Thesis for the degree of Doctor of Philosophy

Urine processing for efficient nutrient recovery and reuse in agriculture

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Abstract

Recycling of nutrients between urban areas and farmland is a critical step towards an ecologically sustainable development. Human urine is the most nutrient-abundant part among the domestic waste components and urine separation has been proposed to achieve maximum recovery and recirculation of nutrients in Sweden. Several types of toilets and small-scale separation systems have been installed and tested. Storage and transportation of large amounts of urine, as well as spreading and hygiene, are the main obstacles in achieving system efficiency. The biggest problem today is reuse of anthropogenic nutrients on arable land.

The objective of this thesis was to evaluate and - if its possible - to optimise three different urine processing techniques:

- Volume reduction and nutrient concentration by partial freezing
- Recovery of nutrients as solid minerals, mainly as struvite
- Capacity and behaviour of zeolite, wollastonite and activated carbon as nutrient adsorbent materials in urine, and

to test plant availability of the resulting fertilizers in short-term pot trials on wheat under climate chamber conditions.

The results are presented in seven (I-VII) papers, which are the basis for the following conclusions:

- A volume reduction and nutrient concentration from human urine can be obtained by partial freezing. By these means more than 80% of both N and P can be concentrated in 25% of the original urine volume. Partial freezing can be used combination with struvite precipitation and adsorbtion.
- By addition of small amounts of MgO to human urine, struvite was obtained and identified as the main component. With struvite precipitation 98-100% of P, 22-64% of K and 2-5.6 % of Ca was recovered. C:a 25% of the N recovery is also due to struvite crystallization.
- Both natural zeolites (especially clinoptilolite) and wollastonite adsorbed ammonium rapidly, and the quality of the minerals, their grain size and the ion strength of the urine are important for the adsorption. Mineral adsorption used in combination with struvite precipitation could recover 64-80% of the N in laboratory tests. The N recovery from fresh and stored human urine was dependent upon both the amounts of MgO and zeolite added. The P recovery was 98-100% and mainly affected by MgO addition, but it was also affected by zeolite addition. Active carbon adsorbed N, reduced smell and colour, but did not adsorb P. Optimum combined recovery of N and P occurred at added concentra-tions of 0.1 g of MgO and 15-30 g of zeolite per liter stored and diluted urine. The optimal additions are dependent on the initial N and P concentrations of the urine.

In the tests on wheat (*Triticum aestivum* L.) the struvite/adsorbent mixtures showed better nutrient availability than the struvite alone, probably due to a synergistic effect between the struvite dissolution and zeolite ion exchange or active carbon release of nutrients. Both struvite, struvite/zeolite mixtures and struvite/active carbon mixtures acted as slow-release fertilizers. In tests with five substrates and five nutrient sources (two of them commercial fertilizers) the struvite/zeolite mixture from urine was similar to DAP and CaP as slow-release P-fertilizer.

Keywords: urine, partial freezing, struvite, zeolite, wollastonite, active carbon, mineral adsorption, nutrient recycling, *Triticum aestivum* L., fertilizer, sustainable, ecosan

Sammanfattning

Näringsämnenas kretslopp mellan stad och land är ett kritiskt steg på vägen mot en ekologiskt hållbar utveckling. Humanurin är den mest näringsrika delen av hushållens avfalls- och avloppsflöden. För att uppnå en maximal näringsåterföring från hushåll till åkrar har urinseparation föreslagits som uthållig avloppslösning i Sverige. Olika toalettyper och småskaliga sorteringssystem har installerats och testats. Lagring och transport av stora mängder urin (utspädd vattenlösning), spridning och hygieniska aspekter vid hantering har hindrat effektiva systemutbyggen. Det största problemet idag är bristande återföringen av hushållens näring till jordbruket.

Syftet för denna avhandling var att utvärdera och – om det är möjligt – optimera tre olika behandlingstekniker för sorterat humanurin:

- Volymminskning och näringskoncentrering med delvis frysning,
- Näringsåtervinning i fast form, som struvit,
- Kapacitet hos zeoliter, wollastonit och aktivt kol som näringsadsorbenter, samt

testa den återvunna näringens växttillgänglighet och beteende i olika substrat genom veteodling i klimatkammare.

Avhandlingen baseras på sju (I-VII) vetenskapliga artiklar innehållande följande kortfattade slutsatser:

- En volymminskning och näringskoncentrering från humanurin kan nås med partiell frysning. Härigenom kan mer än 80 % av näringen (N och P) koncentreras i 25 % av urinens ursprungliga volym. Partiell frysning kan kombineras med struvitfällning och mineraladsorption.
- Genom att tillsätta små mängder MgO i urinen bildades struvit som identifierades som huvudkomponent i fällningen. Med struvitfällning har 98-100 % av P, 22-64 % av K och 2-5,6 % av Ca återvunnits. Ca 25 % av N blev också återvunnen i struviten.
- Såväl zeoliter (speciellt clinoptilolit) som wollastonit adsorberade snabbt ammonium jonerna i urin, och mineralens kvalité, kornstorlek och urinens jonstyrka är viktiga faktorer för adsorptionen. Mineraladsorption använd i kombination med struvitfällning gav 64-80 % N-återvinning i laboratorietester. N-återvinningen från färsk och lagrad urin är beroende av tillsatta mängden MgO och mineral. P-återvinningen var 98-100 % och styrdes huvudsakligen av mängden tillsatt MgO, men de tillsatta mineralerna har också en positiv effekt. Aktivt kol adsorberade N, minskade urinens lukt och färg, men adsorberade inte P. Optimal kombinerad återvinning av N och P nåddes vid tillsatser av 0,1 g MgO och 15-30 g zeolit per liter lagrad och spädd urin (från separerade toaletter). Optimala mängder MgO och adsorbent är beroende av initiala koncentrationer av N och P i urinen.

I odlingstester med vete (*Triticum aestivum* L.) hade struvit-adsorbent blandningarna från humanurin en bättre förmåga att avge näring än bara struvit. Detta kan bero på en synergisk effekt mellan struvitets löslighet och zeolitens resp. aktiva kolets adsorptionsegenskaper. Både struvit och struvit-adsorbent blandningarna fungerade som långsamsläppande gödselmedel. I tester med fem substrat och med fem olika gödselkällor var struvit-zeolit blandningen från humanurin en lika P källa som andra långsamsläppande handelsgödselmedel som DAP och CaP.

List of papers

This thesis is based upon the following papers, referred to in the text by their Roman numerals. Published papers, submitted manuscripts and manuscript are appended.

I. Lind, B-B., Ban, Zs.,Bydén, S. (2001). Volume reduction and concentration of nutrients in human urine. *Ecological Engineering* 16, 561-566.

II. Lind, B-B., Ban, Zs., Bydén, S. (2000). Nutrient recovery from human urine by struvite crystallization with ammonia adsorption on zeolite and wollastonite. *Bioresource Technology* 73, 169-174.

III. Bán, Zs., Dave G. (2004). Laboratory studies on recovery of N and P from human urine through struvite crystallisation and zeolite adsorption. *Environmental Technology* 25, 111-121.

IV. Adamsson M., Ban, Zs., Dave G. (2003). Sustainable utilisation of human urine in urban areas – practical experiences. Peer reviewed paper published in Conf. Proceedings: 2nd International Symposium on ecological sanitation, Lübeck, Germany, April 2003, 643-650.

V. Ganrot, Zs., Dave, G., Nilsson, E. (2004). Recovery of N and P from human urine by freezing, struvite precipitation and adsorption to zeolite and active carbon. (Submitted manuscript)

VI. Ganrot, Zs., Dave, G., Nilsson, E., Li, B. (2004). Plant availability of nutrients recovered as solids from human urine tested on wheat, *Triticum aestivum* L. (Submitted manuscipt).

VII. Ganrot, Zs., Wallin, G., Dave, G., Nilsson, E., Li, B. (2005). Fertilizer value of nutrients (N and P) recovered as solids from human urine compared to two commercial fertilizers and two liquid fertilizers in five substrates tested on wheat (*T. aestivum* L.). (Manuscript*)*

Notes on the authorship:

Ban, Zs. and Bán Zs. is the same name and person, as well as Ganrot, Zs.

To my parents,
Sofia och Stefan Katona

with
deepest gratitude

”The important thing in science is not so much to obtain new facts
as to discover new ways of thinking about them.”
Sir William Bragg

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1. Introduction

1.1. Closing the nutrient loops in a sustainable society

Recycling of materials is essencial in a sustainable society, and some elements (like C, N and P) are more important than others for healthy ecosystem functioning. (Wolgast, 1993; Winblad, 1996).

1.1.1. Past and present solutions are not enough

Conventional forms of both centralized and individual sanitation systems are facing increasing criticism today. The current core problems are (Werner *et al.*, 2003):

- The discharge of organic and inorganic nutrients mixed with thousands of pollutants
- The discharge of hormones and medical residues
- Valuable drinking water is misused as carrier for nutrients and pollutants
- Potable water can only be provided with increasing technical and financial inputs today, due to the pollution and eutrophicaton of the freshwater reserves worldwide.
- Germs are rarely retained or destroyed in conventional sewage treatment plants.
- The valuable nutrients in wastewater are not recycled.
- The need for safe drinking water as well as wastewater treatment is big in many parts of the world.
- The investments, operating and maintenance costs of conventional treatment plants make them unsuitable as solutions for developing countries.
- Centralized sewage systems produce large amounts of sewage sludge.

The main disadvantage of conventional sanitation systems is their linear character. They represent a typical end-of-pipe solution transferring and aggravating a number of problems and turning valuable resources into pollutants. Furthermore, in flush-and-discharge systems a relatively small amount of human faeces is allowed to pollute a huge amount of water. In spite of this, flush-and discharge is universally regarded as the ideal option for urban areas. Almost without questioning it is promoted in cities and towns around the world, even in poor countries, where people cannot afford it and in arid areas, where there is hardly enough water for drinking (Winblad, 1996).

Attempts to develop sanitation infrastructures possessing recycling abilities were made in Sweden since the end of 80´s, especially with the construction of modern urine separation toilets. However, the brake-through for urine separating solutions requires that environmental costs of hygienic risk-elimination, processing and transporting residuals to agricultural areas, etc. will not exceed economical and environmental benefits. With the invention of water toilets and development and installation of subterranean gravity sewer systems sanitary problems were solved, but in a world of water resource scarcity and the growing need for ecologically sound farming systems these resources are lost, being discharged to water, as point discharges (from wastewater treatment plants), causing severe pollution in freshwater or seawater ecosystems (Jenssen, 1996). In addition to this, the agriculture sector is often fertilizer and pesticide intensive and irrigation-based. These two broken loops of conventional sanitation systems and agricultural practices means, that we are feeding more and more algae instead of feeding crops.

1.1.2. Connecting wastewater management and food production

The commercial fertilizers, which are intensively used in agriculture today, require fossil energy and mineral resources (Hodge *et al*., 1994). The current phosphate capacity will be utilized at its maximum production level in 10 years, and economically exploitable reserves are estimated to last ca 100 years from now (Steen, 1998). For nitrogen fertilizers the main production technology is based on the ammonia production with Haber-Bosch process (stripping from air) with a very high energy input. Moreover, artificial fertilizer production includes many different types of fertilizers for different crops and needs today, which requires strong acids and energy intensive processes (Hodge *et al*., 1994; Davis *et al*., 1999).

Then the nutrients are utilized in food production (crops, vegetables, fruit, meat and animal food products) some nutrients (like phosphorous) accumulate in the soil for longer periods, and its leakage is more latent and dependent upon agricultural methods, plant uptake and irrigation. Today, most of nutrients are 'lost' as wastes through food production processes, restaurants, trade, and as domestic waste in our household through kitchen waste, urine and faeces (Table 1).

Table 1. N,P,K budget calculations show that the following nutrients are lost as wastes.
The numerals represent procentual approximations. The table is taken from Jönsson (1994).

Type of waste	Nitrogen	Phosphorous	Potassium
From food production processes in industries and restaurants, etc.	8	10	15
From trade	5	4	6
Domestic (kitchen)	15	26	18
Urine	64	43	52
Faeces	7	22	9

If all nutrients from these wastes are recycled, 35-45% of commercial fertilizer use can be avoided with proper management. The urine alone, can substitute 20-25% of commercial fertilizer used today in food production for people and animal stock (Jönsson, 1994).

1.1.3. The ECOSAN approach

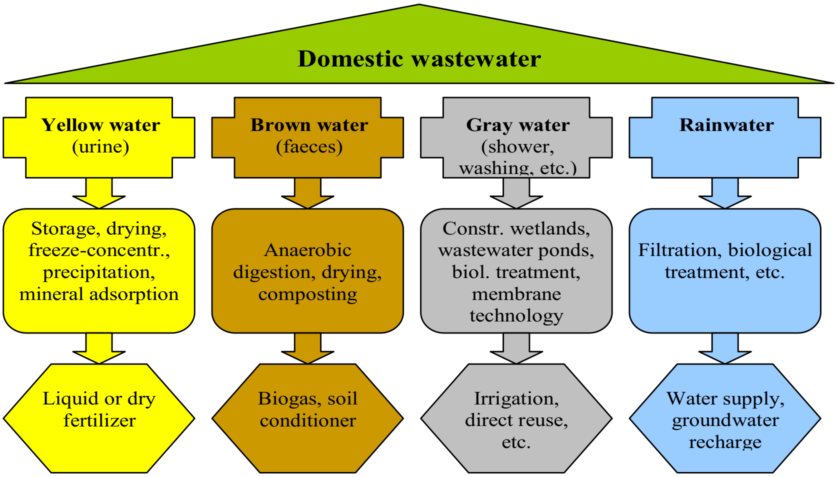
Ecological sanitation (ECOSAN) systems permit the complete recovery of all nutrients from faeces, urine and gray-water, benefiting agriculture and minimizing water pollution, as well as allowing economical use of water and its maximal reuse, particularly for purposes of irrigation. An even broader understanding of the term could also include the use, storage and infiltration of rainwater, treatment and recycling of solid wastes and utilization of the energy content of solid and liquid wastes. These approaches are based on the systematic realization of material flow oriented recycling processes spanning the full range from strictly low-tech solutions to high-tech solutions (Werner *et al*., 2003):

- Elements of ECOSAN system can range from compost latrines, dehydrating latrines with urine separating to complex, mainly decentralized solutions. These may consist, for example, of urine diversion toilets, vacuum transport systems and anaerobic or membrane treatment technologies, combined with near-nature gray-water (domestic wastewater without urine and faeces) treatment, e.g. in wetlands.

- Even if ECOSAN approaches chiefly comprise semi-centralized or non-centralized sanitation systems, they can - in urban areas - also be a combination of centralized sewerage system and sewage treatment plants. However, the treatment technology would then have to be oriented to render the effluent hygienically safe instead of to eliminate the nutrients, and the hygienically safe wastewater would then have to be used for agricultural irrigation (Larsen *et al*., 1996).
- ECOSAN systems offer appropriate and sustainable solutions for different circumstances and demands. They permit acceptable and affordable sanitation for poor and rural areas, as well as for high-income areas and industrialized countries.
- In the ECOSAN model excreta do not “disappear” into pipes, tanks or pits, and their recycling and reuse requires a different kind of engagement and awareness by the users. Holistic strategies for wastewater management and sanitation include direct links with agriculture (especially urban farming) and food security, health care, economics, urban planning and waste management in general.
- ECOSAN systems make the excreta flow in the natural direction, closing the nutrient loop and directing the hormones to soil, just as previously during evolution. Since the excreta are collected in small volumes, practicable and resource efficient sanitation methods can be developed. The hygienic standard of these systems can therefore be higher than that of the present flush-and-discharge system. Furthermore, these systems also increase the possibility of developing accurate treatments for the inactivation of pharmaceutical residues (Jönsson, 2003)

Thus, ECOSAN approaches aim not only at closing the nutrient cycles and rendering them safe, but also at closing local water cycles. It represents a new basic understanding of wastewater handling in which urine (yellow water) and faeces (brown water) and gray-water are not considered as pollutants but instead as useful resources. Yellow, brown, gray, etc. are the “new colors of ecological sanitation” (Otterpohl, 2003; Figure 1).

Figure 1. Possible ECOSAN systems with source-separation, treatments and utilization examples (modified figure after Werner, *et al*., 2003)



1.2. Human urine as a resource

1.2.1 Possibilities

The recycling and reuse of nutrients between urban areas and farmland is a critical step towards ecologically sustainable development. Human urine is the most nutrient-abundant part among the domestic waste components. However, urine makes less than 1% of the total domestic wastewater volumes produced per capita (Jönsson *et al*., 2000). Components of nutrient value in human urine are calculated and presented in Table 2.

Table 2. Some components of nutrient value in human urine (based on Altman and Dittmer: Biological handbook, 1974).

Nutrient compound	Amount excreted (mg/kg body wt day)	Amount excreted (g/ pers. day)*
N -total	215.00	15.05
P -total	12.00	0.84
K -total	34.00	2.38
Mg -total	1.35	0.10
S -total	16.00	1.12
Ca- total	5.75	0.40

* the body weight was taken for a 70 kg as average adult human body weight

Table 3 contains calculations (by Wolgast, 1993) of annually excreted nutrient amounts in human urine and faeces compared to the average fertilizer use to produce ca 250 kg grain (necessary to cover the calories and protein intake of an adult person during one year). An adult person excretes ca 500 l urine and ca 50 l faeces during a year.

Table 3. The nutrients in human excretion and the fertilizer need to produce 250 kg grain/year. (Source: Wolgast, 1993)

Most important nutrients	Urine 500 l	Faeces 50 l	Total	Fertilizer need for 250 kg grain
N	5.6 kg	0.09 kg	5.7 kg	5.6 kg
P	0.4 kg	0.19 kg	0.6 kg	0.7 kg
K	1.0 kg	0.17 kg	1.2 kg	1.2 kg
N + P + K	7.0 kg (94%)	0.45 kg (6%)	7.5 kg (100%)	7.5 kg

The nutrient balance of human urine is appropriate for cereal production. The food we need and metabolize is in balance with the ‘wastes’ we excrete as nutrients during a year and this is enough to produce the next years food (roughly calculated in kg grain). Nitrogen is mainly in form of urea, phosphorous as phosphates and potassium as ion, and micronutrients are also present in balanced form in human urine. Moreover, human urine is free from cadmium and other heavy metals (Palmqvist *et al*., 2003) and in a healthy person urine is sterile, when leaving the body (Table 4).

Table 4. Concentrations of some metals in urine and faeces compared with farmyard manure on organic cattle farms in Sweden (from Jönsson *et al*., 2004).

