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Resource Recovery from Synthetic Nitrified Urine in the Hydroponic Cultivation of Lettuce (*Lactuca sativa* Var. *capitata* L.)

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Abstract: The application of hydroponic cultivation fertilized with biologically nitrified synthetic urine can produce nitrate-rich fertilizer for lettuce (*Lactuca sativa* var. *capitata* L.). The mounting water crisis and depletion of natural resources makes nitrogen recovery from human urine a practical option. Nitrified urine can be used in indoor vertical hydroponic cultivation and is characterized by a high degree of element recovery. Because of its high ammonium content, hydrolyzed fresh urine may be toxic. A nitrification sequencing batch reactor with suspended activated sludge biomass ensured urine stabilization and biological conversion into nitrate-rich fertilizer. The diluted nitrate-rich fertilizer was then supplied for soilless cultivation. The results show that diluted nitrified urine is an excellent source of bioavailable nitrogen and phosphorus and, with proper enrichment with microelements, could replace commercial fertilizers in hydroponic systems. The yield and quality parameters of lettuce cultivated with enriched urine were comparable to those obtained with a commercial fertilizer. The mass balance calculation showed that industry-scale lettuce production can be based on urine fertilizer collected from a few hundred people for a single unit.

Keywords: hydroponics; urine; biological nitrification

1. Introduction

The rapidly growing human population is expected to reach 10 billion at the end of the 21st century [1], 90% of which will live in megalopolises [2]. This coupled with global warming and water scarcity will require new technical solutions such as paradigm shifts in the approach to food production, which in conventional farming, requires substantial amounts of water, fertilizers, pesticides, and land that is constantly being taken for city development. Having an immense amount of space, urban areas, both horizontally and vertically, could be used for unconventional farming, thus redefining the meaning of the expression "concrete jungle".

Vertical farming, which is gaining popularity due to advantages such as on-spot, high-quality food production; independence from weather and seasons; and lower water consumption and required space, is a strong candidate to help the population face upcoming challenges [3]. Out of the possible solutions, hydroponics—a soilless cultivation method that can be used for growing plants in mineral nutrient solutions that use water as a solvent—is considered the future of agriculture [4]. The design of this method minimizes water and nutrient losses. Compared to soil cultivation, this technology reduces the water consumption by 90% [5], nitrogen by 15%, and phosphorus by 38% [6]. Carvalho et al. [7] also emphasize its high productivity and ease of operation, while Maestre-Valero et al. [8] highlight lower specific energy consumption and greenhouse gas emissions. Vertical farming comes with its own challenges that need to be addressed. Maintaining stable conditions and the adequate nutritional solution, gas exchange system, or water sources are only a few examples of the challenges that need to be addressed when considering vertical farming.

Providing adequate nutritional solutions can be troublesome due to preparation, concentration, transport, etc. Therefore, the utilization of renewable and limitless resources that are available on site might be the next step for vertical farming development. Urine qualifies as a source of valuable nutrients and water. Urine is 95% water, almost 90% nitrogen, and 70% phosphorus [9]. If left without treatment and if it is disposed in the environment, then those elements become the main contributing factors to water eutrophication. The same elements, if appropriately used, are essential for crop production. Even though urine is mixed with other wastewater streams in most cases, its separate collection is possible with urine diverting toilets, which are recently drawing more attention. Source-separated urine can act as a liquid fertilizer for both soil and soilless crop cultivation [10]. Unfortunately, its ammonium concentration and high pH make it toxic to plants [11]; in a nutrient solution, ammonium induces nitrification, leading the pH to drop to a level that is unfavorable for plants.

Among soil and soilless types of cultivation, the use of urine as a fertilizer is being increasingly discussed in the literature. Some studies concern soil cultivation plants (cabbage, red beet, cucumber (*Cucumis sativus* L.), wheat (*Triticum aestivum* L.), and okra (Abelmoschus esculentus) being grown using fresh human urine [12–16]. Others deal with stored human urine supplemented with wood ash [17] that is applied to tomato (Solanum lycopersicum) in soil cultivation. There are also studies on the cultivation of wheat (*Triticum aestivum* L.) on freeze–thawed urine along with struvite precipitation and nitrogen adsorption on zeolite and activated carbon [18].

For soilless crop cultivation, there are only a few experiments concerning the use of urine in hydroponic cultivation. One example is for the production of lettuce in outer space [19]. Here, cultivation was supplied with an aqueous solution of 1500 mL of human urine that had been oxidized with 33% H_2O_2 and 150 g of human feces. The use of nitrified urine in hydroponics was previously examined in tomato cultivation (Solanum lycopersicum) [20] and in the cultivation of dwarf tomatoes [21]. Other experiments have been performed on water spinach (*Ipomoea aquatica*) and during tomato (Solanum lycopersicum) cultivation with diluted, pretreated hydrolyzed urine [12,22] as well as on wheat and watercress cultivated with liquid mineralized human feces and urine [23].

In the era of climate change, a growing human population, the intensification of industrialization, and urbanization, the demand for water and food is constantly increasing. Increased agricultural activity makes it necessary to look for new solutions not only for cultivation itself but also for the sustainable production of fertilizers. There is no doubt that the trends of decreasing natural water resources, decreasing agricultural land area, and the increasing consumption of the elements used in the production of fertilizers will not change without changes being made in the management of these resources. Therefore, it is important to test the applicability of the circular economy between sanitation and agroculture.

Activated sludge is a commonly used technology in municipal wastewater treatment. Fully developed activated sludge contains a substantial amount of nitrifying bacteria and can be used to biologically convert urine into a nitrate-rich fertilizer in typical reactors [1].

In this study, a biological wastewater treatment method (fully nitrifying in the activated sludge technology in a sequencing batch reactor) was used to nitrify urine for the hydroponic cultivation of lettuce. New data on lettuce yields and growth parameters show the feasibility of this approach.

