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**Wood ash as a nutrient supplement for *Cucumis sativus* in
an anthroponics system**

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May 13th 2016

Malmö, Sweden

Abstract

Anthroponics is a recirculating soilless agriculture system that uses natural bacterial cycles to convert human biowaste such as urine into plant fertilizer.

A small experiment was conducted in Malmö, Sweden. The method involved building three anthroponic systems, collecting, and ageing urine samples from one healthy individual. The objective was to cultivate *Cucumis sativus* (cucumber) in the anthroponic systems under different dosages of wood ash to observe how it affected plant growth and the chemical composition of the water.

In the beginning of the experiment, System 1 received no amount of wood ash, System 2 received 37g of wood ash, and System 3 received 72g of wood ash. Afterwards, all systems were dosed with 165mL of aged urine every week. It was observed that wood ash supplied Systems 2 and 3 with a higher starting concentration of Potassium (150-200mg/L vs 20mg/L in System 1) and Magnesium (50mg/L vs 15mg/L in System 1), also affecting each system's electrical conductivity level. System 1 had considerably higher Ammonia levels, indicating insufficient nitrification, possibly due to lower pH values throughout the experiment. All systems had an increase in concentration over time of Nitrate, and an increase in concentration of Phosphorus, with the exception of System 1. All systems showed very low concentrations of Iron (<0,06mg/L) considering the optimal concentration for cucumber (~3mg/L). Results from the laboratory analysis also confirmed an Iron deficiency (0,00mg/L) in the water and leaf tissue, as well as a correlation between wood ash and higher amounts of Potassium, Magnesium and Sulphur in the water and leaf tissue. Some Arsenic was found in the water above the reference level (>0,01mg/L), though no definite conclusion could be drawn from its source.

In conclusion, while the experiment was too short to observe fruit growth, System 2 produced the longest vegetative growth, as well as a bigger weight of leaves. While Iron was found lacking both in the water and as Iron deficiency signs in plant leaves, it seems the addition of wood ash has supplied sufficient Potassium and Magnesium in the cucumber plants of System 2 and 3. It is possible that a dosage amount of metal-free un-treated and safe wood ash between the amounts used for System 2 (37g) and System 3 (72g), as well as additional Iron supplementation and pH control, can provide sufficient nutrients for a healthy production of a cucumber plant in an anthroponics system.

Keywords: Anthroponics, cucumber, human urine, hydroponics, nutrient recovery, wastewater treatment.

1. Introduction

Anthroponics can be defined as a recirculating soilless agriculture system that uses natural bacterial cycles to convert human biowaste such as urine into plant fertilizer. As stated in the author's report *Lactuca sativa production in an Anthroponics system* (Sanchez¹, 2015), the main processes behind anthroponics include Ammonia volatilization from urea and the aerobic nitrification of Ammonia to Nitrate. This report is, thus, a continuation of previous experiments on the optimization of anthroponic production (Sanchez², 2015).

The present report has the goal to test different dosages of wood ash and observe their effect in the development of the cucumber plants, both by analyzing water chemistry as well as observing deficiency signs. Wood ash was chosen as wood is a renewable fuel, and the ash from its combustion contains a high concentration of nutrients such as Calcium, Potassium, and Magnesium (Misra *et al*, 1992). Wood ash has also been used successfully in trials alongside human urine for tomato growth in soil (Pradhan *et al*, 2009). It is therefore expected that the results show a clear difference in the amount of Calcium, Potassium and Magnesium between the system without addition of wood ash and the systems with addition of wood ash. Plant development, in terms of height from stem to the tip of the newest leaf and fresh leaf weight, is also expected to reveal a better development in the plants of the systems with the addition of wood ash. It is also expected that no significant metals are present in the water at the beginning and end of the experiment, as well as part of the leaf tissue of the cucumber plants.

The results will enable a greater understanding in the use of wood ash as a nutrient supplement in an anthroponics system, while understanding the ideal wood ash amount and its effect on the underlying anthroponic processes and plant development. The results might have interesting applications for home growers as well as for future scalable systems that use source-separated urine for plant cultivation.

The experiment took place at Hemmaodlat's office. Hemmaodlat is a Swedish Non-governmental organization located in Malmö, Sweden, with the goal of teaching hydroponic and aquaponic concepts. Helping with this experiment was bachelor student Verånika Trollblad, doing her bachelor thesis on this topic for her programme in Physical Geography at Lund University. Lund University also supported the cost of laboratory analyses on the water quality and tissue sampling in this experiment.

