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Impact of different concentrations of nitrified urine in a recirculating nutrient solution on growth, yield and quality of lettuce

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1. Introduction, Knowledge, Objectives

An increasing amount of greenhouse vegetables is produced using hydroponic systems. The used mineral fertilizers are often generated in energy consuming processes (esp. nitrate in Haber-Bosch) or gained from finite resources (esp. phosphate rock). Recovering the respective nutrients from waste streams is a promising option to make crop production more sustainable. Particularly, human urine is rich in plant nutrients. Herrmann & Klaus (1997) estimate that about 80% of nitrogen (N) and 60% of phosphorus (P) in domestic wastewater originate from urine. Considering this, researchers advocate for separation toilets to prevent the dilution of these concentrated nutrients and facilitate their recycling (Larsen et al., 2001). In stored urine, nitrogen is mainly present as ammonium (NH_4^+) and it is thus very volatile. A suitable treatment to stabilize the nitrogen and to mine all potential plant nutrients in urine is nitrification (Udert and Wächter, 2012). Additional treatment steps are distillation, to inactivate pathogens and reduce volume, and activated carbon filtration, to remove pharmaceuticals and hormones (Etter et al., 2015). However, high Na^+ and Cl^- concentrations in nitrified urine are problematic for hydroponic cultivation, because almost all cultivated vegetable crops are in varying degrees sensitive to high salt concentrations in the nutrient solution (see Shannon and Grieve, 1999). To our knowledge, no previous studies evaluate nitrified human urine as a fertilizer in a recirculating hydroponic system. In the current experiment, the objective was to find a preliminary threshold of nitrified urine in the nutrient solution for the salt sensitive species lettuce (*Lactuca sativa* var. *capitata* cv. Salanova 'Descartes RZ') regarding yield and product quality.

2. Material and Methods

The experimental set-up consisted of five nutrient film technique (NFT) channels covered with opaque foil and their respective nutrient solution tanks (200 l). Control treatment was a modified nutrient solution for lettuce according to Göhler and Molitor (2002). The original recipe was adjusted to an electrical conductivity (EC) of 2.0 mS/cm when using tap water. Calcium was adjusted to prevent tip burn. Target concentrations were 163.45 mg/l NO_3-N, 4.67 mg/l NH_4-N, 41.29 mg/l P, 286.73 mg/l K, 146.96 mg/l Ca, 24.31 mg/l Mg, 1.49 mg/l Fe, 360.3 µg/l B, 174.4 µg/l Zn, 31.8 µg/l Cu, 183.1 µg/l Mn and 32 µg/l Mo. In the four

remaining tanks, nitrate salts and other fertilizers were substituted with increasing concentrations of nitrified urine (31%, 45%, 50% and 53% N from nitrified urine). The nitrified urine fertilizer (Aurin) was obtained from Vuna GmbH (Dübendorf, Switzerland).

Table 1. Nutrient concentrations in Aurin fertilizer according to ICP-OES and CFA analysis.

	NO_3-N	NH_4-N	Total N	Cl	Na	K	S	P
[g/l]	32.39	30.69	63.08	35.97	28.29	24.24	5.77	4.30
	Ca	Mg	B	Zn	Cu	Fe	Mn	Mo
[mg/l]	1188.27	214.85	35.33	16.90	7.70	3.60	1.05	0.67

Due to the high ammonium content in nitrified urine (Table 1), ammonium nitrogen concentration was initially set higher than the optimum in all treatments (26% of total nitrogen for the control treatment, and 25%, 30%, 33% and 39% of total nitrogen with increasing Aurin concentration). Two samples of each tank were taken weekly and filtrated. Nutrient (K, Na, S, P, Ca, Mg, Fe, B, Zn, Cu, Mn and Mo) and dissolved nitrogen (NO_3-N and NH_4-N) concentrations were analyzed via ICP-OES and continuous flow analysis (CFA) respectively as described by Dannehl et al. (2015) and modified by Suhl et al. (2016). The chloride (Cl^-) concentration was determined with a hand-held spectrometer (Hach Lange, Düsseldorf/Berlin, Germany). The nutrient solution pH was adjusted to 6.0-6.8 with phosphoric acid and potassium hydroxide.

