P was 16.52 mg/L, the minimum was measured as 0.11 mg/L, and the maximum was measured as 262.40 mg/L. A standard deviation was calculated for extractable P as 23.98 mg/L. The geometric mean of extractable P measurements was 9.59 mg/L. In the solid fraction, as measured in 355 samples, the mean was 6057.00 mg/kg, the minimum was measured as 702.80 mg/kg, the maximum was measured as 27,595.90 mg/kg. A standard deviation and geometric mean were calculated for solid fraction P and were observed as 5135.78 mg/kg and 165.78 mg/kg, respectively.

3.14b 39,000 gal/acre Application Rate

Calculated total P contributions at both 39,000 gallons of septage and 1000 gallons of septage are provided below in Table 3-1e. First year P availability from the solid portion of septage is calculated as 60% for P.

Table 3-1e: Comparison of Phosphorus Additions at Different Application Rates

Parameter	**Minimum** (lbs P)	**Median** (lbs P)	**Maximum** (lbs N)	**Mean** (lbs P)	**Geometric Mean** (lbs P)	**Standard Deviation** (lbs P)
Total P Loading Rate (lbs P/39,000 gal septage)	0.30	35.39	1371.55	58.04	32.79	100.00
First Year P Availability (lbs P/39,000 gal septage)	0.30	12.89	857.05	36.94	21.56	62.61
Total P Loading Rate (lbs P/1000 gal septage)	0.01	0.90	35.17	1.49	0.84	2.56
First Year P Availability (lbs P/1000 gal)	0.01	0.57	21.98	0.95	0.55	1.61

3.1.4c Potassium

As measured in 359 samples, mean extractable K was 7.12 mg/L, the minimum was measured as 0.58 mg/L, and the maximum was measured as 169.4 mg/L. A standard deviation was calculated for extractable K as 17.06 mg/L. In the solid fraction, as measured in 355 samples, the mean was 7917.20 mg/kg, the minimum was measured as 694.40 mg/kg, and the maximum was measured as 28345.70 mg/kg. A standard deviation and geometric mean were calculated for solid fraction K and were observed as 4382.31 mg/kg and 28.78 mg/kg, respectively.

3.1.5 Heavy Metals

Heavy metals are present in septic wastes. Federal standard ceiling concentrations of contaminants exist for the regulation of land applied sewage sludge. These concentrations are contained in 40 CFR Part 503 (EPA, 1994). Domestic septage is comparably weaker than sewage sludge. At this time there are no applicable ceiling concentrations that exist for the land application of domestic septage, as septage is exempted from the 40 CFR Part 503 guideline. For the purpose of comparison, frame of reference, and clarity, the sewage sludge standards are compared with the domestic septage characterization results in Table 3-1f. Of the 23 metals measured, 9 of them have established standards in sewage sludge. This is also available in Appendix C in a larger format. Overall, there were very few heavy metal levels that that exceeded the sewage standards. In particular, there was 1 isolated home that had several heavy metal levels that exceeded the sewage sludge standards.

Table 3-1f: Summary of Heavy Metals in Analyzed Septic System Samples

Metal	Sample Size	Ceiling Concentrations (Table 1 of 40 CFR 503.13) (miligrams per kilogram, dry weight)	Number of Values Above Ceiling Concentrations	Minimum (mg/kg)	Median (mg/kg)	Mean (mg/kg)	Geometric Mean (mg/kg)	Max (mg/kg)	SD (mg/kg)
Al	355	--	--	80.84	1979.55	2647.97	8.56	17701.34	2442.01
As	178	75	1	1.89	3.40	16.42	1.67	**2048.56**	153.23
B	355	--	--	10.93	48.43	78.00	3.65	1766.43	117.16
Ba	355	--	--	13.01	82.18	191.68	5.74	8197.78	655.43
Ca	355	--	--	7150.00	32393.00	34361.00	228.42	140648.00	14988.61
Cd	11	85	0	5.20	6.59	9.97	4.67	41.12	10.39
Co	318	--	--		1.98	33.10	0.87	9772.05	547.86
Cr	350	3000	0	0.83	12.53	17.26	0.73	199.56	19.07
Cu	350	4300	3	2.47	178.17	424.13	3.00	**23214.95**	1412.31
Fe	350	--	--	85.69	2927.41	5862.58	17.41	55423.32	8192.87
K	355	--	--	702.80	4175.50	6057.00	165.78	27595.90	5135.78
Li	321	--	--	1.22	2.44	3.49	1.14	37.70	3.93
Mg	355	--	--	495.30	6076.00	7380.10	21.80	28429.30	5141.36
Mn	355	--	--	4.96	82.27	120.34	3.54	2815.70	222.17
Mo	100	75	3	3.74	6.50	27.50	3.19	**1646.85**	164.79
Na	355	--	--	1298.00	36487.00	59393.00	47.88	326190.00	63510.83
Ni	353	420	1	1.25	6.24	43.85	1.14	**11466.80**	609.79
P	355	--	--	694.40	7363.20	7917.20	28.78	28345.70	4382.31
Pb	343	840	0	1.02	7.34	13.57	0.79	292.85	24.08
S	355	--	--	1664.00	11630.00	13588.00	45.00	74115.00	8671.06
Se	80	100	0	2.45	3.86	10.88	2.19	88.08	17.35
Sr	355	--	--	8.40	44.25	140.21	6.78	7894.74	651.85
Zn	355	7500	0	20.77	952.65	1449.61	12.71	4424.81	785.40

Notes:

1.) The total samples size for all metal analysis was 355 samples. Values that were below the MDL ar enot incuded in the statistical analysis.

3.) **Bold** values indicate values that are above the ceiling concetrations for sewage sludge.

2.) 40 CFR 503.13 ceiling concentration values are for sewage sludge not domestic septage. These values are for comparison purposes only and do not represent an exceedance in the ceiling concentrations.

3.1.6 Statistical Analysis

Nutrients and heavy metals were analyzed for linear relationships between factors using a Pearson's correlation. This method was used to identify if certain factors, including demographic factors or physical parameters, could be an indication of higher or lower nutrient or heavy metal contents. The Pearson's correlation coefficient values range from +1 to -1. The values quantify the direction and strength of a linear relationship between two factors. If the coefficient values are close to zero, then no linear relationship is present. If the values are closer to +1 then a positive linear relationship exists, meaning that an increase in one factor is linked to an increase in the other factor. If the values are closer to -1 then a negative linear relationship exists, meaning that an increase in one factor is linked to a decrease in the other factor. Generally, values of +0.70 and/or below -0.70 indicate fairly strong relationships between the factors. Results of this analysis showed no relationships between heavy metals and other measured parameters. When comparing the nutrient data to other measured parameters, a positive linear relationship was found between the percent solids content, and the total N and P contribution of the septage waste. The correlation coefficients and figures visualizing these relationships calculated in septage are provided in Table 3-1g and Figures 3.1 through 3.4, respectively. No other correlation values indicated relationships between measured factors. As a note, the elevated values observed in the figures are representative of a single septic system. At the time of land application, a truck containing numerous systems combined would be applied to land, which would dilute the overall application of nutrients contained in septage to land.

Table 3-1g: Pearson Correlation Coefficients

Compared Factors	Pearson Correlation Coefficient
Percent Solids ~ Total Nitrogen Contribution 39,000 gal/acre	0.84
Percent Solids ~ First Year Nitrogen Contribution 39,000 gal/acre	0.76
Percent Solids ~ Total Phosphorus Contribution 39,000 gal/acre	0.70
Percent Solids ~ First Year Phosphorus Contribution 39,000 gal/acre	0.69

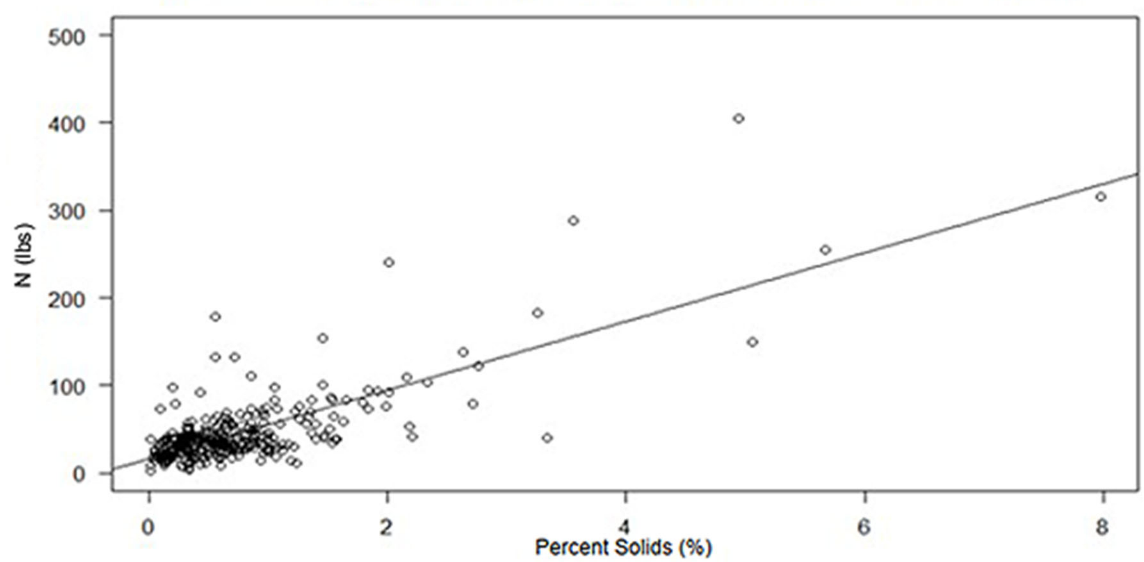


Figure 3.1: First Year Nitrogen Contribution at 39,000 gal/acre vs. Percent Solids