Material	Unit	Cu	Zn	Cr	Ni	Pb	Cd
Human urine	µg/kg ww	67	30	7	5	1	0
Human faeces	µg/kg ww	6667	65000	122	450	122	62
Cattle manure org. farming	µg/kg ww	5220	26640	684	630	184	23

During the past 20 years several types of toilets and small-scale separation systems has already been installed and tested (Palm *et al.,* 2002). Pilot studies and even direct applications (in some costal municipalities) had been started. Exergy analysis showed that the potential for phosphorus and nitrogen recycling is most effective in systems with urine separation (Hellström *et al*., 1997, 2003). A comparison of urine separation systems and conventional treatment systems showed that the main problem regarding efficiency in terms of exergy was related to management aspects such as storage and transport (Hellström, 1998). Other LCA-studies of urine separation systems showed that the transportation of large amount of urine as well as spreading and hygiene are the main obstacles in achieving system efficiency (Larsen *et al*., 1996; Jenssen *et al*., 1997; Kärrman, 2000). LCA studies made by Maurer *et al.* (2003) emphasized that specific attention is needed during spreading in order to quantify any ecological benefit or drawback. Furthermore, nutrient recovery is energetically more efficient than removal and new production from natural resources. This is valid for recovery and recycling of both P and N from urine either directly as liquid fertilizer or by producing struvite (a solid, slow-release fertilizer) (Maurer *et al*., 2003).

1.2.2 Problems

Introducing urine-separating toilets in small communities with people with high environmental awareness and willingness to separate and recycle is not a problem today. However, the acceptance of a new technique is not problem-free in public buildings such as exhibitions, working places, public toilets or lay-bys (motorway WC), where awareness and motivation is not high, and the toilet users or the owners' responsibility is unclear. New behaviour patterns are crucial for these systems to work (Wallin, 2002; Adamsson *et al*., 2003).

However, the biggest problem today is the last step before closing the loop; the reuse of anthropogenic nutrients on arable land. Few farmers in Sweden or elsewhere are involved in this crucial step today for different reasons. Transport and storage are key issues, as well as spreading techniques for urine. This issue is quite big in countries outside the cold climate region or with poorer economical resources like in the developing world. Lack of clear regulation regarding urine spreading for hygienic reasons makes the uncertainties even more problematic. Swedish recommendations are just guidelines and its interpretation may lead to confusions or subjective assumptions (Wallin, 2002).

It is important that the whole system for collection, storage and handling of human urine is constructed to minimize nutrient losses. The experiences from handling and storage of animal manure showed that nitrogen losses can be as high as 30 – 77 % (Misselbrook *et al*., 2004;

Parkinson *et al.*, 2004), and phosphorus losses around 4 %- 30 % (Kirshman, 1998; Parkinson *et al.*, 2004).

In fresh urine the greater part of the nitrogen appears in organic form as urea [$CO(NH_2)_2$]. Urea hydrolyses rapidly into ammonia (NH_3). Loss of nitrogen as NH_3 and toxicity to plants can occur during urine management and application as fertilizer (Blouin, 1979). Urea hydrolysis is catalyzed by the *urease*, an enzyme, which many microorganisms possess. In the hydrolysis pH is increased and ammonium and bicarbonate are produced (Hanaeus *et al.*, 1996):

$$CO(NH_2)_2 + 3H_20 \leftrightarrow 2NH_4^+ + HCO_3^- + OH^- \qquad (1)$$

Ammonium is in equilibrium with dissolved ammonia:

$$NH_4^+ + OH^- \leftrightarrow NH_3\ (aq) + H_2O \qquad (2)$$

The pK_a-value for the equilibrium is 9.3 at 25 ^{0}C.

Dissolved ammonia is in equilibrium with gaseous ammonia:

$$NH_3\ (aq) \leftrightarrow NH_3\ (g) \qquad (3)$$

The decomposition of urea will lead to an increase in concentration of ammonium-ammonia and an increase in pH in urine (from c:a 6 to c:a 9.5) and ammonia will evaporate in contact with air. Ammonia evaporation in the field is dependent also of the soil mineralogical composition, its cation exchange capacity (CEC), soil pH, other salts present, and of the weather conditions, time of spreading, etc. Ammonia evaporation up to 25 hours after urine spreading with different application techniques was studied in a clay soil under cold climate conditions in Sweden. Immediate incorporation of urine in topsoil could minimize the ammonia loss to around 5% (Rodhe *et al.,* 2004).

Experience from handling urine in stock-farming in Sweden has shown that the losses of nitrogen during storage could be minimized by having a low temperature, a low pH-value and avoiding aeration above the liquid surface in storage tanks. However, high pH, high temperature, concentrated form of urine (high N concentration) and long storage periods are favorable for hygienic reasons (Höglund, 2001). Pilot studies on storage conditions (Hanaeus *et al.*, 1996) have shown that dilution of urine seems to promote the conversion of urea to ammonia. During spreading of urine as fertilizer to plants a dilution ratio of 3:1 to10:1 has been recommended (Jönsson *et al.*, 2004). However, dilution is also one of the main reasons why pathogens can survive in urine (Höglund, 2001).

At the pH interval common for fresh urine (pH= 5.6-6.8) phosphorus exists as $H_2PO_4^-$ (pK_a=7,71 at 25°C). Stored human urine has an elevated pH (around 9) and the phosphorus is in HPO_4^{2-} form (pK_a = 12.36 at 25°C). Phosphorus in urine is directly available to plants, and phosphorus loss during urine management is not high (Jönsson *et al.*, 2004).

Macronutrients as K and S in the urine occures as free ions (K^+ and SO_4^{2-}), which are directly available to plants (Jönsson *et al.*, 2004).

1.2.3 Solutions

Direct use as liquid fertilizer

Human urine collected in separating systems can be used directly as a liquid fertilizer (Kirshmann *et al*., 1995; Kirshmann, 1998; Jönsson *et al*., 2000). The fertilizer value of pure urineis similar to NPK 18:2:5 (Lindén, 1997) and for urine mixture (urine mixed with flush water) to NPKS 15:1:3:1 (Palmquist *et al*., 2003). Guidelines for agricultural use of urine and also a LCA of grain production using urine are available (Simons *et al*., 2003; Tidåker, 2003; Vinnerås *et al.*, 2003). Loss of nitrogen through ammonia evaporation during storage and spreading can be reduced from well above 20% to below 10 % with better management and agricultural practice in Sweden (Johansson, 2000; Jönsson *et al.*, 2000; Richert Stintzing *et al*., 2001).

Controlled release into the sewer system

At the Swiss Federal Institute for Environmental Science and Technology a group of scientists developed an integrated wastewater management model adapted to municipal settings (Larsen *et al*., 1996; Larsen *et al*., 2001; Larsen *et al*., 2001). This model relies on urine sorting with small, on-site storage tanks and use of the existing sewer network for transport to a treatment facility at night when the sewers are empty. However, this model has not been tested in practice so far.

Processing urine

There are three major approaches to process the separated urine. These are presented below.

Stabilization

The aim of this technique is to reduce or stop urea hydrolysis and nitrogen losses as ammonia during storage and handling of urine. Experiments were performed to decrease the high pH by nitrifying a part of the ammonia to nitrate, but complete nitrification was not achieved (Hellström, 1998; Hellström *et al*., 1999). About 50% of total ammonia in urine could be stabilized by biological nitrification in biofilms (Udert *et al*., 2003). By addition of acid to urine the hydrolysis can be prevented (Hellström, 1998).

Volume reduction

Several methods have been studied, such as distillation (in space research, Miernik *et al.*, 1991), drying (Hellström *et al*., 1998; Hellström, *et al*., 1999), and partial freezing and freezing-thawing (**I**; **V**).

Production of a solid fertilizer

It is also possible to transform the nutrients in urine in solid fertilizers, and especially struvite formation has been studied (Boistelle *et al*., 1983; **II**; **III**; **IV**; **V**). Struvite is known as slow-release fertilizer since many decades (Bridger *et al*., 1962; Ghosh *et al*., 1996; Schuiling *et al*., 1999). Struvite is produced from wastewater and organic animal waste today in Japan and Europe (Liberti *et al*., 1986, 2001; Momberg *et al*., 1992; Shin *et al*., 1997; Booker *et al*., 1999; Battistoni *et al*., 2000, 2001; Celen *et al*., 2001; Münch *et al*., 2001; Ueno *et al*., 2001; Doyle *et al*., 2002). Animal manure is also stabilized and treated with magnesium to minimize nutrient loss and produce struvite in animal farms worldwide (Rulkens *et al*., 1998; Beal *et al*., 1999; Schuiling *et al*., 1999; Burns *et al*., 2001, 2002; Suzuki *et al*., 2002, 2005; Nelson *et al*., 2003; Kim *et al*., 2004). More recently struvite precipitation from urine separating systems has also been investigated by others (Otterpol, 2003; Ronteltap *et al*., 2003).

By crystallizing struvite from human urine the volume is reduced, the handling is facilitated, hygienisation is improved, and the crystals obtained can also be stored for later use or directly used in agriculture. Handling and spreading can be made with readily available agricultural equipment.

Some natural minerals and materials can be used as nutrient adsorbents and later used as plant fertilizers. Especially zeolites have been intensively studied (Jorgensen *et al*., 1976; Booker *et al*., 1996; Beler-Baykal *et al*., 1996, 1997; Beler-Baykal 1998; Kithome *et al*., 1998; Rozic *et al*., 2000; Ming *et al*., 2001; Jorgensen *et al*., 2003). Zeolites, wollastonite and activated carbon have been investigated in this thesis as nutrient adsorbents (**II**; **III**; **IV**; **V**).

The nutrient value of struvite is known from several studies (Bridger *et al*., 1962, Ghosh *et al*., 1996, Johnston *et al*., 2003). Struvite precipitation, zeolites and other adsorbents in contact with human urine were invesitgated for plant availability and compared with DAP (di-ammonium-phosphate) and CAP (mono-calcium-phosphate) commercial fertilizers (**VI**; **VII**).

1.3. Theoretical backgrounds for urine processing methods

1.3.1 Freezing-thawing as a concentration and volume reduction technique

Freezing as a method in wastewater treatment has been investigated previously (Lorain *et al*., 2001), and sludge dewatering methods has been developed and used, based on this technique (Halde, 1980; Hung *et al*., 1997; Martel, 2000; Örmeci *et al*., 2001; McMinn *et al*., 2003). Sludge treatment by freezing and thawing is a robust and cost saving method in cold climate regions (Hellström, 1998; Martel, 1998). Freeze-concentration can be an efficient method also for soluble pollutant removal from wastewater (van der Ham *et al*., 1998, 2004; Lorain *et al*., 2001; Gay *et al*., 2003). However, freeze-concentration of human urine has not been investigated before.

Freeze-concentration and thaw-release of ions

When ice crystals are under formation, the irregular network structure of liquid water molecules undergoes a re-organization to a stabile regular tetrahedral network. During slow formation of ice from aqueous salt solutions the ions remains concentrated in the liquid part and are excluded from ice crystal formation. By these means the ice is practically salt-free. The higher the crystallization velocity, the more ions follow the water molecules in to the crystal network, but as impurities, not as structural elements in the crystals (Olovsson, 1995).

Freezing point depression occurs because the water in the solution has a lower vapor pressure than that of pure ice. No ice forms under these conditions. At a certain temperature, the so-called eutectic point, the solution is saturated with salt and further cooling results in the formation of pure ice, and salts may precipitate as separate salt crystals. The density difference between ice and a salt makes it possible to separate both solids simply by gravity. This process is used in eutectic freeze crystallization as a very efficient, low energy consuming purification technique in industrial processes and wastewater purification today (van der Ham *et al*., 1998, 2004).

Snow ageing is a constant freezing-melting process, when high level of ionic pollutant release occurs during the snow-melt periods under a few warm spring hours (Olovsson, 1995). The first run-off-water contains very high concentrations of impurities (ionic pollutants), which had been accumulated during the ice and snow building processes (Bengtsson, 1995). Both laboratory and field experiments indicate that 50-80% of the pollutants are released when the first 30% of the snow melts. The very first melt-water may have concentrations about 5 times the values found in bulk snow, and in extreme cases the factors reached up to 10 (Overrein *et al.*, 1981). These theoretical and experimental research results were applied in studying nutrient concentration and volume reduction by freezing human urine (**I** and **V**).

1.3.2 Struvite recovery

Struvite formation

Struvite (magnesium ammonium phosphate hexahydrate) is a colorless crystal, with rhombohedral lattice type. The calculated molar ratio of struvite is 1(P):1(N):1(Mg). Struvite has a low solubility in water (c:a 0,02 g/100ml water), is highly soluble in dilute acidic solutions and highly insoluble in alkaline solutions (Weast *et al.,* 1981). So the most favorable environment for struvite precipitation occurs at pH intervals around 9-10 (Mohajit *et al.*, 1989).

Struvite is precipitated according to the general reaction:

$$PO_4^{3-}\,(aq) + NH_4^{+}\,(aq) + Mg^{2+}(aq) + 6\,H_2O \rightarrow \underset{\text{Struvite}}{NH_4MgPO_4 \cdot 6H_2O\,(s)}$$

When wastewater or urine are used as N and P source for struvite formation the chemistry of precipitation becames even more complex.

Struvite - from problem….

Struvite crystal growth created maintenance and operational problems in wastewater treatment plants from industrial farming in Singapore (Mohajit *et al.*, 1989). Figure 2 illustrates the seriousness of this problem in wastewater pipes.

Figure 2. Struvite precipitated in pipes at a wastewater treatment. (source: www.crystalenv.com/struvite_pipe_256x192.jpg; 2004-12-10)

For humans and animals, kidney stone (struvite and apatite calculi) production can become a serious problem. Urease producing bacteria such as *Proteus* species or *Corinebacterium* can cause urease catalysed hydrolysis of urea which generates ammonium, elevates urine pH and causes hyper-saturation with respect to struvite and apatite with precipitation of Mg^{2+} as struvite, and Ca^{2+} as apatite (Soriano *et al*., 1987; Buffington *et al*., 1990; Downey *et al*., 1992).

…to resource

Crystallization studies for removal and reuse of phosphates by struvite formation, using anaerobic digester supernatant, were performed at a laboratory and pilot scale (Sen *et al*., 1988; Momberg *et al*., 1992). The phosphate removal was substantial (80-98%), and more than half of the ammonia was adsorbed at pH range of 9.5-11.5. Supernatant from anaerobic digestion of piggery wastewater was also investigated for struvite precipitation studies, with similar results (Wrigley *et al*., 1992; Wrigley *et al*., 1992). A method for high removal of both ammonia and phosphates from domestic wastewater with recovery of struvite and use of zeolite was investigated (Liberti *et al*., 1986, 2001). The precipitate obtained in this way contained 92.7 % struvite, 0.3% magnesium phosphate and 7% organics, corresponding to a fertilizer value of $N\text{-}P_2O_5\text{-}MgO = 5\text{-}27\text{-}15$ on the fertilizer market. The cost-effectiveness of this process depends upon the prices on the fertilizer market (Liberti *et al*., 1986).

Recent studies on the thermodynamics of struvite precipitation in source-separated and artificial urine showed, that organic complexing agents do not influence the solubility of struvite and the inorganic complexes formed had little influence on the struvite solubility (Ronteltap *et al*., 2003).

1.3.3 Use of minerals and adsorbent materials for improved nutrient recovery

Zeolites

Zeolites are crystalline, hydrated aluminosilicates of alkali and alkaline earth metals with three-dimensional atomic structures. They are characterized by the ability to lose and gain water reversibly and to exchange certain constituent atoms, also without major change of atomic structure. Along with quartz and feldspar minerals, zeolites are three-dimensional frameworks of silicate (SiO_4^{4-}) tetrahedra in which all four corner oxygen ions of each tetrahedron are shared with adjacent tetrahedra. This framework contains open cavities in the form of channels and cages. Water molecules and extra framework type of cations usually occupy these. If each tetrahedron in the framework contains silicon as its central atom, the overall structure is electrically neutral, as is quartz (SiO_2). In zeolite structures, some of the Si^{4+} is replaced by Al^{3+}giving rise to a deficiency of positive charge. The charge is balanced by the presence of mono- and divalent cations, such as sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), and magnesium (Mg^{2+}), elsewhere in the structure.

The unit-cell formulae for clinoptilolite, a common natural zeolite is:

$$(Na_3K_3)(Al_6Si_{40})O_{96} \bullet 24H_2O$$

Ions within the second set of parentheses are known as structural cations, because with oxygen they make up the rigid framework of the structure. The (Al+Si):O ratio is always 1:2 in the formulae of crystalline zeolites, and the SiO_2 :AlO_2 ratio is always equal to or greater than 2:1. Ions within the first set of parentheses are known as exchangeable ions, because they can be replaced (exchanged) more or less easily with other cations in aqueous solution, without affecting the aluminosilicate framework. This phenomenon is known as ion exchange, or more commonly cation exchange. The exchange process involves replacing one monovalent exchangeable ion in the zeolite by one monovalent ion from the solution or replacing two monovalent exchangeable ions in the zeolite by one divalent ion from the solution. The magnitude of such cation exchange in a given zeolite is known as its cation-exchange capacity (CEC) and is commonly measured in terms of moles of exchangeable cation per gram (or 100 grams) of zeolite, or in terms of equivalents of exchangeable cations per gram (or 100 grams) of zeolite. CEC is a function of time, ion concentration in solutions, temperature and pH (Mumpton, 1984, Semmens, 1984).

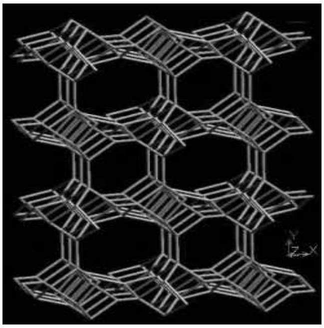
Calculated CECs for some common zeolites are presented in Table 5.

Table 5. The calculated cation exchange capacity (CEC) of some common zeolites, based on theoretical formulae (Mumpton, 1984):

Common name	Unit-cell formulae	CEC
Clinoptilolite	$(Na_3K_3)(Al_6Si_{40}O_{96}) \bullet 24H_2O$	[2.16 meq/g]
Mordenite	$(Na_8)(Al_8Si_{40}O_{96}) \bullet 24H_20$	[2.29 meq/g]
Erionite	$(Na_3Ca_3K_2)(Al_9Si_{27}O_{72}) \bullet 27H_2O$	[3.14 meq/g]
Chabazite	$(Na_6K_6)(Al_{12}Si_{24}O_{72}) \bullet 40H_2O$	[3.70 meq/g]
Phillipsite	$(Na_5K_6)(Al_{10}Si_{22}O_{64}) \bullet 20H_2O$	[3.87 meq/g]

The large cavities and channels of zeolites are generally filled with water molecules that form hydration spheres around the exchangeable cations. If the water is removed, usually by heating the zeolite for several hours at 350-400^{o}C, the molecules with appropriate diameters enter the channels and are readily adsorbed on the inner surfaces of the dehydrated central cavities. Molecules too large to enter the channels are excluded and pass around the outside of the zeolite particle, giving rise to the second well-known property of most crystalline zeolites, molecular sieving. The surface area available for adsorption ranges up to several hundred m^2 per gram zeolite. Figure 3 shows the hollow structure of clinoptilolite.

Figure 3. The clinoptilolite structure. (source: Ch. Baerlocher, L.B. McCusker, Database of Zeolite Structures: http://www.iza-structure.org/databases/)



Zeolites can also adsorb anions on their structural cations, *e.g.* significant amounts of phosphorus to their hydrous oxides of Al structural sites (Sakadevan, *et al.*,1998; Wild *et al.*,1996). Phosphate sorption was also observed by the zeolite used in urine processing (**III**).