This research aims to investigate the impact of diluted nitrified urine, and diluted nitrified urine supplemented with deficient elements (K, Ca, Mg, S, Fe, Mn, Cu, Zn, B, Mo) on lettuce yield and quality parameters (lettuce composition, and photosynthetic pigments content). The results were compared to the reference cultivation carried out with a commercial nutrient solution that is commonly used in large-scale commercial production. This research complements our knowledge on the cultivation of hydroponic lettuce on urine-based fertilizers that was previously conducted by Volpin et al. [24] and El-Nakhel et al. [25]. The cultivation of lettuce based on a fully nitrified stream (~0% NH_4 and 100% $NO_3/(NO_3 + NO_2)$ in effluent) obtained in an activated sludge reactor has not been performed in the past. This study also calculates resource recovery rates based on the production volumes of existing vertical hydroponic indoor farms that grow lettuce in various locations around the world and estimates the required number of urine donors for vertical farms.

2. Materials and Methods

2.1. Urine

The synthetic urine used in this study was prepared based on the recipe from Feng and Wu [26], which includes 0.5 g $CaCl_2{\cdot}2H_2O$, 4.12 g K_2HPO_4, 0.47 g $MgCl_2{\cdot}H_2O$, 0.29 g KCl, 4.83 g NaCl, 1.55 g NH_4Cl, 2.37 g Na_2SO_4, 13.34 g urea, 1.0 g creatinine, and 0.65 g sodium citrate (pH 6.8) per 1000 mL of water. The applied dilution factor of urine to toilet flushing water was 3:1, as defined by Anderson et al. [27], which is a typical dilution factor in source-separated systems. The comparison between elemental composition of the synthetic urine and real human urine is shown in Tables S1 and S2 in the Supplementary Materials.

The synthetic urine solution prepared as stated above was stored in a 180 L tank equipped with a mechanical stirrer and a liquid level sensor.

2.2. Urine Nitrification

Nitrification was carried out in a 150 L pilot scale Sequencing Batch Reactor (SBR), which is shown in Figure 1 (for more details see Janiak et al. [28]). The system was capable of data acquisition and automatically controlling all of the major processes (filling and decanting, pH control, oxygen control, temperature control, mixing, aeration). The inner side of the SBR was made of PVC with thermal insulation that is able to reduce heat loss. An internal heater (ETG-K D50 AISI 316L, Termik, 1.5 kW) controlled the temperature. The external dimensions of the reactor were 58 $\times$ 39 $\times$ 102 cm (length $\times$ width $\times$ height).

At the start-up, the reactor was seeded with 40 L of activated sludge from the Wrocław wastewater treatment plant, conducting full nitrogen removal via nitrification/denitrification. The sludge concentration was 8 $g{\cdot}L^{-1}$. Activated sludge was used as the inoculum for the nitrification bacteria. The SBR was aerated and stirred for the entire cycle (air pump ES/ET 105, Charles Austen Pumps Ltd., 60 W; stirrer HM-191, Kacperek, 370 W). $NaHCO_3$ was used for pH correction and as a source of alkalinity. Sedimentation and decantation were conducted manually every few days when the reactor was full (effluent and influent peristaltic pump TH25, Pompy Dozujące, 1900 $mL{\cdot}min^{-1}$, 18 W, 50 W). No excess sludge removal was conducted. The sludge retention time was more than 100 days. Oxygen concentration was maintained at 3.0 set point $gO_2{\cdot}m^{-3}$. pH was maintained in the range of 5.5–7.0. The temperature set point was 30 $\pm$ 0.5 °C. Nitrified urine was collected in a 50 L tank, where sample collection was conducted for periodic measurements of the effluent quality.

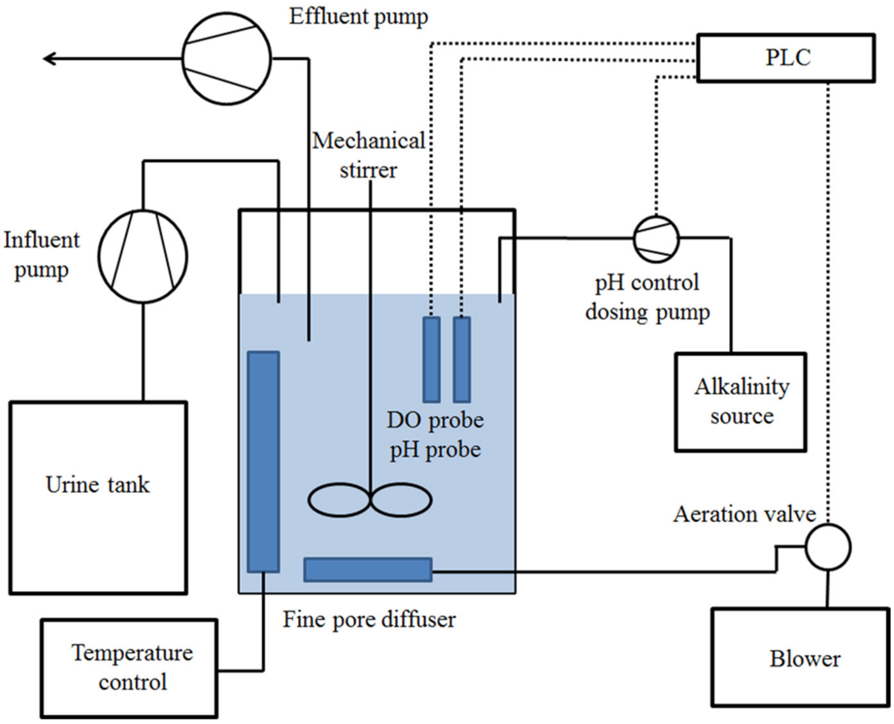

Figure 1. SBR set-up for nitrification of the synthetic urine (for more details see Janiak et al. [28]). PLC—programmable logic controller.

