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Urine as a Nitrogen Source for *Lepidium sativum*: Creation of A Novel Synthetic Urine Testing Model and Product

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ABSTRACT: The Haber-Bosch Process produces a majority of the world's ammonia-based fertilizers. Due to the inherent explosive nature of ammonium nitrate, the requisite fossil fuels, and the massive carbon dioxide (CO_2) emissions, it is time to evaluate other sources of nitrogen to feed the world's 8 billion people. In many studies, the use of human urine as a fertilizer has been described, but this concept is challenging on many levels. This paper describes a novel method to create, test, and store solid synthetic urine as a fertilizer for *Lepidium sativum* (garden cress). Garden cress fertilized with synthetic urine had a significantly higher yield in mass than the control group. Synthetic urine in its solid, powdered form decreases storage and transportation issues compared with large volumes of liquid urine. Additionally, this study demonstrates that solid urine is shelf-stable due to its bacteriostatic properties and low hygroscopicity. Fertilizing with human urine can provide a renewable resource in agricultural systems, reducing the need for the Haber Bosch Process and its consequences.

KEYWORDS: Plant Sciences, Agriculture and Agronomy, Haber Bosch Process, Sustainable Fertilizer, Synthetic Urine.

Introduction

Using nitrogen-based fertilizers for crops is a tremendous asset, as we must now feed 8 billion people. Most of this nitrogen is captured from the atmosphere and converted into ammonia and solid ammonium nitrate. This energy-intensive chemical reaction, the Haber-Bosch process, demands tremendous amounts of non-renewable energy and generates 1.4% of all global CO_2 emissions.[1] Additionally, ammonium nitrate is highly explosive, and it has been used in acts of terror and has resulted in tragic deaths from inadvertent explosions.[2,3]

Using human waste as a fertilizer is a centuries-old technique; however, the modern use of biosolids and sewage sludge is not without controversy. There are growing concerns about the safety of this modality. These include the risk of viral and bacterial pathogens, heavy metals, and toxic organic compounds such as PCBs, dioxins, and even hospital waste.[4] While permitted in the United States and Europe, despite stringent regulations, the processing cost runs in the billions of dollars, and there are still no guarantees about long-term safety.

On the other hand, the process described in this paper results in solid urine that is more sanitary than sewage sludge (bacteriostatic) and comes without the risk of toxic organic and inorganic compounds. Because of the high urea content, urine is an exemplary nitrogen source. The infrastructure to isolate urine from the waste stream has been developed and studied, and when processed, the potential of diluted urine has been analyzed in some Scandinavian countries.[5] In liquid form, however, urine remains susceptible to microbial growth and presents transportation challenges due to the large volumes of liquid that must be delivered to farms. This paper presents a unique process to create a shelf-stable, solid urine fertilizer that can be stored and prepared at the site and time of application. On an environmental level, a long-term goal would be to scale this process up to capture urine city-wide, creating fertilizer and diverting the nitrogen stream. Mechanical vapor recompression technology exists and could perform large-scale urine dehydration cost-effectively and minimize energy use.[6]

Not only is the creation of solid urine unique, but using updated medical-grade synthetic urine for agricultural testing has not been previously described. The process and testing are outlined using *Lepidium sativum* (garden cress). This research complements other studies that have demonstrated increased plant growth when fertilized with human urine.[7]

Methods

Creating Solid Synthetic Urine:

In a well-ventilated fume hood with constant airflow, two liters of distilled water were added to a 2.5-liter non-reactive stainless-steel pan containing individual compounds of medical grade synthetic urine as described in *A New Artificial Urine Protocol to Better Imitate Human Urine* (see Table 1).[8] The fume hood was used to provide a constant airflow to enhance the evaporation of the final product and mitigate the risk posed by any volatile compounds created during the process. The pan was placed on a hot plate with a stir bar set at 95 RPM, maintaining the synthetic urine temperature at 40°C. Throughout the process, safety goggles were worn to protect the eyes. The hot plate temperature was adjusted to 70°C to keep the solution at 40°C. One component, uric acid, is unstable in aqueous solution at 40°C. However, this did not affect the overall plant growth. The main nitrogen source, urea, does not decompose at temperatures less than 60°C. A thermometer monitored the temperature until the urine became too viscous to stir (24 hours). Improved control of the temperature could be implemented for future studies, such as the use of a water bath. The stir bar was removed, and the hot plate temperature was set