There are more than 80 distinct natural zeolite species including several hundred different zeolites. Sedimentary zeolitic rocks of volcanic origin commonly contain 50-95% of a single zeolite species along with varying amount of quarts, potassium feldspar, calcite, gypsum, montmorillonite, etc. The zeolite ore commonly called clinoptilolite is actually a clinoptilolite-rich zeolite, where the clinoptilolite amount is 60-90% of the zeolite content in the ore (Armbruster, 2001).

The clinoptilolite is especially interesting because of its ability to selectively ion exchange NH_4^+ (ammonium ions) in solutions (Jorgensen *et al.,* 1976; Kithome *et al.*, 1998; Dyier *et al.*, 1999; Rivera *et al.*, 2000). This property makes clinoptilolite very interesting in *e.g.* aquaculture, water and wastewater treatment (Wakatsuki *et al.*, 1993; Kalló, 1995; Oldenburg *et al.*, 1995; Booker *et al.*, 1996; Beler-Baykal *et al.*, 1996; Beler-Baykal *et al.*, 1997; Beler-Baykal, 1998; Lahav *et al.*, 1998; Rozic *et al.*, 2000; Jung *et al.*, 2004; Jorgensen *et al.*, 2003). Recycling and reuse of ammonium-loaded zeolites as slow-release fertilizers have been studied and are well known in so called 'zeo-agriculture' (Pond *et al.,* 1984).

Wollastonite

Wollastonite often occurs in nature as a result of the reaction of calcite in metamorphosed limestones and quartz, according to the reaction:

$$CaCO_3 + SiO_2 \rightarrow \underset{\text{Wollastonite}}{CaSiO_3} + CO_2 \qquad \text{(Deer, } et\ al.\text{, 1997)}$$

The Ca atoms are situated inside the tetrahedra making the Si-O chains weakly bounded in all directions. A pure wollastonite looks like a fibrous mineral. The escape or the enclosure of CO_2 (g) during the mineral formation creates abundant pores making the mineral quite porous, and as such, very suitable as an adsorbent for different ions. The $CaSiO_3$ framework is not well charge balanced because of the lack of electron intensity around outmost atoms of the crystalline structure, giving it a positive charge. In an aqueous solution this is surrounded by OH-layers changing the mineral grain surface to negatively loaded. Cations present in the solution can be adsorbed to these negatively charged grain surfaces, and the binding strength is dependent of the size and charge of the cations (Lifvergren, 1997).

Depending upon the chemical composition of the adsorbent, the ion adsorption is influenced by transfer or sharing of electrons, which is a slow and often irreversible process called chemisorption. However, in aqueous solutions the adsorption is dominated by electrostatic adsorption and this type of physical bound is highly dependent upon pH, ionic strength of the solution, ion radius and temperature. High pH, small ion radius and higher temperatures facilitate the process. Increased ion concentrations increase the adsorption capacity. This ion selective and concentration dependent behaviour makes wollastonite interesting for recycling purposes.

Wollastonite has been tested for removing heavy metals from aqueous solutions (Singh *et al.*,1988; Sharma, *et al.*,1990; Sharma *et al.*,1990; Yadava, *et al.*,1991; Lifvergren, 1997) and it was found to be a potential cation adsorbent for removal of industrial pollutants in water. Phosphorus removal by wollastonite was investigated by Brooks *et al.* (2000).

Active carbon (AC)

Active carbon is made from various raw materials that have high carbon content, such as charcoal, wood, lignin, petroleum coke and coconut shell. Nowadays different waste fractions as fly ash, sewage sludge or solid waste is used as raw materials for active carbon production (Chen *et al.*, 2002; Leboda *et al.*, 2003; Nakagawa *et al.*, 2004). Generally, the raw material is converted to porous carbon under high temperatures and pressure through a two-stage process of carbonization and activation. The fissure system in the porous carbon obtained after thermal degradation (carbonization) is extensive, creating a very large surface area in a small carbon package. Activated carbon absorbs gases and particles by a combination of London Force and small electrostatic forces that creates a type of capillary action at a micro level. The character of macro- and microporosity of a certain type of active carbon depends on the activation process chosen (Thomson *et al.*, 2000; Diáz-Terán *et al.*, 2001).

Active carbon and zeolite as ammonia adsorbents have been compared in fish cultures, and both adsorbents were found to be effective on ammonia reduction, but zeolite was considered cheaper and easier to manage (Emaldi *et al.*, 2001). Technologies using carbon based adsorbents (active carbon, fly ash, charcoal, etc.) are widely used for removal of phenolic acids from agro-industrial wastewater (Garcia-Aaraya *et al.*, 2003), removal of synthetic organic chemicals (Matsui *et al.*, 2003), removal of aquaculture therapeutants (Aitcheson *et al.*, 2000), remediation of dyes from industrial wastewaters (Robinson *et al.*, 2001) and heavy metal removal from water and wastewater (Viragaghavan *et al.*, 1991; Viragaghavan *et al.*, 1998; Mizuta *et al.*, 2004). Active carbon based filters are also used in urea and ammonia sorption in medical devices (Giordano *et al.*, 1976; Lehmann *et al.*, 1981).

However, little is known about the behaviour of carbon-based adsorbents in nutrient removal from domestic wastewater. The use of active carbon as adsorbent for total nitrogen recovery from human urine and its fertilizer value has not been investigated before. If it works, carbon based adsorbents can be a potential supplement to zeolite, which is an imported mineral in Sweden.

2. Objectives

The first and major objective of this thesis was to evaluate and to optimise three different urine-processing techniques. These are:

- volume reduction and nutrient concentration by freezing-thawing
- recovery of nutrients as solid minerals, mainly as struvite
- additional recovery of nutrients by addition of natural zeolite, wollastonite and activated carbon and study these materials in contact with urine and in combination with struvite precipitation

A second objective was to test the plant availability of recovered nutrients in short-term pot trials on spring wheat (*Triticum aestivum* L.) under climate chamber conditions.

To achieve this, synthetic human urine, fresh human urine undiluted (collected from colleagues) and diluted and stored (collected from urine storage tanks at Universeum Science Centre) was investigated in laboratory studies.

An additional question emerged and was investigated during the studies: Is it possible to reuse the supernatants from urine processing as dilution water in an aquaculture based wastewater system already in work at Universeum? If so, an entirely closed nutrient- and water loop can be achieved in the Science Centre, with 24 separating toilets in work and an annual visitor number of 500 000. The answer was given by the results of acute toxicity tests to *Daphnia magna* made on the processed urine supernatants.

3. Materials and methods

The pH and conductivity measurements, all the chemical analysis, mineral identification and qualitative analysis, toxicity tests and statistical analysis were made by the author and co-authors, or our laboratory personal with exception of some analysis which were performed by accredited laboratories, such as AnalyCen (Sweden), Analytica (Sweden).

Pot tests with *Triticum aestivum* L. were made with carefully chosen growth media for short-term tests in climate chamber conditions. A technique based on optimal nutrient addition and growth condition for crop cultivation was developed at our laboratory using a modified Ingerstad technique (Ingestad, 1982) for climate chamber testing of plants.

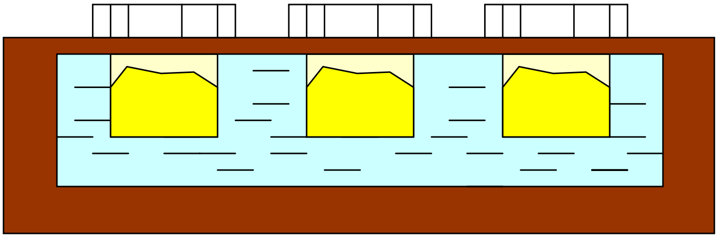
3.1. Freezing-thawing as concentration and volume reduction (I and V)

Human urine was collected from five healthy, young or middle-aged people of both sexes and was used for experiments in fresh form, stored in clean PET bottles in refrigerator (+8°C) and in a freeze (-14°C) and used within 72h for the experiments (**I**). The pH, temperature and conductivity were measured during the experiments.

Two types of methods, **bath freezing-thawing** and **column freezing-thawing** methods were used.

In the **bath freezing-thawing** method the freezing process of the samples was made using a saturated salt (NaCl) solution bath as 'isolator' for the controlled top-freezing (partial freezing) process. 100 ml samples were immersed until the upper half of the volume became frozen. Then the solid phase (ice-bulk on the top) was separated from the liquid phase and analyzed separately. Figure 4 shows the experiment design for partial freezing in a saturated salt bath.

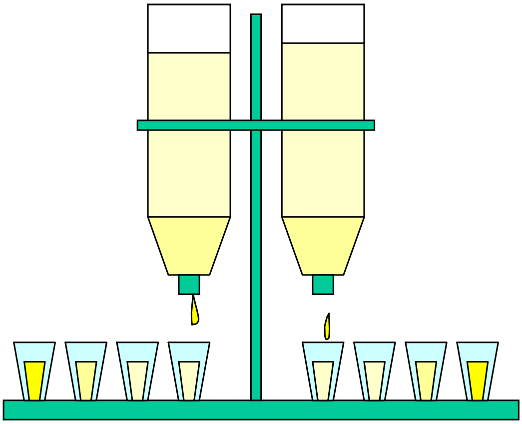
Figure 4. Partial freezing in a saturated salt bath.



The slow thawing process was studied when total samples were frozen and the thawing process was controlled by the salt-bath 'isolator', until half of the sample volume was melted. The solid phase was separated and analyzed.

In the **column freezing-thawing** method freezing and thawing processes were studied to obtain detailed results regarding fractionation and concentration of the ionic compounds and to observe two different ways of thawing. Figure 5 shows the parallel-performed column experimental design.

Figure 5. The parallel-performed two-column experiment design.



First, one-column test was made with 960 ml of synthetic urine and a similar test was repeated with 700 ml of fresh human urine. Fractions of 40 ml respectively 50 ml were collected continuously during the experiments. In a parallel-performed two column experiment 450 ml human urine was used for each column. In one column the urine was directly frozen and kept in the freezer during the preparations of the next column experiment. In the second column the urine was frozen and crushed in small and mostly homogenous ice grains, left to melt for 30 minutes at room temperature (22°C) and than frozen again. This process was repeated twice and in this way a heavy snow-like sample was obtained. Two types of thawing processes were compared with this method. The collected fractions were also compared for eventual differences in ion concentrations. 20 fractions were collected from each column during melting. Some fractions collected from the column were analyzed for total-N and total-P concentration (**I**).

Human urine was collected from the storage tank from Universeum and transported in closed plastic cans to the laboratory for testing. The urine was stored in a refrigerator at +8°C during 3 days before the experiments started. This urine was diluted with about 50% flush-water. Therefore, the concentrations of all solutes were only half of those found in direct collected fresh urine (**V**).

Precipitation and adsorption tests were made as described in **V** followed by the freezing-thawing test. After 72 hours the test beakers were transferred to a freeze-box with –18°C. After 24 h the frozen beakers were bottom-heated in a warm water bath (temperature ca 30-40°C) for 5-10 minutes. Approx. 60% of the total volume were removed as the ice top ('ice' supernatant) from each beaker, removed and collected for chemical analysis and toxicity testing.

3.2. Struvite recovery (II)

The laboratory experiments were based on *the dephosphatisation principles* (Schuiling, 1998, Schuiling *et al.*, 1999) using MgO (98% pure). The normal pH of human urine is usually such that most of phosphate is present as HPO_4^{2-}. When MgO is added the pH is increased and the phosphate equilibrium shifts in the direction of PO_4^{3-} ion and the struvite precipitates:

$$HPO_4^{2-} + NH_4^+ + MgO + 6H_2O \text{ -----------> } NH_4MgPO_4{*}6H_2O + OH^-$$

The Mg:N:P ratio for struvite formation was calculated according to the molar weights as follows:

	Mg	:	N	:	P
	24	:	14	:	31
Simplified:					
	1,71	:	1	:	2,21

The experiments made according to this principle resulted in rapid formation of small crystalline structures, which settled at the bottom of the test tubes. After filtration and drying (at 40°C) of the precipitate a fine crystalline powder was obtained. Synthetic urine and fresh human urine was used for the tests (**II**).

Synthetic human urine containing the most abundant 11 solutes in concentrations equivalent to the average urine of normal healthy men was prepared according to conventional urological methods (Griffith *et al.*, 1976 a, b). Synthetic human urine is free from organic macromolecules and other substances that could eventually enhance or inhibit the nutrient recovery. Fresh human urine was collected from five healthy, young to middle-aged people of both sexes. The tests were made at room temperature (20°C). The pH was measured continuously and MgO additions were adjusted to give pH values ranging from 6.5 to10. The MgO added was more than sufficient to supply the stoichiometric molar ratio M:P, 1.71:2.21. Electron microscope, energy dispersive X-ray analysis (EDS) and X-ray diffraction (XRD) analyses were made for component identification of the precipitates at Geovetarcentrum, Göteborg University (**II**)

3.3. Use of minerals and adsorbent materials for improved nutrient recovery (II, III, IV and V)

Zeolites used

The clinoptilolite-rich zeolite used in the studies on ammonium uptake from human urine originates from Mád, Tokay Hills, Hungary. The chemical composition (wt% after ignition at 900°C) of two, different amounts of clinoptilolite containing zeolites from this region are presented in **II** (Table 1). The zeolite containing over 60% clinoptilolite is called 'clinoptilolite' here and the zeolite containing 50-60% clinoptilolite is called zeolite-mix here (commercial name: Granofilter C 1.5-2.0, producer Geoprodukt Kft., Mád, Hungary). The minerals were not pre-treated or activated in any way, just crushed and sieved to specific grain size fractions. CEC is 1.0-1.2 meq/g (Kallo, 1995) and the exchanged amounts of ammonium for this zeolite theoretically is 18.7- 22.4 mg NH_4^+ /g zeolite. The mix-zeolite with grain size of 1.2-2.0 mm was used in all experiments in **III, IV, V** and **VII.**

Wollastonite used

The wollastonite ore used in the studies on human urine originates from Silverön, Hulta East, Sweden. The chemical composition (wt% after ignition at 900^{0}C) is presented in **II** (Table 1, in **II**). The mineral was not pre-treated or activated in any way, just crushed and sieved to specific grain size fractions.

Active carbon used

The active carbon used in the studies was a high quality commercial charcoal (Chemviron, grain size 0.4-1.4 mm).

General experimental design

Samples of either 25 ml (**II**) or 100 ml (**III, IV, V**) test solutions (human urine of different qualities, synthetic human urine, NH_4Cl-solutions) were used for batch treatments.

The experiments were made at room temperature (20°C) and preparations of stock solutions and all dilutions for analysis were made with double deionised (MilliQ) water.

The beakers with the test solutions, MgO and minerals added were covered and left for 72 h with a short manual stirring once daily. Changes in pH during the experiments were measured by a calibrated Metrohm 605 pH-meter. After 72 h the supernatants were decanted and collected for chemical analysis and toxicity tests.

More specific experimental design were applied according to the aim of the studies, and they are presented in papers **II, III, IV** and **V.**

Toxicity tests

Toxicity tests (**III, V, VI**) with *Daphnia magna* were made on the supernatants according to ISO 6341 (1982). Neonates for testing (6-24 h old) were obtained from our laboratory culture. The dilution water for culture and toxicity tests had a hardness of 250 mg/l as $CaCO_3$ and pH 8.1, and the dilution water was prepared from double deionised water (MilliQ) and ISO 6341-1982 stock solutions. Ambient conditions were 20-22°C and photoperiod of 16 h light and 8 h darkness. All exposures were made in Petri-dishes (id 10 cm) with 50 ml test solutions and 20 daphnids, using a 0.5 dilution factor. In some tests the exposure was made in six-well, plastic Nunc plates (multidish, Brand products), with 10 ml test solutions and 10 daphnids. Dissolved oxygen and pH was recorded at start and after terminating exposure. Immobility was recorded after 24 h and 48 h, and the fifty percent effect concentration (EC50) was determined by probit analysis or moving average angle. Pure dilution water was used as positive controls and a reference toxicant test with potassium dichromate was made, in parallel with all toxicity tests.

Chemical analysis

The supernatants were analysed in our laboratory for total-P and total-N using a DR4000 spectrophotometer with relevant Hach reagents according to the Hach manual (Hach Company, USA, 1997).

Statistical analysis

All obtained data was statistically treated for analysis of variance (one-way ANOVA) and for Spearman rank correlations (two-tailed) using a statistical software package (Crunch version 4, Crunch, Software Corp., Oakland, CA, USA)

3.4. Testing plant availability and fertilizer value of solids recovered from human urine (VI and VII).

Plant availability, crop growth and fertilizer value were tested with short-term (21-33 days) pot tests in climate chamber using common spring wheat, *Triticum aestivum* L. Two different experiments were made and presented in **VI** and **VII** respectively.

Climate chamber conditions

Light regime: 16 h light and 8 h darkness.
Light intensity: 520 mmol photons/m^2 s^1, or approx. 18 770 lux).

Temperatures: 20^{o}C daytime and 16^{o}C nighttime.
Humidity: 75 % daytime RH and 60 % nighttime RH.

Irrigation

Manually with small amounts of deionised water in such a way that the water in the bottom casks or beakers was re-absorbed within 2 h after irrigation.

Growth media and nutrient need

The different substrates used as growth media and the calculated balanced macronutrient need for spring wheat are presented in Table 6 and Table 7, respectively.

Table 6. Different growth media used in the experiments.

Substrate	Amounts (g) used/pot
sand	400g
sand + dolomite	400g + 7.0g
sand + peat + dolomite	200g + 100g + 10g
sand + vermiculite	200g + 100g
vermiculite	200g
K-soil	200g

Table 7. Balanced macronutrient need for cereals, according to Ingestad (1982) and Ingestad *et al*. (1982) and modified for our experiment.

Macronutrients	N	P	K	Ca	Mg	S
Balanced nutrient demand according to Ingestad (weight %)	100	16	80	63	39	9
Nutrient need for one plant (ca 1.4 g dry weight) during approx. 30 days of growth (g)	0.3	0.048	0.24	0.19	0.12	0.03

Plant availability of N and P was tested using struvite and nitrogen equilibrated materials as the only nutrient sources obtained in an earlier experiment on stored human urine (in **V**). Plant growth was investigated for common spring wheat after 21 days in climate chamber having only N and P from 100 ml processed urine as nutrient source (**VI**).

The fertilizer value of nutrients (P and N) recovered as struvite and nutrient equilibrated zeolite (SZM) was compared to four fertilizers (two solid, slow-release commercial fertilisers). Five substrates were also tested in this experiment.

Chemical analysis

The total-P and total-N concentrations were analysed using a DR4000 spectrophotometer with relevant Hach reagents according to the Hach manual (Hach Company, USA, 1997) in our laboratory. pH was measured by a calibrated Metrohm 605 pH-meter at the beginning of the analysis and during struvite dissolution with HCl (**VI**).Conductivity was measured by a calibrated Conductivity meter CDM 3 (Radiometer Copenhagen) (**VII**). For element analysis two samples of the solid fertilizer (SZM) was sent to AnalyCen accredited laboratory, Sweden.

Statistical analysis

Data from **VI** and **VII** were statistically treated as described earlier (on page 25).