2.3. Hydroponic Set-Up

The experiment was carried out in three lab-scale hydroponic modules, each planted with ten seedlings. Each module (shown in Figure 2) had a 40 L nutrient solution tank, which had a scale intended for water level measurements and a cover to prevent water evaporation.

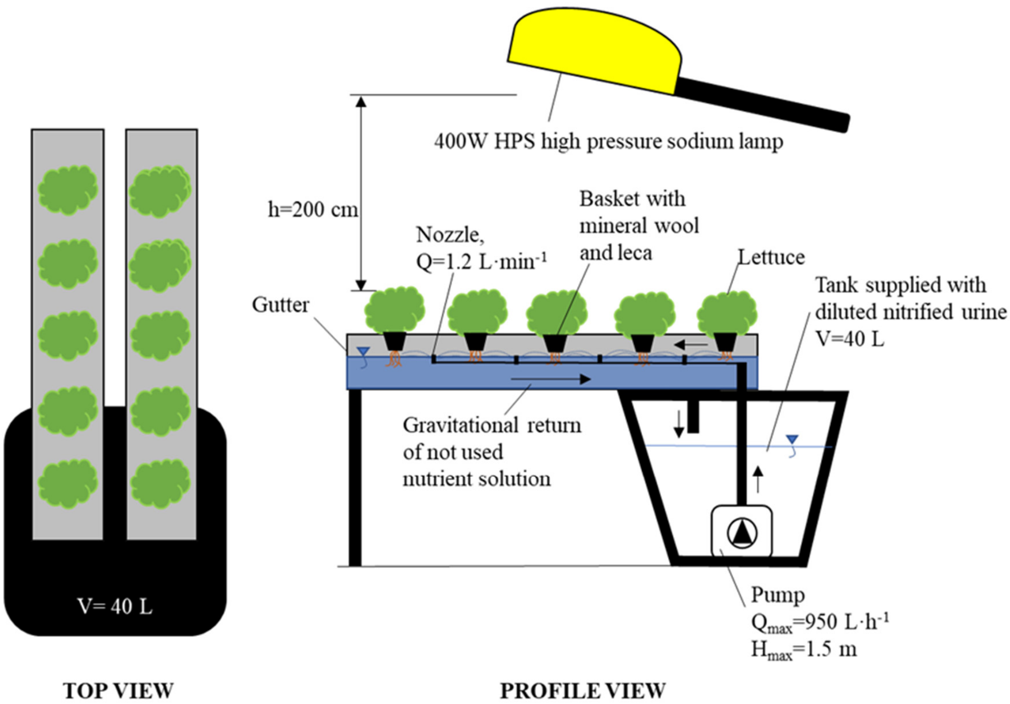

Figure 2. Schematic of a single hydroponic module.

Each module was composed of a pump, a tank with nutrient solution, piping with nozzles, a basket filled with a lightweight expanded clay aggregate (leca), a drainpipe, and a 400 W HPS high-pressure sodium lamp. Recirculation of the nutrient solution in each module was provided by gravitational flow and the drainpipe. The water level in the gutter was kept at a specific level to ensure that the root zone was in constant contact with the solution.

The plant selected for testing was lettuce (*Lactuca sativa* var. *capitata* L.). The seeds were sown in mineral wool, soaked with 1.8 $mS \cdot cm^{-1}$ of nutrient solution with electrical conductivity (EC) with a pH of 5.5, and then incubated until the desired root length was reached. After sowing, the temperature was maintained at 20 °C during the day and at 18 °C at night. Well-grown healthy cuttings with 3–4 proper leaves were designated for planting. The seedlings were grown in a greenhouse with a cycle of 16 h of light and 8 h of darkness under natural light for 4 weeks. After this time, the young plants were placed in pots filled with keramzite and were then transferred to the hydroponic installation for 35 days and were then harvested. Sodium lamps illuminated the plants to prevent shock associated with changing the diurnal cycle [29].

The experimental set-up was composed of three modules. Two modules were supplied with urine-based fertilizer. One module was the reference module and was supplied with commercial fertilizer. The nutrient solution recipes are shown in Table 1. Module 1 (DNU) was supplied with a nutrient solution based on 10-fold diluted nitrified urine stock (see the nitrified urine composition in Table 2). The nutrient solution was prepared by diluting the feedstock (nitrified urine) at a ratio of 1:10 with distilled water. Module 2 (DNUE) had the same nutrient solution base as DNU but was additionally enriched with fertilizers, as shown in Table 1. The nutrient solution of the reference module (Module 3) was prepared in distilled water with commercial fertilizers.

Nutrient solutions prepared with feedstock (nitrified synthetic urine) contained excessive amounts of sodium and chloride and consequently had higher EC in comparison to commercial fertilizers (reference module). It was noted that the higher sodium content might result in higher salt concentrations in the plant tissue and that this might cause, leaf necrosis or a slower growth rate [30,31]. The nutrient solution and undiluted feedstock (nitrified urine) compositions are shown in Table 2.

2.4. Analytical Methods

Measurements of the elements in the undiluted feedstock (nitrified urine) and in the experimental nutrient solutions (DNU, DNUE, Reference) were made according to the following methodology: The nitrate and chloride contents were measured with a Thermo Scientific Orion 4-Star Plus ion meter. The pH and conductivity were measured with the OPC-511 Elmetron meter, and the remaining elements were measured with the ICP PerkinElmer Inc. (Waltham, MA, USA) Optima 2000 DV apparatus.

Table 1. The nutrient solutions recipes.

