Investigating the Impacts of Wastewaters on Lettuce Seed Germination

Contestant Name: Liam Reynolds
ASABE Membership Number: 1061258

Institution: University of Illinois in Urbana
Champaign Degree Date: 05/13/2023
Advisor: Dr. Paul Davidson

Contestant Signature: Liam Reynolds

Ag Mech Student Branch Advisor Signature: Paul C. Davidson

Date of Signature: 5/12/2023

ORIGIN OF RESEARCH

This experimental research originated within the Water Quality Laboratory of the Agricultural and Biological Engineering Department at the University of Illinois in Urbana-Champaign. The Environment Enhancing Energy project in the ABE department has done substantial work with Hydrothermal Liquefaction, and previous undergraduate as well as graduate research has been conducted investigating the viability of Hydrothermal Liquefaction Aqueous Phase as a nutrient source for crop production. It was determined that something needs to be done to this aqueous phase to increase its viability for crop production. A literature review was conducted and it was determined that mixed wastewaters, including aquaponic effluents, could be mixed with HTL-AP to create a complete nutrient source. This initial germination screening experiment was performed to determine initial viability ranges and ideal characteristics for mixed wastewater parameters to be used in future experiments.

ACKNOWLEDGEMENTS

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ABSTRACT

There is an opportunity for agriculture to utilize the many different waste streams in our world to capitalize on what would otherwise be viewed as waste products. Hydrothermal liquefaction (HTL) is an emerging technology for converting wet biomass to bio-crude oil, while aquaponics is a practice tracing back to indigenous communities around the world; both of these technologies can provide the necessary nutrients for crop growth sustainably. Food systems worldwide are actively transitioning to sustainably and efficiently address the many challenges of climate change. Urban agriculture (UA) has the potential to generate localized crops in densely populated areas year-round, but has its challenges including high capital requirements, especially for vertical farming and controlled environment agriculture, as well as being energy intensive due to artificial lighting and fossil fuel-based synthetic fertilizers. This study investigated the potential for aquaponic and HTL effluents to be used in hydroponic systems through a seed germination screening experiment. Buttercrunch lettuce seeds were placed in Ziploc bags on paper towels saturated with the wastewater treatments for 10 days while their total biomass growth was routinely measured from the tip of the root to the tip of the cotyledons. The CHSAS aquaponic effluent with a higher ammonia & nitrate content outperformed the Bevier aquaponic effluent and improved any other source water it was combined with. Results also showed seed germination was not inhibited in the presence of 2-8% solutions of hydrothermal liquefaction aqueous phase (HTL-AP), as a higher percentage may lead to inhibitory effects in plants and a lower percentage may not provide enough nutrients in the proper forms to sustain plant growth. However, the nutrient analyses revealed there is still much to investigate regarding the combination of wastewaters to provide a complete, well-rounded, and sustainable source for hydroponic crop production.

INTRODUCTION

Nontraditional farming techniques including hydroponics, aquaponics, urban indoor controlled-environment agriculture (CEA) operations, and vertical farming have the potential not to replace, but to supplement and reduce strain on the existing food supply chain in addition to increase awareness among consumers of the links between the food supply chain and health (Ackerman, 2012). However, the current state of agriculture in industrialized nations around the world must undergo a transition to increase food production while supporting a wider variety of food sources produced locally and sustainably consistently (Grafton et al, 2015). There are widespread food insecurities and nutrient deficiencies globally, that will only be exacerbated by agricultural losses due to extreme weather events and shifting climates from the increased emissions of greenhouse gases (Aleandratos, 2012). There are many market and non-market benefits to urban agriculture (UA) (Specht et al, 2014; Tornaghi, 2014; Orsini et al, 2013; Mougeot, 2000; Opitz et al, 2016; Shabbir, 1996; IDRC 2006; Kalantari et al, 2017; Goldstein et al, 2016; Ackerman, 2012). The most promising benefit relating to food security and food supply chain improvements are reduced food miles as the food is produced closer to the consumer, increased yields due to vertical and year-round farming, and improved distribution efficiencies including reduced packaging and spoilage (Goldstein et al, 2016). This paper will address another prospective benefit of UA focusing on its sustainability, specifically the assimilation of organic waste for nutrient recycling in urban ecosystems. With the eventual goal of creating a closed-loop urban system, various forms of urban 'waste', whether it is the composting of crop residue or other solid waste, irrigation with nutrient-dense wastewaters, or the conversion of organics to biofuels, can and have been sustainably utilized in many urban farming operations (Kalantari et al, 2017; Goldstein et al, 2016; Ackerman, 2012).

