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Human Urine

Can it be applied as fertilizer in agricultural systems?

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Title

Human Urine- Can it be applied as fertilizer in agricultural systems?

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Abstract

In cities today, vast amounts of nutrients are being wasted. Improvement in nutrient management within agriculture can contribute to a more sustainable society. Reusing nutrients in agriculture could aid in creating a more circular system, where organic fertilizers can be used instead of chemical fertilizers. Urine is a liquid which has a high nutrient content. According to the Swedish environmental protection agency, human urine can replace mineral fertilizers, by using methods such as source separation, where urine is divided from faeces. This is a cheap, effective and sustainable fertilizer management system that can be easily achieved. In this study, urine fertilizers were compared with ecological and conventional fertilizers (NPK and cow manure). The study examined the effect of different urine fertilizers compared with organic and inorganic ones on plant growth, nutrient content, pH value and microbial growth. The plant growth experiment was carried out in the greenhouse facilities in Alnarp, Sweden. The results from the experiment show that cow manure has a better outcome when it comes to plant growth, but Aurin, one of the urine fertilizers, had the highest uptake of nitrate. Non-diluted urine had a stable result in all analyses. According to this study human urine is a fertilizer which can be used in crop cultivation systems, and can deliver good agricultural results.

Keywords
Ecosan, source separation, non-diluted urine, aurin, urine powder, organic fertilizers

Preface

This Master's degree project on urine fertilizers was an initiative of Sweden Water Research and was carried out at the facilities at the Swedish Agricultural University in Alnarp. I would like to thank my main supervisor Sammar Khalil, co supervisor Anna- Karin Rosberg and the research leader at Sweden Water Research, David Gustavsson for setting the prerequisites for the project. A special thanks to Sammar and Anna-Karin who have guided me though the project and have given me contribution of knowledge. I would also want to thank my mother how always supports me and guides me with good advice.

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Julia Filling

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1. Introduction

In cities today, vast amounts of nutrients are being wasted (Zhang et al., 2014). Linear structures are defined as structures where the product or the content is not reused. Today, nutrients are not recycled or reused, thus, the reserve of nutrients is greatly reduced. To obtain a more sustainable society, a change in its structure needs to be done in order for it to become more circular. The increasing fluxes of phosphorus (P) and nitrogen (N) from agroecosystems to the consumer, often ends up in wastewater and amongst its nearby recipients (Bonvin et al. 2015). The nutrients then become a problem instead of a resource. Historically, human excreta and waste was used in agriculture for crop production due to its high nutrient content. Unfortunately, urbanization has created a separation between agriculture and food consumption which has led to the disappearance of the local nutrient loop. In Sweden, human excreta and waste was replaced by mineral fertilizers during the 20th century, however farmers did not want to use human excreta anymore because of the "*yuck factor*" (Saifi & Drake 2008). Nutrients from excreta is flushed down the toilet-together with the faeces and urine to a wastewater treatment plant, where it is either directly let out to sea, or recycled in the form of biogas.

In Sweden today there exists an effective wastewater system, but this system still does not reuse nutrients, which is an important criterion for sustainable management (Hellström *et al*. 2000). According to Werner *et al*. (2003) ecological sanitation methods (EcoSan) can achieve sustainable systems because of their ability to recover nutrients in the wastewater. EcoSan does not favor one specific technique, but has the capability to mimic nature and work as a material- flow-cycle. One system that is based on this approach is source separation methods. Source separation methods are when urine is divided from faeces. By separating the urine from the excreta, one can save 20-40% of domestic water consumption, and can also treat the water depending on its content (Werner *et al*. 2003; Gardner 1997).

To treat and use urine as fertilizer is cheap, effective and sustainable. Urine is produced naturally in our bodies and works as a balancing medium for liquids and salts (Jönsson *et al*. 2004). This means that the amount of urine produced varies over time, depending on the person and circumstances. Factors such as height, weight and diet all have an effect on urine production. Urine contains the most nutrients in the urban waste fraction, and macronutrients such as nitrogen, phosphorus and potassium appear in high amounts (Jönsson 2001; Winker *et al*. 2009). Urine is therefore a plausible alternative as fertilizer. The Swedish environmental protection agency, called the Swedish EPA (2002), have concluded that human urine contains enough nutrients to satisfy crop needs, and can replace mineral fertilizers. In the future, wastewater treatment plants must consider recycling of nutrients. By collecting urine, 50% of the phosphorus, nitrogen, potassium and sulfur can be returned to nature, which would contribute to a more circular stucture (Swedish EPA 2002). In this thesis project, different urine treatments will be examined to see if they can replace conventional fertilizers.

1.1 Research Question

- Is urine an alternative to be used as fertilizer in crop cultivation systems?
- What effect does urine have on plant growth?

1.2 Aim and objectives

The aim of this study is to investige if urine fertilizers are an effective source to be used as conventional fertilizers in the cultivation system.

To establish this, following objectives have been set to fulfill the aim:

- Examine the effect of different urine fertilizers compared with organic and inorganic ones on plant growth and nutrient content in the plants
- Examine the effect of the different fertilizers on the nitrate content in the leaves
- How pH value in the different fertilizers affects the cultivation system
- Examine the effect of the different fertilizers on the microbial growth in the cultivation system

1.3 Hypothesis

Urine fertilizers can supply nutrients necessary for plant development and growth.

2. Background

2.1 Source separation

In recent years, demands to change wastewater systems have increased. With new improved systems, water usage can be radically reduced as conventional flush water toilets are replaced with dry toilets, vacuum systems and separation of domestic wastewater streams (Winker et al. 2009). Source separation toilets contribute to a more sustainable waste treatment. Separation of urine is a promising technique for the new improved wastewater systems (Tidåker et al. 2005). By reusing urine as a fertilizer the nutrient load is reduced and does not contribute to eutrophication in the same extent. The urine fraction in Sweden had approximately 36 000 tons of nitrogen and 3300 tons of phosphorus in the early 21st century, and is probably more today due to the population growth. These numbers can be compared with the total use of mineral fertilizer in agriculture during 2015/2016, which was 196 080 tons of nitrogen and 29 370 tons of phosphorus (Tidåker et al. 2005; SCB 2017).

2.2 EcoSan

EcoSan, or ecological sanitation, is sustainable sanitation systems and is a closed loop approach that aims for the prevention of pollution by reuse of nutrients and organic matter (Schmitt 2003 & Esrey et al. 2001). The approach does not refer to a specific technology, but is used to describe many different systems and technologies that strive to create nutrient loops (Schmitt 2003 & Jönsson et al. 2004). EcoSan addresses both water scarcity and better sanitation and is widely used and implemented in both rural and urban areas.

The sanitation systems are often designed to mimic nature in order to create nutrient loops which are as sustainable as those existing in nature (Jönsson et al. 2004 & Esrey et al. 2001). The systems can be placed in households, where both the water and nutrient cycle is favoured. (See figure 1.) The nutrient loop is benefited because the systems can convert the human waste to a resource, that can be used in agriculture or gardens to produce plants and food.