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to 50°C for complete liquid evaporation in the fume hood. The evaporation process took an additional 48 hours. Once the urine solidified, it was removed from the pan and ground into a fine powder using a mortar and pestle (see Figure 1 for the final product). CAUTION: Safety goggles and gloves were worn as precautions.

Table 1: Components of Synthetic Urine as described in *A New Artificial Urine Protocol to Better Imitate Human Urine*.[8] The recipe lists essential electrolytes, organic waste products such as urea and creatinine, and various salts that contribute to the physiological and chemical properties of urine.

Compounds	Quantity (g) for 100 ml/ of water CT-AU
Urea CH_4N_2O	1.2012
Uric Acid $C_5H_4N_4O_3$	0.0168
Creatinine $C_4H_7N_3O$	0.0452
Trisodium citrate dihydrate $Na_3C_6H_5O_7.2H_20$	0.1471
Salt NaCl	0.3156
Potassium Chloride KCl	0.2237
Ammonium Chloride NH_4Cl	0.0802
Calcium Chloride $CaCl_2$	0.0333
Magnesium Sulfate Heptahydrate	0.0493
$MgSO_4.7H_2O$	
Sodium Bicarbonate $NaHCO_3$	0.0168
Potassium Oxalate $K_2C_2O_4$	0.0018
Sodium Sulfate Na_2SO_4	0.1278
Sodium Phosphate Monobasic Dihydrate $NaH_2PO_4.2H_2O$	0.0562
di-Sodium hydrogen phosphate dihydrate $Na_2HPO_4.2H_2O$	0.0071

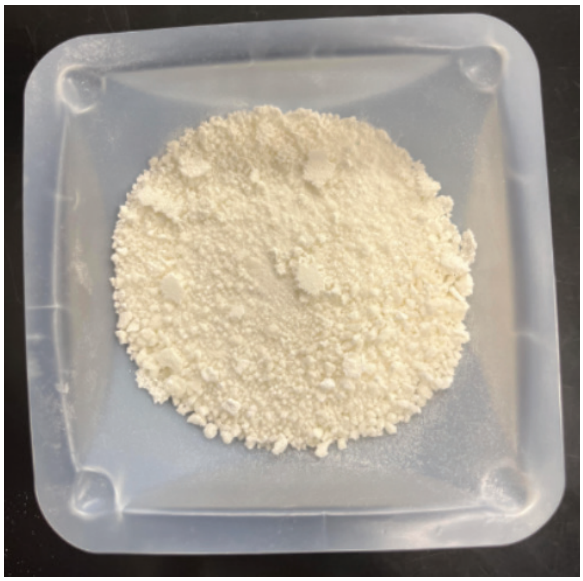

Figure 1: Synthetic urine after dehydration. The dehydration process spanned over 72 hours, resulting in a powdery substance after being ground with a mortar and pestle.

pH of Synthetic Urine:

The pH of the urine solution prior to dehydration was measured and found to be 6.10, within the ideal range for garden cress. pH testing was measured with a calibrated Vernier pH probe. Because varying plants thrive under different pH conditions, additional feasibility titration testing was successfully undertaken using a 100 mL burette with 0.2 M NaOH and HCl. The process was straightforward, and the pH could be adjusted in 0.1 increments up or down. CAUTION: Safety goggles and gloves were worn as precautions.

Serial Dilutions:

Serial dilutions were performed using a 250 mL volumetric flask, distilled water, and a graduated pipette, creating concentrations of 0.025g/mL (equivalent to normal human urine concentration), 0.0025g/mL, and 0.00025g/mL. CAUTION: Safety goggles and gloves were worn as precautions.