Experimental design (VI)

The contents in the beakers contained c:a 40% of the original urine volume (after ise removal). The urine together with the struvite precipitate, adsorbents (se in **V**) were used as nutrient sources for cultivation of common spring wheat (*Triticum aestivum* L.). Plastic pots filled with washed quartz sand were used as growth medium. The pots were placed inside the plastic beakers containing the nutrients in the bottom. Three pots with beakers containing 1 mg, 10 mg and 100 mg of NPK (14-4-21), and one beaker with no fertilizer addition were used as controls. The amounts of NPK were chosen to be comparable with the N and P amounts recovered in the 100 ml batch tests with human urine (as described in Ganrot *et al.*, 2004). The amounts of N used in the pot tests were in the nutrient deficiency range for optimum growth for *T. aestivum* L. (Wild, 1988, Marchner, 1999). Four seeds of *T. aestivum L.* were sowed in each pot, and all pots were placed randomly on mobile banks in a climate chamber. The banks with the pots were periodically rotated in the chamber during the experiment. After 5 days of sprouting the plants were culled to get an equal number of three plants in each pot. After 21 days the plants were harvested, and the average heights of the shoots were recorded. Colour and shoot and root development were also recorded. The whole plants were weighed before and after drying (in an oven at 70°C for 3 days), and the plants fresh weight and dry weight were determined.

After harvest the remaining irrigation water was collected and diluted to 100 ml in each beaker. After one day at room temperature (20°C) samples were collected from the liquid phase and analysed for total-N and total-P concentrations in solution (considered available for the plants) and the acute toxicity was determined for *D. magna*. To the rest of the liquid (48 ml in each beaker) with the struvite precipitate and adsorbents on the bottom, 2 ml 1N HCl was added to dissolve the struvite and analyse the liquid for tot-P and tot-N (considered not directly available fraction for the plants). There are several routinely applied soil phosphorous extraction methods and their uses differ among countries (Kvarnström, 2001). We have chosen a strong acid for the dissolution of the struvite precipitate. Our intention was not to make fertilizing P recommendations for a given crop, but to evaluate the total-P pool, which remained insoluble after the pot trials, to be able to evaluate the struvite behaviour in the system. Toxicity tests were made as described earlier (on page 25).

Experimental design (VII)

The value of different fertilizers combined with different soil substrates were tested by growing spring wheat (*Triticum aestivum* L.) from seed for a period of 33 days in plastic pots (0.3 l). The seeds were soaked in aerated and distilled water at room temperature (20ºC). Three seeds were sowed in each pot at a depth of (~1 cm). The pots were placed on carriages in a climate chamber The carriages were rotated daily in the chamber to achieve similar climate conditions for all plants. The pots were thinned down to one plant per pot by removing the smallest plants after 5 days.

Five nutrient sources and 4 soil substrates were used plus a commercial nutrient enriched soil (K-soil) as a separate control. Alltogether 21 treatments were used, each treatment having 15 pots for three biomass harvests giving 5 replicates per treatment and harvest. Three solid fertilizers; a struvite-zeolite mix with nutrients recovered from urine (SZM); di-ammonium hydrogen ortophosphate (DAP); mono-calcium-phosphate monohydrate (CaP) and two nutrient solutions; one with an optimal nutrient composition (OPT) and a corresponding solution without P (OPTnoP) were used as nutrient sources. The solid fertilizers were supplemented with a nutrient solution aiming at an optimal nutrient composition for wheat seedlings according to Ingestad *et al.* (1982). The stock nutrient solutions were prepared according to Ingestad *et al*. (1982), Ingestad *et al*. (1986) and Ingestad (1987). Deionised water was used for all washings and preparations.

Plants were harvested after 11, 22 and 33 days. Each plant was separated into shoot and root and weighed before and after drying in a ventilated oven (70^{o}C) for 3 days. Average heights of the shoots were recorded at the last harvest by measuring the length from the base shoot base to the top of the uppermost leaf on each tiller. Colour and general appearance of the shoot and the root system were also recorded. After the last harvest the substrates were collected and extracted for further analysis. 10 g well mixed sample of each substrate was shaken with 100 ml deionised water during 24 h and vacuum filtered. The pH and conductivity were determined on all solutions.

For analysis of mineral contents, the dried shoots were cut into small pieces and a sub-sample was grinded in a ball-mill to a fine powder. The N concentration was determined using an elemental analyser (Model: EA 1108 CHNS-O, Fison, Italy). The content of P, K, Ca, Mg, Fe, Mn, Zn, Cu, Mo and Na were analysed at Analytica, Sweden by EPA methods (200.7 ICP-AES and 200.8 ICP-SFMS).

4. Results

4.1. Freezing-thawing as concentration and volume reduction (I and V)

The **bath freezing-thawing** method

A much clear (better) conductivity value division between the ice phase and liquid phase was obtained by the top freezing process, when particles and other impurities are rejected in the liquid phase as advancing ice formation occurs.

In the case of total sample freezing and slow thawing process a considerable difference can be seen between the conductivity values of the ice phase and liquid phase related to the initial conductivity values. This difference may due to the particle or other impurity (sludge) restraining character of the ice structure in the case of ice thawing process.

The **column freezing-thawing** experiment

One-column test: The period of time required for the melting of the 700 ml sample was approx. 7 hours. The first 50 ml fraction was collected after 1.5 hour. Then the thawing process slowly accelerated. The one-column test with synthetic and real urine gave similar results. Approx. 80% of nutrients were collected in the first five samples. Total-P, total-N and carbon content were measured in this case (**I**, Figure 2).

In the case of two, parallel performed column freezing-thawing experiments the period of time for each of them was approx. 5-6 hours (450 ml samples used, 20 ml fractions collected in 12-15 min. intervals).

The first fraction in the first column (bulk-frozen urine) was collected already after 10 minutes and the thawing process was faster -due to the surface melting. In the second column (crushed and re-frozen urine) the first fraction was collected after 50 minutes. The thawing accelerated slowly, in accordance with the theory of snow-melting processes mentioned earlier.

The conductivity showed that the majority of the ions are collected in the beginning. About 70-75% of ionic compounds were collected in the first 10 % of meltout. The early fractions (No 1 till No 6) also contain the highest concentrations of nitrogen and phosphorus (Table 8).

Table 8. Fractionation of ionic compounds in column with bulk-frozen urine.
Results from the total-P and total-N measurements.

<table>
<tr><th colspan="5">Start parameters (450ml)
pH 6.1
Conductivity (mS/m) 1547.2
Temperature +22°C</th></tr>
<tr><td colspan="5">Freezing temperature –14°C
Measurement temperature +22°C</td></tr>
<tr><td colspan="5">Column with bulk-frozen urine</td></tr>
<tr><td>Fraction nr. (20 ml)</td><td>pH</td><td>Conductivity (mS/m)</td><td>Total-P* (mg/l)</td><td>Total-N* (mg/l)</td></tr>
<tr><td>1</td><td>5.6</td><td>8834.8</td><td>165</td><td>2700</td></tr>
<tr><td>6</td><td>6.0</td><td>1547.2</td><td>28.5</td><td>425</td></tr>
<tr><td>13</td><td>5.8</td><td>501.5</td><td>15.5</td><td>230</td></tr>
</table>

* The total-P and total-N analyses was made by AnalyCen Accredited Lab., Sweden

4.1.1. The effect of freezing-thawing on the P recovery

In combination with MgO addition and adsorption on zeolite or active carbon (also abbreviated as AC) the freezing and thawing process has not significantly contributed to the phosphate removal from the urine (**V**)**.** Struvite precipitation accomplished by MgO addition dominated the P removal (Figure 6 and 7).

Figure 6. Total-P in frozen and not frozen supernatants after MgO and zeolite treatment (from **V**).

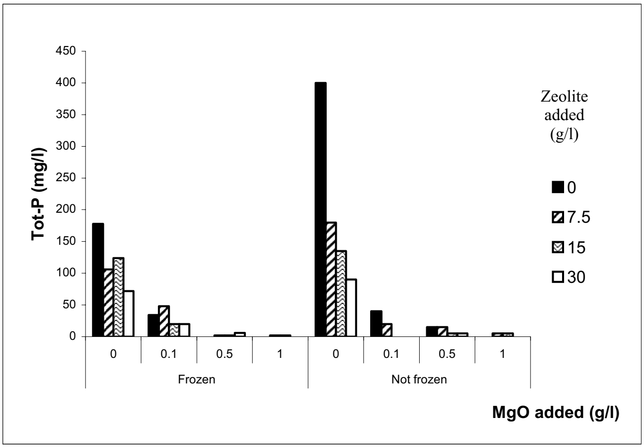
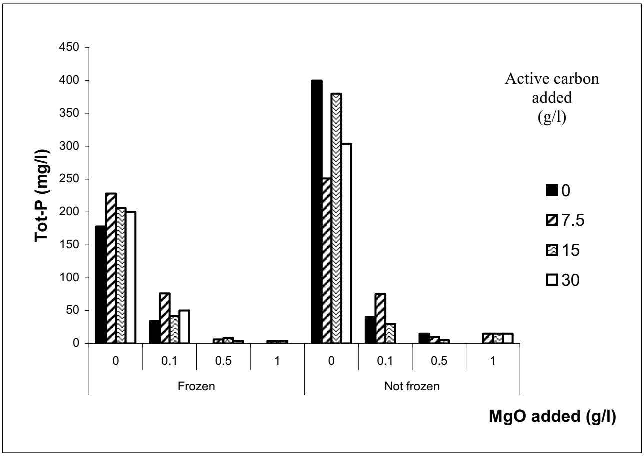


Figure 7. Total-P in frozen and not frozen supernatants after MgO and AC treatment (from **V**).



4.1.2. The effect of freezing-thawing on N recovery

The freezing and thawing process contributed significantly to the nitrogen removal from the urine (**V**). Addition of zeolite contributed to nitrogen removal in freezing-thawing tests and also in combination with MgO addition (far left columns in Figure 8 and Figure 9).

Figure 8. Total-N in frozen and not frozen supernatants after MgO and zeolite treatment (from **V**).

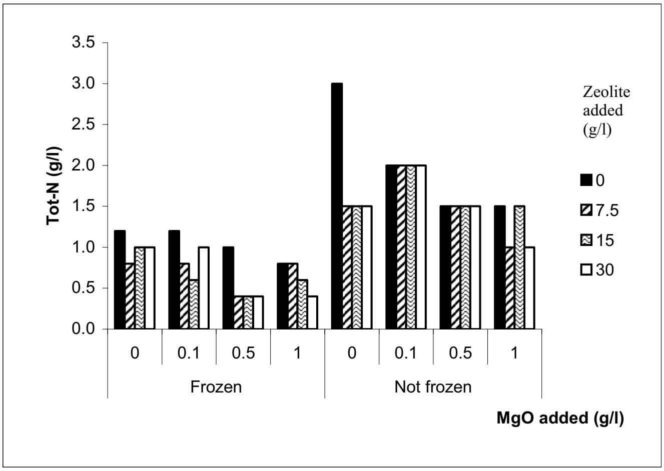
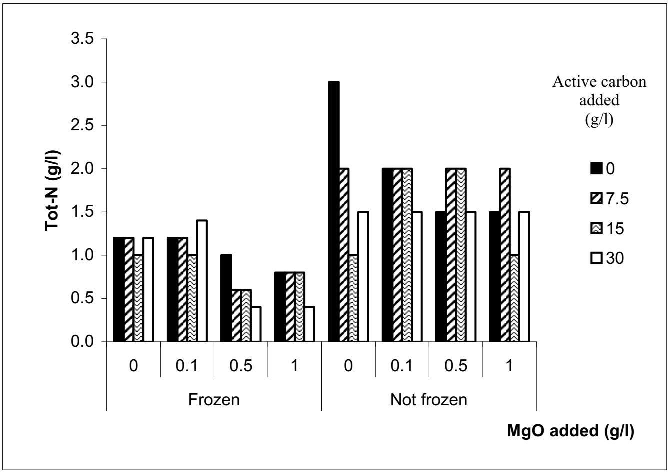


Figure 9. Total-N in frozen and not frozen supernatants after MgO and AC treatment (from **V**).



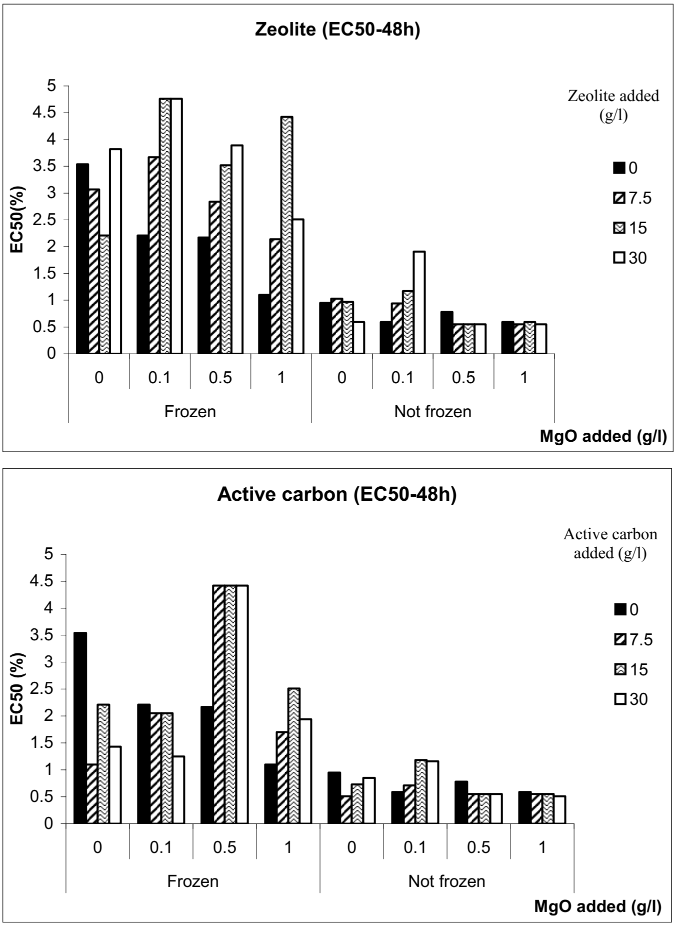
The total N removal was mainly affected by freezing, but additional removal was also found with MgO, zeolite and AC addition. These results suggest that freezing, precipitation and adsorption can be used in combination for N and P recovery from urine.

4.1.3. The effect of freezing-thawing on toxicity

The freezing and thawing process increased the *Daphnia magna* survival significantly in all tests (**V**). The nutrient content in the supernatant was reduced by 60% with this method compared to the tests not frozen. As a consequence, the acute toxicity for *D. magna* was significantly reduced (Figure 10).

In the absence or zeolite or AC the toxicity increased (lower EC50s) by the MgO in the frozen supernatants (black columns in left part). This is probably due to the increased pH values caused by MgO addition and in the resulting higher concentrations of free ammonia. This increased toxicity was counteracted by both zeolite and AC additions in frozen solutions (Figure 10).

Figure 10. Toxicity (48h-EC50) of supernatants in all treatments for *D. magna* (from **V**).



4.2. Struvite recovery (II, III, IV and V)

Paper **II** is dealing with the basic understanding of struvite formation from urine. The test tube experiments using synthetic urine and fresh human urine resulted in a rapid formation of small crystals, which settled on the bottom and wall of the tube. Electron microscope studies revealed small (up to 1mm long) crystals of struvite, and EDS and XRD analysis confirmed that struvite was the major constituent and potassium (K) and calcium (Ca) was included in the crystalline structures (**II**, in Table 2 and Table 3).

Struvite obtained from urine does not have a pure end-member composition, and small amount of Ca, Mn and Fe may substitute for Mg, and K may substitute for NH_4. A more correct formula for struvite is [$(Ca, Mg)(K, NH_4)(PO_4){*}\,6H_2O$]. Together with struvite other minerals, such as montgomeryite [$Ca_4MgAl_4(PO_4)_6(OH)_4{*}12H_2O$], brucite [$Mg(OH)_2$] or epsomite [$MgSO_4{*}7H_2O$], may form depending on pH, the amounts of Ca and other metals available.

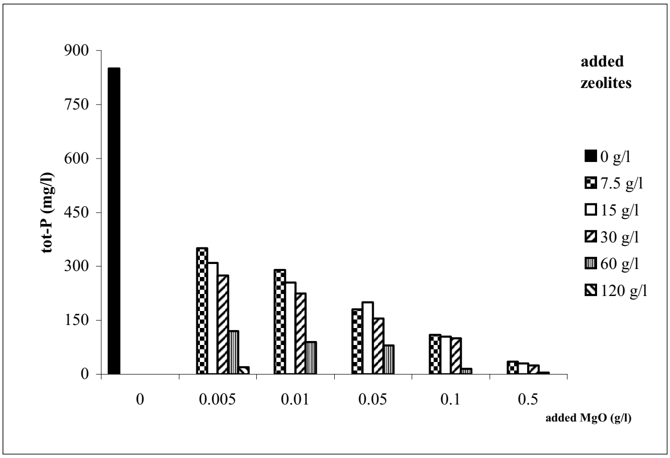
Calculations based on the mass and composition of the precipitates indicated that all P, 22-64 % of K and 2-5.6 % of Ca in the urine were recovered (**II**, in Table 4).With combined precipitation and adsorption 64-80% of nitrogen can be fixed in mineral form, regardless the way of MgO and mineral addition to the urine. Both simultaneous and separate addition gave similar results (**II**, in Table 5).

In papers **III, IV** and **V** experiments on struvite formation were made with different amounts of MgO addition in combination with other methods for nutrient recovery, like freezing-thawing and use of adsorbent materials. The results and the effects of MgO addition (struvite formation) are presented together with the materials or techniques used to optimize the processes.

4.2.1 The effect of MgO addition on P recovery

When the stoichiometric molar weight ratio for struvite formation (Mg:P of 1.71:2.21) was reached in all experiments, the MgO addition caused a rapid decrease of total-P in urine and its precipitation as struvite. When the stoichometric ratio for struvite precipitation was not satisfied, increasing amounts of zeolite had a significant impact on phosphorus reduction (**III**, Figure 2 (a and b), and Table 2). For stored and diluted human urine from Universeum, both MgO addition and zeolite gave a total-P reduction of 95-98% (Figure 11). Struvite recovery in combination with adsorbents and freezing (**V**) also emphasizes the main role of correctly dosed MgO for maximum struvite recovery.

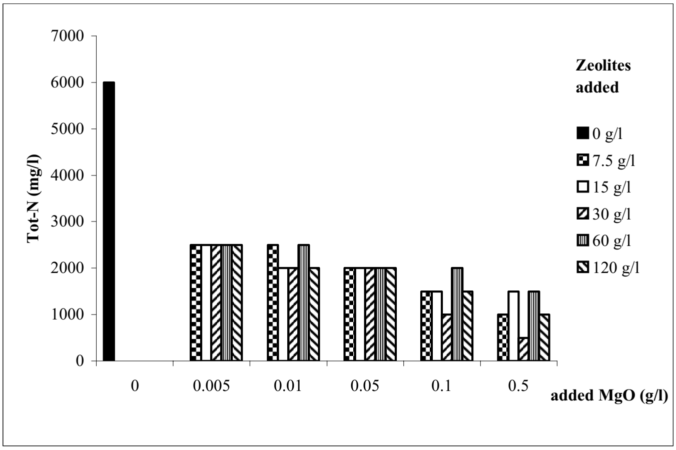
Figure 11. Total-P reduction in urine from Universeum by addition of MgO and zeolite (from **IV**).