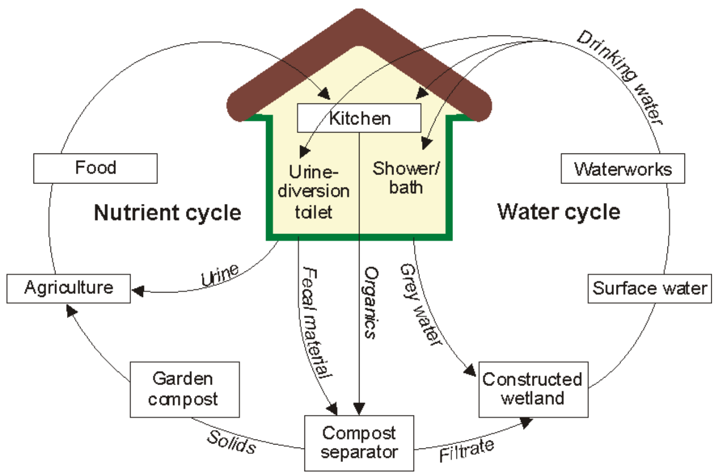

Figure 1: Nutrient loops according to Ecosan standards by Arno Rosemarin, Stockholm Environment Institute, 2003.

To address the water scarcity problem, and to benefit the reduction of polluted water, ecological sanitation minimizes water usage as much as possible. By having systems that minimize water usage, and treat waste, the UN millennium development goals about water supply and sanitation is benefitted (UN 2016). The UN goal is set to "*ensure access to safe water sources and sanitation for all"* (UN 2016). Ecological sanitation systems are made to achieve this goal together with providing hygienic services at a lower cost than conventional sanitation (Esrey et al. 2001). The target of halving the proportion of people without sustainable access to safe drinking water was met in 2010, five years ahead of schedule, but there are still billons of people that lack safe water sources. Water scarcity affects more than 40 percent of the global population, and the number is expected to rise even more (UN 2016). Sanitation is also a problem that still needs to be improved globally. 2.4 billion people lack access to sanitation systems, which leads to open defecation that pollute the waterways. EcoSan have an important role to play in this topic, if the system is used widly and adequately, it can contribute to better access to safe water sources and sanitation (Gensch et al. 2010). By implementing EcoSan in the form of source separation toilets in Sweden, we contribute to technologies that can be used worldwide, which can benefit sanitation, water usage and nutrient reuse.

2.3 Nutritional needs in agriculture

Plants need light, water, soil and nutrients to grow (Jönsson 2004). Plants also need a variety of different substances, and some of these comes from particles in the soil. The elements that are essential for plant growth are nutrients, and these can be divided in to two groups; macronutrients and micronutrients. Macronutrients, nutrients that have high uptake capability for the plant and are mainly taken up from the soil by the roots, are nitrogen (N), phosphorus (P), potassium (K), sulphur (S), calcium (Ca) and magnesium (Mg). Micronutrients are the nutrients that are taken up in very small amounts, but are as essential for plant growth as macronutrients. Micronutrients that are common in plant uptake are boron (B), copper (Cu), iron (Fe), chloride (Cl), manganese (Mn), molybdenum (Mo) and zinc (Zi). Some of the macro- and micronutrients appear naturally in the soil, and some we add with fertilizers (Palmstierna I 1993).

2.4 Agriculture today

In agriculture today, application of fertilizers is very common (Ranasinghe et al. 2016). The human population depends on fertilizers to produce the amount of food that is needed today.– With the increasing population and need for more food, the use of fertilizer is expected to increase by 60 million tonnes by 2050 (Kidd et al. 2017). According to Kidd et al. (2017) does fertilizers have a significant impact on the environment and ecosystem functions and services. Today, fertilizers have a low sustainability level due to problems like nutrient leaching, ground- and surface water contamination, eutrophication, loss of biodiversity and soil acidification (Kidd et al. 2017; Saifi & Drake 2016). The production of fertilizers is also a big environmental problem.

Fertilizers on the market have a high content of nutrients. Macronutrients such as nitrogen, phosphorus and potassium are essential for many plants, and therefore these nutrients appear in all fertilizers (Jönsson et al. 2004). Phosphorus is, for example, a non-renewable resource, and therefore it is of great importance to reuse nutrients, and fertilize in a sustainable manner.

The fertilizer market today is dominated by chemical fertilizers. This kind of fertilizer is very land use effective and can increase the production of crops (Carvalho 2006). Lately, this massive use of chemical fertilizers has been detected as an environmental problem (Camargo & Alonso 2006). The use of these different types of fertilizers cause serious contamination to aquifers, where nitrate is a big contributor to the decrease in water quality (Savci 2012). Water eutrophication and accumulation of nitrate have led to both water and air pollution, accumulation of nitrate can lead to air gases containing nitrogen and sulfur, which contribute to the greenhouse effect. Some chemical fertilizers have also raised problems of environmental contamination with heavy metals, where the metals not only accumulate in soil and plant system, but also in the waterbodies (Rutherford et al. 1995; Savci 2012). Chemical fertilizers, such as NPK, contains heavy metals like Hg, Cd, As, Pb, Cu, Ni, and Cu, which can be absorbed by the plant though the soil; they then enter the food chain, which can affect both humans and other animals (Savci 2012). Due to these problems, there is today a growing interest to use organic fertilizers in agriculture (Ranasinghe et al. 2016).

2.5 Organic fertilizers

Organic fertilizers originate from natural sources and are classified as an eco-friendly option (Muktamar et al. 2016). According to Muktamar et al. (2016) organic fertilizers are also more favourable due to its ability to achieve higher agricultural production compared to other fertilizers. Organic fertilizers are also vital for nature conservation, and by reusing organic matter we contribute to a nutrient cycle.

Livestock manure has been used as a fertilizer for centuries, but was largely replaced by chemical fertilizers when the population started to grow at a higher rate in the 19th century. Since the beginning of the 21st century, manure production has started to increase and the interest in organic fertilizers has been reintroduced (Sheldrick et al. 2005). Manure contains high levels of nutrients and is important for the maintenance of soil fertility (Oenema & Tamminga 2005).

According to Oenema and Tamminga (2005) livestock excrete around 100 Tg nitrogen globally per year, but only 20-40% is reused in agriculture. The reuse of nutrients is not fully realized globally, and according to research it has been proven to be a complicated process to change the current manure management systems (Oenema et al. 2007) New environment technology, and restructuring conglomerations of landless livestock production systems is needed, which require a lot of investments which are not prioritized today.

Another potential organic fertilizer on topic today is human urine. Urine is a liquid that is produced by the human body (Gensch et al. 2010). The liquid contains valuable macronutrients, which comes from the food humans eat. 80 percent of the total nitrogen, 66 percent of the total phosphorous and 80 percent of the potassium humans excrete is contained in urine (Esrey et al. 2001). Human urine has been tested as a fertilizer by many researchers today due to its low level of pathogens and good amount of required nutrients, such as nitrogen, phosphorous and potassium (Esrey et al. 2001 & Ranasinghe et al. 2016). The fertilizer is quick acting and can boost plant growth, but needs to be stored for at least one month before use, to minimize possible health risks (Gench et al. 2010; Jönsson et al. 2004 & WHO 2006). The well-balanced liquid has comparable production results with synthetic fertilizer, and does not rely on the finite supply of non-renewable resources that synthetic fertilizer does (Gensch et al. 2010). By using source separation of urine, a "closed loop" approach is done, and the nutrients from the urine is made into resources instead of becoming pollutants (Jönsson et al. 1997 & Esrey et al. 2001). Urine also has a low level of heavy metals, which is favoured by nature and the environment.