Determining Optimal Concentration of Urine:

The individual dilutions listed above were evaluated to determine the optimal urine concentration as a fertilizer. 75g of nitrogen-poor soil was placed into 8 2x2 planters. Then, 25g of nitrogen-poor soil was mixed with one-half teaspoon (2.5 mL) of cress seeds. This mixture was put into all the planters on top of the 75g of nitrogen-poor soil. Then, each pair was fertilized with different fertilizer dilutions. Two planters were watered with 25 mL of water (control), 25 mL of 0.025 g/mL, 25 mL of 0.0025 g/mL, and 0.00025 g/mL. All planters were given equal sunlight and were kept in a stable environment (50% relative humidity and a temperature of 75°F). The planters were watered daily with 30 mL of water. On day 7 of the experiment, the cress was harvested. 0.025g/mL failed to germinate due to the high salt levels. 0.0025g/mL had the highest yield in mass (See Table 2 for results).

Table 2: Masses of cress for determining optimal synthetic urine concentration. The lack of growth in the 0.025 g/mL concentration demonstrates that it would not be feasible to apply pure human urine. Dilution is required.

Control	0.025 g/mL	0.0025g /mL	0.00025g /mL
1.75g	No Growth	3.14g	2.57g

Further Testing with 0.0025g/mL:

Additional tests were conducted to further test the potential of the 0.0025g/mL dilution. 75 grams of nitrogen-poor soil were placed into a 2x2-inch planter. Then, a mixture of half a teaspoon (2.5 mL) of cress seeds with 25 grams of the same soil type was spread over the base layer in each planter. This procedure was replicated across 24 planters. Half of these planters were fertilized with 25 mL of a 0.0025 g/mL synthetic urine solution, the optimal urine concentration. The remaining planters were watered with 25 mL of water to serve as control groups. All planters were placed in a greenhouse under the same conditions as the previous trial (75°F, 50% relative humidity, and equal sunlight). The planters were watered daily with 30 mL of water. On the seventh day of the 14-day growth period, the experimental group of planters was refertilized with another 25 mL of the synthetic urine solution.

The control group planters were again watered with 25 mL of water. Figure 2 shows a notable difference in height between fertilized cress (left) and unfertilized cress (right).

Figure 2: Cress fertilized with synthetic urine (left) and the control group (right). The picture was taken on day 14 of the experiment. Cress fertilized with a 0.0025g/mL concentration demonstrates a fuller, healthier yield compared to the control.

Harvesting Cress:

Harvesting occurred on day 14 using the method described in *Yield and quality of garden cress affected by different nitrogen sources and growing periods*.[9] The height of each planter was measured in cm, cress stalks were cut close to the soil surface, and leftover soil attached to the stalks was removed carefully. The mass of the cress from each planter was measured individually. The yields of each planter were aggregated and exhibited a stark difference in biomass (see Figure 3).

Figure 3: Yield of fertilized cress (left) and unfertilized (right). The large difference in biomass highlights the enhanced growth and higher output of fertilized cress compared to the unfertilized counterpart.

Testing for Bacterial Growth:

Agar plates were created and streaked with *E. coli* K-12 culture to test bacterial growth. Synthetic urine was placed on the plates with a 15µL scoop, spaced evenly with five samples per plate. The plates were placed upside down in an incubator for 24 hours, examined for inhibition zones using a dissecting microscope, and then placed in the incubator for 24 hours. After re-examination, the plates were discarded in an autoclave after sterilization. CAUTION: Safety goggles and gloves were used while handling the Petri dishes to prevent additional bacterial contamination.