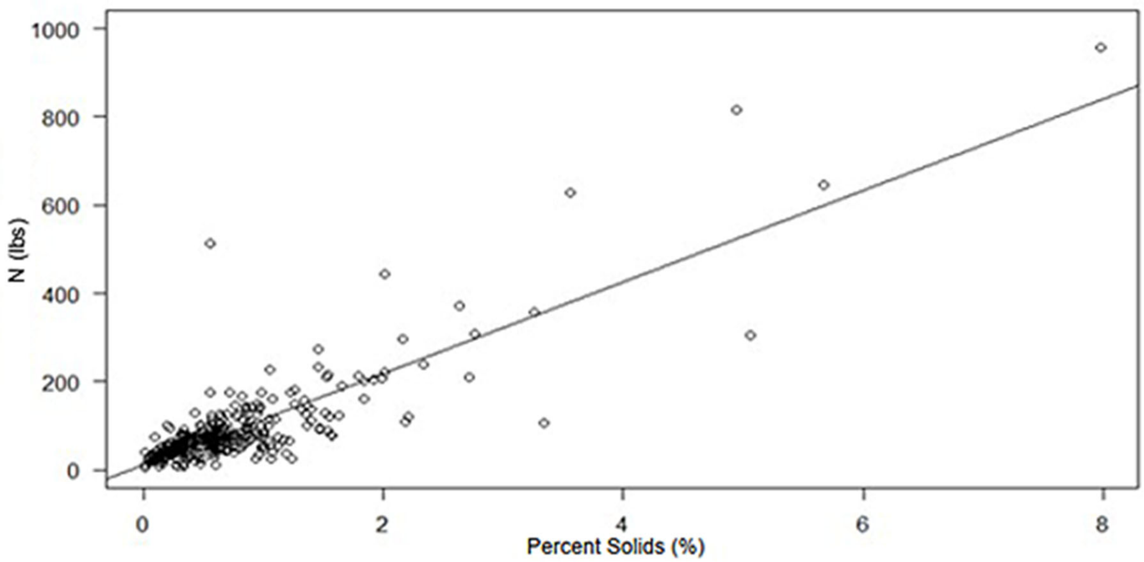


Figure 3.2: Total Nitrogen Contribution at 39,000 gal/acre vs. Percent Solids

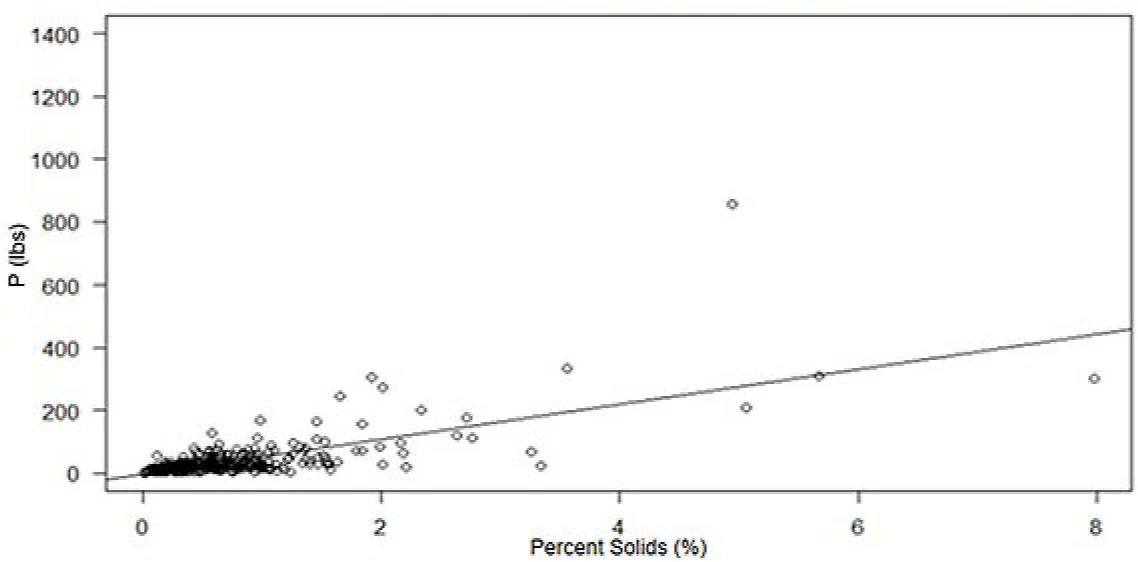


Figure 3.3: First Year Phosphorus Contribution at 39,000 gal/acre vs. Percent Solids

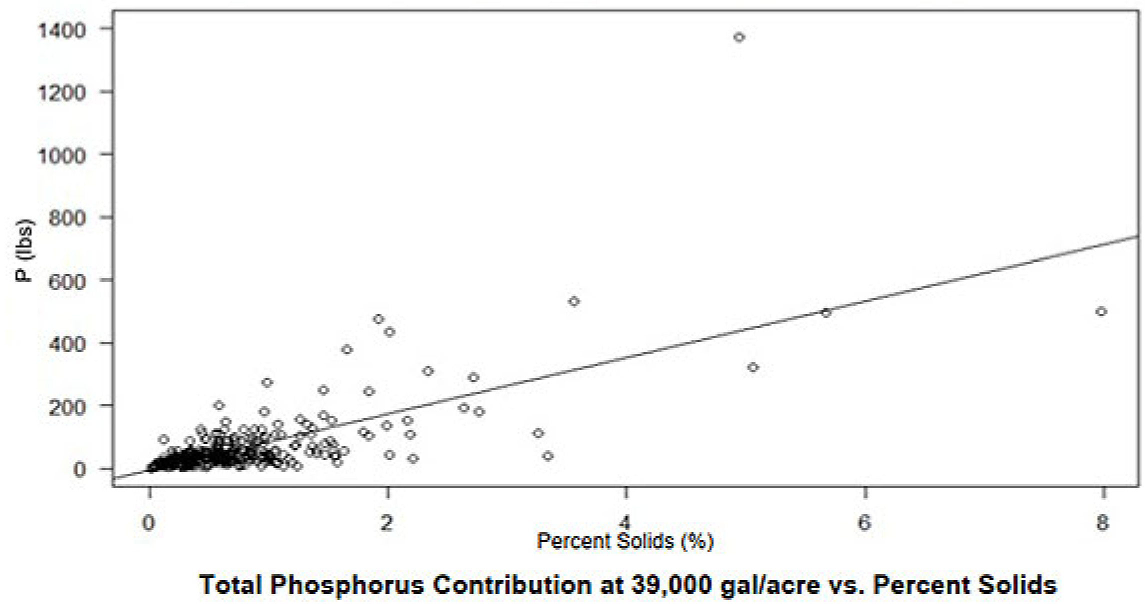


Figure 3.4: Total Phosphorus Contribution at 39,000 gal/acre vs. Percent Solids

3.2 Comparison of Land Application Methods

3.2.1 Plant Harvest Measurements

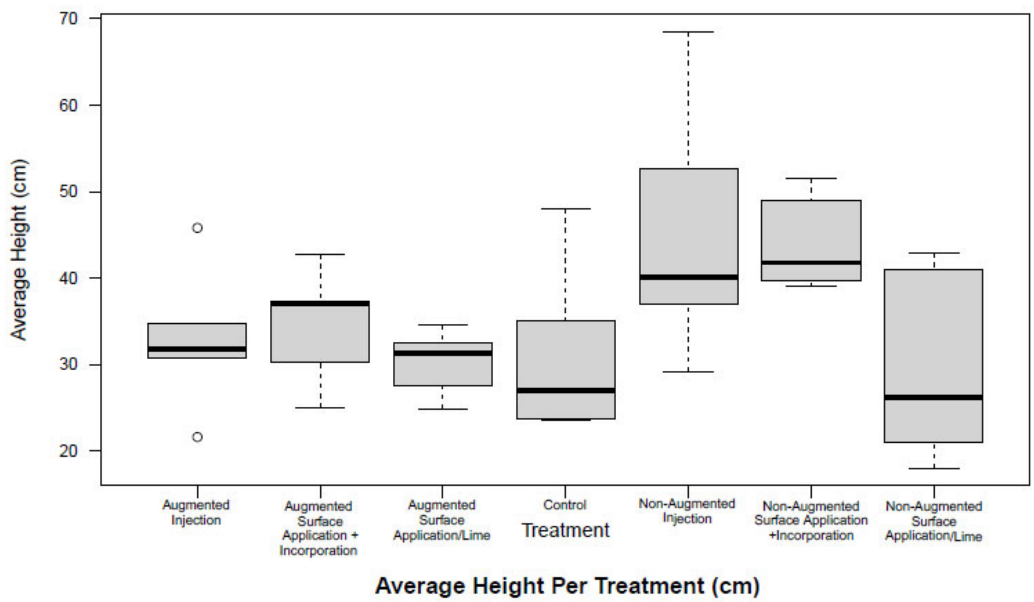
Four measurements were performed at the time of harvest to characterize plant growth and nutrient content influence. These parameters included plant height, 2nd-3rd internodal length, stem diameter, and maximum leaf width (Table 3-2a).

