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An Appraisal of Urine Derivatives Integrated in the Nitrogen and Phosphorus Inputs of a Lettuce Soilless Cultivation System

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Abstract: Reinforcing and optimizing sustainable food production is an urgent contemporary issue. The depletion of natural mineral resources is a key problem that is addressed by recycling mined potassium and phosphorus, and nitrogen, whose production depends on very high energy input. A closed-loop approach of fertilizer use asserts the necessity for efficient management and practices of organic waste rich in minerals. Human-derived urine is an underutilized yet excellent source for nitrogen fertilizer, and, in this study, processed urine fertilizer was applied to greenhouse soilless cultivation of lettuce (*Lactuca sativa* L.) cv. Grand Rapids. Biomass increase, biometric parameters, soil plant analysis development (SPAD) index, minerals, and organic acids content of lettuce were analyzed. From eight different urine fertilizer products generated, K-struvite, urine precipitate-CaO, and the liquid electrodialysis (ED) concentrate supported the growth of lettuce similar to that of commercial mineral fertilizer. ED concentrate application led to the accumulation of potassium (+17.2%), calcium (+82.9%), malate (+185.3%), citrate (+114.4%), and isocitrate (+185.7%); K-struvite augmented the accumulation of magnesium (+44.9%); and urine precipitate-CaO induced the highest accumulation of calcium (+100.5%) when compared to the control, which is an added value when supplemented in daily diet. The results underlined the potential of nitrogen- and phosphate-rich human urine as a sustainable source for the fertilization of lettuce in soilless systems.

Keywords: sustainable fertilization; nutrient recovery; K-struvite; ED concentrate; urine precipitate; source-separated urine; circular economy; food security; waste streams

Citation: El-Nakhel, C.; Geelen, D.; De Paepe, J.; Clauwaert, P.; De Pascale, S.; Rouphael, Y. An Appraisal of Urine Derivatives Integrated in the Nitrogen and Phosphorus Inputs of a Lettuce Soilless Cultivation System. *Sustainability* **2021**, *13*, 4218. https://doi.org/10.3390/su13084218

Academic Editor: Bernhard Huchzermeyer

Received: 10 March 2021
Accepted: 8 April 2021
Published: 10 April 2021

1. Introduction

The natural resources on Earth are the driving factor of humans' welfare. The linear exploitation of these resources is currently scrutinized and requires a strenuous redesign [1]. The urgency entails a new vision concerning resources' overconsumption and losses. In the view of circular agriculture, a resource is detained within the system through recycling and reuse [1]. Food production systems and our consumption customs are unsustainable; therefore, to render it sustainable, implementations are propounded to reinforce it and optimize it through the concept of a circular economy [2], especially with a continuous population growth that drives an expansion in agricultural activity to emulate the pace [2–4].

Global food production has been thriving through extrinsic inorganic fertilizers administration, such as nitrogen (urea and ammonium nitrate), phosphorus, and potassium [5–7]. Nitrogen fertilizers are acquired from ammonia, which is manufactured through the Haber–Bosh process, whereas potassium and phosphorus fertilizers are derived from the mining industry (sedimentary and phosphate rocks, respectively) [6,8]. The production of ammonium nitrate is prone to a downturn over the coming decades, whereby urea is a potential alternative for nitrogen fertilization [7]. The high-grade phosphate rock mines

are predicted to deplete in the upcoming decades [2,5,9–12]. Phosphate is a critical raw material [2], for which there is currently no recoverable product to substitute phosphate rock [11]. Similarly, potassium as a non-renewable resource along with its unbalanced global distribution could jeopardize agriculture in countries where potash is absent [12].

Under continued linear fertilization methods, agriculture will further deteriorate the natural ecosystems [3]. Therefore, an approach of a closed-loop fertility cycle asserts the realization of adequate natural resource management, amelioration of human welfare, and sustainable food security [7]. A circular nutrient management approach, incorporating exhaustive recycling of nutrients encompassed in human excreta into agriculture [13], will require the use of treated wastewaters granting a resource of nutrients (P, K, and N) [14]. The new paradigms of urban water management coupled with sustainable technologies implies the recovery and recycling of nutrients from waste streams in order to be implemented for the production of fertilizers; hence, shifting towards a circular economy of nutrients [9].

Amidst waste streams, source-separated human urine represents a plausible source for recovering nutrients [8–10], where an adult person can produce up to 500 L a year [10,15]. Markedly, ammonia recovery represents a lower energy-demanding alternative to the Haber–Bosch process and results in a lower carbon footprint [9,10]. Furthermore, such recovery suggests together a cost and an energy-consumption reduction in fertilizers production and nitrogen removal from wastewater [16]. The recovery of P represents a sustainable substitute for phosphate mining [10]. Furthermore, the captivating part of nutrient recovery is that it not only closes the nutrient loop but also minimizes aquatic environmental pollution [12]. Interestingly, human urine can be featured as a liquid fertilizer in agriculture [12], and when processed for nutrient recovery as a solid fertilizer, promising results have been reported [17]. Urine is practically sterile, unlike feces [15], and therefore poses a limited risk of spreading microbial diseases [7]. Indeed, human urine embodies a copious supply of macro-nutrients [8,12,18], with a dried solid content of 13% C, 14–18% N, 3.7% P, and 3.7% K [13]; nonetheless, high in salinity [18]. For the recovery of urine, considerable technologies have been adopted, such as (i) membrane distillation, (ii) electrochemical concentration, (iii) stabilization + distillation, (iv) ammonia stripping, and (v) struvite precipitation [9].