Results

Statistical Analyses:

A two-means t-test was conducted, and the results returned as statistically significant. The p-value is less than 0.0001, allowing us to reject the null hypothesis (there is no difference in growth between plants fertilized with the synthetic urine and unfertilized plants) and instead supports the alternative hypothesis (plants fertilized with the synthetic fertilizer have greater growth than unfertilized plants). The city of Harrisonburg, Virginia, has a population of 53,000 people. Assuming that the average person produces 1.4 L of urine daily, enough urine can be collected to fertilize 2,589 acres of cress using the tested regimen after one year.

After conducting a two-means confidence interval test, there is a 95% confidence that the interval 1.96g - 3.99g captures the true average difference between the means of fertilized cress and unfertilized cress. This suggests that, on average, cress fertilized with synthetic urine will be at least 1.96g greater than unfertilized cress.

Two Means Confidence Interval Test:

$$(5.34 - 2.36) \pm 1.8\sqrt{\frac{(1.86)^2}{12} + \frac{(0.6)^2}{12}} = (1.96, 3.99)$$

(difference in means) ± margin of error

As seen in Figure 4 the average mass per planter of fertilized cress is significantly greater than the average mass of unfertilized cress, exemplifying urine's potential as a fertilizer.

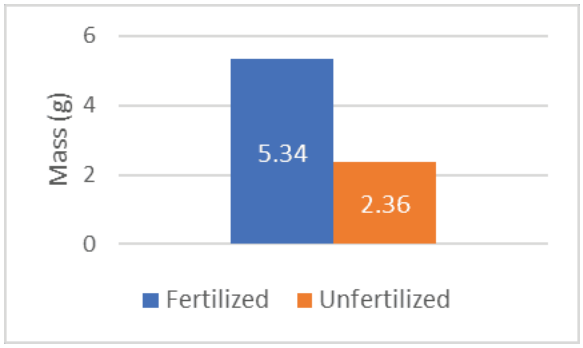

Figure 4: Comparing the average masses per container between fertilized and unfertilized cress. The fertilized cress had an approximately 226% greater yield than unfertilized cress.

Garden Cress:

Lepidium sativum was chosen because of its history in botanical sciences. It is a fast-growing herb that is easily obtainable. Also, as a plant growth model, it is known to be growth-responsive to varying nitrogen levels,[10] as seen in the results. It is a common garnish in soups and sandwiches and adds peppery seasoning. Cress grows best at a pH of 6.0-6.7.[11] After measuring with Vernier pH probes, the synthetic urine met these conditions.

Soil:

Purposefully, the soil used in this experiment was depleted in nitrogen content. It had a history of planting cycles without the addition of nitrogen. Also, it was exposed to the elements, such as rain, for over a decade. Soils are not known to sequester nitrogen, which is a primary reason farmers must resort to the reapplication of fertilizer.[12] Since nitrogen is known for depleting quickly, the soil was tested for nitrogen semi-quantitatively. The nitrogen levels were between N1/Deficient and N2/Adequate. The nitrogen levels rose after applying synthetic urine fertilizer (see Figure 5).

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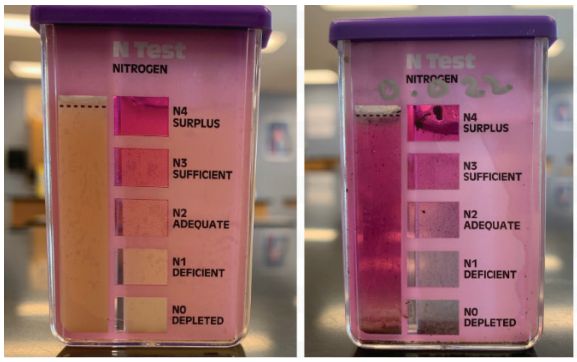

Figure 5: Soil nitrogen levels before and after fertilizer application (deficient/adequate nitrogen levels (left), sufficient/surplus nitrogen levels (right)). The pink color is stronger in intensity in the fertilized soil sample (right), indicating higher levels of nitrogen.

The soil was sent to Virginia Tech, where it was tested at the soil testing lab; the other soil nutrient levels were relatively sufficient. Additionally, the pH was 7.1, near the optimal range of cress growth. See Table 3 for specifics.