3. Methodology

3.1 Fertilizers

Different fertilizers were used in this project. Soil was used as control to compare the urine products. Cow manure and NPK were used as reference fertilizers to ecological, and conventional farming. The nitrogen, phosphourus and potassium content of the fertilizers is presented in table 1. Due to lack of information about other nutrients than N in all the fertilizers, it has been chosen in this study to only focus on the N content in the treatments.

In the experiment, all the fertilizers were applied before the spinach seed was planted. The powder treatments, wood ash with dried urine powder (AS) and biochar mixed with dried urine (DP), was mixed with the substrate before the substrate was wettened. The liquid fertilizers, non-diluted urine (DU) and Aurin (AU), was added after the substrate was wettened with water. The reason for applying the urine fertilizers before the seed was planted, was to get the best fertilizing effect and to avoid ammonia loss (Jönsson et al. 2004). Because that some fertilizers contained urine, it was chosen to only apply the fertilizer into the soil directly and only one at a time.

Non-diluted urine (DU)

In this experiment non-diluted urine was used. One household supplied urine for this study, and was collected in 2014-2015. The urine was stored for at least one year indoors, then at an outdoor storage with a lower temperature since.

Aurin (AU)

Aurin is a fertilizer developed by researchers from the “VUNA” research project (EAWAG 2017). Valuable nutrients are extracted from urine by a biological process whereby the urine is stabilized and the nutrients are bound. The liquid is later vaporized, leaving a high nutrient content fertilizer. From 1000 liters of urine, 50 liters liquid of fertilizer can be extracted. The product is recommended for use for flowers, ornamental plants and lawns.

Wood ash with dried urine powder (AS) & biochar mixed with dried urine (DP)

The treatments where dried urine was mixed with wood ash and biochar was made though experiments by SLU in Uppsala, Sweden. The ash was produced from birch wood that was sieved (<Ø 1mm) and thermally treated in 500 °C for 5 hours in a furnace. The biochar was produced through pyrolysis (450 °C) of chopped willow trees that was sieved to < Ø 1mm.

For the production, one liter of urine was concentrated with 30 gram of either ash or biochar mixture. The experiment was done 36 times, where they in each experiment added 30 milliliter urine in the same initial amount (30g) of biochar/wood ash. The products were then dried.

Cow Manure (CM)

Cow manure was used as a reference in this study. The cow manure is from the company Weibulls. The fertilizer is suitable for smaller plantations and for gardens. The cow manure is a natural fertilizer which is KRAV labeled and approved for organic farming (Weibulls 2014).

NPK (F)

NPK was also used as a reference in this study. NPK stands for nitrogen (N), phosphorus (P) and potassium (K), which is the three main elements in the fertilizer. NPK is a synthetic chemical fertilizer and is produced industrially. It can be used in a wide range of applications, and is used in many parts of the world (Jordbruksverket 2016).

Soil (S)

The control treatment was a nutrient poor soil. The soil was a substrate in all of the treatments, but was not mixed with anything in the S- treatment.

Table 1. The nutrient content in the used fertilizers in percentage.

Treatment	Nitrogen (%)	Phosphorus (%)	Potassium (%)
Aurin	4,2	0,4	1,8
Cow Manure	2	1,5	1,7
NPK	11	5	18
Soil	-	-	-
Wood Ash mixture	10	-	-
Biochar mixture	6,5	-	-
Non-diluted urine	1,1	-	-

3.2 Pot trials

In this study, a literature review and a plant growth experiment has been done. The plant growth experiment was carried out in the greenhouse facilities at the Swedish University of Agricultural Sciences (SLU) located in Alnarp. Spinach (Spinacia oleracea) was used as a model crop. The plants were planted in 1,5-liter pots with an area of 0,01 m^2. The following treatments were used: S (Control with only substrate, soil with low nutrient content), DU (Substrate mixed with non-diluted urine), AU (substrate mixed with Aurin), AS (Substrate mixed with wood ash containing dry urine powder), CM (Substrate mixed with cow manure), F (substrate mixed with NPK) and DP (Substrate mixed with biochar containing dried urine). Each treatment was used with four replicates using one seed in each pot. The duration of the experiment was four weeks.

3.3 Calculation of the amount of fertilizer

The calculation is based on the total nitrogen available during the growth period and the standard use of fertilizer for leafy green production per ha in Sweden. The calculation used in this project is as follows:

$$F_{ha} = \frac{N_{ha}}{N_{tonne}}$$ (Equation 1)

Where F_{ha} is the amount of fertiliser applied per hectare and N_{ha} is the amount nitrogen used per hectare. N_{tonne} is the available nitrogen in one tonne fertilizer.

$$F_{pot} = \left(\frac{A_{pot} \times N_{ha}}{A_{ha}}\right) \times 1000$$ (Equation 2)

Where F_{pot} is the amount of fertilizer added per pot (g), A_{pot} is the area of the pot and A_{ha} is the area in hectares.

In table 2, the results of the calculations is presented. The amont of nitrogen added depended on the results from the results.

Table 2. Amount of nitrogen to be available for plant uptake during the growth period and the amount of each fertilizer added per pot. The fertilizers are abbreviated as CM (cow manure), F (NPK), DU (non-diluted urine), AU (aurin), AS (Wood ash with dried urine powder) and DP (biochar mixed with dried urine).

	Fertilizer					
	CM	F	DU	AU	AS	DP
Total N (%)	2	11	1.1	4.2	10	6,5
Readily available N (% of N-tot)	10	100	100	100	100	100
Available N-tot growth period (% of N-tot)	0.8	10	0.11	4.2	15	15
Amount fertilizer (kg/ha)	12500	1000	9090.9	909.1	66.67	66.67
Fertilizer added (g/pot)	12.98	1.04	9.44	1.6	0.069	0.069

3.4 pH control

Initially, the soil pH was ca 5,5. With the different treatment this pH changed, but stayed in the interval between 5,3-6,7 except the non-diluted urine that had a mean value of 8,32. The pH control was made because a very high or low pH can affect the growth of the plants due to that the nutrients will not be available for the plant to absorb. The interval between 5,3-6,7 is optimal for the plant, so no pH change was made. The pH of 8,32 is in fact too high, but to change the pH out in the field, where the product will be used for commercial cultivation, is usually not done. To lower a pH on larger scale is not done today, so it was chosen to do the experiment without changing the pH so the experiment can be used as an initial fact basis for further research.

The method used for the control was the international standard ISO 10390:2005, IDT, which is an instrumental method. The control was done as follows 30 ml of the substrate (soil + treatment) was measured in a measuring cylinder, and dropped 5 cm above the table so it became more compact. The measuring cylinder was then filled up to 30 ml again and 150 ml distilled water was added. The samples were on a shaking machine for one hour. The pH measurement was done by using a pH-meter on the liquid in the mixture. Three pH measurements were done for each treatment.