Thus far, the exploration of nitrogen- and phosphate-rich human urine to grow lettuce in soilless systems, has received little attention. In this work, the performance of urine derivatives (UDs) was compared with commercial fertilizers, in a short growing cycle of *Lactuca sativa* L. The results showed that UDs were suitable for integration in future complete growing cycles and could be a component for creating a circular cultivation method whereby nutrients were recycled and reused for the production of food.

2. Materials and Methods

2.1. Solid and Liquid Urine Derivatives Preparation

All the UDs used for this study were prepared prior to the beginning of the experiment. Then, the dilution and the addition of the rest of the fertilizers were done during the growing cycle just before the fertigation in order to maintain a steady level of the different forms of nitrogen. The initial composition of the derivatives is mentioned in Table 1, while the used methods are briefly described below. In addition, an overview of the preparation methods and the main N compound can be found in Table S1 in the Supplementary Materials.

Table 1. Control (NPK + TE) and urine derivatives initial composition and concentration.

Treatments	Total N	NH_4^+-N	NO_2^--N	NO_3^--N	PO_4^{3-}-P	K^+	SO_4^{2-}	Ca^{2+}	Mg^{2+}	Na^+	Cl^-
Stabilized urine-low pH	6396.0	502.1	1.4	12.4	258.7	2648.4	912.6	-	-	2719.6	6826.8
Stabilized urine-high pH	5610.0	403.3	4.6	9.0	3.0	1620.0	791.4	10.2	110.0	2506.7	3431.2
Hydrolyzed urine	3376.0	3300.7	0.0	0.2	87.2	1354.0	531.1	-	-	1360.6	2227.7
ED concentrate	4500.0	12.0	122.8	3885.5	74.8	1853.5	225.0	-	-	8776.2	3919.4
Aurin	42,000.0	20,346.0	0.0	21,654.0	1746.0	14,943.0	-	-	-	17,000.0	31,000.0
K-Struvite	5.2	4.2	0.0	0.0	61.0	42.4	-	17.7	15.3	52.9	6.5
Urine precipitate-NaOH	147.1	22.1	0.0	0.0	20.6	52.3	-	2.4	0.54	115.5	100.4
Urine precipitate-CaO	81.2	7.5	0.1	0.1	15.5	35.3	0.0	22.6	0.1	29.4	66.9
NPK 20-10-20 + TE	200.0	80.0	0.0	120.0	100.0	200.0	-	-	1.5	-	-

Liquid derivatives (stabilized urine-low pH, stabilized urine-high pH, hydrolyzed urine, ED concentrate, and Aurin) are expressed in mg/L, and solid derivatives (urine precipitate-NaOH, urine precipitate-CaO) and control NPK + trace elements (TE) are expressed in mg/g. ED: electrodialysis.

2.1.1. Hydrolyzed Urine

Urine was collected and stored at room temperature for several weeks. During storage, urea, the main nitrogen compound in fresh urine, hydrolyzed into TAN (total ammonia nitrogen) and (bi)carbonate. As a result, the pH increased to ~9.2, triggering precipitation of calcium and magnesium salts (mainly struvite, hydroxyapatite, and calcite, according to the literature) [19–21]. Hence, TAN was the predominant nitrogen compound, and precipitation of phosphate with calcium and magnesium lowered the phosphate concentration in the hydrolyzed urine.

2.1.2. Stabilized Urine-Low pH

Based on the method of Saetta and Boyer [22] and Ray et al. [23] that was slightly modified, fresh urine was stabilized immediately after collection by adding 75 mmol HCl L^{-1}. Because of the low pH (<2), enzymatic urea hydrolysis was inhibited. Hence, urea was the main nitrogen compound in the stabilized urine at low pH.

2.1.3. Stabilized Urine-High pH

Fresh urine was stabilized immediately after collection by increasing the pH to 12.2–12.7 with NaOH (0.111 mol L^{-1}) and CaO (0.111 mol L^{-1}). Because of the high pH, enzymatic urea hydrolysis was inhibited [24]. Hence, urea was the main nitrogen compound in the stabilized urine at high pH. In contrast to the stabilized urine at low pH, precipitation occurred at high pH, explaining the lower phosphate content of the stabilized urine at high pH. Because of the addition of calcium (by the use of CaO), more phosphate was removed compared to the hydrolyzed urine (where calcium and magnesium are limiting).

2.1.4. Electrodialysis Concentrate

Electrodialysis (ED) concentrate is a urine derivative produced by a combination of precipitation, nitrification, and electrodialysis [8]. First, NaOH was added to dilute fresh urine in a precipitation reactor in order to remove bivalent cations by controlled precipitation (at pH 11). Subsequently, the urine was treated in a nitrifying bioreactor in order to remove the organics and to convert all the urea and TAN into nitrate. Next, the nitrified urine was concentrated with electrodialysis. Due to full nitrification, all nitrogen was present as nitrate, and part of the phosphate was removed in the precipitation step.

2.1.5. Aurin

Aurin (Vuna GmbH, Dubendorf, Switzerland) is a commercial concentrated ammonium nitrate (NH_4-N/NO_3-N: 1/1) fertilizer made from stored human urine, using partial nitrification and distillation [25,26].

2.1.6. K-Struvite

The precipitate was obtained from urine by removing all NH_4-N (below 50 mg N/L), adding an equivalent molar amount of Mg^{2+}, and increasing the pH to 10. The crystal retention time in the reactor was 1 h [27].

2.1.7. Urine Precipitate-NaOH

NaOH (4.4377 g L^{-1}) was added to fresh urine immediately after collection to induce precipitation (pH of 12.5–12.7). Urine precipitate (rich in phosphorus) was harvested by dewatering the precipitation sludge in a drying oven at 105 °C [24].