Hydrothermal liquefaction is an emerging process that has shown potential in converting wet biomass into a renewable and sustainable fuel source in the form of bio-crude oil (Gollakota et al, 2018). Gollakota et al. (2018) summarize HTL as "the thermochemical conversion of biomass into liquid fuels by processing in a hot, pressurized water environment for sufficient time to break down the solid biopolymeric structure to mainly liquid components." Jesse and Davidson (2019) point out that although this bio-crude oil is the main product of HTL, the aqueous phase byproduct of HTL, or Hydrothermal Liquefaction Aqueous Phase (HTL-AP), has the potential for use in crop production. HTL-AP has irrigation potential due to the destruction of pathogens by the high temperatures and pressures associated with HTL, while essential plant nutrients (i.e., N-P-K) remain (Leng & Zhou, 2018; Huang & Yuan, 2015; Peterson et al, 2008). Jesse and Davison (2019) additionally point out there may still be heavy metal contamination in addition to potential genetic material or pharmaceutical residues in HTL-AP due to the origin of the feedstock. They determined both “raw and various treated HTL-AP meet US EPA guidelines for wastewater reuse for crop irrigation in terms of heavy metals and *E. coli* and coliforms”. However, in another study, Jesse et al. (2019) demonstrated that diluted HTL-AP source waters alone are not a sufficient source of nitrogen and phosphorus for the hydroponic production of lettuce; with nitrogen specifically, it was present in raw HTL-AP, but only 0.03% was in the plant-available form of nitrate. This nutrient deficiency led to the growth of less biomass, found to be correlated to higher concentrations of arsenic at levels higher than the maximum levels allowable under the US Department of Agriculture due to lower total biomass, as shown by total dry yield. Therefore, although HTL-AP has some potential for irrigation use as a nutrient source, it must be supplemented with sufficient nutrients to minimize the risk of metalloids while also maximizing yields.

A sustainable source of these supplemental nutrients can be found in aquaponics, a highly engineered water-based agriculture system, utilizing internal nutrient recycling from fish effluent, through the co-cultivation of fish with plants in hydroponic sub-systems (Bartelme et al., 2018). Aquaponics may be a sustainable nutrient source because the production method is based on the concepts of minimal water use and nutrient reuse through recirculating aquaculture systems (RAS), leading to minimal impact on environmental water quality compared to traditional agricultural production methods (Blidariu & Grozea, 2011). Additionally, incorporating aquaponics into urban food production systems provides both a source of protein as well as fresh produce; aquaponics has also been shown to positively impact community and economic development in urban areas (Goodman, 2011). Aquaponics is not without its own needs for supplemental nutrient sources, often in the form of chemical fertilizers, but work has been done in researching plant growth-promoting microorganisms (PGPMs) to alleviate this supplementation by emphasizing PGPMs in system design (Bartelme et al., 2018).

For this study, aquaponic effluent was used as a supplemental source of nutrients for hydroponic systems as an important source of micronutrients along with PGPMs. Previous research by Goddek et al. (2016) found aquaponic sludge processed via anaerobic digestion positively increased plant growth over that of aerobic digestate, as well as the control system. It was hypothesized this was due to increased ammonium, dissolved organic matter, humic acid, and PGP rhizobacteria and fungi. Carvalho et al. (2018) found although hydroponic systems with both wastewater and chemical fertilizers as nutrient sources were no different than the positive control, the system with wastewater as the sole nutrient source required additional nutrient supplementation. Egbuikwem, et al. (2020) also determined there was a positive indication for the reuse of mixed wastewater in hydroponics. They confirmed a need to further investigate the benefits and limitations of such water.