3.5 Analyses

Plant growth

The plant biomass was determined at harvest through weight and leaf area measurements. Both fresh and dry weight of both leaves and root were measured. The leaf area was determined by a plant image analysis where all the leaves from all the treatment was measured.

Chlorophyll

Chlorophyll fluorescence is measured to give information about the state of photosystem II (PSII). The light energy absorbed by the chlorophyll molecules in the leaves can undergo three different fates depending on the changes in the efficiency of photochemistry and heat dissipation (Maxwell & Johnson 2000). The light energy can either be used to drive photosynthesis, excess energy as heat or be re-emitted as light.

To measure the photosynthetic performance, the leaves was put in the shade for 20 minutes, then exposed to a high intensity short duration flash. During the flash, the maximum fluorescence (Fm) was measured. This value was compared to the steady state yield of fluorescence in the light (Ft) and the yield of fluorescence in the absence of photosynthetic light (F0). The comparison between these values gave data about the efficiency of photochemical quenching and the performance of PSII (Maxwell & Johnson 2000). This method can give information to what extent PSII is using the energy absorbed by the chlorophyll, and to what extent it is being damaged by the excess light to the plant. By having this information, the tolerate level of environmental stress can be interpreted.

Nutrient Content

The nutrient content in both leaves and substrate was determined after harvest. All analyses were done at LMI laboratories in Helsingborg, Sweden. For the leaves, SAP analyses were used, and for the substrate, the Spurway method was applied. All replicates from the treatment were pooled before the analyses, resulting in one analysis per treatment.

Microbial Analyses

For the microbial analyses, the viable count method was applied. See figure 2. This method is used in cell culture to determine the number of living cells in a culture. The whole root was placed in 20 ml tubes, mixed with 9 ml of the detergent solution. The tubes were placed on a shaker for 20 minutes to separate the microflora from the roots. 1 ml of the suspension was then taken from each tube to dilution series with 9 ml Nacl in order to determine the bacterial and fungal concentrations in the different treatments. Tryptic soy agar (TSA) was used for enumeration of bacterial flora, and the dilutions 10^3- 10^5 was used. Malt extracted agar (MA) was used for enumeration of fungal flora and the dilutions 10^0- 10^2 was used.

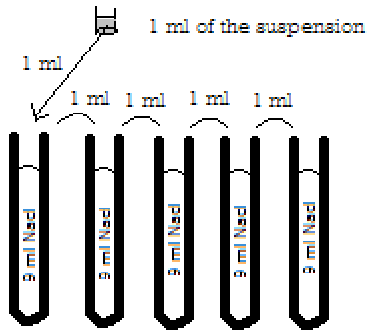

Figure 2. Dilution steps in the viable count dilution series. Steps 10^0-10^5 was done.

Statistical analyses

The statistical analyses were done with one-way ANOVA with 95% confidence. Statistical significant difference in the means of the treatments was determined with the Tukey Pairwise Comparisons method with confidence level.

4. Results

4.1 Weight

According to figure 3, the fresh weight of leaves from cow manure was significantly higher compared to soil, wood ash with dried urine powder, biochar mixed with dried urine and aurin. Aurin had the smallest and fewest leaves, and therefore also the lowest values. Non-diluted urine, wood ash with dried urine powder, NPK, biochar mixed with dried urine and aurin did not differ so much from the control (S), as the cow manure did.

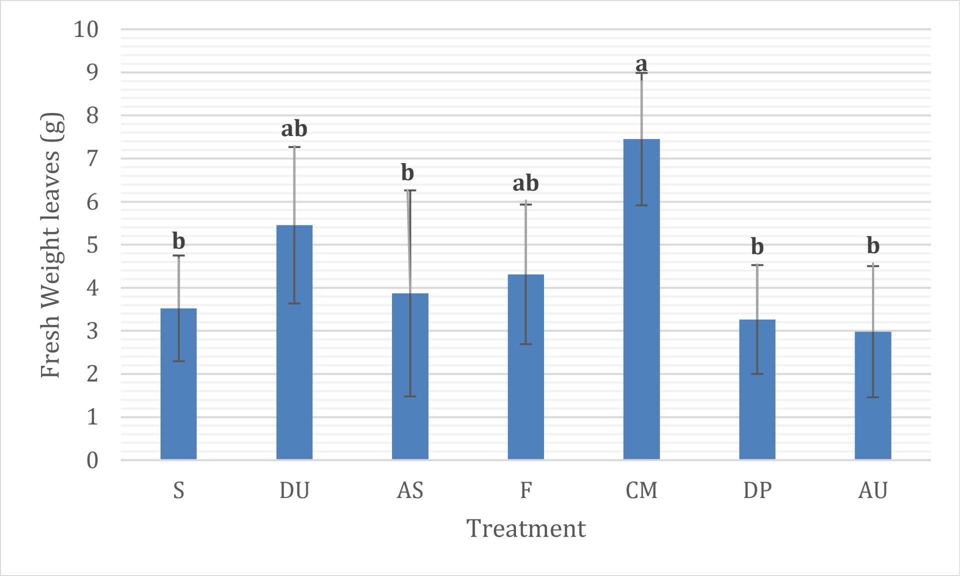

Figure 3. Fresh weight of leaves from the different treatments. Different letters at the top of each bar indicate significant difference between the treatments using ANOVA. Each bar represents a mean of 5-6 replicates. The treatments are abbreviated as S (control soil), CM (cow manure), F (NPK), DU (non-diluted urine), AU (aurin), AS (Wood ash with dried urine powder) and DP (biochar mixed with dried urine).

The fresh weight of the roots relates to the fresh weight of leaves when it comes to the treatment that had the highest weight. According to figure 4, cow manure had significantly higher values than NPK, aurin and wood ash with dried urine powder. The control was similar to non-diluted urine and biochar mixed with dried urine.

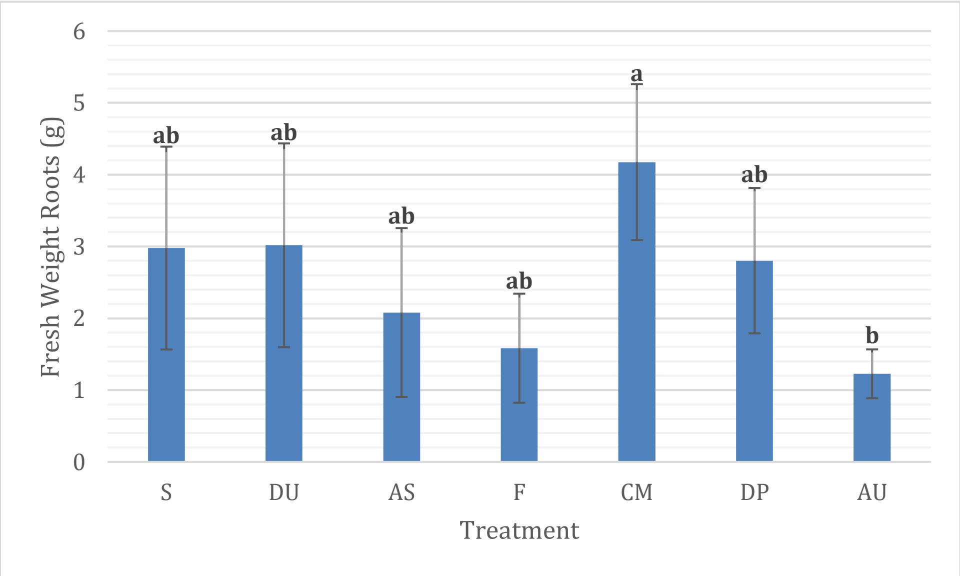

Figure 4. Fresh weight of roots from the different treatments. Different letters at the top of each bar shows that there is a significant difference between the treatments using ANOVA. Each bar represents a mean of 4-5 replicates. The treatments are abbreviated as S (control soil), CM (cow manure), F (NPK), DU (non-diluted urine), AU (aurin), AS (Wood ash with dried urine powder) and DP (biochar mixed with dried urine).