Table 3-2a: Summary Statistics for Plant Harvest Measurements

Treatment	Augmented (Y/N)	Average Height (cm)	Average Internodal Length (cm)	Average Maximum Leaf Width (cm)	Average Stem Diameter (mm)
Surface Application/Lime	N	29.22	3.44	17.90	2.32
Surface Application/Incorporation	N	43.78	12.14	14.85	2.09
Injection	N	44.59	6.44	23.21	1.78
Augmented Surface Application/Lime	Y	30.33	3.78	21.38	2.08
Augmented Surface Application/Incorporation	Y	34.42	6.96	19.33	2.14
Augmented Injection	Y	32.75	9.08	23.13	2.25
Control	N	30.69	5.60	18.92	2.03

3.2.1a Plant Height

Plant height is the height of the plant measured from the base to the terminal leaflet. Taller plant height can be associated with higher levels of available N, but overall plants that are well supported with nutrients tend to grow taller. The average plant height summary statistics per treatment are listed in Table 3-2a and are visualized for each treatment in Figure 3.5. Across all treatments the summary statistics for average plant height are as follows: the minimum plant height recorded was 18 cm, a median of 34.50, a maximum of 68.50 cm, with a mean value of 35.13 cm and a standard deviation of 10.21 cm. When comparing the values per treatment listed in Table 3.2a, the treatment with the tallest average plant height was the non-augmented injection (44.59cm). The shortest average plant height came from the non-augmented surface application (29.22cm). A tabulated summary of all harvest measurements per pot is available as Appendix D.



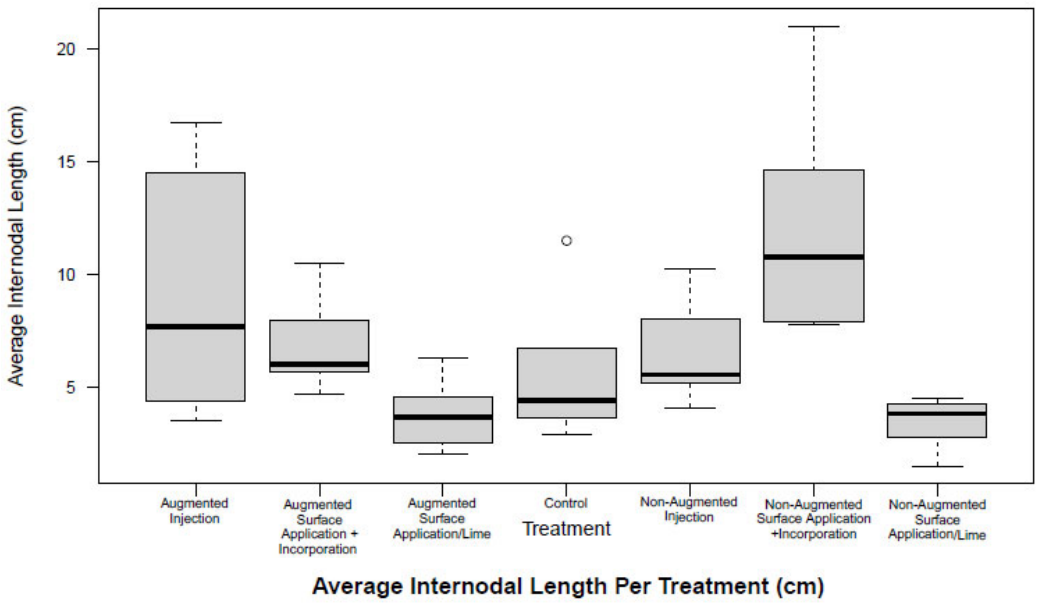
Notes: “o” is an identified outlier

Figure 3.5: Average Plant Height Per Treatment

Plant height was statistically analyzed using an ANOVA. This statistical test tested the null hypothesis that the mean plant height for each treatment was the same, and the alternative hypothesis that the average plant height for at least one treatment was different. The hypothesis test determined with 95% confidence that a statistical difference between the average plant heights across at least one treatment was different ($F = 3.144$, $P = 0.014$). A Tukey HSD test was used post hoc to determine where the differences lied. The results of this test were inconclusive. The lowest recorded adjusted p-value was 0.068 when comparing the average plant heights between the non-augmented surface application and the non-augmented injection. It is worth noting that an adjusted p-value from a Tukey HSD test is a corrected p-value that takes into account the multiple comparisons made in the test. This is designed to help control Type I error rate and is a more stringent criterion for statistical significance than traditional p-values, which works to exclude random outlier data. In conclusion, there are no statistical differences between the mean plant heights across all treatments based on the variability shown in Figure 3.5.

3.2.1b Internodal Length

Internodal length is the measure of distance between nodes 2 and 3 on the plant. A node is where plant leaves develop and extend from the plant stem. A larger internodal length is indicative of a healthier plant and (potentially) a higher N content. The average internodal length on a per treatment basis is tabulated in table 3-2a and visualized across each treatment in Figure 3.6. Across all treatments the summary statistics for average internodal length are as follows. The minimum recorded was 1.5 cm, a median of 5.3cm, a maximum of 21 cm, with a mean length of 6.78 cm and a standard deviation of 4.29 cm. When comparing values for each different treatment the maximum average internodal length was found in the non-augmented surface application and incorporation treatment (12.14 cm), while the minimum average internodal length was observed in the non-augmented surface application and lime treatment (3.44 cm). Statistical analysis was not deemed necessary for this measured parameter. A tabulated summary of all harvest measurements per pot is available as Appendix C.

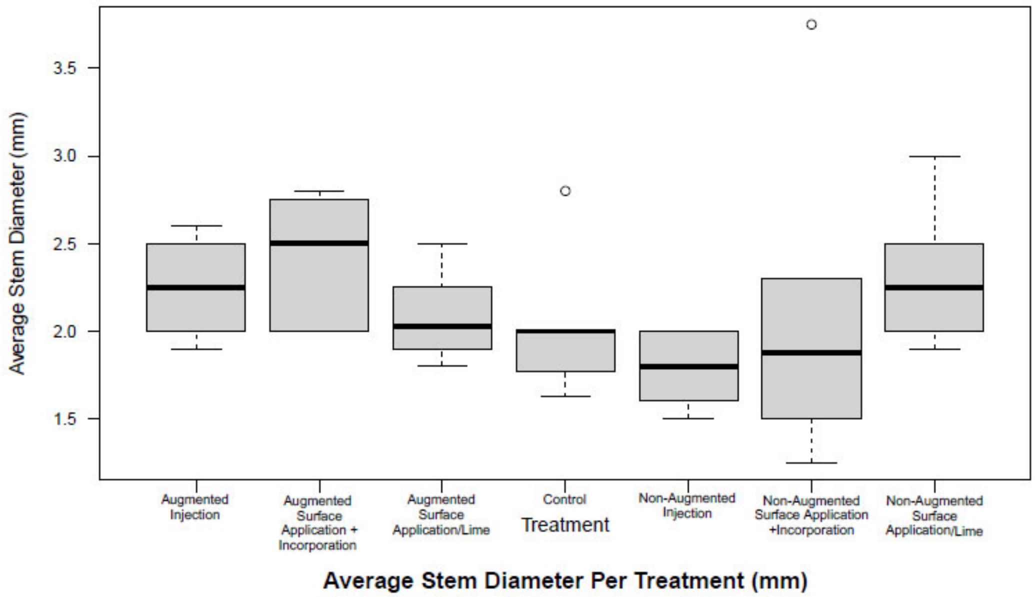


Notes: "o" is an identified outlier

Figure 3.6: Average Internodal Length Per Treatment

3.2.1c Stem Diameter

Stem diameter is the measure of the plant diameter 1 cm above the soil surface. A larger stem diameter is indicative of a healthier plant. The average stem diameter on a per treatment basis is tabulated in table 3-2a and visualized across each treatment in Figure 3.7. Across all treatments the summary statistics for average stem diameter are as follows. The minimum diameter recorded was 1.25 mm, a median of 2.0 mm, a maximum of 3.75 mm, with a mean diameter of 2.132 mm and a standard deviation of 0.47 mm. When comparing values for each different treatment the maximum average stem diameter was found in the augmented surface application and incorporation treatment (2.41 mm), while the minimum average stem diameter was observed in the non-augmented injection treatment (1.78 mm). Statistical analysis was not deemed necessary for this measured parameter. A tabulated summary of all harvest measurements per pot is available as Appendix D.