4.2.2 The effect of MgO addition on N recovery

Theoretically, the maximum amount of N that can be recovered in the struvite chemical structure is 38% from stored urine at Universeum (Adamsson *et al.*, 2003). The MgO addition contributed to the total N recovery, and the zeolite addition contributed to additional N recovery (**IV**), see Figure 12.

Figure 12. Total-N reduction in urine from universeum by addition of MgO and zeolite (from **IV**).



Freezing was the major factor for N recovery, but also MgO addition contributed to additional N recovery in combination with adsorption on zeolite or AC (**V**).

4.3. Use of minerals and adsorbent materials for improved nutrient recovery (II, III, IV and V)

4.3.1 Ammonium uptake

The results from ammonium uptake by clinoptilolite, zeolite-mix and wollastonite are presented in Figures 13-15 (modified from **II**). Ammonium uptake from the NH_4Cl solutions was studied in relation to grain size, ion concentration and contact time. Higher sorption at smaller grain sizes was shown at both low and high ammonium concentrations. The ammonium sorption was stabilized after a few minutes and showed 70-80% uptake from low concentration solutions for the clinoptilolite, about 50-60% for the zeolite-mix and about 50% for the wollastonite. The high concentration gave about 40-50% uptake for the clinoptilolite, about 20% for the zeolite mix, and about 30% for the wollastonite, illustrating the fact that sorption is decreasing with increased competition for exchangeable sites on the minerals (Sand, *et al.*, 1978; Pond, *et al.*, 1984).

Figure 13. Ammonium uptake (concentration change in %) in 0.5 g clinoptilolite from 25 ml 0.02 M and 1.0 M NH_4Cl-solution (from **II**)

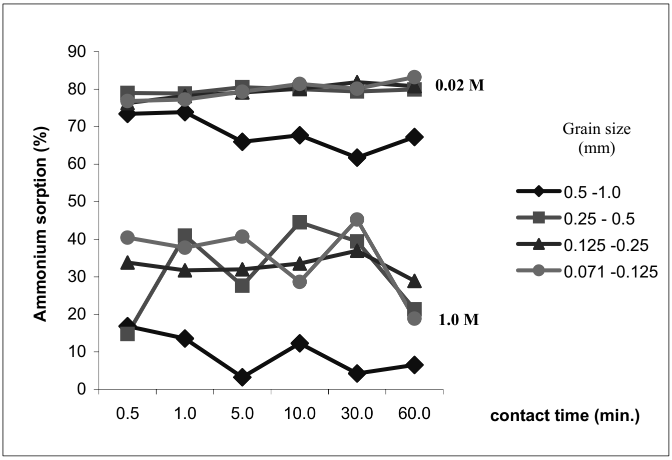


Figure 14. Ammonium uptake (concentration change in %) in 0.5 g zeolite-mix from 25 ml 0.02 M and 1.0 M NH_4Cl-solution (from **II**).

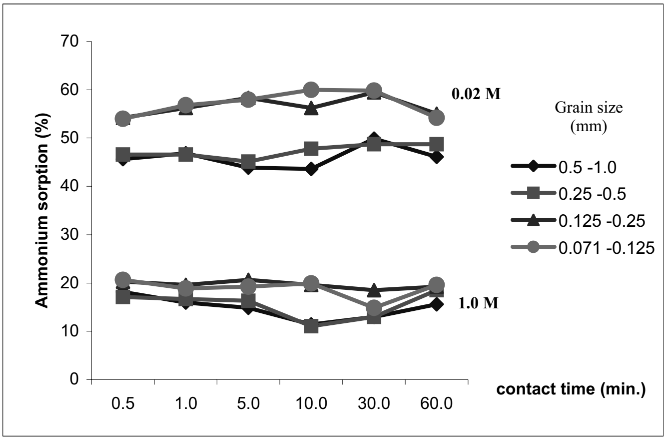
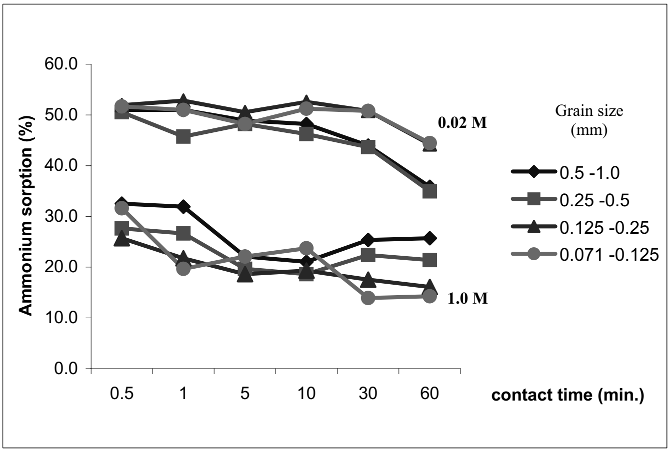


Figure 15. Ammonium uptake (concentration change in %) in 0.5 g wollastonite from 25 ml 0.02 M and 1.0 M NH_4Cl-solution (from **II**).



With combined precipitation and adsorption 64-80% of nitrogen can be fixed in mineral form. Both simultaneous and separate addition gave similar results (**II**, in Table 5)

In **III** and **IV** results from simultaneous struvite precipitation and adsorption tests on zeolite are presented. Effects on pH, P recovery and N recovery were studied.

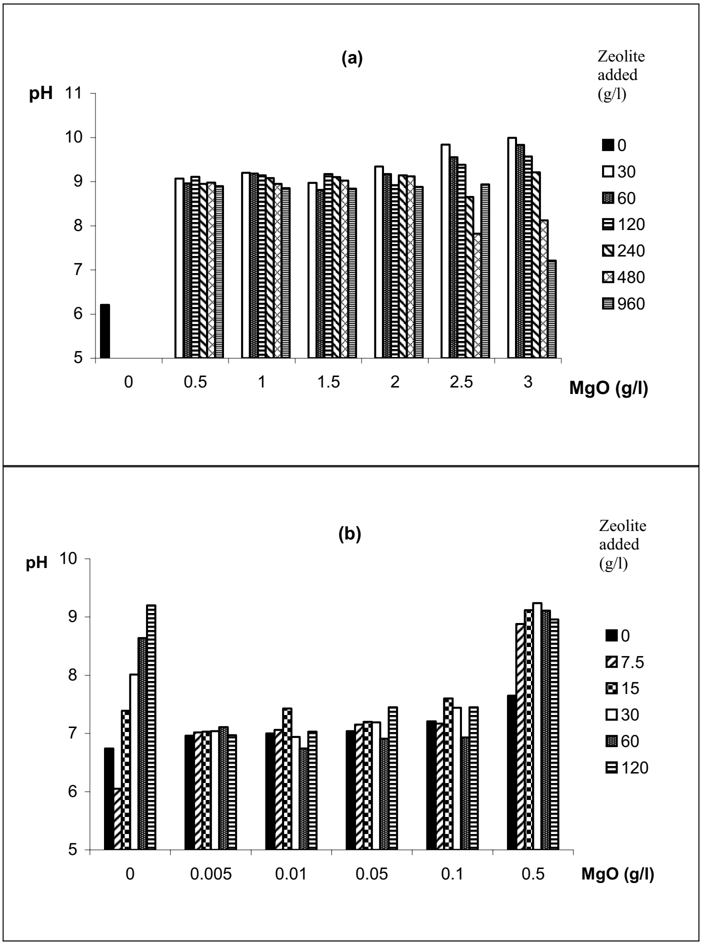
4.3.2 Adsorbent impact on pH

A significant increase in pH occurred at MgO additions of 0.5 g/l or higher amounts. Zeolite had an opposite, pH-decreasing effect Figure 16a.

No significant changes in pH in the low concentration tests were seen as a result of addition of zeolite, but marginal pH changes resulted from addition of small amounts of MgO (Figure 16b, first columns in each group). This observation is also supported by the analysis of variance (**III,** in Table 2) and the Spearman rank correlation test (**III,** in Table 3**).** When zeolite alone was added to the urine samples (Figure 16b, first group of columns) the tested amounts of zeolite significantly influenced the sample pH by increasing it from 6.7 to 9.2.

In **V**, the pH value was between 9.2 and 9.5 in all experiments. Thus, the adsorbents were not affecting the pH at the concentrations used.

Figure 16. Supernatant pH in high (a) and low (b) concentration tests (from **III**)

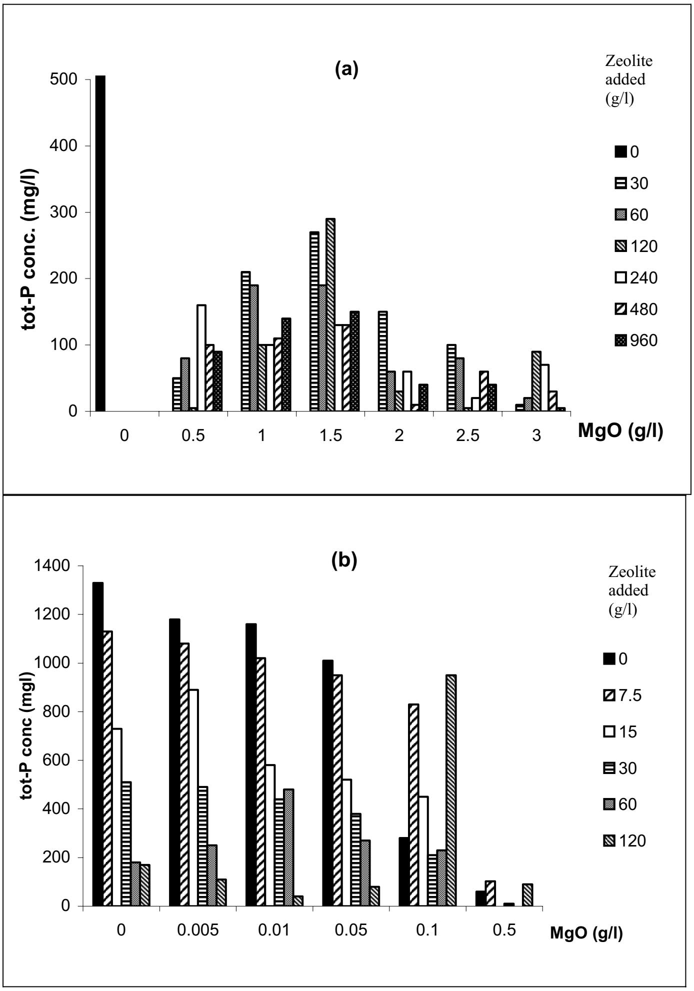


4.3.3 Adsorbent impact on P recovery

After struvite precipitation the supernatant pH decreased in the high concentration test (**III**). This decrease was observed in the samples with the highest zeolite content, where also the phosphorus concentration was reduced both by struvite precipitation and zeolite adsorption (Figure 17a).

In the low concentration test (**III**), where the stoichiometric ratio of Mg:P of 1.71:2.21 for maximal struvite precipitation was not satisfied, increasing amounts of added zeolite had a significant impact also on the phosphorus reduction (Figure 17b). In this experiment the pH was too low for maximal struvite recovery, and the phosphate removal was affected by both zeolite and MgO.

Figure 17. Total P in supernatants in high (a) and low (b) concentration tests.

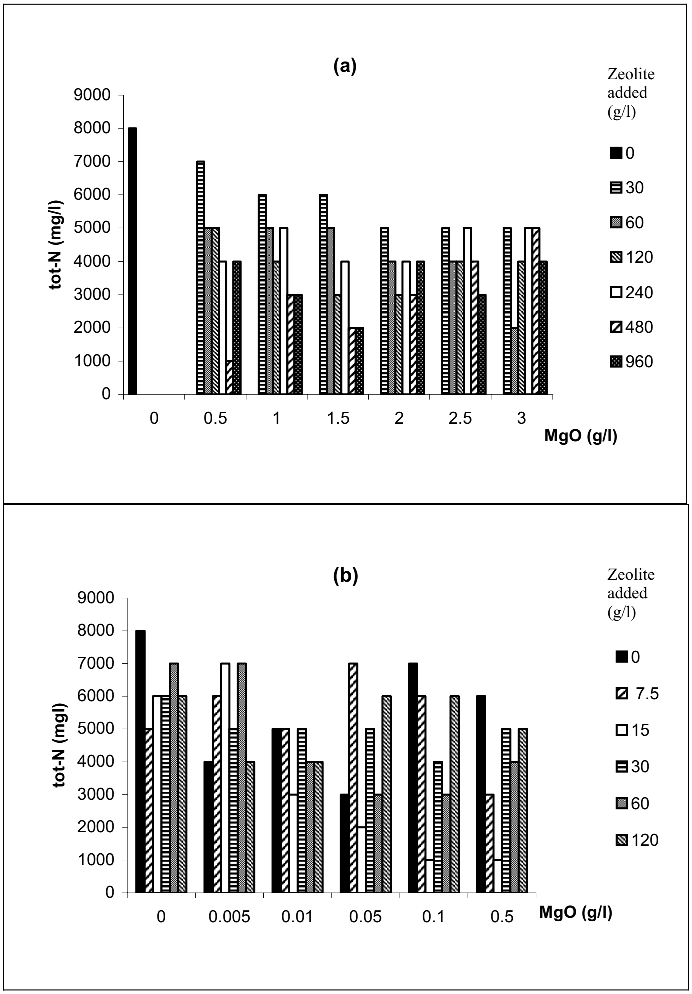


In **V** the P removal was affected by zeolite and freezing, especially when no MgO was added (Figure 7 on page 30). It was observed in earlier experiments that the zeolite itself works as a phosphate adsorbent (**III**). No significant effect of AC on P removal was found in these experiments.

4.3.4 Adsorbent impact on N recovery

The results on total-N reduction are presented in the Figure 18(a and b). In the high concentration test the N reduction was controlled by the zeolite (**III**, in Table 1). Depending on the added amount of natural zeolite and MgO the N recovery varied in the low concentration test, but the total-N reduction cannot be ascribed to either MgO or zeolite, and statistically their influence was not significant (**III**, in Table 2 and Table 3).

Figure 18. Total N in supernatants in high (a) and low (b) concentration tests.

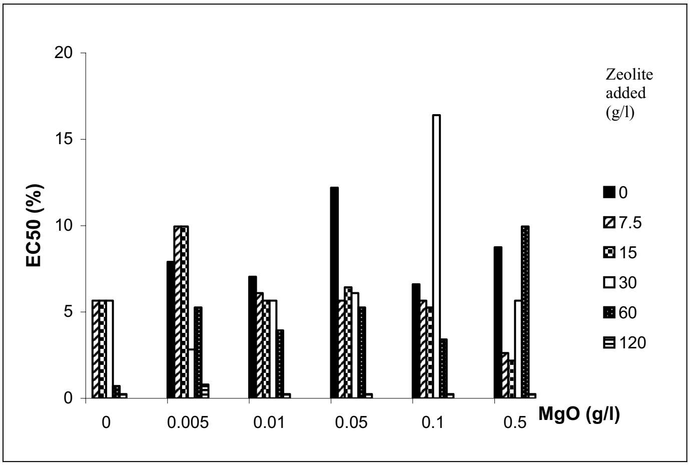


In **V**, the reduction of N was mainly affected by freezing and to some extent by MgO addition. However, the results suggests that also zeolite and AC reduced N in non-frozen samples, and both zeolite and AC reduced N in frozen samples. The maximum reduction of N, from 3.0 g/l to 0.3g/l, was found in frozen supernatants with both MgO (0.5 g/l and 1.0 g/l) and zeolite or AC (all amounts) treatments.

4.3.5 Toxicity of supernatants

The toxicity of the supernatants in the low concentration test to *Daphnia magna* (EC50 % after 48 h) is presented in **III** and Figure 19. Addition of small amounts of MgO contributed to a moderate increase in pH of the supernatant, with an ammonium-ammonia equilibrium change. When the nitrogen removal was low, an increased toxicity to *D. magna* would be expected. However, an increased addition of both zeolite and MgO also increased the pH in the supernatant, which resulted in an overall increase in toxicity for *D. magna* (**III**, in Table 2). Zeolite was also tested separately in dilution water, and the result shows that zeolite itself is not toxic for *D. magna* (not presented here).

Figure 19. Acute toxicity (48h-EC50) of supernatants (from **III**).



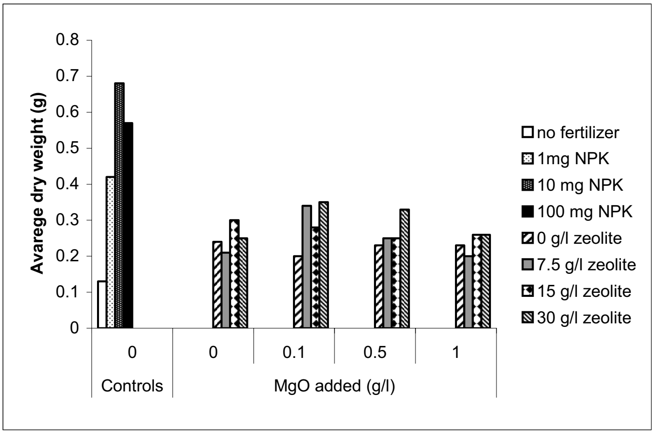
The results on toxicity of the supernatants to *Daphnia magna* (EC50 % after 48 h) in paper **V** are presented in Figure 10 on page 32. Toxicity was mainly affected by freezing, which increased EC50. The effects of MgO and zeolite were more complex with minor, but significant interactions with freezing. However, no significant effect of AC was found.

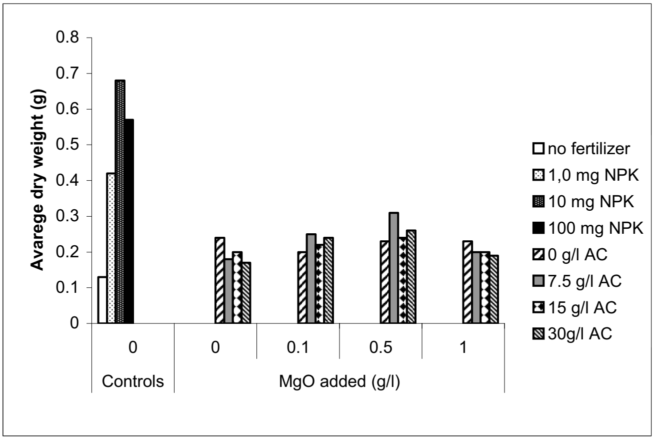
4.4. Plant availability and fertilizer value of solids recovered from human urine (VI and VII)

The addition of zeolite or AC modified the effect of MgO for both N and P availability, as described in **VI**. The growth responses (biomass and height) were limited by the lack of N, and no N was detected (< 1mg/l) in the irrigation water collected after harvest (after 21 days of growth), and no toxicity for *Daphnia magna* was detected at any combination of MgO, zeolite and AC used. However, some N remained in the insoluble solid phase (not plant available).