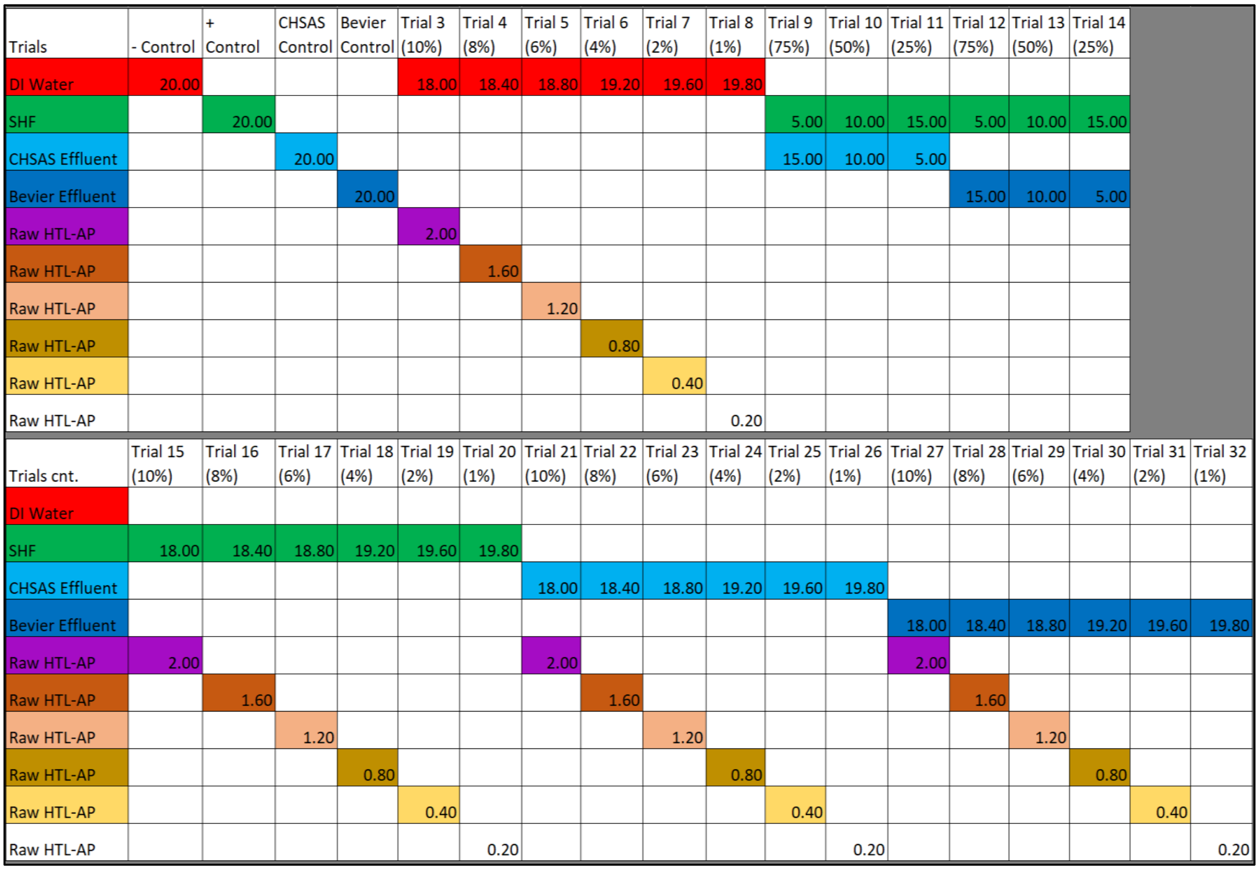
When utilizing nutrient analysis and spectroscopy to determine what makes a viable nutrient source, there have been few studies investigating the impacts of various nutrients and compounds on the actual germination of seeds as opposed to the growth of crops after successful germination. The work of Arancon et al. (2012) investigated the effects of soaking lettuce and tomato seeds in various concentrations of compost teas before analyzing differences in germination. They found germination percentage increased linearly with compost tea concentration and soaked trials outperformed unsoaked trials. Ahmed et al. (2018) determined nitrogen nanobubbles had a significant positive effect on both germination percentage and hypocotyl length for lettuce seeds. This aligns with the compost tea results as compost tea is largely composed of carbon and nitrogen species. The impact of certain micronutrients as well as biological treatments, comparable to PGPMs, were studied by Postic et al. (2021). They determined the biological treatment had the largest positive impact on germination percentage followed by the mixed treatment and then the Zinc and Boron treatments.

The overarching aim of this investigation and future research in this area should focus on increasing the sustainability of nutrient sources for UA to reduce the environmental impacts of local food systems. The **overall objective** of this study is to further investigate the effects of mixed wastewaters, namely HTL-AP, chemical fertilizers, and aquaponic effluent sources, on the initial germination of buttercrunch lettuce seeds. The ***specific objectives*** of this study are to:

1. Assess the final germination proportion, germination rate, total biomass growth, and growth rate of lettuce seeds in the presence of wastewater treatments.
2. Characterize each wastewater to provide context around the impact on seed germination and initial growth period.

MATERIALS AND METHODS

This study investigated three different source wastewaters and two controls: HTL-AP, aquaponic effluent from CHSAS, aquaponic effluent from Bevier Cafe, a positive control containing standard hydroponic fertilizer (SHF), and deionized (DI) water as the negative control. Not counting the two control trials, a total of 32 different combinations of the various wastewaters were created as pictured in Figure 1.



Trials	- Control	+ Control	CHSAS Control	Bevier Control	Trial 3 (10%)	Trial 4 (8%)	Trial 5 (6%)	Trial 6 (4%)	Trial 7 (2%)	Trial 8 (1%)	Trial 9 (75%)	Trial 10 (50%)	Trial 11 (25%)	Trial 12 (75%)	Trial 13 (50%)	Trial 14 (25%)
DI Water	20.00				18.00	18.40	18.80	19.20	19.60	19.80						
SHF		20.00									5.00	10.00	15.00	5.00	10.00	15.00
CHSAS Effluent			20.00								15.00	10.00	5.00			
Bevier Effluent				20.00										15.00	10.00	5.00
Raw HTL-AP					2.00											
Raw HTL-AP						1.60										
Raw HTL-AP							1.20									
Raw HTL-AP								0.80								
Raw HTL-AP									0.40							
Raw HTL-AP										0.20						

Trials cnt.	Trial 15 (10%)	Trial 16 (8%)	Trial 17 (6%)	Trial 18 (4%)	Trial 19 (2%)	Trial 20 (1%)	Trial 21 (10%)	Trial 22 (8%)	Trial 23 (6%)	Trial 24 (4%)	Trial 25 (2%)	Trial 26 (1%)	Trial 27 (10%)	Trial 28 (8%)	Trial 29 (6%)	Trial 30 (4%)	Trial 31 (2%)	Trial 32 (1%)
DI Water																		
SHF	18.00	18.40	18.80	19.20	19.60	19.80												
CHSAS Effluent							18.00	18.40	18.80	19.20	19.60	19.80						
Bevier Effluent													18.00	18.40	18.80	19.20	19.60	19.80
Raw HTL-AP	2.00						2.00						2.00					
Raw HTL-AP		1.60						1.60						1.60				
Raw HTL-AP			1.20						1.20						1.20			
Raw HTL-AP				0.80						0.80						0.80		
Raw HTL-AP					0.40						0.40						0.40	
Raw HTL-AP						0.20						0.20						0.20

Figure 1: Volume of each source water used to create each combination. A total of 20 mL was made of each trial. The colored cells have the mL of component effluents in the column below each Trial number.