2. Materials & Methods

Three cycled anthroponic systems used in the author's report *Lactuca sativa production in an Anthroponics system* (Sanchez¹, 2015) were used to plant the *Cucumis sativus* seedlings (Figure 1). The overall materials such as containers, pumps, hoses and lights as well as the construction are detailed in the mentioned report. The seedlings were started in a mix of 30% coco fiber, 30% perlite and 30% vermicompost humus. The wood ash was sourced from a private house and was achieved by the combustion of birch (*Betula*) and ash tree (*Fraxinus*) wood.

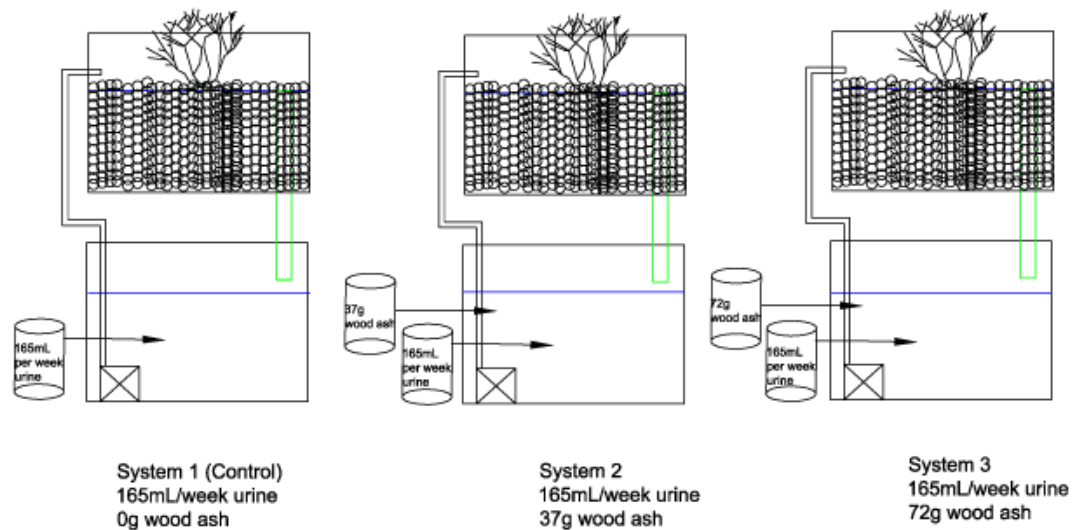


Figure 1: Experiment overview with the different systems.

More detailed information about the systems can be seen in Table 1. The urine was collected from a healthy individual and under no type of medication. The amount of urine used per week was 165mL per system, based on the conclusions of a previous report (Sanchez², 2015). The urine was stored in a jar containing crushed and dehusked watermelon seeds, and before its addition the urine had its pH monitored to ensure a value greater or equal than 9, rendering it safe for use (Sanchez³, 2016).

Table 1. Main parameters in the experimental anthroponic systems.

System	1	2	3
Total Water volume (L)	30-35	30-35	30-35
Pump flow rate (L/h)	650	650	650
SSA media (m²/m³)	250	250	250
Biofilter volume (L)	19,5	19,5	19,5
BSA (m²)	4,875	4,875	4,875
Aged urine (mL/week)	165	165	165
Wood ash (g)	0	37	72
Experiment duration (days)	28	28	28
Plant growing area (m²)	0,11	0,11	0,11
Number of plants in grow box	1	1	1
Plant species	<i>Cucumis sativus</i>	<i>Cucumis sativus</i>	<i>Cucumis sativus</i>

pH and Electrical Conductivity (EC) were measured with calibrated HM Digital waterproof PH-200 and calibrated HM Digital waterproof COM-100. Other parameters including Ammonia, Nitrate, Phosphorus, Potassium, Magnesium and Iron were measured with a HI Multiparameter Photometer and the respective test kits (Ammonia Medium Range HI93715A-0 & HI93715B-0, Nitrate HI93728-0, Phosphorus HI93706A-

0 & HI93706B-0, Potassium Medium Range HI93750A-0 & HI93750B-0, Magnesium HI93752A-0 Mg & HI93752B-0 Mg, and Iron High Range HI93721-0).

To control the pH to an acceptable range of 5,5 – 6,5 (Resh, 2005), a commercial solution containing 59% phosphoric acid (Hesi pH – minus BLOOM commercial solution) was used to lower pH, while slaked lime (Calcium Hydroxide) was used to increase pH.