2.1.8. Urine Precipitate-CaO

CaO (6.155 g L^{-1}) was added to fresh urine immediately after collection to induce precipitation (pH of 12.2–12.6). Because of the addition of calcium, more phosphate could be precipitated compared to the NaOH addition. Urine precipitate (rich in phosphorus) was harvested by dewatering the precipitation sludge in a drying oven at 105 °C [24].

2.2. Plant Material, Growth Conditions, and Experimental Design

A thirty-two-day experiment was conducted at ILVO, Melle, Belgium (51°0′ N, 3°48′ E) from 17 November until 18 December 2017 in a heated polycarbonate greenhouse. Initially, lettuce seeds (*Lactuca sativa* cv. Grand Rapids TBR, West Coast Seeds, Delta, British Colombia, Canada) were germinated on a capillary mat (Aquamat capillary matting, Premier Netting, Norfolk, UK) for 24 h. The capillary mat's physicochemical parameters can be found in detail in Kyriacou et al. [28] study. Then the germinated seeds were transferred on Grodan® propagation cubes placed in trays and covered with a dome for the first seven days. After 14 days in a growth chamber, lettuce seedlings were transplanted into 2 L pots containing 100% Grodan® mini rockwool grow-cubes (Grow Magic Hydroponics, Fulham, London, UK) with a density of 12.5 plants/m^2. The greenhouse temperature was around 22 ± 2 °C and 16 ± 2 °C during day and night, respectively, and the relative humidity (RH) ranged between 35 and 65%. The natural day length was sustained with metal halide lamps that provided 100 µmoles s^{-1} m^{-2} at canopy level when undamped sunlight intensity measured lower than 685.5 µmoles s^{-1} m^{-2}. The treatments were arranged in a randomized complete block design with three replicates per treatment. Each experimental unit/replicate consisted of five plants, accounting for a total of 135 plants.

2.3. Plants Manual Fertigation and Nutrient Solutions Electrical Conductivity

The nutritional requirements of lettuce cv. Grand Rapids were calculated based on the exportation of the same cultivar used in the MELiSSA ACSA experiment [29] performed at the MELiSSA Pilot Plant (MPP) located at the Autonomous University of Barcelona. Total nitrogen was the limiting dilution factor of the liquid derivatives, whereas phosphorus was the limiting dilution factor of the solid derivatives. The latter was dissolved by the addition of 2 M H_2SO_4 prior to mixing with the other components. Supplementary bivalent fertilizers were added to the UDs in order to normalize the NPK concentrations across the UD nutrient solutions. The concentrations of the different elements of the UDs after dilution are listed in Table S2. Rainwater was added to prepare the final nutrient solutions, which were applied manually every other day by means of a laboratory beaker. NPK 20-10-20 + trace elements (TE) dissolved in rainwater were used as a control treatment. The pH of the nutrient solutions derived from liquid derivatives was adjusted to reach a value of 6.0 before the application, either by the addition of sulfuric acid (1 M) for (i) hydrolyzed urine, (ii) stabilized urine-high pH, and (iii) ED concentrate or potassium hydroxide (1 M) for (i) stabilized urine-low pH, and (ii) Aurin. Whereas the pH of all the nutrient solutions deriving from solid derivatives was adjusted to reach a value of 6.0 by the addition of potassium hydroxide. The electrical conductivity (mS cm^{-1}) of each nutrient solution was as follows: Control 1.2, stabilized urine-low pH 1.1, stabilized urine-high pH 1, hydrolyzed

urine 2.1, ED concentrate 2, Aurin 2.1, K-struvite 1.7, urine precipitate-NaOH 2.7, and urine precipitate-CaO 2.2.

2.4. Soil Plant Analysis Development (SPAD) Index, Biomass Determination, and Growth Analysis

Before harvesting, the soil plant analysis development (SPAD) index was measured by means of a portable chlorophyll meter SPAD-502 (Konica-Minolta, Osaka Japan). Fifteen leaves were randomly measured and averaged to a single SPAD value for each replicate. Major veins, leaflet margins, and damaged areas were avoided during the measurements.

At harvesting, fresh weight was determined, and the number of leaves per plant was also recorded. Consecutively, the leaf area of each plant was estimated using ImageJ software version 1.50 (Wayne Rasband, National Institute of Health, Bethesda, MD, USA). Plant leaf tissues were dried at 70 °C for 72 h [30] and were subsequently weighed in order to determine the corresponding dry biomass and leaf dry matter content (%).

2.5. Total Nitrogen, Mineral, and Organic Acids Content Analysis

Dried leaf tissues were ground in a Wiley Mill, passed through an 841 µm screen, and then processed for total nitrogen, mineral, and organic acids profile analysis. The total nitrogen concentration of the leaf samples was determined by the Kjeldahl method [31], following mineralization with sulfuric acid (96%, Carlo Erba Reagents, Milan, Italy) in the presence of potassium sulfate and low concentration of a copper catalyst. The anions (NO_3^-, PO_4^{3-}, SO_4^{2-}, and Cl^-), organic acids (malate, tartrate, oxalate, citrate, and isocitrate), and cations (K^+, Ca^{2+}, Mg^{2+}, and Na^+) in the leaf tissues were separated and quantified by ion chromatography (ICS-3000, Dionex, Sunnyvale, CA, USA) coupled to a conductivity detector using an IonPac CG12A guard column and IonPac analytical column for cations as well as an IonPac AG11-HC guard column and IonPac AS11-HC analytical column for anions and organic acids [30,32]. All minerals and organic acids were expressed as g kg^{-1} dw, except for isocitrate that was expressed as mg kg^{-1} dw. As for nitrate, it was expressed as mg kg^{-1} fw based on each sample dry matter percentage.