Trials 1 and 2 served as the “CHSAS control” and “Bevier control” for data analysis purposes as these trials contained 100% of their respective aquaponic effluent. These combinations were chosen to align with similar combinations as well as unexplored combinations within past literature. Specifically, the HTL-AP dilution concentrations were chosen to go higher and lower than the concentration used by Jesse et al. (2019) of 2.5% to investigate the range of its known inhibitory effects. Furthermore, aquaponics effluent supplemented with outside fertilizer is common in industry (Bartelme et al, 2018). Finally, perhaps the synthetic nutrients or the PGPMs in the aquaponics effluents could serve to supplement the HTL-AP with the needed form of N or provide the microorganisms necessary to make them bioavailable to plants respectively.

The trials were observed and measured for a period of 10 days, March 23 to April 1, 2022, where each trial contained a triplicate of buttercrunch lettuce seeds inside of a Ziploc sandwich bag with a 2" diameter circle of 2-ply paper towel for each seed. Each paper towel circle was saturated with 1 mL of the corresponding wastewater before placing the seeds inside and sealing the bags. All of the labeled bags were then placed on two levels of a metal shelving rack, with an overhead cover, inside the Hydraulics Lab of AESB, where they were maintained at 70 °F for 10 days. The day the trials were prepared and placed in the lab is considered Day 0. Since this study was conducted in a shared lab space, a sign was placed by the lights for them to be turned off when the lab was not actively in use. To account for the remaining amount of intermittent fluorescent light coming in, the plastic bags were randomly returned to different positions after measurements were taken each day. Each day starting on Day 1, each trial was checked daily for the number of seeds germinated, the average root length of each trial via triplicate, and the time of cotyledon emergence for 10 days. Pictures were also taken of the trials each day to note observances of any rotting, mold, or un-germinated seeds. The radicals and stem growth often occurred in non-linear shapes to minimize the risk of contamination and physical damage to the seedlings. The growth was measured on the outside of the Ziploc bag from the tip of the radicle to the bottom of the cotyledons with a ruler. Table 1 below provides details of the various source waters, including how they were created or collected.

Table 1. Description of Controls and Source Waters.

Source Water/Process	**Description**
DI Water	Standard deionized water (negative control)
Industry Standard Hydroponic Fertilizer (SHF)	General Hydroponics Flora Series hydroponic fertilizer for "aggressive vegetative growth" solution, consisting of Flora Grow (396; 2-1-6), Flora Micro (264; 5-0-1), and Flora Bloom (0-5-4; 132) measured in (mL/100 L; N-P-K). This solution was created in-house in the water quality lab of the AESB. (positive control)
Aquaponic Effluent from the Chicago High School for Agricultural Sciences (CHSAS)	Collected from the system at the CHSAS; the system was a series of deepwater culture beds growing leafy greens and tomatoes, the system also contained 4 large swim tanks that housed tilapia. This aquaponic water was collected by submerging a 5-gallon bucket horizontally into the fish swim tanks until it was full. This sample was obtained on 09/2020 and stored refrigerated for approximately 6 months.
Aquaponic Effluent from the UIUC's Bevier Cafe Aquaponic System	Sourced from the system run by Bevier Cafe in the UIUC greenhouses to supplement their food supply. This system consists of an ebb and flow system made up of three leca-filled drain beds growing tomatoes, herbs, and leafy greens as well as a swim tank that housed koi at the time of sample collection. The sample was collected in the same method as the CHSAS sample, horizontal submerging until full. This sample was obtained on 02/2021 and stored in refrigeration for approximately 1 month.
Hydrothermal Liquefaction Aqueous Phase (HTL-AP)	This nutrient-rich effluent or wastewater was collected from the UIUC pilot HTL plant with Kraft salad dressing as the feedstock. The exact specifications of the HTL batch are as follows: HTL-AP Sample: September 21, 2020, Kraft Salad Dressing Bucket 2 of 3 Feedstock Volume Ran through the System: 20 gallons Temperature Range: 240 - 280°C Pressure Range: 1600 - 1800 psi Feedstock Flow-rate Range: 0.14 - 0.18 GPM

These source waters and their combinations were chosen to serve as a wide-ranged screening experiment to identify the trials that outperformed the positive control and those that underperformed the negative control. These combinations will then be analyzed to identify the ideal water characteristics for future wastewater growth experiments and identify any potential inhibitory water characteristics hindering future experiments. Each source water was characterized to provide a baseline understanding. The characterization included measurements for total nitrogen (TN), ammonia-nitrogen (NH_3-N), nitrate-nitrogen (NO_3-N or NO_3^-), and chemical oxygen demand (COD) following HACH methods 10072, 8038, 8039, and 8000, respectively. For each measurement, at least triplicates were analyzed. Nitrate-nitrogen and ammonia-nitrogen readings were performed using a HACH DR/2010 spectrophotometer (Loveland, Colorado, U.S.A.), while a HACH DR/3900 (Loveland, Colorado, U.S.A.) was used for the remaining nutrient measurements. The pH was measured with a standard 3-point calibration using the Accumet AE150 machine by Fisher Scientific (Hampton, New Hampshire, United States). The electrical conductivity (EC) was measured using the PCTSTestr 50 meter by Oakton (Vernon Hills, Illinois, United States). The results are presented as the average of the readings with their respective standard deviations. It should be noted there is likely a large interference with the Total Nitrogen measurement for HTL-AP as it is less than the Nitrate and Ammonia measurements combined.