Module 1 Diluted Nitrified Urine (DNU)	**Module 2 Diluted Nitrified Urine Enriched (DNUE)**	**Module 3 Reference Commercial Fertilizers (Ref)**
4 L undiluted nitrified urine	4 L undiluted nitrified urine	24 g $CaNO_3$ (15.5% N, 18.5% Ca)
Topped up with distilled water to 40 L	12 g $CaNO_3$ (15.5% N, 18.5% Ca)	40 g Peters Orange fertilizer with ICL Special Fertilizers (16% N, 5% P_2O_5, 25% K_2O, 3.4% MgO, 0.1% Fe, 0.04% Mn, 0.01% B, 0.01% Cu, 0.01% Zn, 0.001% Mo).
	10 g K_2S (44.8% K, 17% S)	
	1 g Hortisol Micro (7% Fe, 4% Mn, 0.8% Zn, 0.4% Cu, 0.05% Mo, 0.01% Co)	
	Topped up with distilled water to 40 L	Topped up with distilled water to 40 L

Table 2. Nutrient composition of the feedstock (nitrified synthetic urine) and in the three experimental modules (measured value±measurement error, N = 1).

Parameter	Unit	Undiluted Feedstock	Module 1 Diluted Nitrified Urine (DNU)	Module 2 Diluted Nitrified Urine Enriched (DNUE)	Module 3 Reference
pH	-	6.50 ± 0.15	7.40 ± 0.15	7.35 ± 0.15	7.15 ± 0.15
EC (electrical conductivity)	$mS{\cdot}cm^{-1}$	25.60 ± 0.05	2.70 ± 0.05	3.12 ± 0.05	1.85 ± 0.05
$N\text{-}NO_3^-$	$mg{\cdot}L^{-1}$	2140 ± 5	214 ± 5	261 ± 5	168 ± 5
$N\text{-}NH_4^+$	$mg{\cdot}L^{-1}$	0	0	0	82 ± 5
P	$mg{\cdot}L^{-1}$	174.0 ± 0.5	17.4 ± 0.5	17.4 ± 0.5	22.0 ± 0.5
K^+	$mg{\cdot}L^{-1}$	906 ± 2.5	90.6 ± 2.5	200.6 ± 2.5	199.6 ± 2.5
Ca^{2+}	$mg{\cdot}L^{-1}$	160 ± 1.5	16 ± 1.5	70.6 ± 1.5	105.2 ± 1.5
Mg^{2+}	$mg{\cdot}L^{-1}$	64.6 ± 0.2	6.5 ± 0.2	21.5 ± 0.2	20.4 ± 0.2
Na^+	$mg{\cdot}L^{-1}$	5900 ± 0.5	590.0 ± 0.5	590.0 ± 0.5	5.2 ± 0.5
Cl^-	$mg{\cdot}L^{-1}$	1830 ± 1.5	183 ± 1.5	184.5 ± 1.5	5.2 ± 1.5
SO_4^{2-}	$mg{\cdot}L^{-1}$	1890 ± 5	189 ± 5	265 ± 5	152 ± 5
Fe	$mg{\cdot}L^{-1}$	0.20 ± 0.02	0.02 ± 0.02	1.75 ± 0.02	2.05 ± 0.02
Mn	$mg{\cdot}L^{-1}$	0.20 ± 0.05	<0.02	1.00 ± 0.05	0.94 ± 0.05
Cu	$mg{\cdot}L^{-1}$	0.20 ± 0.05	0.02 ± 0.05	0.10 ± 0.05	0.08 ± 0.05
Zn	$mg{\cdot}L^{-1}$	0.20 ± 0.05	0.02 ± 0.05	0.20 ± 0.05	0.21 ± 0.05
B	$mg{\cdot}L^{-1}$	1.90 ± 0.05	0.19 ± 0.05	0.25 ± 0.05	0.26 ± 0.05
Mo	$mg{\cdot}L^{-1}$	trace	trace	0.05 ± 0.01	0.06 ± 0.01

Humidity and air temperature were measured with an indoor Bioterm weather station equipped with a thermometer, barometer, and hygrometer. The air temperature was on average 21.7 ± 0.8 °C, and the air humidity was 40.2 ± 9.3% on average. The nutrient solution parameters (pH, EC and temperature) during the experiments were measured with Hach probes (Intellical PHC101 for the pH and temperature and Sension+EC5 for EC) and a HQ40D multi meter. The measurement accuracy for the temperature was 0.1 °C and –0.01 for the pH.

The lettuce was weighed with an analytical balance Axix AD 1000 (0.01 g). The roots, leaves, and stems were separated, and their dry matter content was determined by the drying and weighing method after the material was dried to a constant weight at 105 °C. To determine the lettuce composition, leaf samples weighing approximately 1 g from a given number of lettuces (7–10) were taken and mixed into one averaged sample. The content of chlorophylls a and b and the carotenoids were measured using the spectrophotometric method with the Spectroquant Pharo 300 apparatus from Merck. The nitrogen content in the plants was determined by the Kiejdahl method with the Gerhard Vapo Dest 20 apparatus, and the content of the remaining elements was measured after material mineralization in a Milestone Ethos UP mineralizer using the ICP method. Every lettuce composition measurement was conducted in triplicate.

The significance level of the fresh mass weight measurements was checked with the t-Student test for two averages while using a normal distribution for $\alpha = 0.05$.

2.5. Nutrient Recovery from Humans for Cultivation of Lettuce

The assimilation for a given element per plant was calculated as an average dry mass of the edible parts (leaf+steam, Table 3) multiplied by the element content (Table 4).

Table 3. The effect of different nutrient solutions on the lettuce yield (average ± SD). FM—fresh mass, DM—dry mass, N—number of lettucces.

Module Number	Leaf FM	Leaf DM	Stem FM	Stem DM	Root FM	Harvest Index
-	g·plant^{-1}	% FM	g·plant^{-1}	% FM	g·plant^{-1}	%
Module 1 Diluted Nitrified Urine (N = 8)	29.14 ± 11.52	4.71 ± 0.03	4.96 ± 1.53	8.68 ± 0.37	3.27 ± 0.45	90 ± 3
Module 2 Diluted Nitrified Urine Enriched (N = 10)	52.73 ± 10.22	4.21 ± 0.02	8.39 ± 1.90	8.25 ± 0.05	2.69 ± 0.45	96 ± 1
Module 3 Reference (N = 7)	57.47 ± 9.55	4.06 ± 0.04	8.84 ± 1.37	8.06 ± 0.03	2.62 ± 0.59	96 ± 1

Table 4. Content of macro- and microelements and photosynthetic pigment content in leaves averaged sample in the three treatments (measured value ± measurement error). DM—dry mass, FM—fresh mass. N—number of lettuces.