The average weight of *T. aestivum L.* after 21 days of growth is presented in Figure 20. The results are presented for the different treatments described previously (**V**). The average dry weight after 21 days was only half as much as that of the plants getting the optimum concentration of balanced NPK fertilizer as nutrient source (controls). This indicates a lower or slower nutrient availability from the recovered nutrients than from the highly soluble NPK used.

Figure 20. Average dry weight of wheat after 21 days fertilized with struvite and urine-equilibrated materials as nutrient sources (from **VI**).





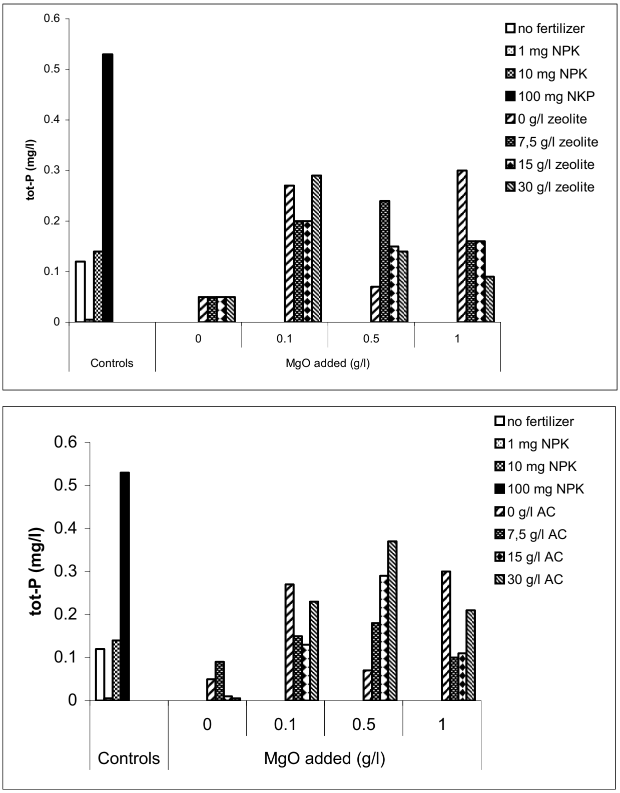
The average shoot heights after 21 days were negatively influenced by the highest MgO addition (**VI**, in Table 1). The root systems of the plants growing in the pots with high additions of MgO were also affected in a negative way resulting in small roots with fine, short root hair structure.

The availability of nutrients from struvite (the main P-source here) and from the nutrient-loaded materials (the additional N-source here) can be estimated if the remaining amounts of nutrients are measured after harvest. In the last irrigation water tot-P and tot-N was measured in both the liquid phase (available fraction for the plants) and in solid phase (not dissolved, not directly available fraction). After 21 days of growth the plants were in the early tillering stage. How much more nutrients that can be available from the solid phase later on was not tested in this study.

The plant available (water soluble) total-P contents in the last irrigation water are presented in Figure 21. The P present in the last irrigation water in all treatments indicates that there was plant available PO_4^{3-}-P present for plants during 21 days of growth. The ANOVA shows that

P is affected by MgO addition (struvite formation), but that the adsorbents (zeolite and AC) had no effect (**VI**, in Table 1).

Figure 21. Total-P in the irrigation water collected after 21 days with struvite and urine -equilibrated materials as nutrient sources (from **VI**).



The plant available total-N measurements showed zero total-N concentrations in the last irrigation water in all treatments, which indicates an efficient N-uptake by the plants, and it also shows that the experiment was conducted under an overall N deficiency for plants in all treatments and the controls. The positive controls with NPK demonstrated that ambient conditions (temperature, light, substrate and water), as well as the biological material (the quality of seeds and plants) were adequate. The N deficiency in the last irrigation water was also verified by the toxicity test made on *D. magna*, which showed 100% daphnid mobility and survival after both 24 h and 48 h in both the non-diluted and 50% diluted irrigation water (data not presented here). The toxicity of urine containing irrigation water is known to be due to its ammonia content (Adamsson, 1999).

The not directly available amount of nutrients, which remained at the bottom of the beakers, were dissolved by addition of HCl after the pot trials, and their concentrations are presented in Figure 22 (a and b) for total-P, and Figure 23 (a and b) for total-N. The amount of not directly available phosphorus was negligible for all concentrations of the NPK, and it increased in proportion to MgO addition indicating the low solubility of struvite (**VI**, in Table 2). At most of the MgO doses the concentration of remaining N and P decreased with increasing addition of adsorbents. Thus, the non-available N-pool followed the same pattern as the non-available

P-pool, indicating the slow-release of nutrients (N and P) from struvite (**VI,** in Table 2) and Figures 22-23.

Figure 22. Total-P remained in solid phase after 21 days with struvite and urine equilibrated materials as nutrient sources (from **VI**).

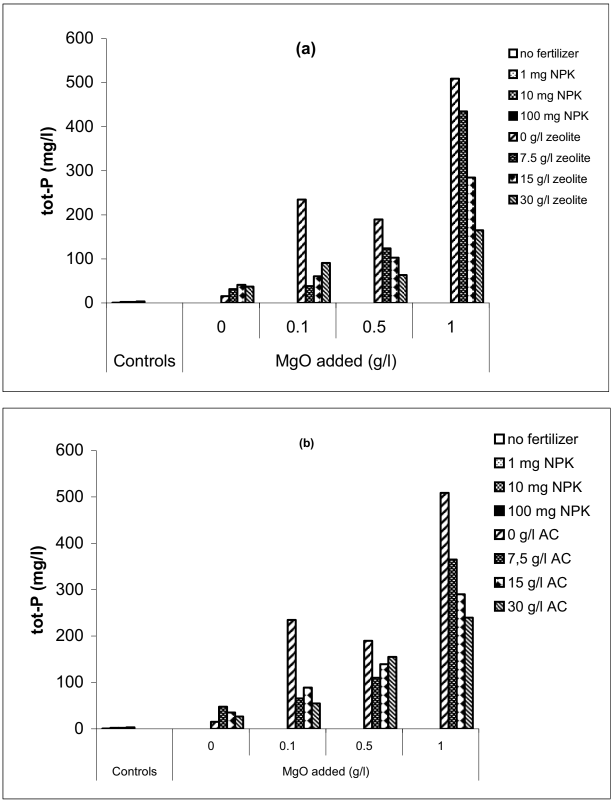
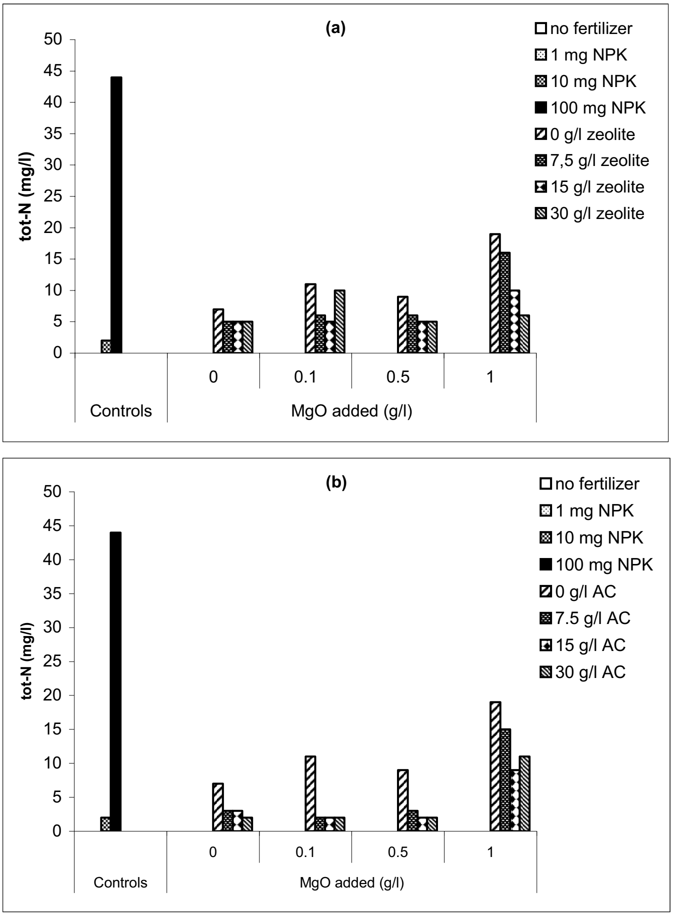


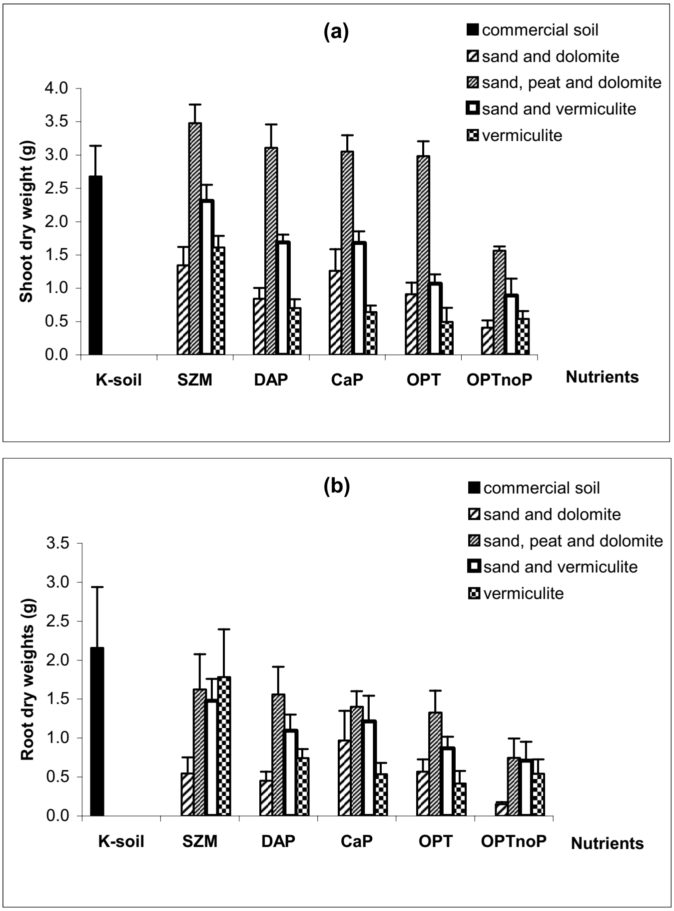
Figure 23. Total-N remained in solid phase after 21 days with struvite and urine equilibrated adsorbents as nutrient sources (from **VI**).



In the case of solids recovered from urine compared with commercial fertilisers (**VII**), the average shoot and root dry weights were very similar when identical substrates were used (Figure 24 a, b). The dry weight was generally higher in the pots having SZM as nutrient source. The lowest dry weight was found in the treatment without P (OPTnoP). The plant biomass was significantly influenced by the substrate (**VII**, in Table 4) and it was highest in the combined sand, peat and dolomite substrate. When solids recovered from urine were compared with commercial fertilisers (**VII**), the average shoot and root dry weights were very similar when identical substrates were used (Figure 24 a, b). The dry weight was generally higher in the pots having SZM as nutrient source. The lowest dry weight was found in the treatment without P (OPTnoP). The plant biomass was significantly influenced by the substrate (**VII,** in Table 4) and it was highest in the combined sand, peat and dolomite substrate. The root and shoot development are correlated (**VII**, in Table 4 and Table 10). The root development is affected mainly by substrate type, and the fertilizer affects mainly the shoots. The root/shoot ratios after 33 days and the relative growth rate between days 22 and

33 calculated for each treatment are presented in Table 5, in **VII**. The results show a similar plant development, when the same substrate was used with different fertilizers.

Figure 24 (a, b). Average biomass (dry weight) of shoots (a) and roots (b) of 33 days old wheat plants grown in different substrates and nutrient sources. K-soil, commercial nutrient enriched soil; SZM, struvite-zeolite mix with nutrients recovered from urine; DAP, di-ammonium hydrogen ortophosphate; CaP, mono-calcium-phosphate monohydrate; OPT, nutrient solution with an optimal nutrient composition; (OPTnoP), nutrient solution with without P.



The plant available P and N was extracted with water from substrates after the final harvest (after 33 days) and the result is presented in Table 9. After 33 days of growth the plants were in the late tillering stage. The total-P present in the extracted solution from all treatments indicates a sufficient plant available PO_4^{3-}-P for 33 days of growth. The total-P was extremely low in OPTnoP treatment. The P availability is mainly affected by the nutrient and to some extent also by the substrate type (**VII,** in Table 6 and Table 7). The plant available total-N was mainly affected by the substrate, and in the treatment with K-soil no total-N was detected in substrate solution (Table 9). Thus, the N availability at the end (33 days) was significantly affected by the substrate type (**VII**, in Table 7), but not affected by the nutrient source.

Conductivity was also only affected by substrate, but not by nutrient source, while pH was equally affected by both substrate and nutrient source (**VII,** in Table 7).

Table 9. The pH, conductivity, plant available total-P and total-N in the substrate extracted with water after 33 days of spring wheat growth. For abbreviations see Figure 24 (a, b).

Nutrient	Substrate	pH	Conductivity (µS/cm)	Tot-P (mg/l)	Tot-N (mg/l)
K-soil	K-soil	7.8	120	5.5	0
SZM	S+D	8.0	270	12.5	20
	S+P+D	8.0	195	21.5	10
	S+V	8.0	275	20.0	20
	V	8.7	330	15.5	30
DAP	S+D	7.5	220	7.0	30
	S+P+D	7.4	100	8.0	10
	S+V	7.8	250	26.0	20
	V	8.0	435	23.0	30
CAP	S+D	7.7	140	9.0	10
	S+P+D	7.5	115	9.5	0
	S+V	8.0	175	18.0	10
	V	8.1	385	14.5	40
OPT	S+D	7.6	180	6.5	10
	S+P+D	7.6	100	5.5	0
	S+V	7.9	205	9.0	20
	V	8.0	450	10.0	40
OPTnoP	S+D	7.2	225	0.5	20
	S+P+D	7.3	340	0.5	30
	S+V	7.3	235	0.5	20
	V	7.7	410	2.5	40

The nutritional status of the spring wheat (measured in the above ground dry matter) for each treatment after 33 days of growth reveals the nutrient availability from fertilizers used, and the effect of substrates on nutrient source as well as on the plant development. The mineral nutrients in the plant shoots were affected differently by the nutrient source and by the substrates. The status of N and Fe was affected by the nutrient source, but not by the substrates. However, the N availability was mainly affected by the substrate, as described before. The P, Mg, Mn and Na status was affected by both factors, while the Ca, Zn, Cu and Mo status and the micronutrient status was only affected by the substrate type (**VII,** in Table 9). Root development was influenced by the substrate, which indirectly affects the mineral balance in the shoot, as is the case for substrates containing vermiculite with high P and Mg content and resulting in lower Zn content in the plant shoot (**VII,** in Table 8 and Table 10). The nutrient status in the shoot was normal for all treatments, with some variations in the case of K, Mg, Zn, Cu and Mo, but never achieving toxic or deficiency levels (**VII,** in Table 10). The fertilizer affected the N, P, K and Na content, and the substrate affected Ca, Mn, Zn, Mo and Na status.

5. Discussion

5.1. Effect of freezing

Urine volume reduction by freezing-thawing is an effective and simple way for nutrient concentration and recovery. More than 80 % of both nitrogen and phosphorus can be concentrated in 25% of the initial volume. This can be achieved both by slowly freezing part of the urine volume or slowly melting a completely frozen amount.

This separation process was effective also in combination with other urine processing methods studied, like struvite precipitation and adsorption. After freezing and thawing the solutes are concentrated in the remaining liquid phase, leaving the ‘ice-supernatant’ almost free from impurities. This significant reduction of ions was confirmed by the total-N and total-P analysis and by the statistical calculations. The increased survival of *D. magna* in the acute toxicity tests made on the ‘ice-supernatants’ confirms this. In combination with precipitation and adsorption this method can radically improve the nutrient recovery from urine separating systems. Freezing and thawing alone or in combination with treatment with MgO and adsorbents could significantly decrease the toxicity for *D. magna,* due to the high total-N removal from the supernatants.

5.2. Struvite recovery

By adding small amounts of MgO to different urine solutions (synthetic urine, fresh, concentrated morning urine, fresh midday urine with lower nutrient content and stored urine diluted with tap-water collected from separated toilet system), struvite was obtained and identified as the main component. Together with struvite, other minerals, such as epsomite [$MgSO_4{*}7H_2O$], brucite [$Mg(OH)_2$] and montgomeryite [$Ca_4MgAl_4(PO_4)(OH)_4{*}12H_2O$], may also form (sometimes even newberyite), depending on the amounts of other divalent or trivalent metal cations available, initial pH of the urine.

Struvite precipitation was probably not affected by minerals added to urine for additional nutrient recovery. Thus, struvite precipitation and addition of different adsorbents can be made simultaneously. The freezing-thawing process did probably not influence the struvite formation either.

5.3. Adsorption on minerals and materials used

5.3.1 Total-P reduction

Struvite is the main precipitated compound from urine. However, zeolite can also adsorb considerable amounts of phosphates to their hydrous oxides on Al structural sites (Vaughan 1978; Wild *et al*., 1996). Sakadevan *et al*. (1998) reported a P adsorption capacity of 2.15 g/kg for clinoptilolite rich zeolites. Our tests also confirm the P adsorption by zeolite. Thus, the recovery of P is a result of both MgO and zeolite addition. The adsorption of phosphates by wollastonite was not investigated taking in consideration the fact, that wollastonite was tested earlier by others exclusively for cation sorption. Active carbon as phosphate adsorbent has not been investigated before, and consequently we cannot compare its efficiency in this case with other studies. However, active carbon might work as PO_4^{3-} adsorbent in our tests, due to its external surface and mesoporous texture (Díaz-Terán *et al*., 2001), but its structure and the chemical character may be affected by the carbon deposit from which the AC originates and the sort of pre-treatment (activation) this adsorbent went through (Leboda *et al*., 2003; San Miguel *et al*., 2003). However, the P reduction of AC was not significant in this study.

5.3.2 Total-N reduction

During the 72 h equilibration time, ca 60% of the nitrogen content in urine is expected to be hydrolysed (Adamsson *et al*., 1996; Haneaus *et al*., 1996) and available as ammonium (NH_4^+) for uptake by the adsorbent, or for precipitation in the form of struvite. In our experiments with urine from Universeum this urine was collected from a storage tank (diluted urine already stored for several days or weeks) and was further stored in the laboratory for 3 more days before the tests. Therefore, we expected an urea hydrolysis of over 80% in this case, even if it was not analysed.

Struvite precipitation will decrease the ammonium concentration and the zeolite will act as cation exchanger for the remaining ammonium uptake from the solution. The clinoptilolite rich natural zeolite used in all experiments has good ammonium selectivity and the total-N recovery varied between 60-80 %. The highest result was observed using diluted urine. It is known from earlier experiments that the NH_4^+-exchange capacity of natural zeolites is negatively affected by high ion concentrations or by the presence of Mg^{2+} or Ca^{2+} in the solution (Vaughan, 1978). This may be the reason why the total-N uptake in urine with higher ion concentrations (fresh urine, especially morning-urine) was not higher than 60%.