The parameters in Table 2 were measured to evaluate the effects of the various wastewater combinations on the initial germination and growth of buttercrunch lettuce (*Lactuca sativa var. capitata*) seeds. The final germination proportion was calculated per trial. The total root and shoot biomasses were measured and the growth rates were also calculated. The day of cotyledon emergence was additionally recorded as the germination rate.

Statistical analyses consisted of two-tailed student t-tests with unequal variance to determine significant differences in performance in the Germination Rate and the Total Biomass Produced. Average performance in each category of analysis was compared relative to the control waters.

Table 2. Characterization of each source water.

	Water Quality Parameters					
Source Water	pH	Electrical Conductivity (ms/cm)	Nitrate (NO3-N) (mg/L)	Ammonia (NH3-N) (mg/L)	Total Nitrogen (TN) (mg/L)	COD (Chemical Oxygen Demand) (mg/L)
DI	6.40 ± 0.74	0.021 ± 0.009	0 ± 0	0 ± 0	0 + 0	0 + 0
SHF	5.01 ± 0.06	2.136 ± 0.047	56.40 ± 1.65	36.58 ± 1.12	208.27 ± 1.57	38.00 ± 3.31
CHSAS Aquaponic Effluent	7.07 ± 0.14	0.739 ± 0.014	3.31 ± 0.05	0.51 ± 0.04	19.80 ± 0.33	58.93 ± 2.94
Bevier Aquaponic Effluent	7.87 ± 0.06	0.572 ± 0.010	0.57 ± 0.06	0.12 ± 0.01	1.47 ± 0.30	15.60 ± 3.05
HTL-AP	3.99 ± 0.06	5.018 ± 0.047	9.21 ± 0.45	64.18 ± 3.68	38.29 ± 0.43	8,532.00 ± 84.17

RESULTS AND DISCUSSION

All treatments were compared relative to the 4 unmodified source waters the negative control of deionized (DI) water, the positive control of SHF, the CHSAS aquaponic control of undiluted source water, and the Bevier aquaponic control also of undiluted source water. The trials were compared using four primary parameters; final germination proportion, germination rate, total biomass produced, and growth rates. For data analysis purposes, the trials were split into three distinct groupings in every category: aquaponics which consisted of the four unmodified source waters, and Trials 9-14, HTL-AP which consisted of the positive (SHF) and negative (DI) controls along with Trials 3-8 and 15-20, and combinations which consisted of the two aquaponic source waters (CHSAS and Bevier) as well as Trials 21-32.

Final Germination Proportion

The goal is to maximize percent germination because any non-germinated seeds are lost products and must be replaced. Due to differences in seed genetics and viability, it is expected that 100% germination is unlikely, however, all of the unmodified source waters, DI, SHF, 100% CHSAS, and 100% Bevier achieved 100% germination. Therefore, there were no significant inhibitory effects on germination for the source waters. Any decrease in germination percentage relative to these controls indicates there is likely some inhibitory compound or compounding inhibitory effects in trials that did not achieve the desired germination percentage. In the first data grouping, neither of the 25% aquaponic waters mixed with SHF matched the control germination percentage. This is possibly due to a lack of nutrients as not enough aquaponic nutrients were available for plant uptake, however, as these trials were combined with the positive control, SHF, which has a higher nutrient content as seen in Table 2, there should have been sufficient nutrients necessary. On the other hand, both the 75% and the 50% aquaponic waters mixed with SHF matched the controls at 100% germination. Therefore, for germination percentage purposes, SHF use in hydroponic systems could be cut by up to 75% and supplemented with various aquaponic waters with no decrease in germination percentage. When it comes to the second and third groupings of data, all 4 trials where the unmodified source waters were mixed to create a 10% HTL-AP dilution match the controls with a 100% germination percentage. This indicated any inhibitory effect HTL-AP has on plant growth does not inhibit germination when it is used to supplement various hydroponic nutrient sources by up to 10%. On the other side of the spectrum, only 2 of the 4 HTL-AP trials diluted to make a 1% concentration matched the controls at 100% germination percentage, the SHF and CHSAS mixtures, while the 1% DI and Bevier trials did not achieve 100% germination percentage. This may be due to a nitrogen deficiency, specifically nitrate, and ammonia, as there are fewer of these nutrients in the DI and Bevier source waters compared to the SHF and CHSAS source waters. If the CHSAS trials that were mixed with HTL-AP to create 8%, 6%, 4%, and 2% HTL-AP solutions are discarded, then all trials where source waters were mixed with HTL-AP to create 8%, 6%, 4%, and 2% solutions would have matched the controls at 100% germination percentage. These trials with CHSAS as the source water could indicate CHSAS has an inhibitory effect on final germination proportion, however, the earlier analysis of the first grouping of data illustrates CHSAS alone, or supplemented with SHF does not affect final germination proportion. Therefore, either these trials had a higher-than-normal percentage of non-viable seeds, or there is a compounding inhibitory effect of CHSAS mixed with HTL-AP; the latter reasoning is also less likely than the former as the 10% and 1% HTL-AP with CHSAS trials matched the controls at 100% germination percentage. Therefore, it is recommended HTL-AP can be used to supplement DI water, SHF, and various aquaponic waters at least up to 10% with no negative effects on final germination proportion. However, 1% HTL-AP solutions could be nutrient deficient depending on the nutrient content of the source water.