Parameter	Module 1 Diluted Nitrified Urine (DNU) (N = 8)	Module 2 Diluted Nitrified Urine Enriched (DNUE) (N = 10)	Module 3 Reference (N = 7)
-		(in g·kg DM^{-1})	
N	45.7 ± 0.3	46.5 ± 0.3	49.4 ± 0.3
P	7.53 ± 0.03	7.58 ± 0.03	6.30 ± 0.03
K	66.30 ± 0.25	85.00 ± 0.25	81.70 ± 0.25
Ca	5.91 ± 0.05	8.82 ± 0.05	14.90 ± 0.05
Mg	3.23 ± 0.02	3.14 ± 0.02	4.02 ± 0.02
Na	50.70 ± 0.02	42.8 ± 0.02	0.70 ± 0.02
Cl	2.88 ± 0.05	3.90 ± 0.05	4.30 ± 0.05
S	15.60 ± 0.02	17.70 ± 0.02	4.60 ± 0.02
		(in mg·kg DM^{-1})	
Fe	42.5 ± 2.5	72.8 ± 2.5	278 ± 2.5
Mn	11.1 ± 2.0	36.8 ± 2.0	182 ± 2
Cu	10.9 ± 0.25	11.5 ± 0.25	11.3 ± 0.25
Zn	56.6 ± 2.5	70.8 ± 2.5	105.0 ± 2.5
B	33.1 ± 1.5	38.3 ± 1.5	42.3 ± 1.5
		(in mg·100g FM^{-1})	
Chlorophyll A	24.87 ± 1.5	49.98 ± 1.5	55.83 ± 1.5
Chlorophyll B	8.9 ± 0.5	18.7 ± 0.5	21.5 ± 0.5
Carotenoids	53 ± 5	156 ± 5	190 ± 5

3. Results

3.1. Yield

Table 3 presents the effect of nitrified urine fertilization on the yield of lettuce, which is expressed as the content of fresh biomass and dry weight, and on the harvest index (ratio of edible parts mass to total mass).

The lettuce yield that was fertilized with diluted nitrified urine (Module 1) was on average 29.14 ± 11.52 g·plant^{-1}. It was about half that of the enriched diluted nitrified urine solution (Module 2: 52.73 ± 10.22 g·plant^{-1}) and the reference solution (Module 3: 57.47 ± 9.55 g·plant^{-1}). A similar trend was found for stem biomass, where the yield value for DNU-fertilized crops was 43.9% and 40.9% lower than that of Module 3 and Module 2, respectively. The percent dry mass in the leaves differed significantly ($\alpha = 0.05$) between all of the tested groups. The highest value was for the DNU solution, followed by the DNUE solution. In contrast, the smallest value was recorded for the nutrient solution based on mineral fertilizers (control).

Regarding the stems, the lowest biomass content was in the lettuce grown in the DNU solution (4.96 $\pm$ 1.53 g), and the highest biomass content was recorded for the reference solution (8.84 $\pm$ 1.37 g). The use of diluted nitrified urine fertilizer increased the crop roots by 24.8% (3.27 $\pm$ 0.45 g) compared to the reference solution (2.62 $\pm$ 0.59 g). Fertilization with nitrified urine reduced the harvest index by 6% (90 $\pm$ 3%) compared to the reference solution and the DNUE solution, which were equal at 96 $\pm$ 1%. Overall, the difference between all of the results obtained for the DNUE (module 2) and the Reference (module 3) was not statistically significant (t-Student test, $\alpha = 0.05$). The difference between the DNU (module 1) and the other two modules was statistically significantly different. The difference between module 1 in comparison to the Reference was lower than the difference between Module 1, and Module 2.

3.2. Lettuce Composition

The composition of the experimental nutrient solutions impacted the nutritional status of the lettuce, which is expressed here as the content of micro- and macroelements in the biomass. The concentration of micronutrients in the nutrient solutions was correlated with the content of these components in the plants.

The results from the analysis of macro and microelements in the averaged leaf samples from each module are shown in Table 4.

In general, the lettuce cultivated in Module 1 was characterized by a lower content of N, K, Ca, Mg, S, Fe, Mn, Zn, and B and a higher content of Na and S compared to the Reference (Module 3), with a significant difference being observed in the pigment content (Chlorophyll A, B, and Carotenoids) compared to the other two modules. The pigment content was significantly higher in the Reference. The composition of the lettuce from Module 2 was generally closer to the Reference treatment, with the exception of the content of Ca, Na, S, Fe, and Mn, which were deficient in Module 2 compared to the Reference.

3.3. Nutrient Recovery in the Lettuce from the Urine

Table 5 shows the assimilation of the individual elements obtained in this experiment, which were calculated per plant on the basis of the data from Tables 3 and 4. The complete calculations are presented in the Supplementary Materials. In the case of the DNU treatment, the entire assimilation can be classified as recovery since all of the elements originate from urine. For the DNUE treatment, only part of the assimilated elements originates from the urine. The proportion of the assimilation from urine and fertilizer is assumed to be identical to the proportion in the solution. For example, in DNU, the nitrogen content was 214 g $N \cdot m^{-3}$, while in DNUE, it was 260.5 g $N \cdot m^{-3}$. Therefore, 82% of the nitrogen assimilated by the plants in DNUE comes from urine and is recovered, while 18% comes from fertilizer. Generally, the lower elemental recovery from DNU occurred due to the significantly lower yields. Consequently, the supplementation of a urine nutrient solution produces a larger yield, as this increases element recovery and leads to commercially attractive crops. As for K, Ca, and Mg, their amounts added with fertilizer are considerably higher (ca. 1.5$\times$, 5$\times$ and 3$\times$) than their levels in urine (see Table 1). Thus, the majority of plant assimilation is based on the added fertilizer. Please note that in Tables 4 and 5, sodium is omitted, as practically all of the sodium in the lettuce originated from the added buffer $NaHCO_3$ to increase the pH. Consequently, the recovery of sodium from urine is not significant in this experiment.