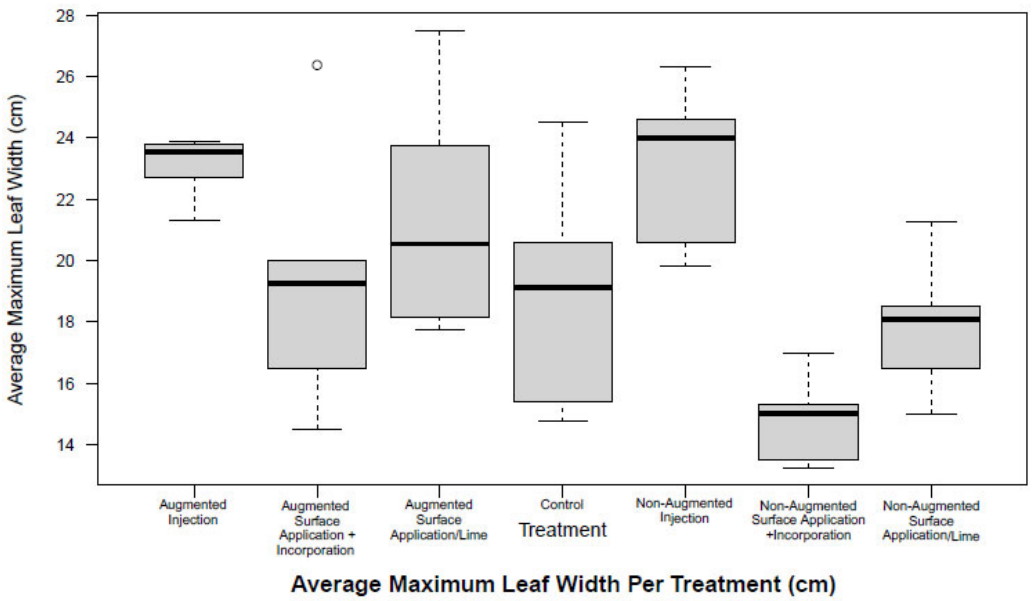


Notes: “o” is an identified outlier

Figure 3.7: Average Stem Diameter Per Treatment

3.2.1d Maximum Leaf Width

Maximum leaf width is the measure of length from leaf tip to leaf tip of the largest leaflet present. A large maximum leaf width is indicative of a healthier plant and a higher nitrogen content. The average maximum leaf width for each pot is tabulated in Appendix D and visualized across each treatment in Figure 3.8. Across all treatments the summary statistics for average maximum leaf are as follows. The minimum width recorded was 13.25 cm, a median of 20.0, a maximum of 27.50 mm, with a mean maximum width of 19.83 cm and a standard deviation of 3.90 cm. When comparing values for each different treatment, the maximum average maximum leaf width was observed in the non-augmented injection treatment (23.21 cm), while the minimum average maximum leaf width was observed in the non-augmented surface application and incorporation treatment (14.85 cm). Statistical analysis was not deemed necessary for this measured parameter. A tabulated summary of all harvest measurements per pot is available as Appendix D.

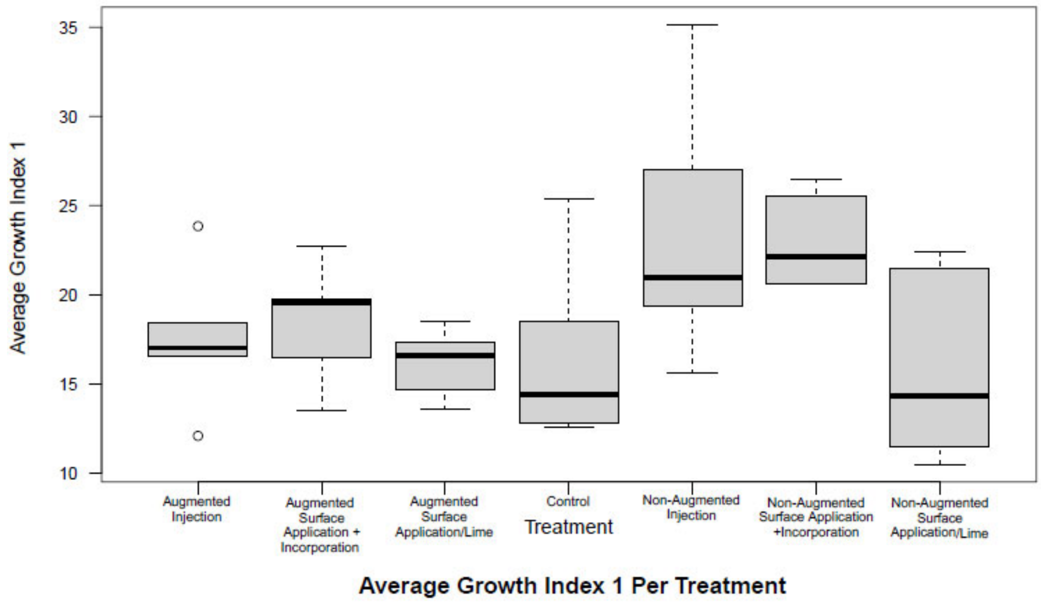


Notes: "o" is an identified outlier

Figure 3.8: Average Maximum Leaf Width Per Treatment

3.2.2 Growth Indices

Two growth indices were calculated to enumerate plant growth in each pot. Growth index 1 (GI1) is the product of the average height and average stem diameter divided by 2. Growth index 2 (GI2) is the product of the average internodal length and average maximum leaf width divided by 2. These factors provide insight to plant success by comparing horizontal versus vertical growth parameters. The summary statistics for both growth indices one and two are provided on a per pot basis in Appendix D. A boxplot for each growth index is provided as Figure 3.9 and Figure 3.10., respectively. Across all treatments the summary statistics for average growth index one is as follows. The minimum value recorded was 10.50, a median value of 18.40, and a maximum of 35.15. The mean was calculated as 18.63 with a standard deviation of 5.05. Across all treatments the summary statistics for average growth index two is as follows. The minimum value recorded was 8.25, a median value of 12.88, and a maximum of 20.25. The mean was calculated as 13.17 with a standard deviation of 2.68. A tabulated summary of all harvest measurements per pot is available as Appendix D.



Notes: "o" is an identified outlier

Figure 3.9: Average Growth Index 1 Per Treatment

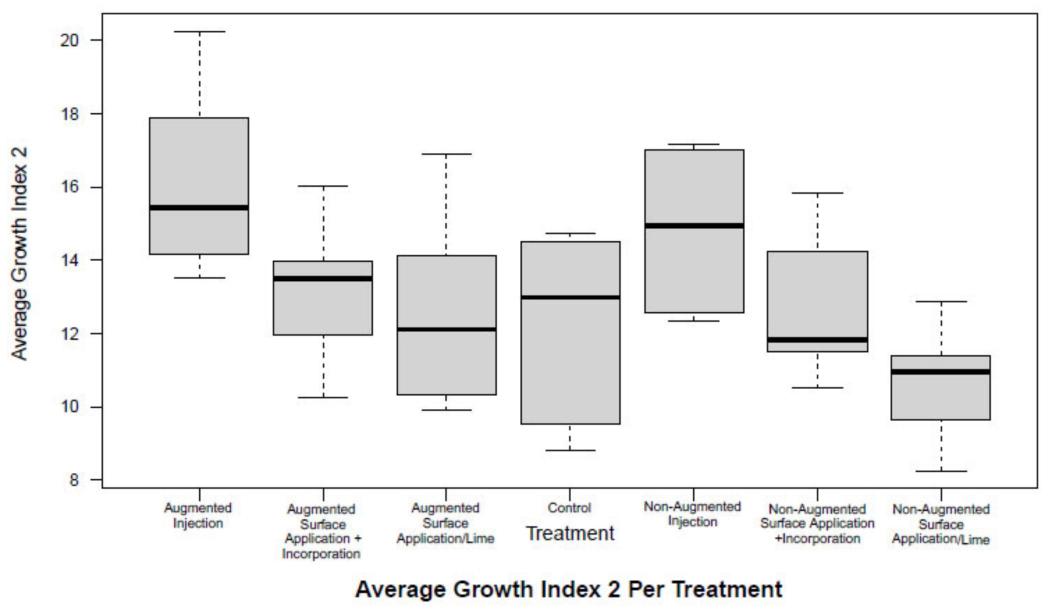


Figure 3.10: Average Growth Index 2 Per Treatment

Both growth indices were statistically analyzed using an ANOVA. Two ANOVA's were performed and tested the null hypothesis that the mean GI1 and GI2 values for each treatment were the same, and the alternative hypothesis that the mean GI1 and GI2 values for at least one treatment were different. The hypothesis test for GI1 determined with 95% confidence that a statistical difference between the average GI1 values across at least one treatment was different (F = 3.06, P = 0.016). A Tukey HSD test was applied to determine where the differences were. The results of this test for GI1 determined there were no significant differences between the average GI1 values across all treatments. The lowest recorded adjusted p-value was 0.08 when comparing the average GI1 values between the non-augmented surface application and the non-augmented injection. The hypothesis test for GI2 determined with 95% confidence that a statistical difference between the average GI2 values across at least one treatment was different (F = 3.75, P = 0.005). A Tukey HSD test was used post hoc to determine where the differences lie. The results of this test for GI2 determined statistical differences in two treatment comparisons. Statistical differences in GI2 values exist between the non-augmented surface application and augmented injection treatments (adj. P = 0.003) as well as the non-augmented surface application and non-augmented injection treatments (adj. P = 0.043). The injection methods produced higher values of GI2 than the non-augmented surface application.

3.2.3 SPAD

SPAD is a measure of chlorophyll content. A “SPAD” meter (KONICA MINOLTA, SPAD-502 Plus) measures light reflectance and quantifies the “greenness” of a plant. This is an indication of chlorophyll content and thereby nitrogen content in plants. SPAD readings for each pot were recorded at two intervals throughout the trial. The first measurement was conducted at a plants’ halfway point (3 weeks growth) and lastly at harvest (6 weeks growth). A summary of the average SPAD readings for each pot is tabulated in Appendix D and is visualized across each treatment in Figure 3.11. Across all treatments the summary statistics for average SPAD readings are as follows. The minimum recorded was 27.18, the median 36.05, and maximum of 44.50. The mean SPAD reading was 36.31 with a standard deviation of 4.64.