Germination Rate

To minimize the production time of hydroponic crops, it is important to have a quick turnaround time from the initial imbibing of water into the seed to the emergence of the cotyledon(s), indicating successful germination. In this study, the negative control (DI) took 3.33 days on average to achieve germination while the positive control (SHF) took 3.67 days. The CHSAS control took 3.67 days on average to achieve germination while the Bevier control only took 3 days. While these results indicate a higher nitrogen concentration delays the time it takes to achieve full germination, the higher nitrogen concentration when supplemented with SHF decreased the time it took for full germination for the CHSAS trials. Zhang et al. (2020) investigated the effect of various NH4 and NO3 concentrations on the final germination proportion and germination rate of eight semi-arid grassland species. They determined up to 20 mM N increased the final germination proportion, but 40 mM N had no effect. Additionally, the mean germination rate decreased with 20 and 40 mM N illustrating higher concentrations of nitrogen can have inhibitory effects on the success of germination and how quickly it is achieved. The 75% CHSAS matched the positive control time to germination while the 50% and 25% CHSAS trials matched, or were even faster than, the negative control time until germination. The 25% Bevier mixture germinated faster than the positive control (SHF) but still took longer to germinate than the negative control (DI). The 75% and 50% Bevier mixtures took longer to germinate than the positive control as well as the Bevier control. This could be due to the lower nutrient concentrations in the Bevier source water, although this claim is contrary to the control trials' time to germination, which indicates a lower nutrient concentration decreases the time to germination. In the second and third groupings of data regarding the HTL-AP with DI trials, the 8% through 2% solutions matched or did better than the positive control, or it took 3.67 days or less to germinate fully. Compared to the HTL-AP with SHF trials, only the 1% dilution did better than the positive control. These results align with the previous findings indicating a higher nitrogen concentration increases the time to germination and the SHF with HTL-AP trials were too high in various forms of nitrogen which delayed the time to germination. Regarding the Combination Trials, the 6, 2, and 1% HTL-AP solutions with CHSAS did better than the positive and negative controls while the 8 and 4% HTL-AP solutions with Bevier only matched the positive control at 3.67 days until full germination. Statistically, there is a significant difference between the averages across all combination groups of the 10% and 1% HTL-AP solutions. A student two-tailed t-test with unequal variance reveals on average, the 1% HTL-AP solutions were significantly faster to germinate than the 10% HTL-AP solutions. This same t-test was used to compare the rest of the HTL-AP dilution averages with all of the various source waters, but no significance was able to be found. There is a strong ($R^2 = 0.8126$) trend between increasing HTL-AP concentration and increasing time to full germination, as pictured below in Figure 2. The error bars in this figure indicate further trials and larger data sets are needed to confirm and refine the model, however, the days to full germination can be estimated as -0.2238*[HTL-AP] + 4.38944 for buttercrunch lettuce seeds utilizing some concentration of HTL-AP in the source water.