Table 5. Assimilated elements per edible mass of lettuce produced in the DNU treatment (diluted urine), where all assimilation is recovery, and for the DNUE treatment, where added micronutrients were added; only the elements from the urine fraction are considered as recovery.

Parameter (g/Lettuce Edible Mass)	Diluted Nitrified Urine (DNU)	Diluted Nitrified Urine Enriched (DNUE)	
		Assimilated from the Urine Content (Recovery)	Assimilated from the Added Supplements
N	0.082	0.111	0.024
P	0.014	0.022	0.000
K	0.120	0.112	0.136
Ca	0.011	0.006	0.020
Mg	0.006	0.003	0.006
Cl	0.028	0.052	0.000
S	0.005	0.008	0.003

4. Discussion

4.1. Yield and Lettuce Composition

There were no statistically tested (t-Student test, $\alpha = 0.05$) significant differences in any of the parameters as seen in Table 2 when comparing DNUE (module 2) and the Reference (module 3) treatments (u in critical area (-1.96 to 1.96, $u = -0.93$ for fresh mass and -0.97 to 0.27 for other parameters). In contrast, the DNU treatment (module 1) differed significantly, and all of the yield parameters (FW, DW) were lower (u outside critical area). The small yield of leaves and stems of the lettuce fertilized with the DNU solution when compared to the DNUE and the Reference solution is due to the deficiency in the micro-nutrients. The higher pH in the treatments than the pH that would be normally found in standard hydroponic cultivation might negatively affect the availability of some elements [32]. For instance, a pH higher than 5.8 affects the availability of K, P, Ca, and Mg more than the availability of Mn, Zn, Cu, and Fe [33]. As shown in Table 6, only N, P, S, and B are sufficient in the DNU solution, while other elements are either insufficient or are in excess when comparing its composition to that of the Reference, which is a commercial and optimized nutrient solution. The uptake of these elements may also be limited in the DNU and in the DNUE solution at a higher pH, as confirmed by the research completed by Roosta and Hamidpour [34]. Both the lower chlorophyll A and B contents and yield can be attributed to potassium, iron, magnesium, and zinc deficiency. The lack of these elements disturbs the nitrogen cycle in plants, inhibits chlorophyll synthesis (chlorosis), and leads to a lower yield [35–37]. Considering that at least 1.5 g $Mg \cdot kg^{-1}$ is necessary for optimal plant growth [38], the magnesium (assuming complete bioavailability) in DNU was sufficient for the growth of approximately 170 g. This assumption is in accordance with the obtained yields. Calculations for other elements led to similar results. The lettuce yields were probably further limited by the lack of calcium. However, during the experiment, no visible symptoms of calcium deficiency, such as necrotic leaf margins, were observed [39]. Generally, all of the lacking elements contribute to a lower yield, and in most cases, their inaccessibility can also be seen in the composition of lettuce leaves (Table 3). The low concentrations of most of the nutrients in DNU stimulated root growth at the expense of shoot development [40]. Thus, lettuce cultivated in DNU was characterized by the lowest harvest index. The above-mentioned deficiencies cannot be overcome by different treatment approaches. The only solution is micro-nutrient supplementation, as urine itself (both synthetic and real human urine) lacks those elements. Please see Table S1 in the Supplementary Materials for a comparison of the compositions of synthetic and real human urine.

Table 6. Nutrient composition in nutrient solution—abundance ("−"—element in insufficient concentration, "+"—element in sufficient concentration, "++"—excess of given element).

Relative Elemental Abundance	DNU Treatment (DNU)	Zabel [21]	Yang [22]	Volpin [24]	The Reference Treatment (Module 3)
N/N	1(+)	1(+)	1(+)	1(+)	1
P/N	0.08(+)	0.07(+)	0.01(+)	0.07(+)	0.088
K/N	0.42(−)	0.22(−)	1.38(++)	0.27(−)	0.8
Ca/N	0.07(−)	0.51(+)	0.01(−)	0.01(−)	0.42
Mg/N	0.03(−)	0.02(−)	0.03(−)	0.001(−)	0.08
Na/N	2.78 (++)	0.33(++)	3.48(++)	0.30(++)	0.021
Cl/N	0.86(++)	0.57(++)	6.57(++)	0.43(++)	0.021
SO_4/N	0.88(+)	0.21(−)	4.27(++)	0.24(−)	0.61
Fe/N	0.0027(−)	No data	0.0003(−)	No data	0.011
Mn/N	0.0000(−)	No data	0.0000(−)	No data	0.004
Cu/N	0.0001(−)	No data	0.0005(+)	No data	0.0003
Zn/N	0.0001(−)	No data	0.0006(+)	No data	0.0008
B/N	0.0009(+)	No data	0.0020(+)	No data	0.001

4.2. Effects of Excessive Sodium and Chloride

Two elements—sodium and chloride—were in excess in the DNU and DNUE solutions compared to the concentrations in the Reference solution. Both sodium and chloride are micronutrients in the human diet, and therefore, their increased concentration in lettuce leaves does not pose a threat to human health, especially if lettuce consumption does not exceed 100 g weekly per person [41]. Excess sodium assimilation in the lettuce resulted in Na concentrations above 4.0% in the DNU and DNUE treatments compared to 0.70% in the Reference treatment. For comparison, in the studies by Długosz-Grochowska [42], the sodium content in lettuce leaves was 0.54–0.93% Na, and in the study by Kleiber [43], it was 0.91–1.19%. High concentrations of sodium might result in leaf necrosis and a decrease in edible dry weight, leaf length, and leaf width as well as a decrease in the photosynthetic rate and stomatal conductance [30].