The amount of wood ash to add for this experiment was calculated on empirical tests. To reach an ideal Potassium concentration of 350mg/L for cucumber production (Resh, 2005) for the amount of water in the systems (30-35L), different amounts of wood ash were weighed and mixed in tap water, followed by testing the level of Potassium in each solution. From this it was possible to calculate that around 37g of wood ash was enough to achieve a concentration of 350mg/L in a volume of water of 30-35L. As a way to observe if a higher amount of wood ash would result in significant improved cucumber plant development, it was decided to approximately double the amount of wood ash to 72g for the remaining system (System 3).

Water samples of around 300mL were collected from all systems before the addition of the cucumber seedlings (5th April 2016) and at the last day of the experiment (27th April 2016) and sent for laboratory analysis of its concentrations in Arsenic, Calcium, Cadmium, Iron, Potassium, Magnesium, Phosphorus, Lead, and Sulphur. All leaves from all cucumber plants were collected and dried, before also being sent for tissue analysis of the same parameters mentioned above. The analyses were made on the 10th of May 2016 by Sofia Mebrahtu Wisén of the Biology Institute of Lund University, at Ekologihuset in Lund, Sweden.

3. Results

3.1. On site results

Upon adding the wood ash, the water turbidity increased in the first few moments for Systems 2 and 3 (Figure 2), returning to a clear colour the following day.



Figure 2: Water colour in System 1 (left), System 2 (center), and System 3 (right) immediately after the addition of wood ash in Systems 2 and 3.

All cucumber plants experienced some vegetative growth during the duration of the experiment (Figure 3). The plants were measured from the base of the stem to the tip of the newest leaf, with the plant in System 2 having the biggest length (48cm) compared to the plants in Systems 1 and 3 (28cm each). The plant in System 2 also

had the biggest wet leaf weight (15g), that is, the fresh weight of all leaves in the plant, compared to the plants in Systems 1 and 3 (8g each).



Figure 3: Evolution of cucumber plants in the anthroponic experiment units. From left to right, the dates are: 4th April 2016, 12th April 2016, 20th April 2016, and 26th August 2016.

The analysis of pH (Figure 4), Electrical Conductivity (Figure 5), Ammonia (Figure 6), Nitrate (Figure 7), Potassium (Figure 8), Magnesium (Figure 9), Phosphorus (Figure 10) and Iron (Figure 11) levels gave some expected results, with the exception of Ammonia which seemed to indicate the Ammonia Oxidizing Bacteria in System 1 were not operating as optimally as in Systems 2 and 3.

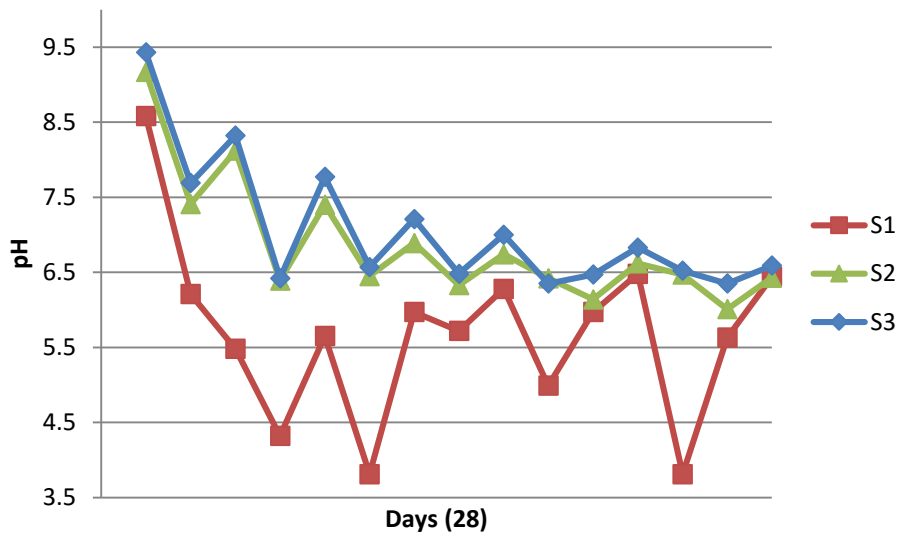


Figure 4: Evolution of pH levels over a period of 28 days in all three anthroponic systems. Target pH was in the range of 5,5 – 6,5. The date was not plotted directly in the X axis as some values were measured twice in the same day.