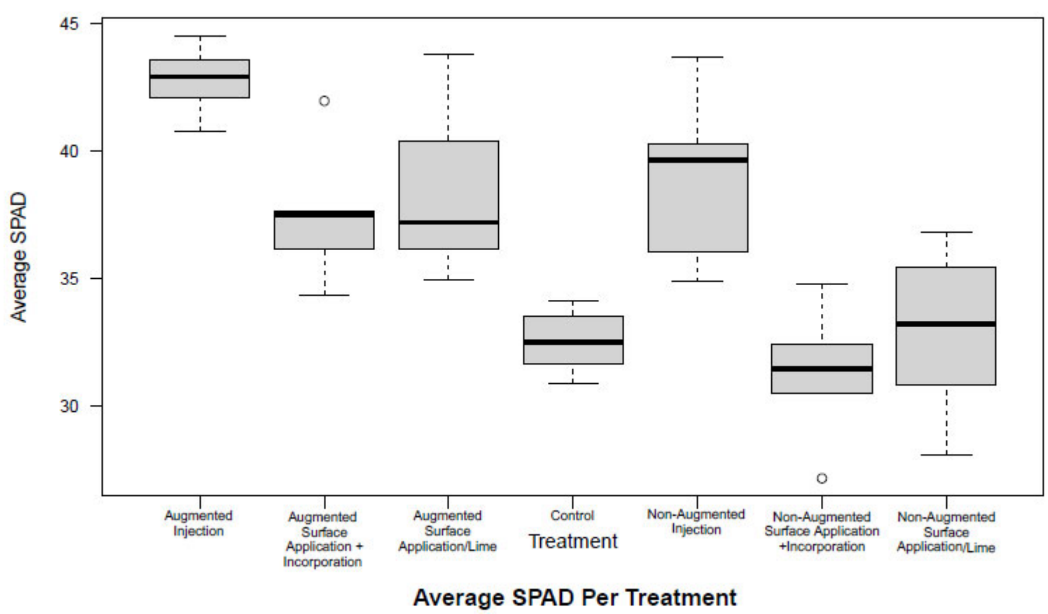


Figure 3.11: Average SPAD Readings Per Treatment

Notes: “o” is an identified outlier

Average SPAD readings were statistically analyzed using an ANVOA. This statistical test tested the null hypothesis that the mean SPAD reading for each treatment was the same, and the alternative hypothesis that the mean SPAD reading for at least one treatment was different. The hypothesis test determined with 95% confidence that a statistical difference between the average SPAD readings across at least one treatment was different ($F = 15.43$, $P = 0.01 \times 10^{-6}$). A Tukey HSD test was used post hoc to determine where the differences lie. The results of this test were conclusive. Statistical differences were found in 12 different treatment comparisons. These differences are tabulated below in table 3-2b

Table 3-2b: Tukey HSD Results Comparing Mean SPAD Readings by Treatment

Treatments Compared			**adj. P**
Non-Augmented Surface Application	vs.	Augmented Surface Application	0.017
Surface Application and Incorporation	vs.	Augmented Surface Application and Incorporation	0.006
		Augmented Surface Application	0.009×10^{-1}
Non-Augmented Injection	vs.	Control	0.002
		Non-Augmented Surface Application	0.004
		Non-Augmented Surface Application and Incorporation	0.002×10^{-1}
Augmented Injection	vs.	Control	0.001×10^{-3}
		Non-Augmented Surface Application	0.034×10^{-4}
		Non-Augmented Surface Application and Incorporation	0.001×10^{-4}
		Augmented Surface Application and Incorporation	0.030
Control	vs.	Augmented Surface Application	0.009
		Augmented Surface Application and Incorporation	0.047

In summary, average SPAD readings were statistically higher in both injection treatments when compared to the average SPAD readings in other treatments. This suggests that injection of septage into the ground will lead to higher levels of chlorophyll and nitrogen contents in a plant, as previously reported (Ebeling and Rwatambuga, 2011).

3.2.4 Dry Weight

Plant dry weight is a measure of physical plant mass after drying. This is a measure of how much plant material each pot produced as weighted on a per plant basis, which is the main measurement for determining plant growth and health. Not all pots in the greenhouse experiment germinated the same number of plants (which is a common occurrence for plant trials), so results are weighted on a per plant basis. A summary of the plant mass produced on a dry weight basis is provided in Table 3-2c and is visualized across each treatment in Figure 3.12. Across all treatments the summary statistics for average dry weight on a per plant basis were as follows: the minimum mass recorded was 0.02 g, the median 0.64 g, and maximum of 1.63 g. The mean dry weight mass was 0.68 g with a standard deviation of 0.36 g. A tabulated summary of all harvest measurements per pot is available as Appendix D.

Table 3-2c: Total Dry Weight Harvested Per Treatment

Treatment	Augmented (Y/N)	Total Number of Plants Harvested	Total Dry Weight (g)
Surface Application/Lime	N	13.00	8.41
Surface Application/Incorporation	N	13.00	5.74
Injection	N	22.00	23.24
Augmented Surface Application/Lime	Y	19.00	12.76
Augmented Surface Application/Incorporation	Y	12.00	7.81
Augmented Injection	Y	27.00	21.83
Control	N	16.00	6.99

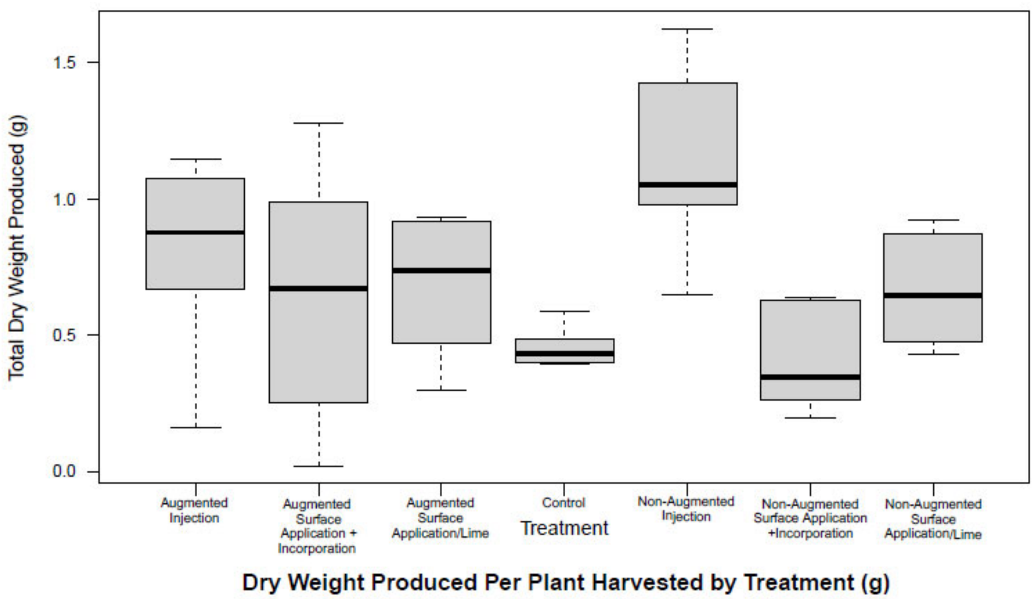


Figure 3.12: Dry Weight Per Plant Harvested by Treatment

Average dry weight measured on a per plant basis was statistically analyzed using an ANOVA. This statistical test tested the null hypothesis that the mean dry weight per plant for each treatment was the same, and the alternative hypothesis that the mean dry weight per plant for at least one treatment was different. The hypothesis test determined with 95% confidence that a statistical difference between the average SPAD readings across at least one treatment was different ($F = 3.73$, $P = 0.005$). A Tukey HSD test was used post hoc to determine where the differences lie. The results of this test were conclusive. Statistical differences were found in 2 different treatment comparisons. These comparisons included the non-augmented injection vs the control and the non-augmented injection vs the non-augmented surface application and incorporation. The total dry weight produced by each treatment was also observed. These results are tabulated in Table 3-2d.

Table 3-2d: Total Dry Weight Harvested Per Treatment

Treatment	Augmented (Y/N)	Total Number of Plants Harvested	Total Dry Weight (g)
Surface Application/Lime	N	13.00	8.41
Surface Application/Incorporation	N	13.00	5.74
Injection	N	22.00	23.24
Augmented Surface Application/Lime	Y	19.00	12.76
Augmented Surface Application/Incorporation	Y	12.00	7.81
Augmented Injection	Y	27.00	21.83
Control	N	16.00	6.99

3.2.5 Carbon and Nitrogen Percentage

Total C and N percentages were measured in the dry plant material. This quantifies what percent of the dry material is made up of each element. A summary of the percentages on a per pot basis is available in Table 3-2e. Figures comparing the values per treatment are shown in Figure 3.13 and 3.14., respectively. It was hypothesized that the liming of septage waste to a pH of 12 or greater would reduce the N contents when applied to land. This practice is done to control pathogens in the septage waste before land application. This N reduction was theorized due to the documented N loss as ammonia (which is a gaseous form of N) when the pH is raised above 8.2. The surface application methods, both augmented and non-augmented, were limed to a pH of 12 for a minimum of 30 min per EPA 503 and NR 113 guidance. A tabulated summary of C and N measurements per pot is available as Appendix D.