4.2 Leaf Area

The leaf area was measured to establish the biomass. The results presented in figure 5 show that cow manure had the biggest leaf area, followed by non-diluted urine. Aurin and biochar mixed with dried urine had the smallest area. There was a significant difference between cow manure, biochar mixed with dried urine and aurin.

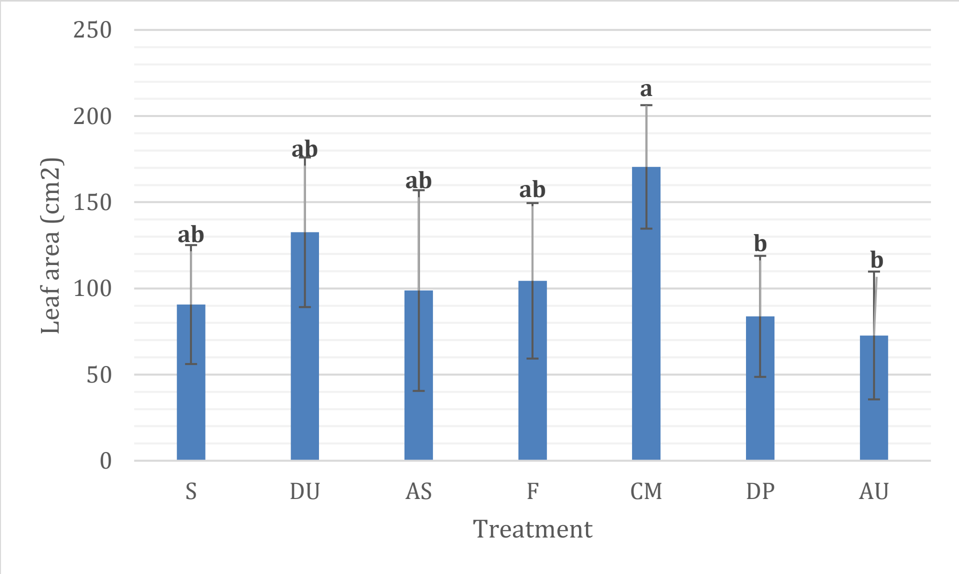

Figure 5. Leaf area from the different treatments. Different letters at the top of each bar indicate significant difference between the treatments using ANOVA. Each bar represents a mean of 5-6 replicates. The treatments are abbreviated as S (control soil), CM (cow manure), F (NPK), DU (non-diluted urine), AU (aurin), AS (Wood ash with dried urine powder) and DP (biochar mixed with dried urine).

4.3 Chlorophyll

Chlorophyll fluorescence was measured and results given in Fv /Fm. Fv / Fm has an optimum between 0.79-0.84. The results, presented in figure 6, shows that all treatments had a lower value than 0.79. Aurin was significantly different from the other treatments, with a mean value of 0,275.

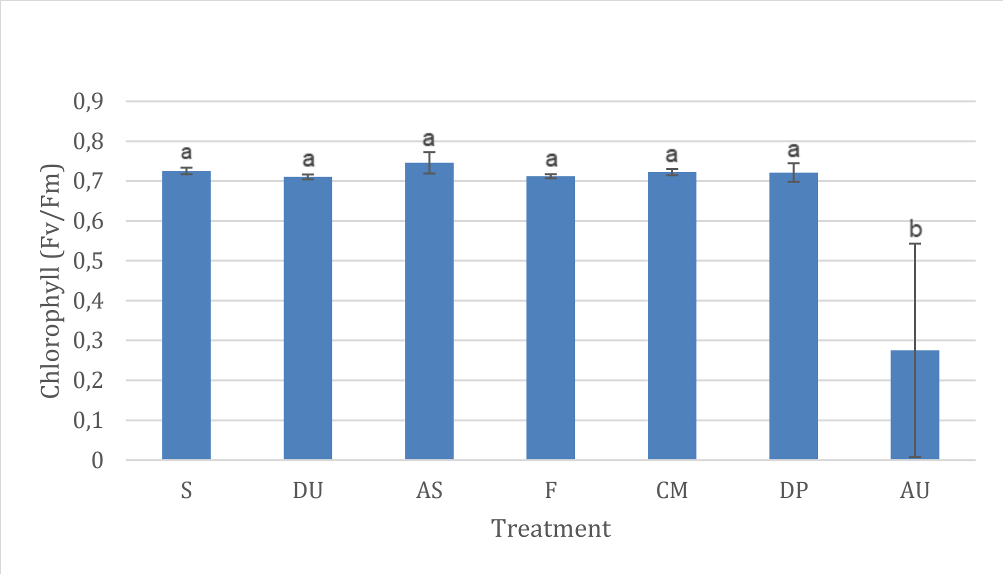

Figure 6. Chlorophyll results from the different treatments. The letter at the AU bar shows that there is a significant difference between the AU treatment and the other treatments using ANOVA. Each bar represents a mean of 3 replicates. The treatments are abbreviated as S (control soil), CM (cow manure), F (NPK), DU (non-diluted urine), AU (aurin), AS (Wood ash with dried urine powder) and DP (biochar mixed with dried urine).

4.4 Nutrient Content

In table 3, nutrients contained in the leaves is presented. According to the results, the leaves that received the cow manure treatment had most nutrients, except for Manganese and Sulfur. Wood ash with dried urine powder had the least amount of nutrients in almost every group.

Table 3. Micro- and macronutrients in the leaves after the experiment. The treatments are abbreviated as S (control soil), CM (cow manure), F (NPK), DU (non-diluted urine), AU (aurin), AS (Wood ash with dried urine powder) and DP (biochar mixed with dried urine).

Nutrients (mg/l)	Treatments						
	CM	AU	DP	DU	AS	F	S
P	970	790	590	670	590	820	630
K	9900	6900	6900	6800	6900	7400	6400
Mg	950	740	580	630	590	620	560
S	160	150	86	100	99	110	85
Ca	12	25	9.8	10	9.4	9	8.6
Mn	3.3	14	4.4	4.6	3.0	12	4.9
B	1.4	0.85	0.57	0.7	0.55	1.2	0.65

Cu	0.28	0.13	0.14	0.14	0.13	0.14	0.14
Fe	1.4	0.97	1.2	1.3	1.2	1.1	1.2
Zn	11	8.4	8.1	6.9	7.1	10	7.3
Si	29	20	20	23	21	20	21

The nitrate results in figure 7 shows that the plants with aurin as treatment had the highest uptake of N, followed by NPK and non-diluted urine. Cow manure had the lowest value in nitrate in the leaves.