2.6. Statistical Analysis

Group means from all data were analyzed using the analysis of variance (ANOVA) via the SPSS 20 software package (IBM, Armonk, NY, USA). Duncan's multiple range test (DMRT) was performed for mean comparisons on each of the significant ($p < 0.05$) variables measured. For the determination of the interrelationship across the growth parameters—yield, physiological traits, and mineral composition with reference to the integration of UDs treatments—a principal component analysis (PCA) was applied, implementing the suitable function PCA from the same SPSS software.

3. Results

3.1. Agronomic Performance and SPAD Index

Based on Table 2, the three UDs that showed similar agronomic performance (leaf number, leaf area, and fresh biomass) like the control NPK + TE, were two solid UDs (urine precipitate-CaO and K-struvite)and one liquid UD (ED concentrate). K-struvite treatment registered the highest leaf number $plant^{-1}$ (17), leaf area (1379 cm^2), and fresh biomass (44.37 g), but these values were not significantly different from those obtained in the other three treatments. Amongst these latter, only ED concentrate had the lowest SPAD index and dry biomass, registering 23.2% and 24.6% less than the others on average, respectively, while Aurin and urine precipitate-NaOH had a high SPAD index just like the best-performing UDs, but yielding on average 50% less. The highest dry matter was recorded in hydrolyzed urine treatment (7.79%) which was among the worst-performing UDs, yielding only a fresh biomass of 7.88 g $plant^{-1}$.

Table 2. Leaf number, leaf area, fresh and dry biomass, dry matter, and SPAD index of lettuce as influenced by the nutrient solution integrated by diverse urine derivatives.

Treatments	Leaf Number (no. plant^{-1})	Leaf Area (cm^2 plant^{-1})	Fresh Biomass (g plant^{-1})	Dry Biomass (g plant^{-1})	Dry Matter (%)	SPAD Index
Stabilized urine-low pH	13 ± 0.22 bcd	565 ± 111 bc	17.38 ± 3.62 b	0.88 ± 0.27 de	4.85 ± 0.52 ef	14.94 ± 0.26 bc
Stabilized urine-high pH	12 ± 0.80 de	387 ± 76 cd	10.33 ± 2.54 c	0.59 ± 0.14 e	5.74 ± 0.26 cde	13.96 ± 0.86 cd
Hydrolyzed urine	10 ± 0.48 e	324 ± 31 d	7.88 ± 0.82 c	0.60 ± 0.06 e	7.79 ± 0.14 a	15.89 ± 0.60 ab
ED concentrate	16 ± 1.73 ab	1283 ± 85 a	38.18 ± 2.05 a	1.81 ± 0.05 b	4.76 ± 0.13 f	13.13 ± 0.60 d
Aurin	13 ± 0.19 cd	620 ± 20 b	18.78 ± 0.82 b	1.30 ± 0.11 cd	6.92 ± 0.51 b	17.24 ± 0.64 a
K-struvite	17 ± 0.33 a	1379 ± 127 a	44.37 ± 3.38 a	2.51 ± 0.30 a	5.64 ± 0.25 def	17.50 ± 0.73 a
Urine precipitate-NaOH	14 ± 0.68 bcd	776 ± 36 b	23.14 ± 0.88 b	1.52 ± 0.07 bc	6.59 ± 0.10 bc	16.98 ± 0.28 a
Urine precipitate-CaO	15 ± 1.47 abc	1308 ± 70 a	42.78 ± 1.69 a	2.33 ± 0.07 a	5.46 ± 0.07 def	16.89 ± 0.50 a
Control 20-10-20 + TE	16 ± 0.48 ab	1290 ± 18 a	42.78 ± 0.96 a	2.49 ± 0.09 a	5.86 ± 0.18 cd	16.77 ± 0.25 a
Significance	***	***	***	***	***	***

All data are expressed as mean ± standard error, n = 3. *** significant at $p \leq 0.001$. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$). dw: dry weight. ED: electrodialysis. TE: trace elements.

3.2. Mineral Composition and Nitrate Concentration

As listed in Table 3, total nitrogen was not statistically different between the best-performing treatments, except for ED concentrate, which registered the lowest percentage of 4.24. On the other hand, plants irrigated with ED concentrate treatment exhibited a high accumulation of K, Ca, and Na, and a minor accumulation of Mg, Cl, and SO_4 in comparison to other treatments. While PO_4 was not significantly different between the best-performing treatments, K accumulated better in ED concentrate and stabilized urine-low pH treatments, same as Ca, which highly accumulated in urine precipitated-CaO. Moreover, k-struvite treated plants were characterized by the highest accumulation of Mg and by a low accumulation of Na, while Cl accumulation was depicted at its highest in the worst-performing treatments like stabilized urine-low/high pH and hydrolyzed urine.

Table 3. Leaf mineral analysis of lettuce as influenced by the nutrient solution integrated by diverse urine derivatives.