To improve uptake, higher amounts of zeolites can be used, but it is a matter of cost efficiency. Pre-treated zeolites are more efficient adsorbents and can probably be used in much smaller amounts, but this is also a matter of cost for activation of zeolites (high temperature and high vacuum) as well as handling and management of this product. Wollastonite as ammonium adsorbent showed almost the same results as the zeolite, with an average ammonium uptake of 50 %. Active carbon has been investigated before as an adsorbent for ammonium and other nitrogen containing organic substances (Emaldi *et al*., 2000; Li *et al*., 2003; Matsui *et al*., 2003), but not as an adsorbent used for such a high nitrogen concentrations as in urine. In our experiment AC turned out to have a comparable total-N adsorbent efficiency with the zeolite. However, zeolite is specialised for ammonium adsorption and AC seems to be a generalist in this case. During testing we observed a difference between these two materials. The solutions treated with AC had lost their original yellow colour and the typical urine odour, becoming colourless and almost odourless. These physical properties were not changed so much when zeolite was used. The question is if AC adsorbs the same type of nitrogen compound from urine as zeolites does. It is possible that AC adsorbs ammonium, but also larger organic molecules containing nitrogen, *e.g.* the pigment bilirubine from urine. However, this question was not investigated further in this study.

5.3.3 Toxicity reduction

Previous studies of urine toxicity for *Daphnia magna* were made in our laboratory with the aim of studying human urine treatment in aquaculture systems (Adamsson, 1999). It was found that human urine, fresh or stored, is highly toxic for *Daphnia magna*. The solution had to be diluted to an initial urine concentration of 0.5 % in order to be used as an algal nutrient solution and a culture medium for *D. magna*. The main causes for urine toxicity were identified as high pH in combination with high concentrations of ammonia-nitrogen (NH_3-N) (Adamsson *et al*., 1996; Adamsson *et al*., 1998).

In this study freezing was most effective for reducing toxicity. Addition of MgO increased toxicity (lowered EC50s), but addition of zeolite or AC counteracted this increase, especially in frozen solutions (Figure 11). Part of this could be due to the presence of ammonia-nitrogen from the NH_4^+- NH_3 equilibrium in the system due to the increased pH values.

Altogether, freezing-thawing alone or in appropriate combination with treatment with MgO and adsorbent can significantly decrease the toxicity for *D. magna*, mainly due to the high total-N removal.

5.4. Plant availability and fertilizing value of solids recovered

The two different plant experiment gave differing results, both having its own strength and weakness for plants testing under otherwise identical climate chamber conditions.

Experiment (**VI**) was designed with nutrient limiting conditions in order to detect differences among the treatments. The nutrient source was only the solids recovered from 100 ml urine processed with different methods presented earlier.

Experiment (**VII**) was designed to provide an optimal and balanced nutrient supply from five different sources (among others SZM from urine) in order to detect similarities among the sources.

5.4.1 Experiment (VI)

Crop growth

The biomass production of the plants having nutrient supply from the treated urine was only half as much as that of the plants using balanced NPK fertilizer as nutrient source (controls). This indicates a lower nutrient availability from the recovered nutrients, than from the highly soluble NPK used. Both struvite and zeolites are known to be slow release fertilizers (Bridger *et al*., 1962; Pond *et al*., 1984; Sikora *et al*., 1989; Ghosh *et al*., 1996). As such, they are not containing and releasing enough nutrients recovered from 100 ml urine per pot for optimal plant growth during the first 3 weeks of intensive growth period for *T. aestivum* L. In the case of the pots having struvite and NH_4-zeolites as nutrient sources the average dry weights of the plants indicated a slightly higher biomass production than in those treated by struvite and N-loaded AC. This may be explained by a synergistic effect of the zeolite/struvite mixed mineral system on nutrient solubility.

Earlier research have shown that non-calcareous soils fertilized with different ammonium-zeolite/apatite mixtures enhanced P dissolution from the apatite (Lai, *et al.*, 1986; Allen, *et al.*, 1993,1995) and increased the biomass production of greenhouse-grown sorghum-sudangrass (*Sorgum bicolour* L.) by a factor of 1.6 (Barbarick *et al.*, 1990). Higher zeolite/apatite ratios gave higher P availability, because of a decrease in the soil pH, dissolution of apatite rock and more available NH_4^+ from zeolite in the plant-soil interactions during growth. The system is based on the principle that zeolites can sequester Ca^{2+} released by the dissolution of apatite, thereby leading to further dissolution of the apatite (Lai *et al.*, 1986). In our case a similar nutrient carrying system with zeolite/struvite mixture and high Mg^{2+} ion content and a high zeolite: struvite ratio (7:1 for 15 g/l zeolite used and 15:1 for 30 g/l zeolite used) may increase the nutrient availability as a result of the synergistic effect from the mineral system. In the case of AC, the nutrients are not recovered by cation exchange and chemisorption, but by a physical adsorption in the porous structure of the material. Desorption in the water microenvironment of root-substrate system with slight pH changes is easier in this case. However, no similar previous experiments or research results can confirm this.

The pH decreased during the pot trials from 10.0 to 8.0 on average (data not presented here), with the greatest decrease at high zeolite additions, where pH was between 7.6 and 8.0 after harvest. In the treatments with high MgO the pH decreased only to 9.0 after harvest, causing small and short root system development and a generally lower shoot height for the plants. The direct and indirect effects of high soil pH are well described in Marschner (1999).

Nutrient uptake

Apart from environmental factors like light, temperature and water, biomass production and healthy plant development is highly dependent on the quality and amount of nutrients applied. The former can be tested and different treatments compared in a climate chamber, if the other factors are optimally regulated for plants. In this study the amounts of N was found to be in the deficiency range for *T. aestivum* L. However, the pot trials were performed to estimate the behaviour of the nutrients recovered as solids from small amounts of human urine, regardless of optimal nutrient supply.

The NaCl content of fresh human urine is about 150 mM (calculated from Altman *et al.*, 1974). In our treatments the 50 % diluted urine (NaCl concentration approx. 75 mM) was concentrated by freezing-thawing. The plants growing in the system (except for the controls) were exposed to approx. 120 mM NaCl concentration. Plant performance may be affected by salinity-induced nutritional disorders (Hu *et al.*, 1997, Grattan *et al.*, 1999). In our experiments a complex interaction between the slow-release fertilizers (struvite and zeolite or AC), the saline conditions from urine concentration, a high pH and a generally low N addition may take place, resulting in low dry weight and height for the plants compared with the controls.

Plant nutrient analysis was not made. However, visual observations on plant development indicate some N and P deficiency symptoms on the plant leafs as yellow apex on old leaves and reddish stem on the plants treated only with struvite or only with AC. Plants treated with zeolites, struvite/zeolite mixture or struvite/AC mixture showed no such visual deficiency symptoms.

5.4.2 Experiment (VII)

Plant biomass and development

The biomass production of the plants having their nutrient recovered from (SZM) was similar to those receiving commercial P-fertilizers (DAP and CaP), when identical substrates were used. This indicates that the nutrient availability from SZM is as good as that from the other solid fertilizers. A slightly higher biomass obtained in pots having SZM as fertilizer may be explained by the difficulties to calculate a very exact nutrient addition (especially for N and other macronutrients from urine). Another explanation may be a better P and N availability from SZM compared to DAP and CaP, due to the synergistic effect of the zeolite/struvite mixed mineral system on nutrient solubility. The nutritional status of the shoots indicate that there was less nutrient available for the plants in the CaP and the OPT treatment as well, but not in the DAP. That means that there also are other factors than nutrient availability that contributes to the differences in biomass between treatments. However, the fact that the nutrient status of the plants from the SZM treatment were among the highest could be explained by earlier research showing that non-calcareous soils fertilized with different ammonium-zeolite/apatite mixtures enhanced P dissolution from the apatite (Lai *et al.,* 1986; Allen *et al*., 1993,1995) and increased the biomass production of greenhouse-grown sorghum-sudangrass (*Sorgum bicolor* L.) by a factor of 1.6 (Barbarick *et al*., 1990). Higher zeolite/apatite ratios have resulted in increased P availability, because of a decrease in the soil pH, dissolution of apatite rock and more readily available NH_4^+ from zeolite in the plant-soil interactions during growth. The system is based on the principle that zeolites can sequester Ca^{2+} released by the dissolution of apatite, thereby leading to further dissolution of the apatite (Lai *et al*., 1986). In our study a similar nutrient carrying system with zeolite/struvite mixture and high Mg^{2+} ion content and high zeolite:struvite ratio (15:1 for 30 g/l zeolite used) may increase the nutrient availability as a result of an interaction with the mineral system. The plant biomass production was lower in the pots receiving substrates containing vermiculite. A lower biomass production may be explained by the impact of this clay on the nutrient availability. Vermiculite is extensively used in greenhouses as root media, fertilizer or pesticide carrier or soil conditioner. It is well known for its high K and NH_4-fixation and high P adsorption on the hydroxyaluminium-interlayer in the vermiculite structure (Corey, 1990; McBride, 1994; Saha *et al*., 1997, 1997; Scherer *et al*., 1999; Chappell *et al*., 2000; Evangelou *et al.,* 2002). In our study (**VII**) the nutrient addition was calculated to be sufficient for the plant, regardless of the substrate type. A higher retention of N and P in the substrate containing vermiculite can be the cause of lower biomass production. The pH and conductivity of the solutions extracted from the substrates with vermiculite was the most elevated in the experiment (pH 7.7-8.7 and conductivity 330-450 μS/cm). This indicates some difficulties for nutrient acquisition of the plants grown in vermiculite, although the final nutrient status in the plants was not lower than in the plants from several other substrates.

The lowest biomass production was obtained in the pots treated by the nutrient without P (OPTnoP). Severe P deficiency symptoms were also visible on these plants, like dark-green leaves and magenta coloured stems.

The relative growth rates (R_G) of the plants were similar to plants growing at optimised nutrient supply (OPT) in the case of the same substrate types, highest in the case of SZM fertilizer and lowest for the plants grown under P deficiency (**VII**, in Table 5).

A proper balance between root and shoot is important for a healthy plant development. The root/shoot ratio may differ depending on environmental conditions around the plant and the type of cultivars (Shangguan *et al.*, 2004). In early stages of wheat growth the root/shoot ratio is near 1.0, but at anthesis this can fall to 0.1 (Brar *et al.*, 1996). For winter wheat tested in climate chamber at sufficient water and nutrient additions and with optimal environmental factors a root/shoot ratio of 0.5 was found to be optimal (Shangguan *et al.*, 2004). A similar root/shoot ratio was found in our study in the treatments having sand, peat and dolomite (S+P+D) as substrate, which also had the highest biomass production. In the substrates containing vermiculite the root/shoot ratio was high (0.7-1.1) and the biomass production was the lower. High root/shoot may lead to reduced nutrient and water use, in spite of root redundancy (Shangguan, *et al.*, 2004).

Nutrient source and substrate behaviour

The availability of nutrients from the fertilizers and the impact of substrate can be estimated, if the water-soluble and plant available amounts of nutrients are measured in the extracted substrate after the last harvest. The solid fertilizers showed very similar qualities as P sources. Both DAP and CaP are known slow release fertilisers, and the SZM obtained from urine showed the same P source behaviour, with slightly better biomass production, probably due to the increased nutrient availability of the zeolite/struvite system, as described above. The water soluble, plant available P content in the extracted solutions indicated also a substrate impact in the case of vermiculite, probably due to its nutrient adsorption, as described above. The main N source for all treatments originates from the supplementary nutrient solutions added, containing 93% of NO_3-N as an easy available N source for plants (Ingestad *et al.*, 1986). The N was sufficient everywhere, except for K-soil, which did not receive supplementary nutrient solution. In a commercial soil the nutrient content must be sufficient and balanced for any type of cultivars and growth conditions (**VII**). In our case the P was sufficient, but the N was not enough for the 33 days long growth of one wheat plant (**VII**). In spite of a good biomass production (Figure 24 (a, b)), the plants in the pots with K-soil showed N deficiency symptoms, like light green leaves and stem, chlorotic old leaves and yellow apex on the youngest leaves. The high root/shoot ratio also indicates N deficiency. The plant N status was in the deficiency range (**VII**). Thus, the N from 200 g K-soil was simply not sufficient for one plant during 33 days of growth.

Impact of nutrient source and substrate on the plant nutrient status

The primary macronutrient content (N, P, K) in the above ground dry matter is directly dependent of the fertilizer macronutrient supply, and the majority of treatments were in the sufficiency range (exceptions were discussed above). However, Ca was in excess in the substrates having dolomite for pH adjustment, but these levels were not in the toxic range for wheat. High Ca in the substrate may inhibit Fe, Zn and Mn uptake (Wild, 1988; Marchner, 1999). In our case, the plants grown in the substrate with sand and dolomite showed the lowest Mn and highest Fe status (**VII**, in Table 10). This negative relationship between Fe and Mn in plant tissue is normal (Vitosh *et al.*, 1994). The Mn plant contents in our treatments were in the sufficiency range. High Fe contents in plant tissue were found in several treatments, but they were not in the toxic level for wheat. The lowest Fe tissue content was found in the substrate with sand, peat and dolomite, which others also had observed (Vitosh *et al.*, 1994). The lowest Zn status was found in plants grown in substrates with vermiculite. The Zn availability for Plants is reduced by the high substrate P level (Vitosh *et al.*, 1994), and this is the case with the substrates with vermiculite. Cu and Mo were in the sufficiency range in all treatments, and the spring wheat is not sensitive to the Na levels found in the tissue.

6. Conclusions

Effective fractionation and concentration of nutrients from human urine can be obtained by a simple process of freezing-thawing. By these means more than 80% of both N and P can be concentrated in 25% of the original urine volume.

Freezing-thawing in combination with struvite and adsorbents used has an overall positive effect on additional nutrient recovery from human urine, especially on N recovery.

By addition of small amounts of MgO to human urine, struvite was obtained and identified as a main component together with montgomeryite, brucite and epsonite depending on pH, the amounts of Ca and other metals available. Thus, a more correct formula for struvite is $[(Ca, Mg)(K, NH_4)(PO_4)_* 6H_2O]$ when it is obtained from such a complex solution as human urine.

With struvite precipitation 100% of P, 22-64% of K and 2-5.6 % of Ca was recovered from synthetic urine. Theoretically, approx. 25% of the N recovery is also due to struvite crystallization.

The pH for maximal struvite recovery from human urine is 9.0-9.5.

Both natural zeolites (especially clinoptilolite) and wollastonite adsorbed ammonium from NH_4Cl-solutions rapidly (< 1 minute). The quality of minerals, the grain size and the ion strength of the solution affect the adsorption.

Mineral adsorption in combination with struvite precipitation could recover 64-80% of the N in laboratory tests with synthetic human urine.

Nitrogen recovery from fresh and stored human urine was more complex and was affected both by the amounts of MgO and zeolite added.

To some extent P recovery through MgO addition was affected also by zeolite addition to both fresh and stored and diluted human urine. In stored and diluted human urine additional P recovery is affected by zeolite addition, by not by active carbon addition.

Maximum combined recovery of N and P occurred at added concentrations of 0.1 g of MgO and 15-30 g of zeolite per liter stored and diluted urine. The optimal additions are dependent on the initial N and P concentrations of the urine.

In stored and diluted human urine both zeolite and active carbon acted as N adsorbents. However, they may act differently and affect different N compounds. Active carbon effectively reduced color and smell.

The acute toxicity to *Daphnia magna* of the remaining supernatant solutions, after N and P recovery from fresh human urine, was affected by both MgO and zeolite addition. High concentrations of zeolites increased the toxicity of the supernatant. However, zeolite itself was non-toxic for *Daphnia magna*, so the increase was probably related to the increased pH followed by increased ammonia toxicity. In stored and diluted human urine high concentrations of added MgO increased the acute toxicity of the supernatants to *Daphnia magna*, but addition of zeolite or active carbon reduced it. Also freezing reduced acute toxicity for *D. magna* of the supernatant, which correlated with reduced ammonia concentrations.

The N recovered from 100 ml stored and diluted human urine as struvite, loaded zeolite or AC and freezing, was not enough to support optimal growth of 3 wheat plants during 21 days. The whole experiment was, therefore, made under N deficiency. However, the struvite/adsorbant mixtures showed better nutrient availability than the struvite alone, probably due to a synergistic effect between the struvite dissolution and zeolite ion exchange or AC release of nutrients. Thus, struvite, struvite/zeolite mixture and struvite/AC mixture all acted as slow-release fertilizers.

The nutrients recovered from urine as struvite/zeolite mineral (SZM) used in proper amounts acts as a good P source, fully comparable with DAP (di-ammonium-phosphate) and CaP (mono-calcium-phosphate) slow release commercial P-fertilizers. These fertilizers were also similar as P sources to the liquid fertilizer used in the experiments.

7. Final remarks

7.1. Possible sustainable use of processed human urine (yellow water)

Yellow water processing in the of ECOSAN approach has provided answers and solutions to present shortcomings of urine separating systems.

Earlier project evaluations of urine separating systems revealed the following general shortcomings:

- Storage and handling (in cities and on farms)
- Transportation of urine as limiting factor for reuse
- Spreading on arable land with nutrient losses and need for new agricultural management
- Hygiene (including fear of spreading pharmaceutical residues and hormones to food production and in the environment)
- Acceptance and socio-cultural approaches of liquid urine handling
- Energy use and economy

The results obtained in this thesis were from laboratory studies, and they have, of course, a limited predictive value when it comes to practical applications.

What can be concluded already today from the urine processing is the following:

- Storage need and handling problem, transport and hygienic aspects can be partially resolved or at least much improved by **freeze-concentration.** A factor 4 can be achieved in volume reduction, with 80% of all nutrient recovery and possible reuse as concentrated liquid urine fertilizer. This concentration level implies a direct improvement toward smaller storage volume needs everywhere. Smaller volume means reduction of transport need probably with the same factor. Concentrated urine is known to be a quick pathogen killer (Jönsson *et al*., 2000) making the hygienic requirements easier to achieve (with shorter storage time or no storage need at all). To be economically and energetically competitive, all stored urine from cities must not be transported longer distances than 200-220 km from the collection places today (Jönsson *et al.*, 2000). However, in a future sustainable agriculture, wastewater resources need to be transported over longer distances to avoid accumulation of high nutrient levels in arable soils in the circumference of cities and towns (Kirchmann *et* *al.,* 2000), and to open up the possibilities for all farmers to change course toward sustainable use of anthropogenic resources.