Table 3: Properties of the soil used. lb./A = pounds per acre, H = High, VH = Very High, SUFF = sufficient, L = Low, meq = milliequivalent.

Analysis	Result	Rating
P (lb./A)	68	H
K (lb./A)	198	H-
Ca (lb./A)	3680	VH
Mg (lb./A)	243	VH
Zn (ppm)	3.6	SUFF
Mn (ppm)	34.7	SUFF
Cu (ppm)	1.1	SUFF
Fe (ppm)	9.2	SUFF
B (ppm)	0.7	SUFF
S.Salts (ppm)	128	L
Soil pH	7.1	-
Buffer Index	6.60	-
Est.-CEC (meq/100 g)	10.4	-
Acidity (%)	0.0	-
Base Sat. (%)	100.0	-
Ca Sat.	88.0	-
Mg Sat. (%)	9.6	-
K Sat. (%)	2.4	-
Organic Matter (%)	4.1	-

Why use Solid Urine?:

Currently, scientists are studying larger-scale methods that use urine in liquid form, which adds complexity to storage and transportation. Such methods are being analyzed in Sweden and Burkina Faso.[13] However, dehydrating laboratory-grade, synthetic urine leads to a stable shelf substance that is easily reconstituted for use. The dehydrated urine was tested for hygroscopicity and did not gain mass/absorb water over eight weeks (Placed in a 30% humidity environment at room temperature). As a solid, it is easier to transport and store, and does not require to be maintained in a low-humidity environment. This was an interesting finding and somewhat counterintuitive based on the salt content of urine.[14]

Additionally, solid urine's susceptibility to bacterial growth was tested. When diluted in liquid form, bacteria grew unrestrained. However, when solid, the synthetic urine demonstrated a zone of inhibition against *E. coli* K-12 (see Figure 6). This is most likely due to the hypertonic state of the fertilizer, which correlates with the common use of salts as a preservative.

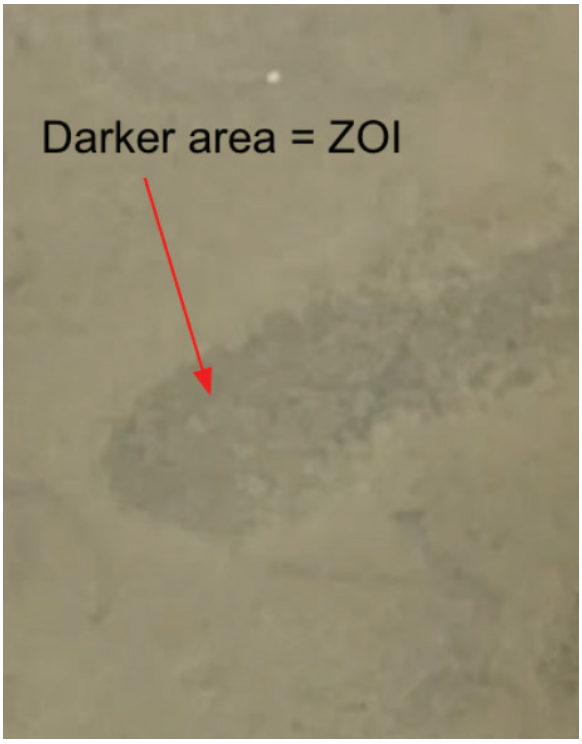

Figure 6: Zone of inhibition around solid urine fertilizer surrounded by extensive *E. coli* K-12 growth. The zone of inhibition extended away from the solid urine location, potentially displaying antibacterial properties.

Other current research emphasizes removing salts to isolate urea. The extraction methods are impractical on a large scale. Multiple steps requiring ethanol evaporation, recrystallization, and vacuum filtration require many resources.[15] This research demonstrates that these are unnecessary steps. Diluting the urine leads to an optimal urine concentration per milligram of water, with enough nutrients to boost plant growth and a low enough salt concentration for plants to germinate and grow.