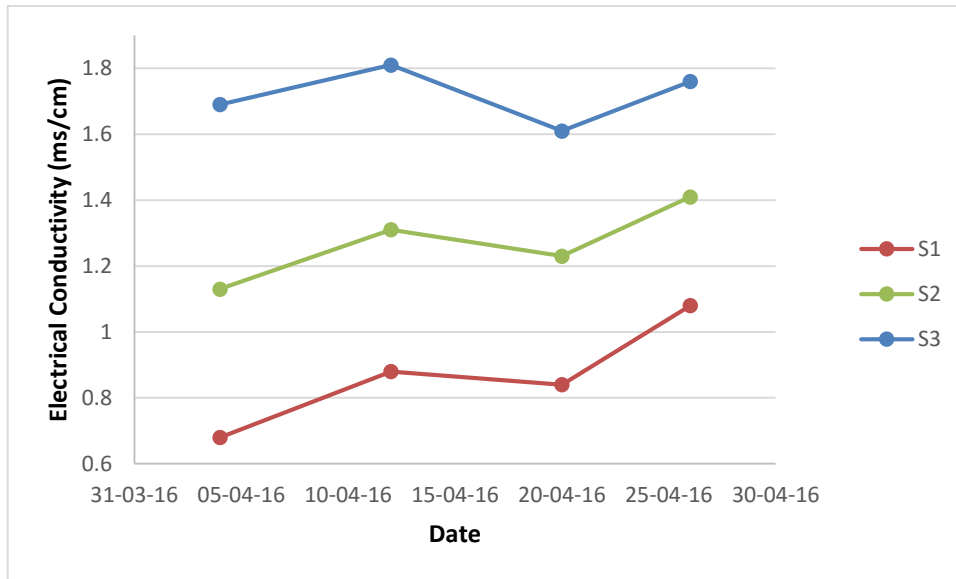


Figure 5: Evolution of Electrical Conductivity levels over a period of 4 weeks in all three anthroponic systems.

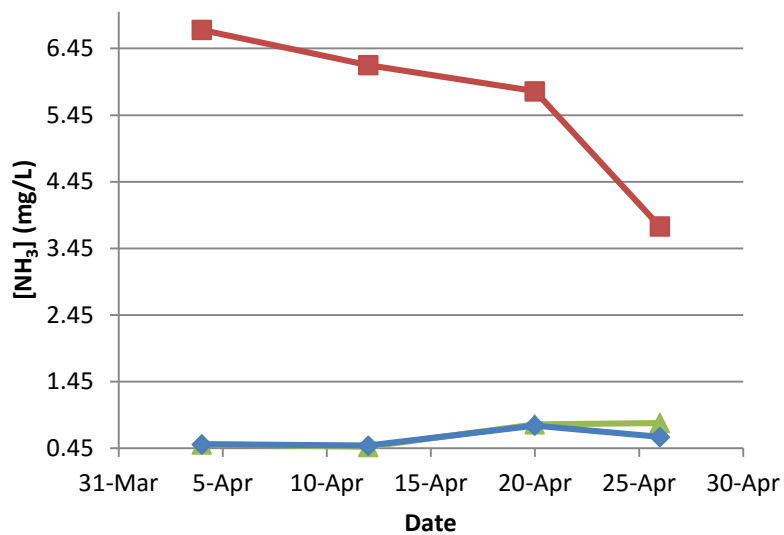


Figure 6: Evolution of Ammonia levels over a period of 4 weeks in all three anthroponic systems.