Excessive chloride in urine-based nutrient solutions is generally caused by the urine composition itself; only changes in human dietary habits (lower salt consumption) can lead to a lower chloride content. On the other hand, the high sodium content was mainly related to the large amounts of $NaHCO_3$ used for pH stabilization during nitrification (see Point 2.2). Without $NaHCO_3$ dosing, only approximately 50% of ammonium would be nitrified, leading to an unsuitable nutrient solution composition. Therefore, an overabundance of sodium can be avoided when only partially nitrified urine is used as a nutrient solution. The high ammonium concentration resulting from this approach could significantly influence plant growth as severely as excessive sodium [24,25]. Volpin et al. [24] treated urine in a membrane reactor without alkalinity correction and then created a concentrated urine product in membrane distillation. That product was further diluted to suit lettuce. The results showed that partially nitrified urine leads to very low yields, significantly lower than those obtained with nitrified urine in these studies (16.1 ± 2.9 g and 29.14 ± 11.52 g respectively). In turn, El-Nakhel et al. [25] cultivated lettuce using electrodialysis (ED) concentrate obtained from fully nitrified urine and using commercial urine-based fertilizer acquired from partial nitrification. That fertilizer contained N in a proportion of NH_4-N/NO_3-N of 1/1. The yield of lettuce grown in ED concentrated from fully nitrified urine was 51% higher. Other solutions to the problem of excess sodium in the production of synthetic nitrified urine are the implementation of denitrification or mixing urine with feces prior to treatment. During denitrification, OH^- ions are created to balance NO_3^- transformation to N_2 while the addition of feces directly provides missing alkalinity. Both approaches would therefore supplement the process with the required alkalinity for pH stabilization. The drawbacks are nitrogen loss during denitrification or the implementation of feces collection system, which is much harder to maintain than a urine collection system.

It is, however, clear that excess levels of those two elements do not result in lower yields, as the mass and composition of the lettuce cultivated on the DNUE solution are comparable to the Reference treatment (see Tables 2 and 3). This indicates that lower yields of plants cultivated in DNU solution are only related to the element deficiencies mentioned above. Furthermore, the toxicity of sodium ions was probably compensated for by a high content of potassium and calcium ions [32]. Therefore, both the yield (Table 2) and chlorophyll content (Table 3) in the lettuce leaves fertilized with the DNUE solution with a relatively high concentration of Ca and K were not affected by sodium.

4.3. Nutrient Content Levels

The nitrogen content in the biomass, regardless of the treatment solutions, was within the range given in the literature [25,44–46]. Similar to nitrogen, the phosphorus content in each module was also in accordance with the scientific literature [46–48] and did not exceed the toxicity limit for lettuce (>10 g·kg DM^{-1}). Therefore, neither element deficiencies nor abundances caused problems in nitrogen and phosphorus fixation.

The potassium content in each module was within the limits given by other researchers [46,47,49] for conventionally grown lettuce. The reduced value for the lettuce fertilized with the DNU solution was due to the significantly lower concentration (45%) of this element in the starting nutrient solution when compared to the DNUE and Reference.

The lower magnesium content in the leaves of the plants fertilized with the urine-based solutions could be due to both the low concentration of this ion in the DNU solution and the ionic antagonism (between magnesium and potassium ions) in the DNUE solution [50]. Nevertheless, the magnesium content was in the optimal range in all of the samples samples, which, according to the literature [45,47], is between 2.3 and 7.3 g·kg DM^{-1}.

The calcium content in the leaves of the lettuce was within the optimal range given by White and Brown [51] (5–10 g·kg DM^{-1}) and by Długosz-Grochowska et al. [42] (16.5–23.9 g·kg DM^{-1}). The differences in the amount of calcium assimilated by the lettuce (for DNU and DNUE, 40% and 59% of what has been assimilated in the Reference, respectively) were due to significant differences in the concentration of this ion in the different treatments (for DNU and DNUE, 15% and 67% of what has been available in the Reference, respectively).

A strong correlation between the composition of the fertilizer and the nutrient content in the leaves was also observed for iron, manganese, and zinc. The poor composition of the DNU solution and its relatively high pH contributed to the poor absorption of these components. Thus, Fe and Mn (Module 1) had the lowest contents corresponding to (42.5 mg Fe·kg^{-1}) and Mn (11.1 mg Mn·kg^{-1}), respectively. Despite the use of a chelated form of iron and its similar concentration in the DNUE and Reference solutions, the lettuce leaves from the former had almost four times less (72.8 mg Fe·kg^{-1}) of this component than the latter and less than the values reported in the literature by [43] Kleiber et al.: above 100 mg Fe·kg^{-1}. Similar observations were made for manganese, the content of which in the lettuce fertilized with the DNU and DNUE solutions was significantly lower than it was in the reference lettuces. This is despite the fact that the DNUE concentration of Mn was comparable to the Reference. Lettuce is one of the species that has a high demand for manganese, with levels of 480 mg Mn·kg^{-1} [43]. Kozik et al. [52] provided average values from 176.6 to 348.1 mg Mn·kg^{-1} when the concentration in the starting nutrient solution was 10–60 mg·L^{-1}. Therefore, only the reference lettuce that was fertilized with mineral fertilizer had a manganese content that was close to the optimal values that are provided in the literature.

The zinc content in the lettuce leaves was 56.6, 70.8, and 105.0 mg Zn·kg^{-1} for modules 1, 2, and 3, respectively, which are similar to the published range of 49–77 mg Zn·kg^{-1} [43]. Therefore, the nutritional status of the lettuce with zinc in this study may be appropriate.

The copper content in the lettuce leaves ranged from 10.9 to 11.5 mg Cu·kg^{-1} in all of the modules. Despite the differences in the pH and the concentration of copper between the treatments, the copper content in the lettuce did not differ from the literature data [53].