Treatments	Total N (g kg^{-1} dw)	PO_4 (g kg^{-1} dw)	K (g kg^{-1} dw)	Ca (g kg^{-1} dw)	Mg (g kg^{-1} dw)	Na (g kg^{-1} dw)	Cl (g kg^{-1} dw)	SO_4 (g kg^{-1} dw)
Stabilized urine-low pH	51.1 ± 0.92 ab	17.13 ± 1.56 bcd	75.63 ± 3.81 a	3.65 ± 0.97 a	1.63 ± 0.17 c	15.11 ± 1.01 c	53.32 ± 5.33 a	6.31 ± 0.34 ab
Stabilized urine-high pH	52.9 ± 2.5 a	14.25 ± 1.55 de	67.70 ± 1.26 b	1.88 ± 0.33 b	1.24 ± 0.03 cde	11.84 ± 0.18 de	41.16 ± 1.46 b	5.45 ± 0.63 b
Hydrolyzed urine	53.3 ± 0.30 a	14.76 ± 0.59 de	63.94 ± 2.78 b	1.36 ± 0.14 b	1.02 ± 0.07 de	7.70 ± 0.67 f	44.21 ± 2.48 b	6.99 ± 0.48 ab
ED concentrate	42.4 ± 0.42 f	18.38 ± 0.58 abc	78.66 ± 2.44 a	3.64 ± 0.89 a	1.54 ± 0.24 cd	32.06 ± 2.06 a	21.22 ± 1.34 d	3.37 ± 0.62 c
Aurin	47.6 ± 0.92 cd	15.62 ± 0.63 cde	64.04 ± 1.03 b	1.18 ± 0.16 b	0.93 ± 0.09 e	10.01 ± 0.10 ef	28.35 ± 1.18 cd	6.51 ± 0.24 ab
K-struvite	50.7 ± 0.10 abc	20.48 ± 0.31 a	65.96 ± 0.96 b	1.47 ± 0.14 b	3.52 ± 0.28 a	9.03 ± 0.42 f	7.63 ± 0.68 e	6.81 ± 0.17 ab
Urine precipitate-NaOH	47.7 ± 0.60 cd	17.99 ± 1.10 abc	51.08 ± 2.82 c	2.03 ± 0.13 b	2.53 ± 0.12 b	25.56 ± 1.14 b	29.22 ± 2.74 c	7.26 ± 0.44 a
Urine precipitate-CaO	46.5 ± 0.82 e	13.68 ± 0.56 e	62.02 ± 0.94 b	3.99 ± 0.47 a	2.17 ± 0.14 b	13.49 ± 0.11 cd	27.59 ± 0.59 cd	6.11 ± 0.34 ab
Control 20-10-20 + TE	4.82 ± 0.03 bcd	20.07 ± 0.66 ab	67.11 ± 0.96 b	1.99 ± 0.24 b	2.43 ± 0.25 b	1.08 ± 0.04 g	5.35 ± 0.60 e	6.25 ± 0.68 ab
Significance	***	***	***	**	***	***	***	***

All data are expressed as mean ± standard error, n = 3. **, *** significant at $p \leq 0.01$ and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test ($p = 0.05$). dw: dry weight. ED: electrodialysis. TE: trace elements.

Across the best-performing UDs, nitrate accumulation (Figure 1) was significantly the highest in ED concentrate (4656 mg kg^{-1} fw) followed by k-struvite (4138 mg kg^{-1} fw), almost 1.4- and 1.3-fold, respectively, higher than the control (3241 mg kg^{-1} fw), whereas urine precipitate-CaO treatment induced a lower nitrate accumulation (2192 mg kg^{-1} fw) around 32.4% less than the control. As for the worst-performing UDs, nitrate accumulation was on average 133 mg kg^{-1} fw.

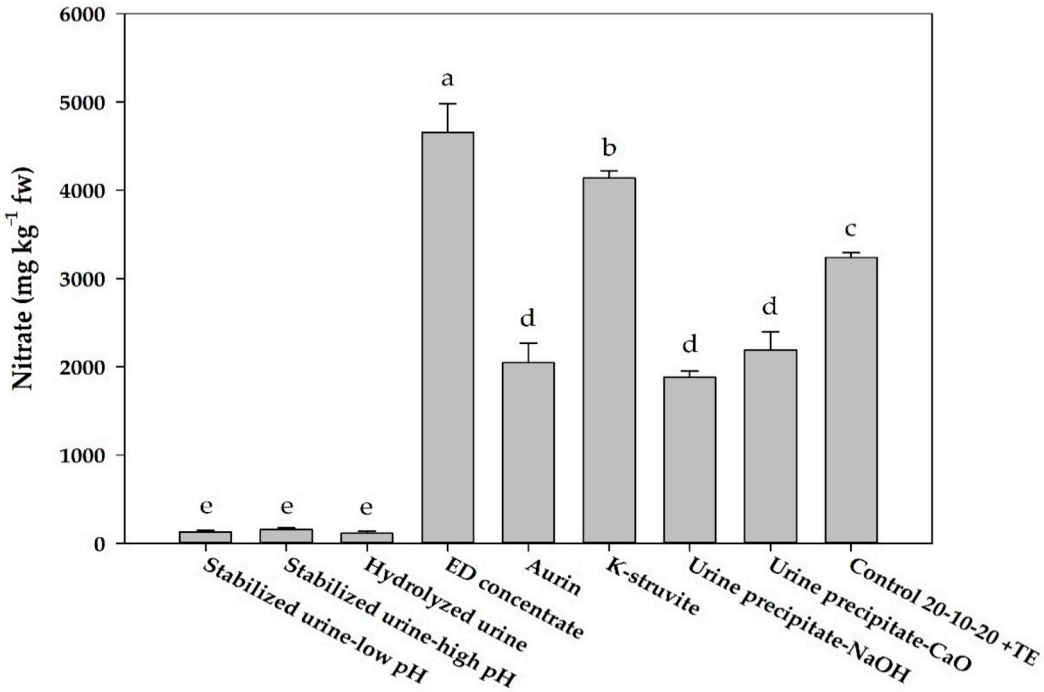

Figure 1. Lettuce nitrate content as influenced by the nutrient solution integrated by diverse urine derivatives. Different letters (a–e) indicate significant differences according to Duncan's test (p = 0.05). Vertical bars indicate mean values ± standard error, n = 3. Fw: fresh weight. ED: electrodialysis. TE: trace elements.