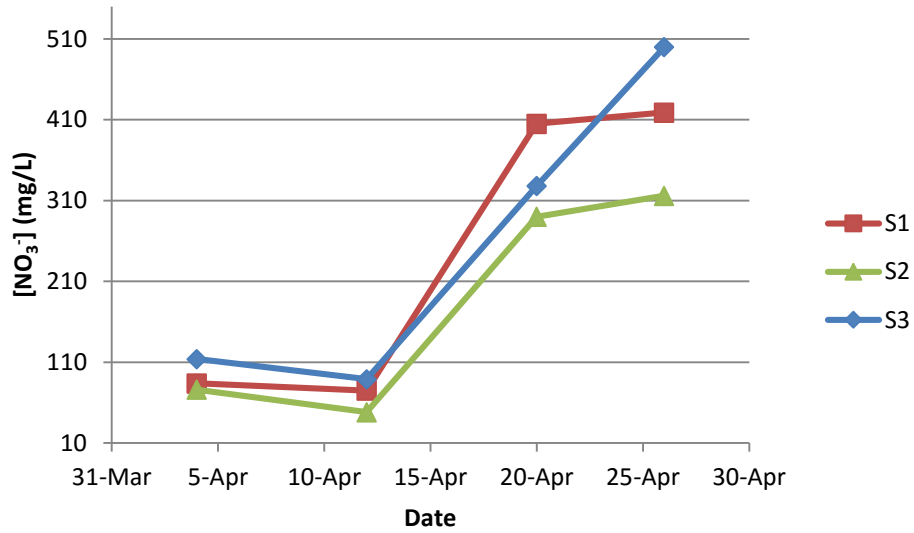


Figure 7: Evolution of Nitrate levels over a period of 4 weeks in all three anthroponic systems.

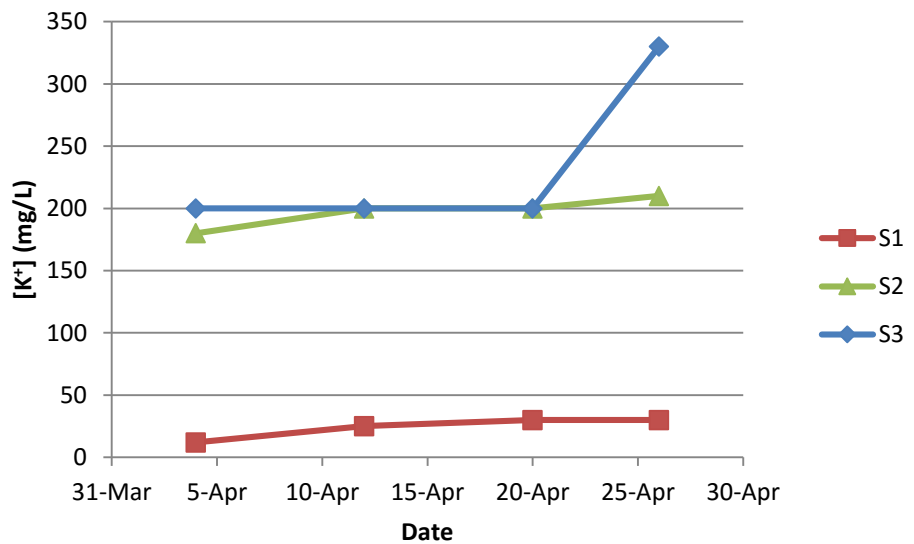


Figure 8: Evolution of Potassium levels over a period of 4 weeks in all three anthroponic systems. Values for Systems 2 and 3 during 12th of April and 20th of April were over the range of the HI Multiparameter Photometer (>200mg/L), so the following sample was prepared with a corresponding 20x dilution in the test done the last week (26th April).

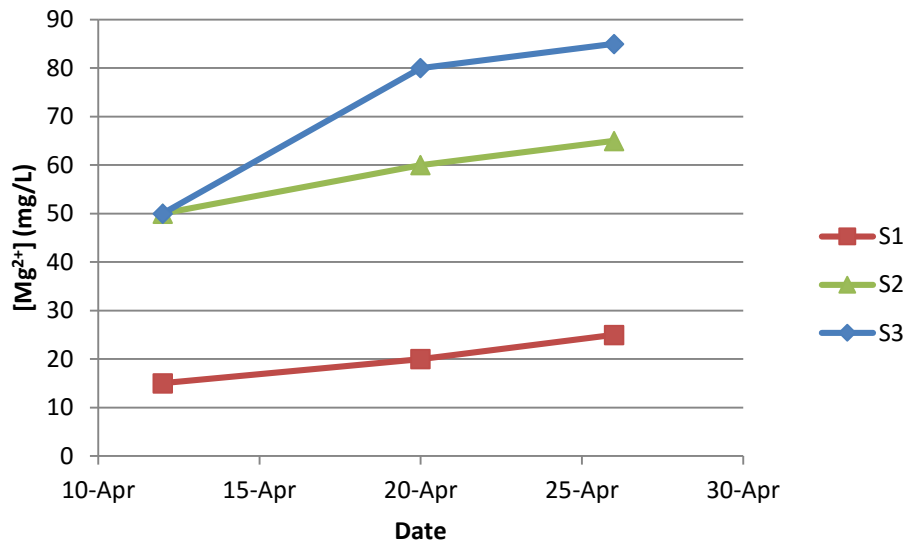


Figure 9: Evolution of Magnesium levels over a period of 3 weeks in all three anthroponic systems.