Lettuce seeds (*Lactuca sativa* var. *capitata* cv. Salanova 'Descartes RZ', Rijkzwaan, Netherlands) were sown on October 19, 2017 and after 15 days transplanted into rock wool cubes in the experimental setup (n=24). Mean air temperature in the Venlo greenhouse was 12.1 °C for the total duration of the experiment. Plant diameters were measured weekly. Leaf numbers were counted after transplanting to the greenhouse and right before harvest. Relative chlorophyll content was measured with a SPAD meter (Konica Minolta, Osaka, Japan) on seven occasions. Plants were harvested on January 22, 2018. Fresh weights were taken (n=23/24), and after measuring the leaf area some lettuce heads (n=7/8) were dried for 72 h at 105 °C to determine the dry weight of samples.

For mineral element and secondary compound analysis, four composite samples (n=4) per treatment were taken and frozen in liquid nitrogen, using the halves of four lettuce heads. Samples were freeze dried and ground to fine powder (Retsch, Haan, Germany). For Ca, Fe, K, Na, P and S analysis via ICP-OES and C and N analysis via an elemental analyzer, samples were prepared and analyzed as described in Dannehl et al. (2017), referring to Dannehl et al. (2015). Proline concentration was determined from 15 mg of the sample according to Bates (1973). Carotenoids and chlorophylls were extracted from 10 mg of the sample with 0.5 ml of MeOH THF solution (1:1 v:v, three times) and analyzed on an Agilent Technologies 1290 Infinity UPLC coupled with an Agilent Technologies 6230 TOF LC/MS using reference compounds (trans-β-carotene, cis-β-carotene, lutein, neoxanthin, chlorophyll a and chlorophyll b) as described by Baldermann et al. (2013). Phenolic acids and flavonoids were analyzed from 20 mg of the sample based on the method described by Mewis et al. (2011) and modified by Förster et al. (2015). Data were analyzed with SAS 9.4 software for Windows (SAS Institute Inc., Cary, NC, USA). The statistical model considered the block design with the factor nutrient solution and position along the channel as a factor of interference. For each parameter least squares means (LSMs) and standard

error means (SEMs) were estimated assuming homogeneity of variances (except for dry matter) and a Tukey test was performed with $p<0.05$.

3. Results

Average sodium and chloride concentrations, and thus EC value, increased with concentration of nitrified urine. Significant differences between treatments were observed for fresh weight, leaf number and plant diameter. For dry weight, dry matter and leaf area, no significant differences between treatments were observed (Table 2). The relative chlorophyll content in the highest concentration of nitrified urine measured just before harvest was significantly higher than the control treatment. Also, carotenoid and chlorophyll analyses revealed a significantly higher concentration of chlorophyll b and trans-β-carotene in the highest nitrified urine concentration (Table 2). Neoxanthin, lutein, cis-β-carotene, and chlorophyll a were also detected but showed no significant differences. Regarding phenolic acids and flavonoids, caffeoyltartaric acid, caffeoylquinic acid, caffeoylmalic acid, dicaffeoyltartaric acid, dicaffeoylquinic acid and quercetin-3-O-(6''-O-malonyl)glucoside were detected, but no significant differences were found between the treatments (data not shown).

Table 2. Least squares means of yield (SEM in brackets, different letters indicate significant differences between treatments regarding parameter in first column; Tukey's test $p < 0.05$).

		Unit	Control	31%	45%	50%	53%
Nutrient solution	Aver. Na^+ conc.	mg/l	47.6	69.1	76.6	81.8	84.0
	Aver. Cl^- conc.	mg/l	72.7	122.3	193.3	226.9	265.1
	Aver. EC value	mS/cm	1.98	2.11	2.22	2.31	2.33
Yield parameters	Fresh weight	% of control treatment	100^{a} (± 1.97)	98.18^{ab} (± 1.97)	91.63^{b} (± 2.01)	93.55^{ab} (± 2.01)	92.06^{b} (± 2.01)
	Dry weight		100^{a} (± 3.60)	96.89^{a} (± 3.60)	92.29^{a} (± 3.91)	90.39^{a} (± 4.29)	88.43^{a} (± 3.91)
	Dry matter		100^{a} (± 4.03)	92.55^{a} (± 0.36)	96.69^{a} (± 2.27)	97.76^{a} (± 3.81)	100.79^{a} (± 6.80)
	Leaf area		100^{a} (± 3.34)	94.14^{a} (± 3.34)	87.54^{a} (± 3.73)	86.24^{a} (± 3.73)	85.90^{a} (± 3.73)
	Leaf number		100^{a} (± 2.45)	90.02^{ab} (± 2.45)	84.93^{b} (± 2.64)	86.41^{b} (± 2.64)	87.61^{b} (± 2.64)
	Plant diameter		100^{a} (± 0.77)	98.02^{ab} (± 0.77)	96.54^{b} (± 0.79)	97.33^{ab} (± 0.79)	95.44^{b} (± 0.79)
Pigments	Rel. chlorophyll content	SPAD	25.4^{b} (± 0.25)	25.5^{b}	26.13^{b}	26.6^{ab}	27.63^{a}
	Chlorophyll b	µg/ g dw	4893.4^{b} (± 126.88)	4506.0^{b}	4712.4^{b}	4681.7^{b}	5600.4^{a}
	Trans-ß-Carotene	µg/ g dw	919.4^{b} (± 24.11)	929.4^{b}	893.6^{b}	924.2^{b}	1052.5^{a}