Synthetic medical-grade urine was used in this study.[8] Common components used to model urine as a fertilizer have not been updated in decades. Currently, scientists continue to use

models that stem from the source: *The Primary Cause of Infection-Induced Urinary Stones*.[16,17] However, the more biologically accurate, medical-grade urine model better helps determine the exact concentration of solid urine to use, resulting in an NPK ratio of the medical-grade synthetic urine using molar calculations of 24:2:5.

Discussion

Future Growth and Implementation:

In nature, a ubiquitous enzyme called urease converts urea into ammonia and carbon dioxide. Because ammonia is a gas under standard temperature and pressure, it can be lost to the environment. Besides the urease produced by garden cress, the model does not include environmental urease. Further studies could add urease to the synthetic urine to simulate a real-world situation. In this setting, it would be possible to identify methods to denature the enzyme to halt degradation before application. Some methods currently in use include acidifying the urine during collection. This approach has been researched in Sweden.[5]

Compared to commercial fertilizer, urine has a relatively low phosphorus content (24:2:5 NPK ratio). Future studies could plan to adjust the phosphorus content to optimize root growth. Additionally, comparing the urine fertilizer to standard commercial fertilizer and studying a more common commercial crop, such as soybeans, would provide greater information on its real-world application. Like cress, soybeans create their urease and would represent a good model, albeit slower growing.

Researchers in Sweden have discovered that a local community produces enough urine to offset its need for commercial fertilizers.[5] However, applying liquid urine to crops is illegal in many E.U. countries because of its odor and promotion of bacterial growth in storage. Additionally, multiple different methods are used to harness waste. Some siphon individual elements from sewage plants, whereas others discard urine entirely, even though "it contains 80 – 90% of the nitrogen (N), 90% of the potassium (K) and 50% of the phosphorus (P) in household sewage."[18] Such problems, however, could be resolved by dehydrating urine. It is odorless, bacteriostatic, and contains an abundance of nutrients. By spreading awareness of the potential of dehydrated urine, harnessing urine as a fertilizer could become a normalized method of fertilization.

Unexpected Outcomes:

Originally, the initial plans were to fertilize the cress with synthetic urine in its solid, unpowdered form. ¼ tsp. and ½ tsp. of urine crystals were placed on top of the soil, and after multiple days, the cress seeds failed to germinate. By this point, the control group was halfway through its growth cycle. After further research, it was determined that it was due to the urine's high salt content.[19] To combat this, further research resulted in a new method of fertilization with serial dilutions. Realistically, inoculating fields with urine's dry form would not be practical as well, as it would be impossible to control its concentration in rainfall-dependent systems. By reconstituting and diluting synthetic urine, the salt concentrations were lowered while maintaining an adequate level of nutrients. In situations where there is poor rainfall, there is a theoretical risk of salt accumulation affecting plant growth. Despite this, Vermont farmers have used urine as a fertilizer for twelve years without noted complications.[20]

Conclusion

This study explores the potential of using human urine as a sustainable nitrogen source for agriculture, focusing on developing a synthetic solid urine model to fertilize *Lepidium sativum* (garden cress). Given the environmental and safety concerns associated with traditional ammonia-based fertilizers produced by the Haber-Bosch Process, this research aims to find a safer, more sustainable alternative. It outlines the creation of a solid urine fertilizer, storage, and testing methods. It highlights the benefits of solid over liquid urine, including reduced risk of microbial growth and easier transportation. The results indicate that garden cress fertilized with the optimal concentration of synthetic solid urine showed significantly higher yields than the control group, proving the effectiveness of urine as a nitrogen source. This approach addresses the challenges of using liquid urine and demonstrates a sustainable path forward in agriculture. With the global population growing and the environmental impact of traditional fertilization methods becoming increasingly unsustainable, this research illustrates the potential of human urine as a fertilizer, demonstrating its potential to contribute to sustainable agriculture practices.

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