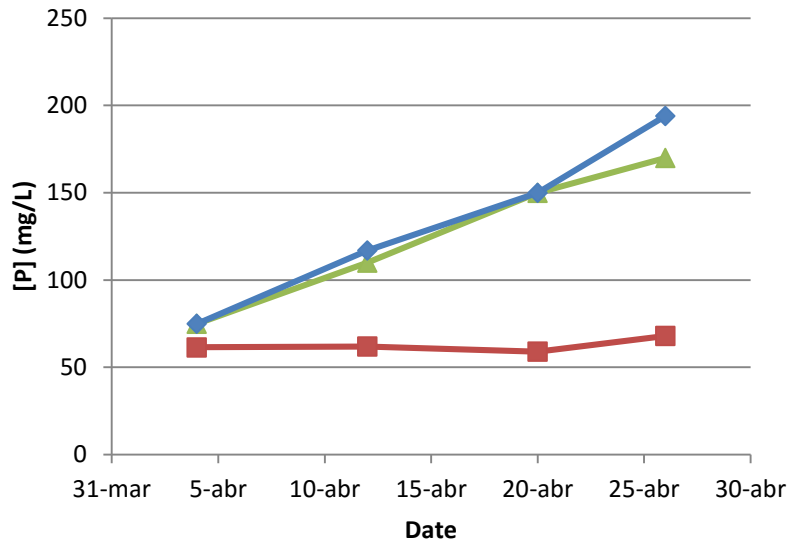


Figure 10: Evolution of Phosphorus levels over a period of 4 weeks in all three anthroponic systems.

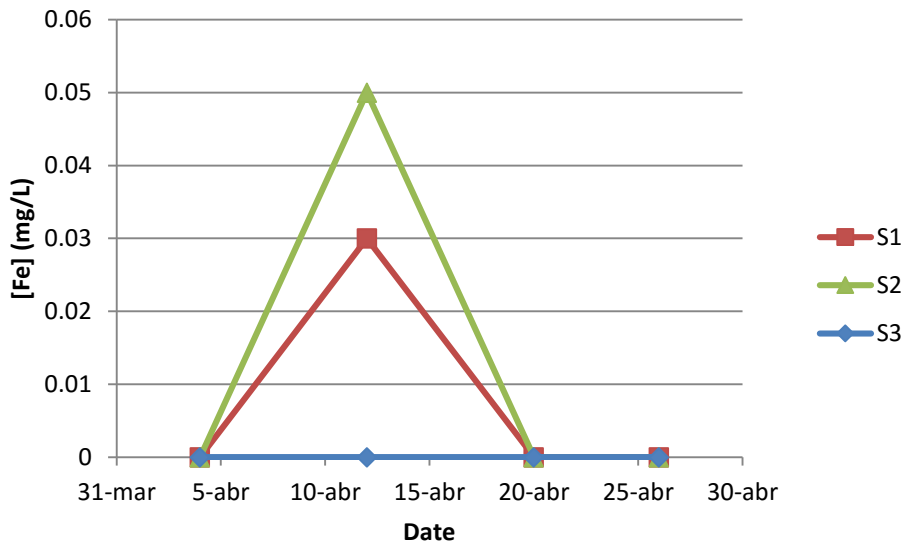


Figure 11: Evolution of Iron levels over a period of 4 weeks in all three anthroponic systems.

Some nutrient deficiency signs were observed during the experiment (Figure 12), particularly in the plants from Systems 1 and 3. These signs included necrosis of old leaves and yellowing of both old and new leaves.



Figure 12: Close-up on plants from System 1 (left), System 2 (center) and System 3 (right), showcasing some nutrient deficiencies. 26th April 2016.

3.2. Laboratory results

The results from the laboratory analysis at Lund University were received on the 10th of May 2016, comprising the dry leaf tissue sample analysis and water quality from the beginning and the end of the experiment. Parameters tested include Arsenic (As), Calcium (Ca), Cadmium (Cd), Iron (Fe), Potassium (K), Magnesium (Mg), Phosphorus (P), Lead (Pb) and Sulphur (S). The values of the tissue analysis were initially expressed in $\mu\text{g/g}$, but were then recalculated as a % so as to compare with existing reference values (Table 2).