Table 3-2e: Average Carbon and Nitrogen Percentages Per Treatment

Treatment	Augmented (Y/N)	Carbon (%)	Nitrogen (%)
Suface Application/Lime	N	41.78	2.77
Suface Application/Incorporation	N	41.91	2.30
Injection	N	42.44	2.33
Augmented Surface Application/Lime	Y	42.15	4.09
Augmented Suface Application/Incorporation	Y	42.10	4.01
Augmented Injection	Y	42.67	3.79
Control	N	42.01	2.40

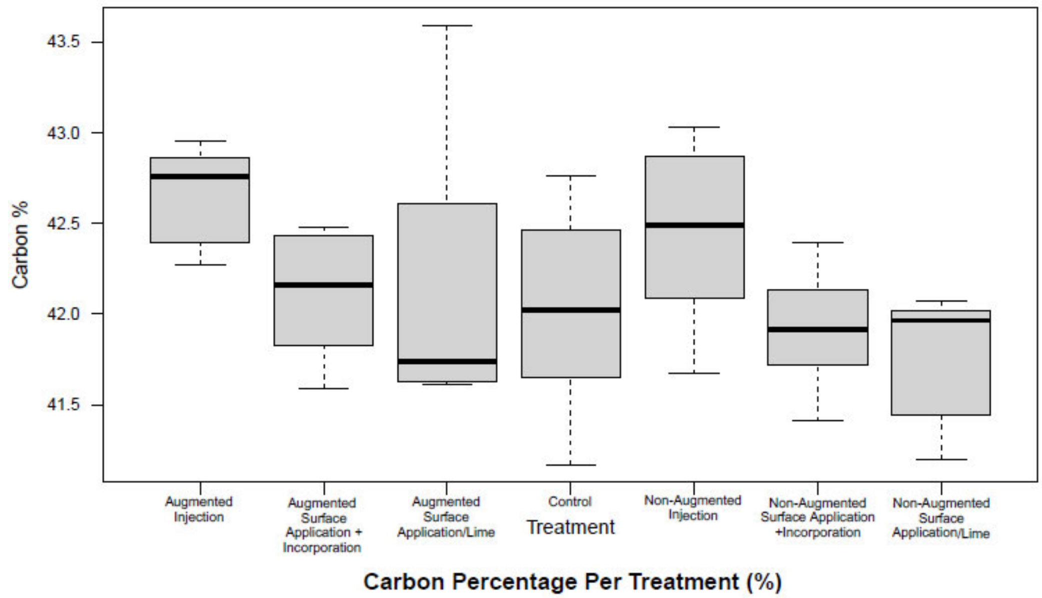
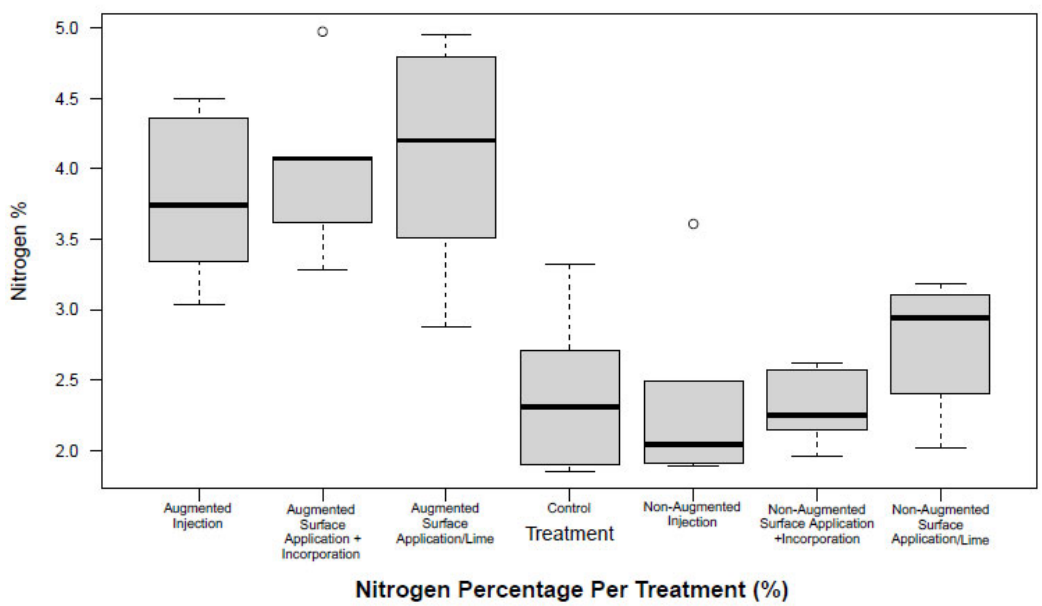


Figure 3.13: Percent Carbon Per Treatment



Notes: "o" is an identified outlier

Figure 3.14: Percent Nitrogen Per Treatment

Both percent C and percent N were statistically analyzed using ANOVA techniques. Two ANOVA's were performed and tested the null hypothesis that the mean C and N percentages for each treatment were the same, and the alternative hypothesis that the mean C and N percentages for at least one treatment were different. The hypothesis test for percent C determined with 95% confidence that no statistical difference is present between the average percent C values across all treatments ($F = 2.27$, $P = 0.059$). The hypothesis test for percent N determined with 95% confidence that a statistical difference between the average percent N values is present in at least one treatment ($F = 3.75$, $P = 0.005$). A Tukey HSD test was used post hoc to determine where the differences could be observed. The results of this test determined statistical differences in 11 treatment comparisons. These differences are tabulated below in Table 3-2f.

Table 3-2f: Tukey HSD Results Comparing Mean Percent Nitrogen Values Per Treatment

Treatments Compared			**adj. P**
Augmented Surface Appliaciton	vs.	Control	$0.004x10^{-1}$
		Non-Augmented Surface Appliaction	0.008
		Non-Augmented Surface Appliaction and Incorporation	$0.001x10^{-1}$
		Non-Augmented Injection	$0.002x10^{-1}$
Augmented Surface Appliaction and Incorporation	vs.	Control	0.001
		Non-Augmented Surface Appliaction	0.025
		Non-Augmented Surface Appliaction and Incorporation	$0.007x10^{-1}$
		Non-Augmented Injection	$0.009x10^{-1}$
Augmented Injection	vs.	Control	0.005
		Non-Augmented Surface Appliaction and Incorporation	0.002
		Non-Augmented Injection	0.003

These results show a statistically higher percent of N values in harvested plants grown after an augmented N application rate was applied. This is an expected result as 120 lbs N was applied for the augmented treatments versus 28.2 lbs N for non-augmented treatments. No differences were observed in treatments that were limed.

3.2.2 Comparison of Harvest Measurements Across Different Land Application Methods

A tabulated and figurative summary of harvest/nutrients\ measurements on a per application method basis was provided. The objective of this trial was to primarily analyze different land application methods and their N yields. It was hypothesized that the injection treatments would increase overall N yields within the plants. When considering the percent N values it was not the application method that proved to create a significant difference, but rather the presence or absence of augmentation within the treatment. However, multiple differences between application methods were observed. Firstly, in pots where an injection method was applied, more plant matter was physically produced. The augmented and non-augmented injection methods grew the most plants, 27 and 22 respectively. Another measurement where differences in application methods were present was in SPAD readings. Pots where an injection method was performed had statistically higher SPAD readings versus the other application methods. This suggests that injection application methods positively impacted the overall chlorophyll content of the plants.

Overall, the results of this plant growth trial are inconclusive. Though there are indications that injection methods have a positive impact on the plants grown, measurable differences between the N content in plants across different treatment methods are not present.

4.0 DEVIATIONS FROM PROPOSAL

As proposed this study contained three components:

1. A 2-season field effort collecting 360 septic system samples across 36 Wisconsin counties and subsequent analysis of these samples for all parameters described. Analysis was to include a characterization of the nutrients, virus and pathogens, and the full EPA heavy metals list.

2. Evaluate the differences in N contents across different land application methods using a greenhouse trial.

3. An investigation was to take place regarding the N contents of a septage being actively surface applied to land at the beginning of application, during application, and at the end of an application event as a septage truck was applying septage to a land base; 54 septage samples were to be collected from application trucks (9 per year over 2 years) as land application was taking place.

Due to the project's constraint in the deadline to complete all analyses within the calendar year of 2022, the surface land application N evaluation from application trucks (#3) was removed from the study due to the inability to conduct all the analyses on a shortened timeframe and the inability to schedule surface application events. Viral and bacterial pathogen measurements (#1) began in May of 2022. These analyses were quickly dropped due to the extreme variability in initial sample analyses, the validity of results, and the extreme amount of time and other logistics required to conduct the sampling and analyses; it was determined that this aspect of the study was not feasible nor relevant to provide any factual results. Lastly, mercury was dropped from the number of heavy metals to analyze as a part of this study due to time and expense.

5.0 CONCLUSIONS

Wisconsin septic waste is regulated by volume based on the nitrogen (N) contents of the waste. This regulation concludes that when 39,000 gallons of septage waste is applied to an acre of land, this is equivalent to 100 lbs of N. As measured in this study, which focused on household septic tank waste, the total N content in Wisconsin septic tank waste is lower than previously thought. When 39,000 gallons of septage is applied in the first year to land, a geometric mean of 34.59 lbs of N is applied with the assumption that only 30% of the solid organic fraction N is available in the first year; however, more N will become available in subsequent years on the same land but at a lesser amount. This is an overall reduction in N content than previously thought per EPA and WDNR guidelines.