3.3. Organic Acids Content

As listed in Table 4, lettuce plants irrigated with ED concentrate were clearly characterized by a high accumulation of organic acids, namely, registering the highest accumulation of malate, citrate, and isocitrate, which were 33.7, 25.0, and 81.6%, respectively, higher than what was registered in the closest treatments (mainly in urine precipitate-NaOH); whilst the lowest accumulation of organic acids was noted in the hydrolyzed urine treatment. As for tartrate, it was almost equal between all the treatments except for Aurin, which registered a low value of 1.45 g kg^{-1} dw; while oxalate registered the highest value of 3.59 g kg^{-1} dw in stabilized urine-high pH treatment.

Table 4. Organic acid content of lettuce as influenced by the nutrient solution integrated by diverse urine derivatives.

Treatments	Malate (g kg^{-1} dw)	Tartrate (g kg^{-1} dw)	Oxalate (g kg^{-1} dw)	Citrate (g kg^{-1} dw)	Isocitrate (mg kg^{-1} dw)
Stabilized urine-low pH	21.32 ± 2.03 cd	3.58 ± 0.65 a	2.92 ± 0.27 b	4.38 ± 0.30 c	113.9 ± 9.79 b
Stabilized urine-high pH	22.60 ± 1.38 cd	3.80 ± 0.36 a	3.59 ± 0.24 a	3.51 ± 0.07 d	90.17 ± 12.8 bc
Hydrolyzed urine	4.95 ± 0.40 g	2.55 ± 0.80 abc	2.04 ± 0.05 c	1.32 ± 0.06 g	39.00 ± 12.0 d
ED concentrate	46.54 ± 3.09 a	2.94 ± 0.15 ab	2.71 ± 0.29 b	6.24 ± 0.26 a	206.9 ± 30.6 a
Aurin	10.45 ± 0.38 f	1.45 ± 0.04 c	2.06 ± 0.16 c	2.07 ± 0.09 f	64.60 ± 1.51 cd
K-struvite	17.94 ± 1.20 de	2.46 ± 0.28 abc	2.14 ± 0.02 c	3.37 ± 0.17 de	81.47 ± 9.20 bc
Urine precipitate-NaOH	34.81 ± 1.22 b	2.19 ± 0.11 bc	2.61 ± 0.09 bc	4.99 ± 0.05 b	112.1 ± 1.94 b
Urine precipitate-CaO	25.31 ± 0.19 c	2.51 ± 0.27 abc	2.09 ± 0.13 c	3.43 ± 0.21 de	94.33 ± 8.67 bc
Control 20-10-20 + TE	16.31 ± 1.41 e	2.15 ± 0.38 bc	2.13 ± 0.02 c	2.91 ± 0.14 e	72.43 ± 8.14 bcd
Significance	***	*	***	***	***

All data are expressed as mean ± standard error, n = 3. *, *** significant at $p \leq 0.05$ and 0.001, respectively. Different letters within each column indicate significant differences according to Duncan's multiple-range test (p = 0.05). dw: dry weight. ED: electrodialysis. TE: trace elements.

3.4. Principal Component Analysis

A principal component analysis was operated for SPAD index, biometric measurements, and mineral and organic acids composition parameters. The first three principal components explained 83.3% of the cumulative variance, with PC1 accounting for 43.8%, PC2 for 31.2% (Figure 2), and PC3 for 8.3% (data not shown). PC1 positively correlated with fresh and dry biomass, leaf number and area, nitrate, PO_4, and Mg, whereas it negatively correlated with NH_4, total N, and Cl. On the other hand, PC2 positively correlated with malate, citrate, isocitrate, oxalate, tartrate, calcium, potassium, and sodium and negatively correlated to the SPAD index, dry matter, and sulfate. PCA in our study was effective in plotting UD treatments and the corresponding plants' response. The score plot of the PCA presented information on the behavior of lettuce in regard to the different UDs. K-struvite and urine precipitate-CaO, identically to the control, presented similar biometric values (fresh biomass, dry biomass, LN, and LA) and mineral accumulation (nitrate, PO_4, and Mg). ED concentrate treatment was located on the upper right quadrant, characterized by a high accumulation of Na, K, Ca, and organic acids (isocitrate, citrate, and malate); whereas, the lower left quadrant depicted Aurin and hydrolyzed UDs, which were distinguished by a higher dry matter percentage and SO_4 leaf concentration. This PCA enabled the identification and grouping of the best UDs, which could be candidates for the integration in soilless systems fertilization.