Table 2. Dry leaf tissue analysis of cucumber leaves of all three systems after the experiment expressed as % with the exception of Iron, which is expressed as µg/g. The reference values presented refer to the deficient, and optimal values for greenhouse cucumbers (Haifa, 2014)

System	As (%)	Ca (%)	Cd (%)	Fe (µg/g)	K (%)	Mg (%)	P (%)	Pb (%)	S (%)
S1	0,00	3,24	0,00	78	4,14	0,88	1,28	0,00	0,73
S2	0,00	3,81	0,00	68,15	5,22	2,21	1,50	0,00	0,50
S3	0,00	2,27	0,00	63,66	5,08	1,90	1,00	0,00	0,39
Deficient reference values	-	<1.2	-	-	<2.35	<0.37	<0.47%	-	-
Optimum reference values	-	2,2-2,4%	-	85-300	3,2-4,5%	0,4-0,7%	0,6-0,9%	-	0,3-0,6%

Regarding the water quality results (Table 3), these were expressed in mg/L and were compared with optimal values for hydroponic cucumber production as well as maximum water quality values in the case of Arsenic.

Table 3. Water quality of all three systems before and after the experiment expressed in mg/L. The reference values presented refer to optimal hydroponic cucumber production (Resh, 2005) as well as maximum water quality values in the European Union (Persson, 2015).

System/Date	As (mg/L)	Ca (mg/L)	Cd (mg/L)	Fe (mg/L)	K (mg/L)	Mg (mg/L)	P (mg/L)	Pb (mg/L)	S (mg/L)
S1 5 th May 2016	0,0108	43,97	0	0	17,21	13,87	49,08	0	15,12
S2 5 th May 2016	0,0074	35,54	0	0	170,9	33,05	80,94	0	22,6
S3 5 th May 2016	0,0117	25,03	0	0	283,9	38,9	72,81	0	38,44
S1 27 th May 2016	0,0244	121,3	0	0	35,28	19,49	52,39	0	19,44
S2 27 th May 2016	0,0101	73,07	0	0	215,7	62,77	149,9	0	30,6
S3 27 th May 2016	0,0193	47,87	0	0	302,7	79,32	159,6	0	47,53
Optimal reference values	<0,01	200	-	3	350	50	50	-	150-200

4. Discussion and conclusions

4.1. pH & Ammonia

During the whole experiment it was necessary to control the pH in all systems. In System 1, there was a tendency for the pH to decrease as the nitrification process occurred. In Systems 2 and 3, there was a small tendency for pH to increase due to the addition of wood ash. It seems that the addition of wood ash effectively cancelled the acidification of the water due to nitrification. However, the usage of wood ash required the use of phosphoric acid to keep the pH level at an optimal range for cucumber plant.

It is likely that the lower pH in System 1 affected not only the availability of Ammonia, but also the nitrification process. Nitrification is done by Ammonia oxidizing bacteria (such as *Nitrosomonas*) and by Nitrate oxidizing bacteria (such as *Nitrobacter* and *Nitrospira*). *Nitrosomonas* have an optimum pH range of 6.0 – 9.0 (JGI, 2016), and their growth becomes inhibited at a pH below 6.0 (Fumasoli *et al*, 2015). *Nitrobacter* have an optimum development at a pH range of 7.6 – 8,2 (Grunditz & Dalhammar, 2001) with a lower limit at a pH of 6.5 (Boon & Laudelout, 1962). *Nitrospira* have an optimum pH range of 8.0 – 8.3 (Blackburne *et al*, 2007). On several occasions the pH level dropped considerably below 6.0, which would inhibit *Nitrosomonas*, and explain the high Ammonia levels in System 1 (>6mg/L vs <1mg/L in Systems 2 and 3).

4.2. Electrical conductivity

Electrical conductivity was relatively stable in all three systems, but with a clear distinction between each system. Since electrical current is transported by the ions in solution, the conductivity increases as the concentration of ions increases. In this case, a greater amount of ions was present in System 2 than System 1 given the addition of 37g of wood ash, and an even greater amount of ions was present in System 3 than System 2, since a greater amount of wood ash added. It is also visible that there was a slight tendency for electrical conductivity to increase over time in System 1, where a lower pH might have affected nutrient uptake by the plant, and therefore the amount of ions present in the water.

4.3. Nitrate

In all three systems there was an accumulation of Nitrate over time, suggesting either that more plants could be added in the same growing area, or that some other factor was limiting the plants' uptake of Nitrate.