Though the standards are N based, more attention has shifted to phosphorus (P) in recent years. The average P contributions from septic tank waste land application as measured in this study is a geometric mean of 21.56 lbs of P, with the assumption that only 60% of the solid organic fraction P is available in the first year; however, more N will become available in subsequent years on the same land but at a lesser amount. These values should be considered when applying septage to land. The ~35 lbs of N to ~22 lbs of P ratio is essentially a 3:2 ratio. Crops do not take up N and P in these ratios, as each crop has its own distinct characteristics. It is quite likely that several options need to be evaluated with further research.

Septage waste applied to land can also apply heavy metals to land. Heavy metals are known to cause harm in the environment at elevated levels. Levels observed in septage are generally low in comparison to EPA standards for sewage sludge. As reported, very few collected samples exceeded the EPA heavy metal limits for sewage sludge. These values are used for comparison, but it should be noted that there are no applicable State regulations relating to ceiling concentrations for heavy metals in septage waste that is applied to land.

The greenhouse/land application trial conducted provided inconclusive results, indicating that no single method of application was better than the other methods of application across most measurements. It was hypothesized that septage injection with augmentation of N would stand alone to produce the best plant growth, but this was not observed. It should also be noted that the greenhouse trial used 1 type of plant and 1 type of soil, even though Wisconsin has a diversity of crops being grown and a multitude of different soil types.

For all results presented, further research is warranted. While 360 septic tank samples is a large dataset in which to base recommendations, there are of almost 800,000 septic systems in Wisconsin. This study also only sampled septic tanks from private households, and not other systems such as holding tanks or POWTS using aerobic treatment components. Some examples for further research include:

- Determining the impacts of the status quo relating to N application rates to fields in determining the potential runoff and P impacts from land application fields.
- Similarly, determining the impacts of the status quo relating to N application rates to fields in determining P issues when the field is internally drained (ie no runoff to offsite locations).
- Determining the best crops for harvesting P from fields to allow for additional septage application without creating additional P issues.
- Determining if different soil types could allow additional septage contributions that in effect could neutralize P.

- Determining the impact of liming on the soils ability to buffer septage applications for nutrients.
- Determining if the use of nutrient management plans, such as those currently exempted by s. ATCP 50.04 and s. NR 151.07, Wis. Adm. Code, are appropriate. Currently there are exemptions for septage application under specific situations from these two agricultural land regulations.
- Determining if field rotations of septage applications to allow the addition of N only or high N/low P additions on non-septage application crop years to balance the concentrations of P being applied to fields is productive.
- Determining if alternative sampling methods and requirements of stored septage waste for N content (and potentially P content) could be useful to determine land application rates of septage, in accordance with using crop rotation techniques with the addition of commercial fertilizers.

6.0 ACKNOWLEDGEMENTS

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APPENDIX A

University of Wisconsin – Stevens Point
Protocol for Protection of Human Subjects in Research

Informed Consent to Participate in Human Subject Research

Dr. Robert Michitsch, a Professor of Soil and Waste Resources in the College of Natural Resources at the University of Wisconsin-Stevens Point, would appreciate your participation in a research study designed to determine the levels of physical parameters, macro and micronutrients, heavy metal contents and select microbial analyses of your household septage. The purpose is to reassess current regulations regarding the land application of septage resources in Wisconsin. This study is being conducted on behalf of the Wisconsin Liquid Waste Carriers Association (WLWCA), the Wisconsin Department of Natural Resources (WIDNR), the Wisconsin Department of Safety and Professional Services and the University of Wisconsin-Stevens Point. You are being asked to complete an anonymous information sheet that should take-up no more than 5-10 minutes of your time, as well as to allow me (or a certified representative) to collect a 1-liter sample of your septage material for full analyses, and potentially a small sample of your household tap water for pH analysis.

I anticipate no risk to you, or your household, as a result of your participation in this study other than the inconvenience of the time to complete the information sheet, and for me and my team to obtain samples of your septage and potentially your household tap water. The information being gathered will never identify you as an individual nor your individual household.

While there may be no immediate benefit to you as a result of your participation in this study, it is hoped that we may gain valuable information to reassess current regulations regarding the land application of septage resources in Wisconsin.

The information that you give us on the information sheet will be anonymous besides from some general demographic information. All completed information sheets will be kept in a secure location in the office of Dr. Robert Michitsch at the University of Wisconsin-Stevens Point.

If you want to withdraw from the study at any time, you may do so without penalty. The information collected from you up to that point would be destroyed.

Once the study is completed, we would be glad to give you a summary of the results.

In the meantime, if you have any questions, please ask me at:

Dr. Robert Michitsch
Soil and Waste Resources, College of Natural Resources
University of Wisconsin-Stevens Point
Stevens Point, WI 54481 (715) 346-4190; rmichits@uwsp.edu

If you have any complaints about your treatment as a participant in this study, please call, email or write:

Dr. David Barry, Chair
Institutional Review Board for the Protection of Human Subjects
University of Wisconsin-Stevens Point
Stevens Point, WI 54481
(715) 346-3953; irbchair@uwsp.edu

College of Natural Resources

University of Wisconsin-Stevens Point Stevens Point, Wisconsin 54481-3897 (715) 346-2853 FAX (715) 346-3624

Septage Sample Information Sheet

Random Reference Code: _____

On what date was your septage sample obtained?: ____________________

When was the last time (in months) that you had your septic tank pumped? ____________________

On average, how often do you have your septic tank pumped (in years)? ____________________

Was a household water sample obtained at the same time?: ____________________

In what City was your septage sample obtained?: ____________________

In what County was your septage sample obtained?: ____________________

Is this a full-time or seasonal home? ____________________

How many individuals normally reside in this dwelling during the year or season?: ____________________

Does a child 2 years of age or younger reside at this home? (Please Circle) Yes No

Does a child 5 years of age or younger reside at this home? (Please Circle) Yes No

Do you frequently have visitors that could impact the contents of your septage system? This could impact the characteristics of your septage. As just one example, children coming home to live with you during the summer if they've been away at school will impact your system if the sample was obtained this summer. Please explain if necessary.

____

____

How many bedrooms are in your dwelling? ____________________

What is the gallon capacity of your septic tank or tanks?: ____________________

If multiple tanks are incorporated, are the chambers either individual tanks or multiple compartment tanks? Can you provide information about the size of each tank?

____

For the tank(s) that are incorporated, where is the sampling manhole located (inlet side, middle, outlet side)? ______

____

Is the absorption area of your septic system considered a conventional drainfield, mound or other?: ______________

Do you incorporate a lift station?: ____________________

What is the age of your septic system?: ____________________

Do you consider yourself an urban, rural or middle-point location?: ____________________

Do you employ extra processing applications, such as filters, a garbage disposal, adding water softening waste, or other techniques to treat or affect your wastewater stream? Please explain if necessary.

____

____

____

Please provide any other information that you feel is relevant. Personal information is not desired.

____

____

____

____

____

APPENDIX B

APPENDIX C

Summary of Heavy Metals Concentrations

Metal	Sample Size	**Ceiling Concentrations** (Table 1 of 40 CFR 503.13) (miligrams per kilogram, dry weight)	Number of Values Above Ceiling Concentrations	**Minimum** (mg/kg)	**Median** (mg/kg)	**Mean** (mg/kg)	**Geometric Mean** (mg/kg)	**Max** (mg/kg)	**SD** (mg/kg)
Al	355	--	--	80.84	1979.55	2647.97	8.56	17701.34	2442.01
As	178	75	1	1.89	3.40	16.42	1.67	**2048.56**	153.23
B	355	--	--	10.93	48.43	78.00	3.65	1766.43	117.16
Ba	355	--	--	13.01	82.18	191.68	5.74	8197.78	655.43
Ca	355	--	--	7150.00	32393.00	34361.00	228.42	140648.00	14988.61
Cd	11	85	0	5.20	6.59	9.97	4.67	41.12	10.39
Co	318	--	--		1.98	33.10	0.87	9772.05	547.86
Cr	350	3000	0	0.83	12.53	17.26	0.73	199.56	19.07
Cu	350	4300	3	2.47	178.17	424.13	3.00	**23214.95**	1412.31
Fe	350	--	--	85.69	2927.41	5862.58	17.41	55423.32	8192.87
K	355	--	--	702.80	4175.50	6057.00	165.78	27595.90	5135.78
Li	321	--	--	1.22	2.44	3.49	1.14	37.70	3.93
Mg	355	--	--	495.30	6076.00	7380.10	21.80	28429.30	5141.36
Mn	355	--	--	4.96	82.27	120.34	3.54	2815.70	222.17
Mo	100	75	3	3.74	6.50	27.50	3.19	**1646.85**	164.79
Na	355	--	--	1298.00	36487.00	59393.00	47.88	326190.00	63510.83
Ni	353	420	1	1.25	6.24	43.85	1.14	**11466.80**	609.79
P	355	--	--	694.40	7363.20	7917.20	28.78	28345.70	4382.31
Pb	343	840	0	1.02	7.34	13.57	0.79	292.85	24.08
S	355	--	--	1664.00	11630.00	13588.00	45.00	74115.00	8671.06
Se	80	100	0	2.45	3.86	10.88	2.19	88.08	17.35
Sr	355	--	--	8.40	44.25	140.21	6.78	7894.74	651.85
Zn	355	7500	0	20.77	952.65	1449.61	12.71	4424.81	785.40

Notes:

1.) The total samples size for all metal analysis was 355 samples. Values that were below the MDL ar enot incuded in the statistical analysis.