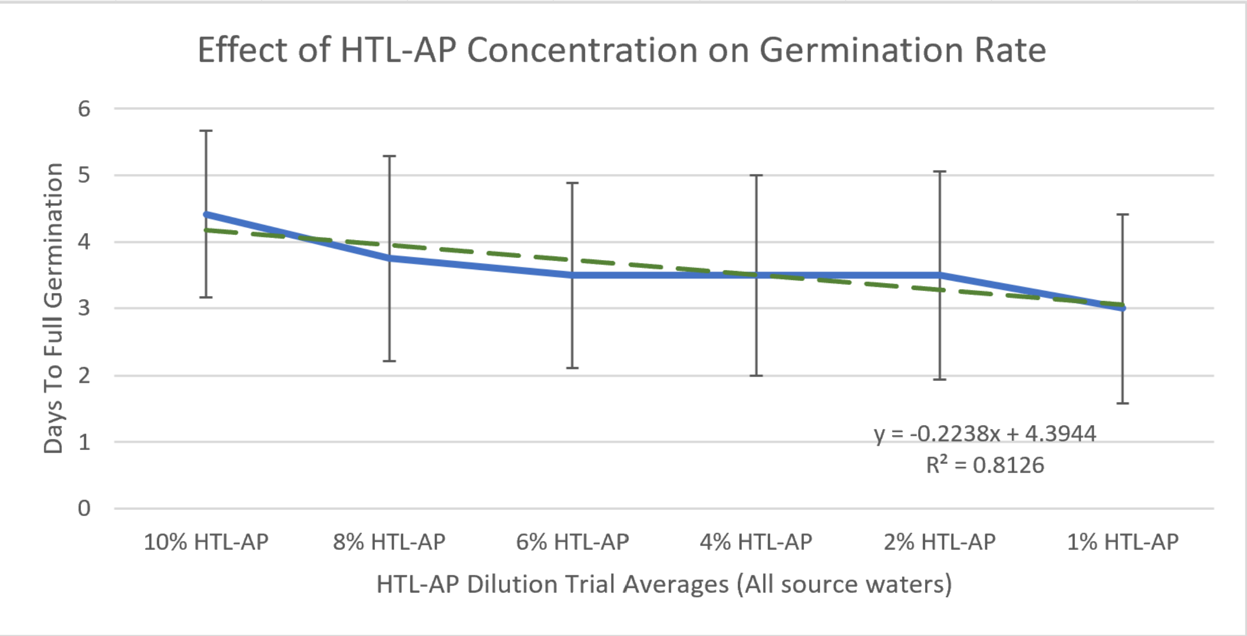


Figure 2. Average Germination Rate for all source water combinations with various HTL-AP Concentrations

Total Biomass Growth

Once a seed has germinated, to get as much marketable product as possible for many crops, including leafy greens, the total biomass produced needs to be maximized as well. In the first grouping of data with the controls, the positive control averaged 47.67 mm of growth over the 10-day growth period while the negative control averaged 37.67 mm of growth. Both the unmodified CHSAS and Bevier source waters (100% aquaponic effluent) surpassed the positive control by producing over 47.67 mm of total biomass growth on average, perhaps due to the presence of PGMPs in the aquaponic source waters. All of the CHSAS trials diluted with SHF also surpassed the positive control, however, all of the Bevier trials diluted with SHF underperformed the negative control meaning they averaged less than 37.67 mm of total biomass growth. Regarding the second and third groupings of data, the 8%, 6%, 4%, and 2% HTL-AP with DI trials surpassed or matched the positive control in total growth while the 1% underperformed compared to the negative control and the 10% mixture was in between the positive and negative control. On the other hand, only the 6% HTL-AP with SHF trial surpassed the positive control while the higher HTL-AP concentrations, 10% and 8% underperformed compared to the positive control, and trials 4%, 2%, and 1% performed between the positive and negative controls. These results indicate all 10% HTL-AP solutions performed worse than lower solutions with the same controls, indicating some degree of inhibitory effects were felt when it comes to total biomass production. When combined with SHF, the 8% HTL-AP mixture may have been too high in nitrogen content whereas when diluted with DI water the 8% HTL-AP surpassed the positive control, indicating it is a viable trial for total biomass production. On the other hand, when not supplemented with outside nutrients, the 1% HTL-AP with DI mixture likely didn’t have enough nutrients to maximize total biomass whereas when supplemented with SHF, it performed adequately. The 6%, 4%, and 2% solutions with both DI and SHF are viable combinations for performing similarly to the controls regarding the total production of biomass. Regarding the HTL-AP and aquaponics combinations, only the 6%, 2%, and 1% HTL-AP with CHSAS trials outperformed the positive control, while all other HTL-AP with CHSAS trials, as well as all HTL-AP with Bevier trials, underperformed relative to the negative control. Interestingly, when comparing the total biomass growth for the Bevier control to the Bevier mixed with SHF trials, the unmodified Bevier water did significantly better in total growth verified by a student 2-tailed t-test with unequal variance. Along these lines, there is also a significant difference between the CHSAS trials supplemented with SHF, and the Bevier trials supplemented with SHF; the CHSAS trials were statistically better verified by the same t-test. Additionally, the HTL-AP trials diluted with DI water also did statistically better than the HTL-AP trials diluted with Bevier aquaponic water. Overall, this indicates the Bevier source water was not ideal for total biomass generation outside of its control, likely due to its low nutrient content. However, the CHSAS aquaponic water performed quite well on its own, when mixed with SHF, and with lower concentrations of HTL-AP indicating aquaponic waters may be a viable nutrient source depending on their composition.

A key takeaway from the total biomass analysis is when diluted with only DI water, HTL-AP can serve as a viable nutrient source for maximizing the total biomass of hydroponic lettuce production when diluted anywhere from 8% to 2% HTL-AP, which is much higher than the previous literature suggests.

Figure 3. Summary of Average Total Biomass Production for the Three Data Groupings