4.4. Potassium & Magnesium

As expected, both Systems 2 and 3 started with a much higher concentration of Potassium (150-200mg/L vs 20mg/L in System 1) and Magnesium (50mg/L vs 15mg/L in System 1), due to the addition of wood ash. An overall trend of Magnesium accumulation in all three systems could indicate that the Magnesium present in urine alone might be sufficient to meet all the plants' nutritional requirements.

4.5. Phosphorus

Phosphorus levels started from a similar level in all three systems, indicating that wood ash did not supply any significant amount of Phosphorus. Over time, the concentration of Phosphorus increased in Systems 2 and 3, while remaining more or less constant in System 1. This could be due to the different pH values in the different systems affecting the availability of Phosphorus ions in the water, and prompting its reaction with other ions to form less soluble compounds.

4.6. Iron

All three systems had very low levels of Iron (<0,06mg/L), and plants in Systems 1 and 3 displayed some form of Iron deficiency signs in their leaves as they yellowed (Resh 2013). This yellowing makes sense as the low levels of Iron are far from the optimal concentration for cucumber growth (~3mg/L) (Resh, 2005). This seems to suggest that neither urine nor wood ash supply an adequate amount of Iron, an issue that must be addressed in future experiments.

4.7. Laboratory tissue sampling and water quality

Having both water quality concentrations and leaf tissue results enabled a comparison between the two to explain if a certain parameter had been absorbed by the plant or if it was not existent in the first place. Both by this comparison and the comparison with the reference values, it is possible to conclude that Arsenic levels are higher than the maximum recommended level, though Arsenic itself was not absorbed by the plant. An explanation for the Arsenic levels could be that Arsenic can be found in some types of wood ash, even those without any wood treatment. Values for Arsenic in un-treated wood ash can be as high as 31mg/kg (Solo-Gabriele *et al*, 2001). This would mean a total of 1,147mg of As in System 2 and 2,232mg of As in System 3, or around 0,03mg/L As in System 2 and 0,06mg/L As in System 3. While the observed values were far lower, it is not unreasonable to expect that the birch (*Betula*) and ash tree (*Fraxinus*) wood where the wood ash was sourced from might have contained some trace amount of Arsenic. It could also be possible that some trace amounts of Arsenic originated from the tap water or the individual's urine rather than the wood ash. However, there were no resources available to test these potential sources for Arsenic.

Other metals such as Cadmium and Lead had no reference information available, though the lack of any positive values in the water quality and leaf tissue indicates that no contamination had occurred. Iron, on the other hand, is an important micronutrient for cucumber, affecting the nitrogen metabolism in both roots and leaves (Borlotti *et al*, 2012), and was found lacking both in the leaf tissue as well as in the water, further confirming that neither urine nor wood ash adequately supply the required Iron.

While nutrients such as Calcium, Potassium, and Sulphur displayed concentrations below the optimal concentration values in the water analysis, such nutrients were all in the range or above the range for optimal reference values in leaf tissue, indicating that the plant had absorbed these nutrients and would likely not show any respective deficiency signs. The remaining nutrients (Potassium, Magnesium and Phosphorus) seemed to correlate with the addition of wood ash, as they were present in higher concentrations in the water of System 2 and System 3. Such correlation did not seem to be visible in the leaf tissue results, which could indicate that other factors affected the plants' absorption of these nutrients.

4.8. Conclusions

In conclusion, while the experiment was too short to observe fruit growth, System 2 produced the longest vegetative growth, as well as a bigger weight of leaves. While Iron was found lacking both in the water and as Iron deficiency signs in plant leaves, it seems the addition of wood ash has supplied sufficient Potassium and Magnesium in the cucumber plants of System 2 and 3. It is possible that a dosage amount of metal-free un-treated and safe wood ash between the amounts used for System 2 (37g) and System 3 (72g), as well as additional Iron supplementation and pH control, can provide sufficient nutrients for a healthy production of a cucumber plant in an anthroponics system.

Future experiments should attempt to optimize the growing process by neutralizing the basification effect of wood ash, as well as providing a sustainable source for Iron supplementation. Even though urine might be enough to grow low-nutrient demanding crops such as lettuce (Sanchez¹, 2015), finding the proper supplementation sources

will enable anthroponics to be used for a wider variety of crops. The source of wood ash is also of vital importance, and future experiments should test different sources of wood ash and test its toxicity in the water quality as well as in the leaf tissue of the plant.

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