No significant difference was found for nitrogen, sulphate and iron concentrations in plant tissue. However, carbon concentration significantly increased with increasing concentration of nitrified urine and phosphorus showed a reverse effect (data not shown). Significant

differences were found between treatments regarding calcium, potassium and sodium concentrations and proline concentration (Figure 1).

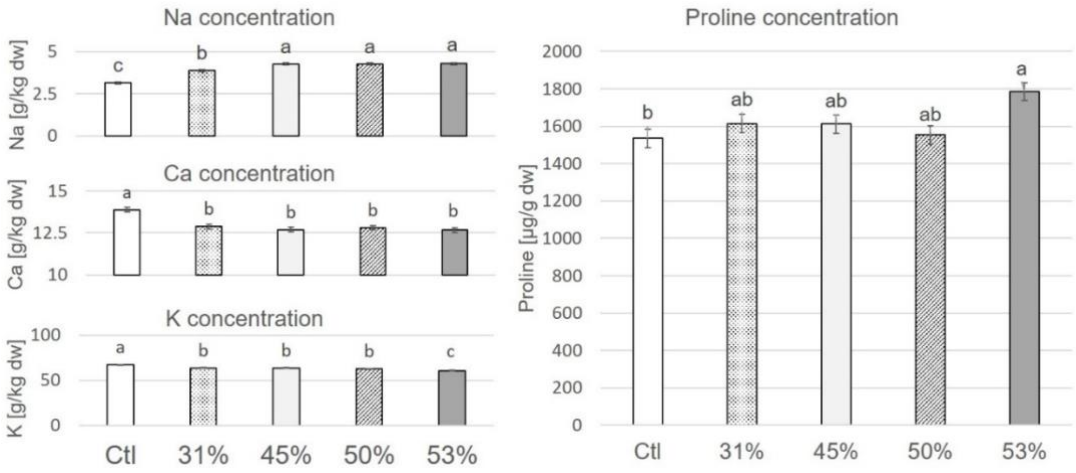

Figure 1. Na, Ca, and K concentrations (left, LSMs) in plant tissue and proline concentration (right, LSMs).

4. Discussion

The observed reduction of fresh weight with increasing concentration of nitrified urine is only of partial statistical significance. Yet, this parameter's result equals a lower marketable produce weight and is especially important for producers when choosing their nutrient solution. The reduction might be caused by osmotic stress and this result is in accordance with findings by Andriolo et al. (2005). Results for dry weight and other yield parameters do not confirm a significant decrease in biomass accumulation due to osmotic stress. Neither did we observe an increase in dry matter with increasing salinity as in Ünlükara et al. (2008). However, osmotic stress is confirmed by a significant proline accumulation with increasing concentration of nitrified urine. It appears, that the given combination of experimental conditions and nitrified urine concentrations only approaches the threshold for many yield parameters. This may be very different in summer times, when transpiration in leaf tissue is much higher. With increasing nitrified urine concentrations, sodium uptake was increased and accompanied by a reduction in potassium concentration in plant tissue. It can be assumed that this was caused by a competitive uptake inhibition on the transporter level. The reduced concentration of calcium, which is taken up passively by the plant, might be due to osmotic stress and a resulting reduction of transpiration. The aforementioned findings of our study regarding mineral content are in accordance with those of Bar-Yosef et al. (2005). A higher concentration of phosphorous in the control treatment plants can be attributed to higher doses of phosphoric acid, which was needed to adjust the pH level in the control solution (organic acids in urine). The significant increase in measured relative and absolute chlorophyll concentration with increasing concentration of nitrified urine along a simultaneous decrease of biomass requires more physiological experimental setups for clarification of reasons.

5. Conclusions

A total of 31% of nitrogen from nitrified urine was identified as a preliminary threshold for lettuce cultivar ‘Descartes RZ’ in a recirculating nutrient solution. No statistical difference was found between this treatment and the control regarding yield and quality parameters, except for calcium, potassium, and sodium concentration in plant tissue. These findings are to be confirmed in an experiment with a greater sample size.

6. Literature

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