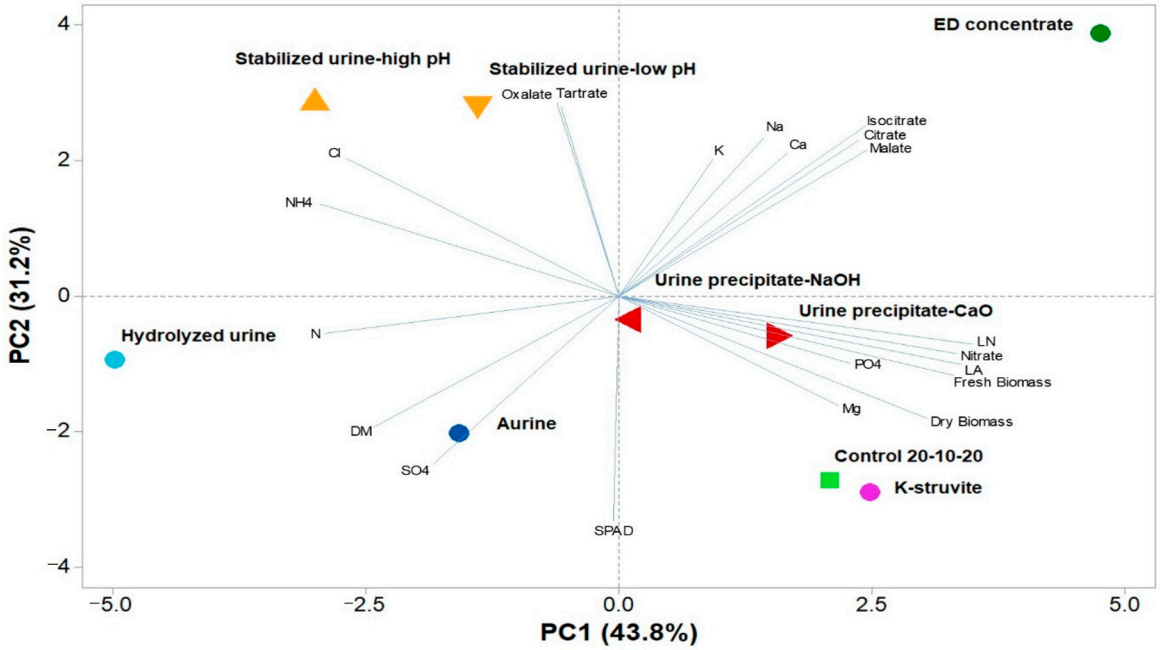

Figure 2. Principal component loading plot and scores of principal component analysis (PCA) of agronomical parameters (SPAD index, fresh biomass, dry biomass, dry matter (DM), leaf area (LA), leaf number (LN)), mineral content (PO_4, K, NH_4, Ca, Mg, SO_4, Cl, Na), total nitrogen (N), and organic acids (Malate, Tartrate, Oxalate, Citrate, and Isocitrate) in greenhouse grown Grand Rapids lettuce cultivated under diverse urine derivatives treatments in soilless systems. ED: electrodialysis.

4. Discussion

Human urine is a potential source of nitrogen, potassium, phosphorus, and trace elements essential for plant growth [33,34]. As demonstrated in this study, UDs supplemented with other minerals were just as effective as control fertilizer to support the growth of lettuce in a hydroponic greenhouse cultivation setup. The best-performing UDs were ED concentrate, K-struvite, and Urine precipitate-CaO. These products did not cause any noticeable morphological changes of lettuce in comparison to the control (NPK + TE), similar to the findings previously reported [35]. K-struvite supported lettuce growth compared to commercial fertilizer in line with previous reports revealing the importance of Mg and P

in struvite [36]. Struvite led to a similar lettuce biomass yield compared to diammonium phosphate [37]. Gonzalez-Ponce et al. [38] indicated that struvite recovered from municipal wastewater was an attractive P source comparable to others sources but concomitantly helped in attenuating the use of phosphate rock and the discharge of P into groundwater resources. Urine precipitate-CaO and ED concentrate derivatives supported growth slightly less effective than K-struvite, presumably due to its higher content in Mg that boosted the growth. The poorly performing UDs were characterized by a high urea concentration as the main N compound, such as stabilized urine-low/high pH, or characterized by high NH_4^+ concentration such as hydrolyzed urine and Aurin. In soilless systems, it was recommended that NH_4^+-N should not transcend 25% of the total nitrogen supply [39]. A too high NH_4^+ supply reduces growth [39,40] and causes leaves to become dark green [40]. Dark green leaves were observed after fertilization with hydrolyzed urine. High ammonium was also correlated with a reduced leaf area, which was possibly due to the implementation of C in producing organic molecules to sustain the osmotic potential instead of boosting leaf expansion processes; knowing that C is a metabolic control linked to the supply of carbohydrates for energy and structural components [40]. In addition, when the NO_3^-/NH_4^+ ratio decreased, leaf area, leaf number, and biomass decreased as well [41], which was evidenced in Aurin treatment. On the other hand, the use of urea as the sole N source in hydroponics resulted in reduced growth [42]. In addition, urea is not immediately available for plants in the same cultivation technique [43], as shown in both stabilized urine treatments. The above findings urge us to manage the N source percentage wisely in the final nutrient solution, with the purpose of efficiently using the UDs that hold a promising application.

Lettuce is known for its high sensitivity to salinity. When the electrical conductivity (EC) of the nutrient solution was higher than 2.6 mS cm^{-1}, plant growth was reduced [44], which was the case of urine precipitate-NaOH treatment (EC = 2.7 mS cm^{-1}) that exhibited almost half of the fresh weight and leaf area registered by the best-performing UDs and the control. Shanon and Grieve [45] explained that the effect of salinity on plant growth is attributed to the reduction of the leaf area and to the influence on photosynthesis and water and mineral uptake. Moreover, salinity effects start at the cellular level (osmotic effect); hence, the shoot growth rate is reduced, inducing smaller and fewer leaves [45]. In addition, Samarakoon et al. [46] stated that an EC higher than 2.5 mS cm^{-1} hampers nutrient absorption due to an increased osmotic pressure. On the other hand, urine precipitate-CaO treatment characterized by a relatively high EC of 2.2 mS cm^{-1} still managed to be amongst the best-performing UDs. The EC effect could be enhanced in different seasons (spring and summer) when the temperature and the solar radiation are higher, thus aggravating the problem of water uptake by plants [44]. As for Aurin and hydrolyzed urine treatments, the reduced yield was mostly linked to the form of nitrogen in the nutrient solution and not linked to the EC values.