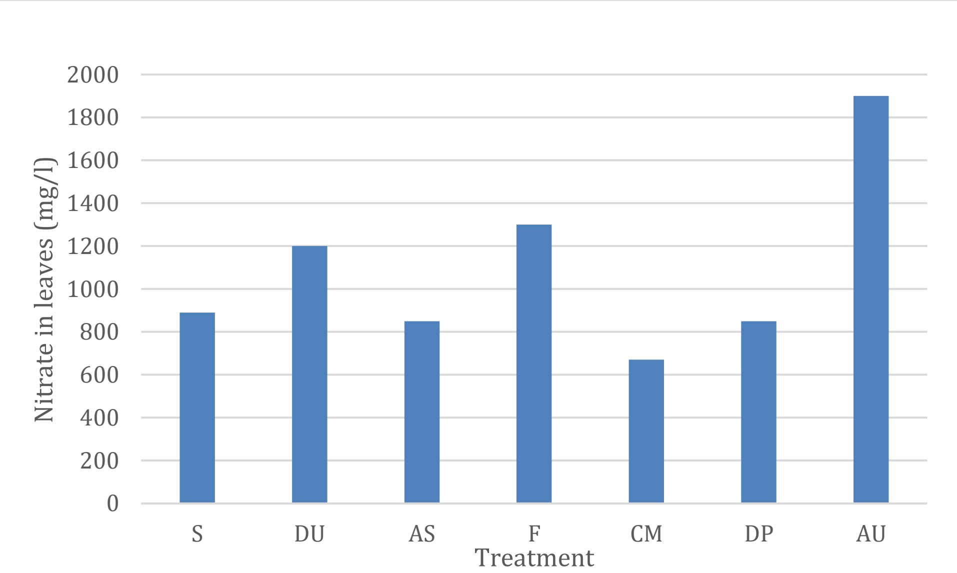

Figure 7. Concentration of N in the leaves after experiment. The treatments are abbreviated as S (control soil), CM (cow manure), F (NPK), DU (non-diluted urine), AU (aurin), AS (Wood ash with dried urine powder) and DP (biochar mixed with dried urine).

In order to determine how much nitrate and ammonium the leaves contained after the experiment, a leaf analysis was done. According to table 4, Aurin had most nitrate in the leaves, followed by NPK and non-diluted urine. Most ammonium had biochar mixed with dried urine, NPK and the soil treatment.

Table 4. The concentration in mg/l of nitrite and ammonium respectively in the leaves of plants treated with S (control soil), CM (cow manure), F (NPK), DU (non-diluted urine), AU (aurin), AS (Wood ash with dried urine powder) and DP (biochar mixed with dried urine).

Treatment	Nitrate (mg/l)	Ammonium (mg/l)
CM	130	1
AU	250	1
DP	120	2
DU	180	1
AS	130	1
F	220	2
S	150	2

The pH values, which are presented in figure 8, were after the experiment in the interval 4,6-5,5. Aurin registered the lowest pH with 4,6 and cow manure registered the highest with 5,5.

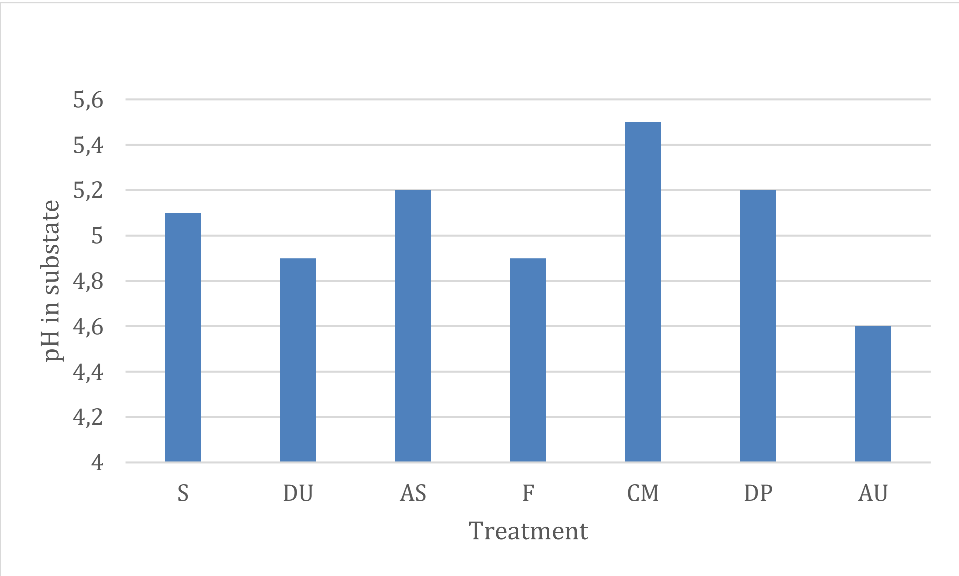

Figure 8. pH values in the substrate from the different treatments. The treatments are abbreviated as S (control soil), CM (cow manure), F (NPK), DU (non-diluted urine), AU (aurin), AS (Wood ash with dried urine powder) and DP (biochar mixed with dried urine).

The nitrogen content in the substrate had a lower value at the completion of the experiment, due to the plant having extracted nitrogen from the substrate. According to table 5, the NPK treatment plants absorbed the most nitrogen, followed by the biochar mixture and aurin.

Table 5. The percentage of nitrogen in the substrate of the different treatments, before and after the experiment. The treatments are abbreviated as S (control soil), CM (cow manure), F (NPK), DU (non-diluted urine), AU (aurin), AS (Wood ash with dried urine powder) and DP (biochar mixed with dried urine).

Treatment	Nitrogen in the substrate before experiment (%)	Nitrogen in the substrate after experiment (%)
AU	4,2	0,47
CM	2	0,44
F	11	0,44
S	-	0,43
AS	10	0,4
DP	6,5	0,34
DU	1,1	0,47

4.5 Microbial Analyses

In the microbial analyses, the concentrations of bacteria and fungi were determined. In the bacterial analysis, the result shows that cow manure and non-diluted urine had the highest amount of bacteria and aurin had the lowest, but there was no significant difference between any of the treatments. The bacterial concentrations of all treatments are presented in figure 9.