Growth Rates

To produce a viable crop product quickly, the initial growth rates of seedlings are important to ensure initial transplanting is a success. The positive control had an average growth rate of 5.3 mm/day while the negative control had an average growth rate of 4.19 mm/day. Both aquaponic controls had a higher average growth rate than the positive control with CHSAS coming in at 5.7 mm/day and Bevier coming in at 5.41 mm/day. Getting into the first grouping of data, the 75% and 50% CHSAS mixed with SHF trials also outperformed the positive control in terms of growth rates. However, the 25% CHSAS with SHF trial, as well as all of the Bevier mixtures with SHF, underperformed relative to the negative control in terms of growth rates. These results may be due to the presence of PGPMs in the aquaponic waters due to their emulation of natural aquatic environments. There are likely more PGPMs in the CHSAS aquaponic source water than in the Bevier aquaponic source water due to the larger nature of the CHSAS aquaponic system. This is why the CHSAS trials outperformed the positive control until they became too dilute, the 25% CHSAS and the Bevier water lacked enough PGPMs to make up for its lower nutrient content. Getting into the second grouping of trials, the 8-2% HTL-AP with DI mixtures surpassed the average growth rate of the positive control while only the 6 and 2% HTL-AP with SHF mixtures surpassed the average growth rate of the positive control. On the edges of the HTL-AP ranges we again see the 1% HTL-AP with DI lacked enough nutrients to provide a sufficient growth rate while the 10 and 8% HTL-AP with SHF trials exhibited toxicity or other inhibitory effects again were unable to produce sufficient growth rate. The 10% HTL-AP with DI along with the 4% and 1% HTL-AP with SHF mixtures had an average growth rate in between the positive and negative control growth rates. Below in Figure 4, a roughly bell-shaped curve that skews towards the center-right for both the HTL-AP mixed with DI trials as well as the HTL-AP mixed with SHF trials although the bell shape flattens out on the right side for the latter. Regarding the last grouping of data, there was not much of a discernable trend in the data as only the 1% HTL-AP mixed with CHSAS outperformed the average growth rate of both of the aquaponic controls. The rest of the HTL-AP trials mixed with CHSAS and Bevier had a lower average growth rate than either of the aquaponic controls. Although it should be noted the 6 and 2% HTL-AP mixed with CHSAS trials as well as the 8% HTL-AP with Bevier trials had average growth rates in between those of the positive and negative controls, not the two aquaponic controls. Overall, the results from the growth rate category highlight the importance of PGMPs and indicate the middle to lower solutions of HTL-AP, from 6-2% have desirable growth rates.

Figure 4. Box & whisker plots of the average growth rates for the aquaponic and HTL-AP trials.

Summary of Results

Table 3 summarizes the results for all four categories of analysis and the three source waters.

Table 3. Summary of Results.

Source Water	Final Germination Proportion	Germination Rate	Total Biomass Growth	Growth Rate
Aquaponics	No significant impact or trends	More nutrients in source water tended to delay germination	PGPMs can make up for lower nutrient content to a certain extent	Higher nutrient contents increase the average rate of growth
HTL-AP	No inhibitory effects on germination even with 10% HTL-AP solutions	Linear model negatively correlated with HTL-AP concentration	10% HTL-AP exhibits significant inhibitory effects on biomass production	8% to 2% HTL-AP solutions with DI & SHF provide viable growth rates; aquaponics inconclusive

It should be noted in future studies, it is recommended that a larger sample size, perhaps triplicates of seed triplicates so 9 seeds per trial, should be utilized for each combination of effluents, as the maximum of 3 data points in this study, assuming successful germination, can only indicate trends to a certain extent of statistical significance. Further, with this small sample size, it is difficult to determine if inhibitory effects are due to seed inviability or source water toxicity. Table 3 highlights the key findings in each of the categories of analysis for source waters relative to the four controls.

CONCLUSIONS

Generally, the water from the CHSAS aquaponics system performed better than the water from the Bevier aquaponics system. This is likely due to the differences between the two systems, namely the larger scale of the CHSAS operation, increased nutrient content as well as the quantity and diversity of PGPMs. However, this second claim needs to be investigated further and verified by testing aquaponic waters from a variety of systems on similar scales and by utilizing some method to quantify PGPMs. Although more work is needed to determine the benefits of PGPMs and their synergistic effects with various nutrient contents and compositions, aquaponic effluents show promise as a viable supplement or even a complete substitute for hydroponic industry-standard liquid fertilizers. HTL-AP performed best as a nutrient source when it was diluted 8% to 2% HTL-AP with all four control waters. A model correlating the increase in HTL-AP concentration to an increase in the time it took to achieve full germination or a decreased germination rate was created. Due to the small sample size and the larger error bars, further research needs to be conducted to better define the relationship between HTL-AP concentration and the rate of germination. Although 10% HTL-AP solutions exhibited some sort of inhibitory or toxic effect regarding total biomass and average growth rate performance, the 8%, 6%, 4%, and 2% solutions of HTL-AP generally performed on par with industry standards when it comes to the total biomass produced and the average growth rate. The HTL-AP with DI combinations have some potential as in this hydroponic production scenario, HTL-AP would be the sole source of nutrients, completely replacing industry standard fertilizers or other alternative nutrient sources. A better understanding of the exact compounds within HTL-AP and the mechanisms of their inhibitory or toxic effects is needed to better understand what actions need to be taken to make HTL-AP a viable nutrient source for hydroponic crop production, as dilution is only one method. Additionally, this study only investigated the impacts of alternative nutrient sources, wastewaters, on the initial 10-day germination and initial growth period of lettuce. Therefore, further studies on the complete growth cycle of lettuce as well as wastewater’s effects on other crops are needed to establish the range of applications these alternative nutrient sources have in hydroponic production systems for either industry or research purposes. Alternative nutrient sources are needed to increase the circularity of global food production systems as well as decrease reliance on chemical fertilizers derived from fossil fuels or mined from the earth.

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