The nitrate content of lettuce subjected to ED concentrate and K-struvite did not exceed the nitrate limits of the lettuce cultivated under conventional methods in the same growing season [47]. The highest nitrate accumulation rate in ED concentrate treatment was likely correlating with nitrate as the main source of nitrogen. As for the plants irrigated with urine precipitate-CaO/NaOH, nitrate accumulation was lower since the nitrogen form was mostly organic. As explained by Colla et al. [47], nitrogen forms notably affect nitrate concentration, which is greatly influenced by nitrate-N, more than by ammonium-N. Gunes et al. [48] were able to lower the nitrate concentration of lettuce cultivated in the nutrient film technique (NFT) by replacing a part of nitrate fertilizer with urea, which was proven in the case of stabilized UDs in this study. Furthermore, the same authors illustrated that a high chloride dose in the nutrient solution could diminish leaf nitrate. The ED concentrate was distinguished by the lowest SPAD index. This parameter is an indicator of chlorophyll content that was reduced under saline stress as a part of senescence response [49].

The accumulation of PO_4 and Mg in lettuce treated with K-struvite corroborated with the results reported by Ryu and Lee [36], while these elements were also abundant in lettuce leaves. Calcium was higher in lettuce treated with stabilized urine-low pH, ED concentrate, and urine precipitate-CaO. These results are in agreement with the findings of Ushakova et al. [35], who used a nutrient solution based on human-mineralized waste to grow lettuce hydroponically on expanded clay aggregates. The same authors also demonstrated the high accumulation of sodium in lettuce leaves (4-fold increase) that was similar to our results, where sodium was on average 14-fold higher than that registered in the control. Corrado et al. [50] showed that when NO_3^-/Cl^- ratio decreased in the nutrient solution, Cl^- leaf content increased, NO_3^- decreased, and vis-versa, which was the case of lettuce in our study. On the other hand, lettuce leaves are packed with antioxidant compounds and macro-nutrients that could sustain the human diet [51]. Up to 17 key minerals that take part in plant development are transferred to human nutrition, where they play a role in averting disorders and maintaining body metabolism and homeostasis [52]. As matter of fact, the best-performing UDs manifested an interesting accumulation of important minerals, such as ED concentrate and urine precipitate-CaO inducing lettuce leaves rich in Ca, and K-struvite treatment inducing leaves rich in Mg.

The accumulation of organic acids could be an adaptive response to Na excess and to osmotic stress [53,54], which was the case of most urine treatments, in particular ED concentrate. Lettuce plants treated with ED concentrate were highly rich in Na resulting in the highest accumulation of malate, citrate, and isocitrate. Furthermore, NH_4 fed plants contained less inorganic anions such as K, Ca, and Mg and organic anions like malate and citrate, when compared to those supplied with nitrate [48], which was the case of Aurin treatment, where half of the N source was ammonium, and the hydrolyzed urine treatment where all of the N source is only ammonium. Moreover, organic acids are important at the cell level, including (i) energy production (Krebs cycle), (ii) biosynthesis of amino acids, (iii) intermediates of photosynthesis, (iv) environmental adaptation [53,55], and maintain ionic balance in plants cells [55], especially with cations flux such as sodium. In contrast, some organic acids could positively affect human health due to their biological activity; malate, tartrate, and citrate exhibit an antioxidative role by their ability to chelate metals [56]. These organic acids were fairly boosted in ED concentrate treatment. Luckily, oxalate concentration in the best-performing derivatives was equal to that of the control, noting that oxalate could reduce the availability of dietary Ca and could enhance the formation of kidney calculus [56].

5. Conclusions

Better-designed nutrient management incorporating recycled nutrients into the production of horticultural crops would contribute to addressing the depletion of natural mineral and energy resources. Nitrogen- and phosphate-rich human urine integrated with commercial fertilizers adapted for the soilless production of lettuce was shown here to have potential. Indeed, several UDs (ED concentrate, K-struvite, and urine precipitate-CaO) in this study performed similarly as mineral fertilizer. In addition, a better accumulation of mineral elements and organic acids, compared to the control, was obvious in the case of ED concentrate treatment (+17.2% K, +82.9% Ca, +185.3% malate, and 114.4% citrate), urine precipitate-CaO (+100.5%), and in the case of K-struvite (+44.9% Mg), which is an added value when added in daily diet. Our results showcased the possibility of curbing the agriculture footprint by exploring waste streams and, hence, creating a closed nutrient loop. Nonetheless, advanced research is needed to reduce NaCl concentrations in UDs due to their drawbacks in horticultural production, especially in soilless systems.

Supplementary Materials: The following are available online at www.mdpi.com/xxx/s1: Table S1—Urine derivatives, preparation, and main N compound; Table S2—Concentrations of the treatments after dilution and before the addition of chemical fertilizers, expressed in mg/L of rainwater.

Author Contributions: Conceptualization, C.E.-N. and D.G.; methodology, C.E.-N. and D.G.; software, C.E.-N. and D.G.; validation, C.E.-N. and D.G.; formal analysis, C.E.-N.; investigation, C.E.-N., D.G., J.D.P., and P.C.; resources, D.G.; data curation, C.E.-N.; writing—original draft preparation, C.E.-N.; writing—review and editing, C.E.-N., D.G., J.D.P., P.C., S.D.P., and Y.R.; visualization, C.E.-N., D.G., S.D.P., and Y.R.; supervision, D.G. and Y.R.; project administration, D.G.; funding acquisition, S.D.P. and Y.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets generated for this study are available on request from the corresponding author.

Acknowledgments: The authors would like to thank Christophe Petit from Horticell for his technical and logistic support during the entire experiment; Chiara Il Grande from CMET for her technical assistance in the laboratory; and Sebastiaan Derese for providing the K-struvite fertilizer. We would like to thank Marie-Christine Van Labeke for some laboratory equipment, Antonio Pannico for the dataset preparation and scientific support throughout the experiment, and Luigi Formisano for the manuscript formatting. This research was supported by the Ugent Endowed Chair Agrotopia.

Conflicts of Interest: The authors declare no conflict of interest.

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