3.) **Bold** values indicate values that are above the ceiling concetrations for sewage sludge.

2.) 40 CFR 503.13 ceiling concentration values are for sewage sludge not domestic septage. These values are for comparison purposes only and do not represent an exceedance in the ceiling concentrations.

APPENDIX D

Summary of Harvest Measurements

Pot Number	Treatment	Application Rate	Total Number of Plants Harvested	Total Number of Beans Harvested	Average Height (cm)	Average Internodal Length (cm)	Average Maximum Leaf Width (cm)	Average Stem Diameter (mm)	Average Growth Index 1	Average Growth Index 2	SPAD Average Total Per Pot	Total Dry Weight (g)	Dry Weight Per Plant (g)	Carbon (%)	Nitrogen (%)
1	Suface Application/Lime	39,000 gal septage / acre	1.00	1.00	18.00	1.50	15.00	3.00	10.50	8.25	32.46	0.48	0.48	41.2	3.18
2	Suface Application/Lime	39,000 gal septage / acre	3.00	9.00	42.90	4.50	21.25	1.90	22.40	12.88	28.08	1.42	0.47	42.07	2.979
3	Suface Application/Lime	39,000 gal septage / acre	2.00	5.00	21.00	3.90	18.00	2.00	11.50	10.95	36.80	1.85	0.93	42.02	2.906
4	Suface Application/Lime	39,000 gal septage / acre	3.00	5.00	29.90	3.75	18.15	2.50	16.20	10.95	33.94	1.29	0.43	41.94	3.101
5	Suface Application/Lime	39,000 gal septage / acre	2.00	7.00	22.50	2.75	16.50	2.50	12.50	9.63	30.84	1.63	0.82	41.44	2.019
6	Suface Application/Lime	39,000 gal septage / acre	2.00	8.00	41.00	4.25	18.50	2.00	21.50	11.38	35.45	1.74	0.87	41.99	2.405
7	Suface Application/Incorporation	39,000 gal septage / acre	2.00	0.00	39.00	8.00	15.30	2.30	20.65	11.65	30.50	1.26	0.63	42.13	2.265
8	Suface Application/Incorporation	39,000 gal septage / acre	2.00	1.00	51.50	14.65	17.00	1.50	26.50	15.83	30.70	0.39	0.20	41.72	2.233
9	Suface Application/Incorporation	39,000 gal septage / acre	4.00	5.00	39.65	7.93	15.05	3.75	21.70	11.49	32.23	2.56	0.64	42.39	2.15
10	Suface Application/Incorporation	39,000 gal septage / acre	2.00	0.00	43.50	7.75	13.25	1.75	22.63	10.50	34.78	0.52	0.26	41.86	2.575
11	Suface Application/Incorporation	39,000 gal septage / acre	2.00	3.00	40.00	21.00	13.50	1.25	20.63	12.00	27.18	0.63	0.32	41.41	2.619
12	Suface Application/Incorporation	39,000 gal septage / acre	1.00	2.00	49.00	13.50	15.00	2.00	25.50	14.25	32.40	0.38	0.38	41.97	1.955
13	Injection	39,000 gal septage / acre	2.00	6.00	37.00	8.00	26.30	1.80	19.40	17.15	39.85	1.96	0.98	41.67	3.608
14	Injection	39,000 gal septage / acre	5.00	9.00	39.43	4.10	20.57	2.00	20.72	12.33	34.87	3.23	0.65	42.43	2.496
15	Injection	39,000 gal septage / acre	3.00	8.00	52.60	5.80	24.20	1.50	27.05	15.00	39.45	4.27	1.42	42.55	1.912
16	Injection	39,000 gal septage / acre	5.00	14.00	29.20	5.30	19.80	2.00	15.60	12.55	36.05	5.59	1.12	43.03	1.889
17	Injection	39,000 gal septage / acre	5.00	8.00	40.80	5.20	24.60	1.60	21.20	14.90	43.65	4.94	0.99	42.87	1.939
18	Injection	39,000 gal septage / acre	2.00	9.00	68.50	10.25	23.80	1.80	35.15	17.03	40.25	3.25	1.63	42.09	2.149
19	Augmented Surface Application/Lime	39,000 gal septage / acre + 84lb ammonium nitrate	4.00	5.00	24.90	4.55	21.00	2.25	13.58	12.78	34.96	1.89	0.47	41.71	4.956
20	Augmented Surface Application/Lime	39,000 gal septage / acre + 84lb ammonium nitrate	2.00	3.00	34.50	6.30	27.50	2.50	18.50	16.90	37.15	1.84	0.92	43.59	2.876
21	Augmented Surface Application/Lime	39,000 gal septage / acre + 84lb ammonium nitrate	4.00	11.00	27.60	2.80	20.10	1.80	14.70	11.45	43.75	3.60	0.90	42.61	3.505
22	Augmented Surface Application/Lime	39,000 gal septage / acre + 84lb ammonium nitrate	3.00	9.00	31.00	4.50	23.75	1.90	16.45	14.13	40.38	2.80	0.93	41.77	4.75
23	Augmented Surface Application/Lime	39,000 gal septage / acre + 84lb ammonium nitrate	3.00	3.00	32.50	2.05	17.75	2.15	17.33	9.90	36.18	1.73	0.58	41.63	4.789
24	Augmented Surface Application/Lime	39,000 gal septage / acre + 84lb ammonium nitrate	3.00	3.00	31.50	2.50	18.15	1.90	16.70	10.33	37.25	0.90	0.30	41.61	3.649
25	Augmented Suface Application/Incorporation	39,000 gal septage / acre + 84lb ammonium nitrate													
26	Augmented Suface Application/Incorporation	39,000 gal septage / acre + 84lb ammonium nitrate	1.00	0.00	25.00	6.00	14.50	2.00	13.50	10.25	34.35	0.02	0.02	42.16	3.287
27	Augmented Suface Application/Incorporation	39,000 gal septage / acre + 84lb ammonium nitrate	4.00	5.00	37.13	5.65	26.38	2.00	19.56	16.01	37.63	2.56	1.28	41.83	4.973
28	Augmented Suface Application/Incorporation	39,000 gal septage / acre + 84lb ammonium nitrate	1.00	0.00	37.00	10.50	16.50	2.50	19.75	13.50	37.50	0.25	0.25	42.48	4.071
29	Augmented Suface Application/Incorporation	39,000 gal septage / acre + 84lb ammonium nitrate	3.00	9.00	42.75	7.95	20.00	2.75	22.75	13.98	36.15	2.01	0.67	41.59	4.08
30	Augmented Suface Application/Incorporation	39,000 gal septage / acre + 84lb ammonium nitrate	3.00	9.00	30.20	4.70	19.25	2.80	16.50	11.98	41.95	2.97	0.99	42.43	3.62
31	Augmented Injection	39,000 gal septage / acre + 84lb ammonium nitrate	5.00	11.00	21.60	3.50	23.50	2.60	12.10	13.50	42.10	3.35	0.67	42.39	4.501
32	Augmented Injection	39,000 gal septage / acre + 84lb ammonium nitrate	4.00	6.00	31.30	8.80	23.60	2.50	16.90	16.20	43.55	4.59	1.15	42.82	3.337
33	Augmented Injection	39,000 gal septage / acre + 84lb ammonium nitrate	4.00	7.00	30.80	4.40	23.90	2.40	16.60	14.15	40.77	0.64	0.16	42.27	4.089
34	Augmented Injection	39,000 gal septage / acre + 84lb ammonium nitrate	5.00	8.00	45.80	6.60	22.70	1.90	23.85	14.65	43.40	4.31	0.86	42.7	3.398
35	Augmented Injection	39,000 gal septage / acre + 84lb ammonium nitrate	4.00	9.00	34.80	14.50	21.30	2.00	18.40	17.90	44.50	3.57	0.89	42.86	4.361
36	Augmented Injection	39,000 gal septage / acre + 84lb ammonium nitrate	5.00	12.00	32.20	16.70	23.80	2.10	17.15	20.25	42.40	5.37	1.07	42.95	3.034
37	Control	Control	4.00	9.00	26.00	4.33	20.60	1.77	13.88	12.47	33.50	1.78	0.45	41.65	2.354
38	Control	Control	2.00	6.00	28.00	6.75	20.25	2.00	15.00	13.50	30.90	0.97	0.49	41.17	1.853
39	Control	Control	1.00	4.00	35.00	4.50	24.50	2.00	18.50	14.50	32.80	0.59	0.59	41.69	3.317
40	Control	Control	2.00	2.00	48.00	11.50	18.00	2.80	25.40	14.75	32.20	0.79	0.40	42.46	1.895
41	Control	Control	4.00	3.00	23.63	3.63	15.40	2.00	12.81	9.51	34.13	1.60	0.40	42.35	2.267
42	Control	Control	3.00	3.00	23.50	2.88	14.75	1.63	12.56	8.81	31.65	1.26	0.42	42.76	2.714