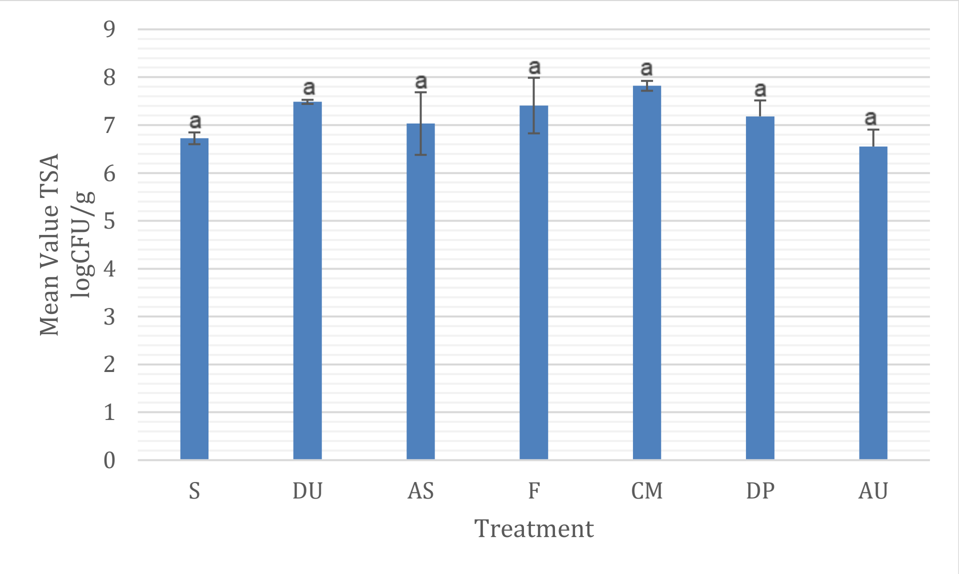

Figure 9. Amount of bacteria in CFU/g. The letters at the top of each bar shows that there is not a significant difference between the treatments using ANOVA. Each bar represents a mean of 2-4 replicates. The treatments are abbreviated as S (control soil), CM (cow manure), F (NPK), DU (non-diluted urine), AU (aurin), AS (Wood ash with dried urine powder) and DP (biochar mixed with dried urine).

The fungi concentrations are presented in figure 10. The results show that Wood ash with dried urine powder had most fungal colonies and biochar mixture the fewest. Overall, there is no significant difference between the treatments.

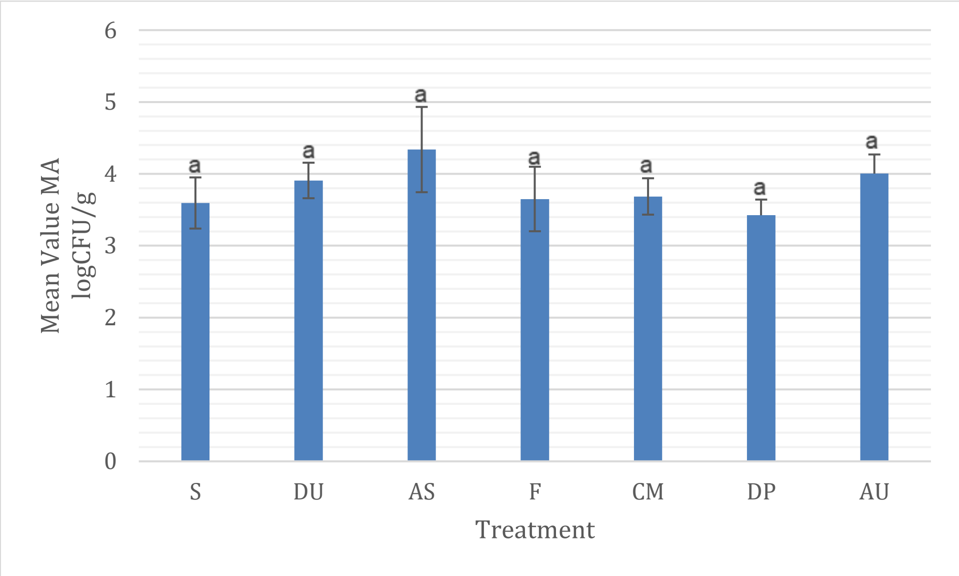

Figure 10. Amount of fungi in CFU/g. The letters at the top of each bar shows that there is not a significant difference between the treatments using ANOVA. Each bar represents a mean of 2-4 replicates. The treatments are abbreviated as S (control soil), CM (cow manure), F (NPK), DU (non-diluted urine), AU (aurin), AS (Wood ash with dried urine powder) and DP (biochar mixed with dried urine).

5. Discussion

To have a closed loop system in agriculture is important because the management today is unsustainable. The nutrients used today comes from non- renewable sources, which will lead to that the nutrients will peter out. By using excreta instead of chemical fertilizers, such as NPK, the agriculture and the environment will be benefit (Andersson 2000). Chemical fertilizers do not have all the substances that plants need; they often contain only nitrogen, phosphorus and potassium, which are only the most essential nutrients a plant requires. NPK does not contain any organic material either, which leads to a drop in the humus content amongst fields. Looking at the human aspect, chemical fertilizer can cause air, soil, and water pollution, which can pose health risks (Savci, 2012). Water pollution is mainly caused by nitrogen that enters the water body by leaching, drainage or flow from agricultural activities (Camargo and Alonso, 2006; Savci 2012 & MISTRA 2004). Nitrogen pollution is a severe problem that affects both drinking water and the aquatic ecosystem.

One solution to this problem is to use cow manure. Cow manure displays satisfactory results in this study and has good potential to replace chemical fertilizers. The problem with cow manure is the large-scale breeding of cows, where the animals produce more manure than agriculture can receive. Nutrients escape both into the waterways, which leads to eutrophication, and into the air in the form of atmospheric nitrous oxide, which is one of the biggest greenhouse gases (Davidsson 2009),

An effective way to avoid problems derived from cow manure, is to balance the agricultural system. Large-scale breeding can be reduced and combined with other sources, such as human excreta.

5.1 Use of human excreta

A 6-year program financed through MISTRA, was initiated in Sweden with the vision: "*Every human being has a right to clean water. For urban areas, our vision is water management where water and its constituents can be safely used, reused and returned to nature"* (Malmquist, 1999). To this vision, urine separation is included, where the urine is reused and returned to nature as fertilizer. Looking at all urban waste fractions, urine is the one substance containing the most nutrients (Jönsson 2001: Esrey et al. 2001 & Ranasinghe et al. 2016). In this study it was also confirmed that non-diluted urine shows good results when it comes to microbial activity, leaf area and weight. The non-diluted urine also had good chlorophyll fluorescence results, compared to the other treatments. This means that non-diluted urine is a good complement to cow manure. It is an available, renewable and sustainable solution, that will benefit the environment, and therefore also humans.

5.1.1 Pros and Cons with urine

Stored urine is an unstable solution, prone to nitrogen losses because of ammonia volatilization, which can lead to strong smells, negative effects on the environment and a lower fertilizer value if it is not handled correctly (Larsen et al. 2013). A lot of research has been done on the subject, and today there is technology available to produce effective P and N fertilizers from source-separated human urine. The stored urine can be stabilized by adding citric acid, but creates negative aspects due to the high-water content, which in turn means high transportation costs. This ultimately affects demand for the product. Stored urine can also contain undesired constituents such as pathogens and micropollutants, which can pose a health risk for farmers. However, source-separated human urine has lately been proven to only have a few pathogens, and is therefore not a high risk for farmers (Pradhan et al. 2007).

Human urine can be considered a good fertilizer in plant production due to its high nutrient content, but also new technology which can remove the negative aspects of the liquid (Ranasinghe et al. 2016; Pradhan et al. 2007). If all urine excreted by humans is collected and recycled, one fifth of the global P demand could be satisfied (Mihelcic et al. 2011). Urine could also by itself be a potential nitrogen fertilizer substitute for agricultural production. 90% of nitrogen is excreted in urine by humans, which make urine a good asset for agricultural purposes (Ranasinghe et al. 2016).