The boron content in the lettuce leaves (33.1–42.3 mg B·kg^{-1}) fell within the range of 15–84 mg B·kg^{-1} given by Sahin et al. [54].

Mampholo et al. [55] reported carotenoid content ranges of 0.066–0.165 mg·gFW^{-1} for different types of lettuce. In this study, the result from Module 1 was slightly lower than this range, while Module 3, on the contrary, was slightly higher. Only Module 2 fell within the mentioned range, which shows the advantageous influence of supplementing the urine with deficient micro-elements.

4.4. Nutrient Content of the Treatment Solutions

The nutrient content of the diluted nitrified urine (DNU) and Reference treatments were compared to other urine-based fertilizers from the literature (Table 6). To achieve comparability, concentrations were recalculated to be proportional to the nitrogen concentration. For example, P = 0.08 means that the P concentration is 0.08 of that of nitrogen. This is a common approach, as fertilizers are typically described by the proportion of N:P:K, which allows the analysis of the abundance of a given element in relation to nitrogen. The references cited in Table 5 vary significantly in the urine treatment approach, which results in differences. Still, some overall conclusions can be drawn. Firstly, in all cases, pure urine cannot provide all of the required elements, and in all cases some, deficiencies occur. In all cases, the urine is overabundant with Na and Cl, which is connected with to high salt consumption. This implies that urine may always require some supplementation in order to maximize crops.

4.5. Nutrient Recovery and the Pros and Cons of Using Nitrified Urine as Fertilizer

The data from existing indoor vertical lettuce farms were collected to better understand the potential benefits of using hydroponic nutrient solutions similar to DNUE in soilless lettuce cultivation. The amount of nutrients saved by using urine as fertilizer together with an estimate of the number of people required to donate urine daily to ensure adequate nutrient supply are presented in Table 7. The elements from urine that are the most are P, K, and Ca. Those elements are mainly excreted mainly in the feces and not in the urine but are also required by lettuce in substantial amounts [56].

Table 7. Data from four indoor vertical farms producing lettuce using DNUE as the nutrient solution; calculated nutrient savings from the use of urine instead of commercial fertilizer; estimate of the number of people required to donate urine daily to ensure adequate nutrient supply.

Farm/Company Name		Local Roots	Jones Food Company and GE	GE and Mirai	Spread Factory
Location		US	UK	Japan	Japan
Number of lettuces cultivated per day *		269	3850	10,000	20,000
Saved amount of each element, kg/year **	N	10.9	156.3	406.0	812.1
	P	2.2	31.1	80.8	161.6
	K	11.0	157.1	408.1	816.1
	Ca^{2+}	0.6	8.2	21.2	42.4
	Mg^{2+}	0.3	3.8	9.9	19.8
	Cl^{-}	5.1	72.4	188.1	376.3
	$SO_4{}^{2-}$	0.8	11.4	29.6	59.1

Table 7. *Cont.*

Farm/Company Name		Local Roots	Jones Food Company and GE	GE and Mirai	Spread Factory
People required to supply the urine ***	N	3	41	106	212
	P	7	96	249	497
	K	12	173	449	898
	Ca^{2+}	6	83	215	430
	Mg^{2+}	3	50	129	258
	Cl^{-}	2	29	75	149
	$SO_4{}^{2-}$	1	11	28	55

* Data taken from [57]. ** Data calculated based on Table 5. *** Data calculated with the assumptions of the mean values of urinary load taken from the review by Jurga et al. [56] and an 100% efficiency of the nitrification process. A 24-h urine collection is assumed.

For all of the farms that were studied, fulfilling the potassium demand was the most difficult. In the case of the largest farm, urine was taken from nearly 1000 people daily. In contrast, for the smaller farms, daily urine collection from a dozen to a few dozen people should not be too problematic as long as there is the appropriate infrastructure in the company area. However, for larger daily demands (e.g., more than 100 people per day), an organized urine collection system is required that will also enable significant water supply to the hydroponic system. Well-known cases of urine collection are the municipalities, eco-villages, and summer houses in Sweden [58]. Swedish urine diversion projects in urban areas include Västervik (*approximately* 230 households), Vaxholm (*approximately* 250 households), Linköping (*approximately* 275 households), Norrköping (*approximately* 300 households), and Tanum (*approximately* 500 households) [58]. Assuming that one household consists of a minimum of 3 people, the above-mentioned examples could provide enough urine for extensive hydroponic lettuce cultivation. It, however, appears that for larger farms with significant feedstock requirements, the possibility of acquiring the raw stream will be crucial to assess the feasibility of using nitrified urine as a fertilizer.

5. Conclusions

This study demonstrates the use of 10-fold diluted synthetic nitrified human urine as a fertilizer source for the hydroponic cultivation of lettuce. The yield and quality of the lettuce grown on nitrified urine was compared to lettuce grown in a standard fertilizer solution and in nitrified urine enriched with micronutrients. After being supplemented with the extra nutrients, i.e., potassium, calcium, and micronutrients, the diluted nitrified urine was efficiently used as a fertilizer for growing lettuce. When growing lettuce with enriched urine, yield and quality were close to those of the reference commercial fertilizer solution. In the case of the cultivation based on 10-fold diluted nitrified synthetic urine alone, much lower yields, a reduced nutritional status, and a high accumulation of sodium and chloride were obtained. It can be assumed that a similar result would have been achieved using real human urine, as the composition of the synthetic urine did not differ from the averaged composition of real urine (Tables S1 and S2 in the Supplementary Materials).

Nitrified urine can be used in indoor vertical hydroponic cultivation and is characterized by a high degree of element recovery; however, an organized daily urine collection system from >100 people would be required for any single industrial scale cultivation.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11112242/s1, Table S1: Comparison between macro-nutrients content in synthetic and real human urine. Table S2: Comparison between micro-nutrients content in synthetic urine and real human urine.

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