To use urine as a fertilizer and spread it to soil has also been proven to have less impact on nearby waters than conventional fertilizer techniques (Tidåker 2005). Agricultural systems that use human urine have many environmental benefits if the technique is used correctly. It leads to less risk for eutrophication in nearby waterways and less impact on global warming compared to mineral fertilizers. Also, by reusing nutrients in the urine, nutrient cycles can be closed. Today large amount of energy on finite fossil resources are used to produce conventional fertilizers (Werner et al. 2003). One example is that phosphorus reserves will be gone in about 60 years at the present rate of consumption. By instead starting to look at our waste as a resource, we can keep the reserves and start working on a circular economy.

5.2 Outcomes

The F treatment was not significantly different from the organic fertilizers, but cow manure and non-diluted urine showed better results in growth. In general, cow manure had the highest uptake and availability of nutrients. This can depend on several factors, like PH, sunlight exposure and root morphology.

5.2.1 Weight and leaf area

Leaf area measure is vital in monitoring growth in plants (Gobron, 2009). The growth of the plant is measured in biomass and shows how well the processes in the plant are working, such as photosynthesis, transpiration and rain interception.

The results for this experiment shows that the leaf area of the cow manure treatment, together with the fresh weight of the leaves, had the highest biomass, followed by non-diluted urine. According to Liu et al. (2014) inorganic fertilizers have the effect to contribute to shorter leaves, while organic fertilizers have longer and wider leaves. This often associates with the total nitrogen concentration in the soil. The urine treatments had various results when it came to growth. Aurin showed unsatisfactory results compared to the control, wood ash with dried urine powder and biochar mixed with dried urine showed similar results to the control and non-diluted urine showed satisfactory results when it came to leaf weight and area. According to these results, the processes favor the cow manure and non-diluted urine treatment, which are organic fertilizers. Even though the other urine fertilizers did not have longer and wider leaves than the control, we can assume that organic fertilizers produce leaves with a higher biomass.

5.2.2 Chlorophyll

Chlorophyll fluorescence has an optimum between 0.79-0.84 Fv / Fm, and lower values indicate that the plant is stressed. The results show that all treatments had a lower value than 0.79. Aurin was significantly different from the other treatments with mean value of 0.275, which is a very low number. This result can indicate that the Aurin treatment can be stressful for the plant and damage the photosynthetic apparatus (Maxwell & Johnson 2000). All treatments were below optimum, but because these had similar numbers this can depend on other factors than the treatments, for example, sunlight exposure.

5.2.3 Nutrient uptake

The quantity of nutrient uptake to the plant by the roots depends on the soil volume, the volume of nutrients in the soil and the root morphology (Adler et al. 2009) According to the nutrient result, the cow manure treatment plants had the highest uptake of most nutrients, which can be linked to bacteria that is found together with roots. Naturally occurring bacteria have proven to stimulate growth, which can lead to the roots more easily is able to pick up the nutrients in the soil (MISTRA 2004). The bacteria result (figure 9) does not show what bacteria is active in the experiment, but it is assumed that the bacteria benefits the plant.

P and K uptake was best in the cow manure treatment, followed by NPK. All urine fertilizers had similar results. Findings from this study show that there was a good uptake of nutrients of the urine fertilizers, compared to the control.

Nitrogen

Nitrogen is the substance that is needed in the highest amount for the plant and is therefore the most limited nutrient for plant growth (Palmstierna I 1993; Jönsson 2004). Nitrogen differs itself from other nutrients, due to the fact that it is very mobile, which means it is not stuck in the soil and awaits to be taken up by the plants and organisms like other nutrients (Palmstierna I 1993). Nitrogen leaches through the ground down to the groundwater, or disappears in to the air in the form of gas. Nitrogen is taken up as ions of nitrate and ammonium by the plant. If the results would show that there was more ammonium than nitrate in the plant, the nitrification process would not have been successful. The soil can have low pH conditions, lack of oxygen or too little organic matter.

In the leaves, the concentration of ammonium was much lower in all treatments compared to nitrate. These results show that the nitrification process was successful. Nitrate is the primary nitrogen form plants take up, and nitrate is more likely to be stored in the leaf cell vacuoles by plants than ammonium (Benton Jr 2003; Van de Leij et al. 1998). The limit of nitrate in spinach in Sweden is 3500 mg/l (Förordning 1881/2006). According to figure 7 all treatments were under this limit. Aurin had the highest results of nitrate in the leaves, with 1900 mg/l, and had the highest nitrogen uptake. The uptake of N by plants is a key process in the global N cycle (Näsholm et al. 2009). It is also vital because it is a large component of chlorophyll and amino acids, which means that both the photosynthesis and proteins are dependent on nitrogen.

The uptake depends on the availability of nitrogen in the soli, the energy input and the metabolism of the plant (Näsholm et al. 2009). In this experiment, all treatments had the same prerequisites with same spinach seeds and energy input. The only factor different in the treatments was the fertilizer compounds, which gave different nitrogen uptake results. According to table 2 Aurin had less available N during the growth period than the majority of the other treatments. In the final results, Aurin had the highest nitrogen uptake in the leaves. This can be contingent on factors as transport mechanisms in the roots and bacterial and fungal availability, which depends on the fertilizer compound (Näsholm et al. 2009). The nitrogen results also indicate that the liquid urine fertilizers lead to higher uptake of nitrate. To use liquid urine fertilizers can therefore be favorable, because it spreads more easily in the substrate and makes the nutrients more available.

5.2.4 Microbial Analyses

The soil microflora plays a significant role in the soil ecosystem. Plant growth is strongly dependent on the microflora and has a key role in the nutrient cycle (Sylvia et al. 1998; Waksman & Starkey 1931). The microflora needs nutrients to grow, and therefore the nutrient content in the soil is important for microbial activity. In the bacterial analysis, the result shows that cow manure and non-diluted urine had the highest amount of bacteria and aurin had the lowest, but there was no significant difference between any of the treatments. That aurin had the lowest bacterial growth is probably because it had a high fungal growth (Waksman & Starkey 1931). The dominated fungal presence results in a low nutrient acquisition by the bacteria which affects the growth in the aurin treatment.

Often, high fungal activity is equal to healthy plant growth, but it can also mean damage to crops depending on the fungi species. In this project, it is assumed that fungi colonies are beneficial for plant growth. The most optimal is to have a high variation in the microbial community, with both fungal and bacterial colonies. This indicates healthy soil. According to the results, non-diluted urine had both a high fungal and bacterial activity.

6. Conclusion

According to this study, non- diluted urine can be used as a fertilizer in crop cultivation systems. The results show that cow manure has better outcomes when it comes to growth and that aurin had the highest uptake of nitrate, but non-diluted urine had stable results in all analyses. One alternative to only use non-diluted urine, is to combine cow manure and urine. By employing a combined system, where nutrients are recycled from both humans and animals, the nutrient cycle can be completed, and the results within agriculture will be as good as possible. With ongoing urbanization, we will gain more access to human urine and this should be seen as an asset. More urine divided toilets should be implemented in cities, so that both water- and nutrient scarcity issues could be minimized. Urine has a high level of plant fertilizing nutrients, contributes to a circular economy, reduces environmental pollution, and is therefore a good potential fertilizer in plant production.

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Appendix

Pictures of the experiment.