- The acceptance of human urine is still a hard issue for many reasons (fear for pathogenes, economical, social, cultural and religious aspects) all across the world. Spreading of urine on arable land is also limited today (Drangert, 2004). In many places this resistance is deeply rooted in social and cultural behaviour, surrounded with *tabus* or low status labeling. Handling of urine is not really accepted as a modern, status giving high-tech sanitation solution, connected with the image of the welfare sanitation systems in developed countries, and it is not considered as a solution giving considerably improvements of the living standard of people in developing countries (Drangert, 2004). Handling of human urine is considered as a 'temporary solution' in most developing countries, unless other products and solutions are developed and can be economically possible. In this aspect the attitude toward handling freeze-concentrated urine is probably equivalent to that of not processed human urine.
- Energy savings and some economy can be improved by freeze-concentration of urine in countries with colder climate, when naturally low temperatures can be used for energy efficiency reasons. Freezing-thawing of urine (or wastewater in general) is a low energy consuming purification technique, simple and robust, applicable for all sizes of systems. This can give considerable economical returns in a system, depending on the chosen freezing technology (Åsblad, 1998). Together with heat exchangers or other technical solutions urine freezing-thawing can be used also in systems designed for hot climate living with a high demand for air conditioning.
- **Nutrient recovery as solids** by struvite precipitation with adsorption on minerals is probably the most efficient way to radically improve the shortcomings of the urine handling system and to solve some problems related to urine handling. C:a 100 % of P, 60-80 % of N, 30% of K, 3 % of Ca, and some S can be recovered in 25-35 g solid mineral mix from 1 l (ca 1 kg) urine. A factor 30 can be achieved in volume reduction with radical impact on storage, transport. Hygienic aspects are facilitated when handling of a solid product, instead of liquid urine. The solids are well-known slow release fertilizers (non burning for plants, not threatening groundwater or surrounding water bodies with leaching, not loosing N to atmosphere) in class with MAP industrial fertilizers. These are probably more easily accepted (economically, socio-culturally) by users worldwide. Struvite production and mineral use is a very simple, robust method, not demanding special equipments or working conditions. The storage tanks used today for urine collection can be used for this purpose with some modifications, to be able to make a simple stirring and emptying of the struvite and minerals after treatment. The added materials are in small amounts. MgO is made of dolomite (found worldwide, not being a scarce resource). The adsorbent type is a choice to be made according to the local possibilities for each situation. Zeolites are present almost everywhere on earth in soils (Scandinavia is one of the few exceptions!), and are also worldwide mined for their special properties and areas of application. Their beneficial effect in agriculture is well known in the field of 'zeo-agriculture'. Zeolite prices are very low on the global market (75-95 USD/tonne; source: www.findstone.com/1202.htm, 2005-02-18). In Scandinavia, other minerals like wollastonite or some clay minerals like bentonite can be used, if zeolite is not an option. Every fertile soil type in every region or part of the world contains inorganic adsorbent components originating from the bedrock weathering. Finding and using them in the first place, is the best choice for the environment and sustainable resource management.
- The energy costs for stirring and drying needed for struvite production and mineral adsorption are considered to be negligible in the total budget of energy costs for running different nutrient recycling sanitation systems (Åsblad, 1998). Dewatering or

drying is dependent on the local possibilities (from simple air-drying to centrifugation, slight heating, etc.). Nutrient recovery from urine by struvite precipitation was evaluated from the energetic point of view and compared with traditional nutrient recovery at wastewater treatment plants. Direct use of urine and nutrient recovered by struvite precipitation are considered to be very efficient solutions and equal in means of their energy value (Maurier *et al*., 2003).

- Nutrient concentration or nutrient recovery as solids from urine also means that a rest solution (called supernatant) most be taken care of in the sanitation systems. In the ECOSAN approach, when the ambition is to close all the nutrient loops, this rest solution from urine processing (containing very small amounts of nutrients or having a pH over 9) can be connected to other loops in the whole sanitation system. The supernatants can be utilized in many ways, depending on the system applied and the local conditions, such as irrigation, dilution for an aquaculture system or simply mixing it with the other household wastewater streams and treatments (infiltration, resorption beds, wetlands, or sent to a centralized sewage system).

7.2. Future research outlook

The studies on yellow water processing revealed many new questions and research topics. Some of them are summarized here:

Further research is needed to develop and optimize the technique of partial freezing at a larger scale. Testing an pilot equipment for method optimization, energy use, cost-benefit calculations is much needed. Experiments with a combination of other urine processes together with freezing and LCA are a necessity. The possibilities of eutectic freeze crystallization for urine sorting systems should also be investigated.

Testing other minerals than those studied here can be important for finding the best options for specific regions to be able to use their own natural resources and spreading them on the arable land in a sustainable way. In this sense, some clay minerals can be of interest and could be tested along with the wollastonite, for Scandinavian conditions.

Testing different forms of activation of minerals or adsorbents to increase the adsorption or CEC is an important research issue. It is known that zeolites can be pre-treated (activated) in different ways for this purpose. Testing and evaluation of these methods could be imprtant for selection of the most cost-efficient and sustainable materials.

To be able to evaluate the real costs and find possible upcoming problems regarding struvite precipitation (with or without additional adsorbent use) it is necessary to conduct tests on pilot equipments and pilot plants placed in different environments (from one family houses to more centralized urine collecting systems). It is of outmost importance to test and evaluate the struvite process as part of different ECOSAN system solutions. Only in this way it is possible to draw conclusions, make recommendations about how, when or if the struvite precipitation is a desirable nutrient recovery method or not.

Further research is needed to evaluate the fertilizer value and behaviour of struvite and struvite+asorbents compared with urine spreading and other commercial fertilizers or animal manure in long-term field studies.

Closing the nutrient loops can be reached only by a high level of acceptance and everyday use of urine or the nutrients recovered from urine. For this reasons research and financing should be oriented not just for behaviour studies on liquid urine acceptance and attitudes to spreading. These studies must include and evaluate also the attitude toward struvite or other fertilizers made by urine processing.

One of the major concerns is the spreading of pharmaceutical residues and hormone mimicking substances on arable land with wastewater, urine or faeces use as fertilizers. This is an emerging, new field of research and some studies and evaluations are already made on wastewater and toilet waste sorting systems. A large part of these substances are eliminated via urine and can be spread via urine irrigation on arable land. It is important that even products from urine processing (struvite, minerals) are included in these tests.

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Short communication

Volume reduction and concentration of nutrients in human urine

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Abstract

Urine-separation toilets have been proposed as a means of achieving maximum recovery and circulation of nutrients not contaminated by hazardous compounds such as heavy metals. However, the use of human urine is problematic with regard to management, storage and transportation. In this pilot study we present a simple method that can be used to reduce the volume substantially and hence improve the management. By freezing urine at a temperature of − 14°C, approximately 80% of the nutrients can be concentrated in 25% of the original volume. This step can be vital to the development of storage and transport.

Keywords: Human urine; Concentration; Urea; Nutrient recovery; Freezing; Melting

1. Introduction

Eco-cycling of nutrients between urban areas and farmland is a critical step towards ecologically sustainable development. With today's linear flows most of the nutrients are lost as waste through food production processes, trade, restaurants and as domestic waste and wastewater. The emission of nitrogen and other nutrients from domestic sewage is currently causing severe eutrophication problems in recipient waters all over the world.

Commercial fertilisers, used intensively in agriculture, are produced with a high level of fossil energy use and from finite mineral resources, sometimes polluted by cadmium or radioactive elements such as uranium (Hodge and Popovici, 1994). If all nutrients in domestic waste are recycled, the use of commercial fertilisers can be reduced by 35–45%. The urine alone can replace 20–25% of commercial fertiliser currently used in food production for people and animal stock (Jönsson, 1994). Nutrient recovery and recycling from human urine can be an important part of

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the eco-cycling society of the future. Calculations of the nutrient balance show that the food we need and metabolise is in balance with the 'waste' we eliminate as nutrients during a year (Wolgast, 1993). Since 88 to 98% of the nitrogen, 65–71% of the potassium and 67–68% of the phosphorus in toilet wastewater originates from the urine (Kärrman, 1995), together with a large amount of micro-nutrients present in balanced concentrations (Wolgast, 1993), new ideas of eco-cycling based on urine separation have been proposed to achieve maximum recovery and circulation of nutrients not contaminated by hazardous compounds and to reduce eutrophication in freshwater and coastal ecosystems. Urine-separating toilets/systems have been developed and installed in several eco-villages around the world, including different parts of Sweden. However, the experience gained is to a certain extent controversial. The systems are sensitive to disturbances in the storage and transportation of the urine. The demand for distribution guarantees and quality- and hygiene-related problems are threatening to end many of the new experiments (because of its high nutrient content, urine is an excellent medium for bacterial growth).

Life cycle assessment (LCA) studies of conventional as well as urine-separating wastewater systems show that a urine-separating system has many advantages with regard to factors such as emissions and nutrient recovery (Bengtsson et al. 1997). Exergy (energy efficiency) analyses of sewage treatment systems show that phosphorus and nitrogen recycling efficiency is highest when urine-separation techniques are used (Hellström and Kärrman, 1997). However, according to LCA studies of urine-separation systems, the transport of large amounts of urine, as well as spreading and hygiene are important obstacles to achieving system efficiency (Jenssen and Etnier, 1996; Larsen and Gujer, 1996; Höglund and others 1998). The main problem regarding exergy efficiency is related to management — the storage and transportation of the urine (Hellström, 1998). Large volumes of urine are needed to fertilise farmland and there are problems related to the collecting and transportation of these larger quantities.

Urine is the key component in the process of nutrient recovery from domestic wastewater. In this paper we present a simple method to reduce substantially the volume of urine by freezing and thawing and still recover the majority of the nutrients. The method may be an important step to improve the efficiency of urine-separating wastewater systems.

2. Concentration of nutrients in human urine

Human urine is a complex water solution containing nutrients as highly diluted compounds. Sodium chloride (NaCl) and urea [$CO(NH_2)_2$] are the main compounds, although urine also contains, for example, potassium (K), calcium (Ca), sulphate (SO_4) and phosphorus. Phosphorus is available as superphosphates ($H_2PO_4^-$ or HPO_4^{2-}) and potassium as an ionic component (K^+). The majority, around 80% of the total nitrogen in fresh urine, appears in organic form as urea [$CO(NH_2)_2$] (Altman and Dittmer, 1994). The rest is ammonia-N from inorganic nitrogen compounds and organic-N bound in matrix. The daily excretion of urea by an adult varies between 11.8 and 23.8 g/pd and the ratio urea-N: total-N is about 0.8 (Fittschen and Hahn, 1998).

When the temperature is lowered and ice crystals are being formed, the irregular network structure of such a solution undergoes reorganisation into a stable, regular tetrahedral network. With the formation of ice it becomes almost impossible for elements other than water molecules (impurities, salts in water solution) to be incorporated into this crystalline structure (the exception is ammonium-fluorite, which has a very similar crystalline structure) (Olovsson, 1995). During slow ice formation from aqueous salt solutions, the ions and chemical compounds remain concentrated in the liquid part and are excluded from the ice crystal formation. The ice is practically salt-free. The higher the crystallisation velocity, the more components follow the water molecules into the crystal network. However, they do so as impurities in pores and not as structural elements in the crystals.

Ionic separation also occurs during the melting process. Both field and laboratory snow-metamorphism experiments confirm that 50–80% of the pollutants are released when the first 30% of the snow melts (Overrein et al., 1981; Bengtsson, 1995). The first meltwater may have ion-concentrations that are around five times the values found in bulk snow. In extreme cases the factor can be as much as 10.

There are thus processes available for fractionation and concentration of ionic compounds in human urine. When stored, urea is subject to chemical hydrolysis and biological decomposition (Hanaeus et al., 1996):

$$CO(NH_2)_2 + 3H_2O \rightarrow 2NH_4^+ + HCO_3^- + OH^- \quad (1)$$

The ammonium is in equilibrium with the dissolved ammonia:

$$NH_4 + OH^- \leftarrow \rightarrow NH_3\ (aq) + H_2O \quad (2)$$

The pKa-value for the equilibrium is 9.3 at 25°C.

The dissolved ammonia is in equilibrium with the gaseous ammonia:

$$NH_3(aq) \leftarrow \rightarrow NH_3(g) \quad (3)$$

The decomposition of urea will lead to an increase in the concentration of ammonia and an increase in pH in the urine. Ammonium is directly available for the biota and thus a key component in the nitrogen cycle

3. Material and methods

This study was undertaken using human urine collected from five healthy, young or middle-aged people, and synthetic urine prepared from 11 solutes according to conventional urological research (Griffith et al., 1976a,b). Both types of urine were stored in PET-bottles in a refrigerator (+8°C) or a freezer (−14°C) for up to 72 h. All equipment was acid-rinsed before use. Samples were immediately transported in closed bottles for analysis by AnalyCen Nordic AB, accredited laboratory, Göteborg.

Two types of freezing and melting methods were performed to concentrate the nutrients. The first was a bath freezing-melting method, where the freezing and melting was controlled by an isolation bath of saturated NaCl. The second was a column freezing-melting method, where columns of the samples were frozen completely in different ways and then melted. Temperature, conductivity, pH, total-N and total-P were measured.

In the bath freezing-melting method samples were placed in an isolation bath and put into the freezer, where the freezing process started from the top of the samples, leaving the lower part as liquid. After 3, 5 h (at −14°C) the solid phase (ice-bulk at the top, half of the total volume of the sample) was separated from the liquid phase (solution in the bottom of the plastic pot) and analysed separately. One sample volume comprised 100 ml and the measured variables were pH, temperature and conductivity. The experiment was undertaken to study ion separation during the freezing process. Six samples were studied.

A similar method was used in the next experiment, when the samples were frozen completely in the bath and the melting process was slow at room temperature (21°C), controlled by the salt (NaCl) bath isolator until half of the sample volume had melted (approximately 2.5 h). The same parameters as above were measured. The purpose of the experiment was to study ion separation during slow melting. Six samples were studied.

In the column freezing-melting method, the melting of frozen columns (in 1 l PET-bottles) was studied. The purpose was to observe ion separation during the melting process.

After initial tests with freezing and melting of 700 ml columns of fresh human urine a series of four parallel column experiments were performed, each with 450 ml of urine. Two of the columns were homogeneously frozen while the other two were frozen and crushed into small and mostly homogenous ice grains that were left to melt for 30 min at room temperature (21°C) and then frozen again. This process of freezing and crushing was repeated twice and a snow-like sample was obtained. The column was then placed at

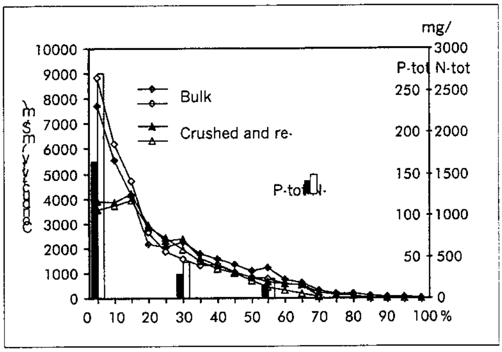

Fig. 1. Conductivity (left axis) and P-total and N-total (right axis) from the melting of the 450 ml frozen columns of human urine. The bottom axis show the accumulated sampling (20 ml) until 100% of the columns volume were collected as melted 20 ml samples.

room temperature in the laboratory to melt completely. Using this method it was possible to observe, in parallel, two different types of melting processes and to compare the collected fractions with regard to possible differences in ion concentrations. Twenty fractions (each of 20 ml) were collected from each column during melting. The fractions were collected at roughly 15-min intervals, even longer depending on the melting process. The variables measured were pH, temperature and conductivity, complemented with total-N and total-P analyses.

Additional experiments using synthetic urine were performed using the column-freezing-melting method. Twenty-four samples of 40 ml were taken from a frozen volume of 960 ml. pH, conductivity, tot-P, tot-N and tot-C were measured in all samples.

4. Results and discussion

The results from the column freezing-melting experiment are presented in Fig. 1. The results show a significant concentration of ions in the first melted fractions. In Fig. 1 it can be seen that about 78% of the ions measured as conductivity are present in the melt-out of the first 100 ml (22% of the volume). This picture is confirmed by the total-N and total-P analyses. There is, however, a difference between the bulk-frozen columns and the crushed columns. The first fractions in the bulk-frozen columns were collected after just 10 min, whereas the first 20 ml fraction took 50 min to melt in the crushed columns. The melting accelerated more in the crushed columns and the last fraction was taken almost simultaneously in all four columns.

The column freezing-melting experiments with synthetic urine show similar results. Of all the measured constituents 80% were collected in the first five samples, see Fig. 2. It is also evident that

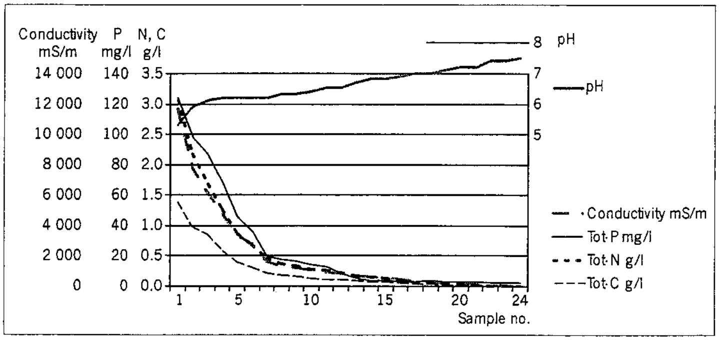

Fig. 2. Conductivity, total phosphorus, nitrogen, carbon content and pH in 40 ml samples (1–24), collected from melting of a frozen column of 960 ml synthetic urine.

there is a very good correlation between the conductivity and the total P, N and C content.

The results from the top-freezing experiments and the results from the total sample bulk-freezing and slow-melting experiments (bath freezing–melting method) both showed that many of the ion compounds were concentrated in the liquid phase. The separation process seemed to be effective and the ion concentration doubled in the liquid phase compared with the initial samples. The remaining ice phase was very low in ion content, measured as conductivity.

It is concluded from these experiments that effective fractionation and concentration of nutrients (potassium, nitrogen and phosphorus) can be obtained by a simple process of freezing and melting. More than 80% of both nitrogen and phosphorus can be concentrated in 25% of the volume. The concentration may be obtained both by slowly freezing part of the sample or by slowly melting a completely frozen sample.

5. Conclusions

Separate management of human urine opens up the possibility of effective recovery and circulation of nutrients not contaminated by hazardous substances. In this study, simple methods of concentration of nutrient compounds in human urine were presented. The freezing-melting method makes it possible to capture 80% of the nutrients in 25% of the volume. This can be an important step towards improving urine management with regard to storage and transportation. With the concentration method a 6 m^3 tank is sufficient for collecting urine from 400 people during two months. The remaining unenriched portion of the urine may be disposed of to a recipient, preferably a wetland recipient.

The method certainly needs to be further developed and optimised. However, we believe that the technique presented for the recovery of nutrients from separated urine can have a role to play in the urban sanitary system of the future, which will almost certainly be much more diverse than it is today.

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II

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BIORESOURCE TECHNOLOGY

Nutrient recovery from human urine by struvite crystallization with ammonia adsorption on zeolite and wollastonite

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Abstract

Urine-separation toilets are a possible route for achieving maximum recovery and recycling of urine nutrients not contaminated by hazardous compounds such as heavy metals. However, the direct use of human urine as agricultural fertiliser is problematic and controversial with regard to hygiene, storage, transport and spreading. In this paper, simple methods for capturing the nutrients in urine by transformation into solid mineral form are presented. On the addition of small amounts of MgO to synthetic or natural human urine most of the phosphorous and significant amounts of the potassium and nitrogen were precipitated, with crystalline struvite $[Mg(K, NH_4)(PO_4) \cdot 6H_2O]$ as a major component together with montgomeryite, newberyite, brucite and epsonite. Nitrogen recovery could be improved by adsorption. Clinoptilolite, wollastonite and a natural zeolite all showed excellent adsorbent properties in contact with ammoniacal solutions. In combination with struvite crystallisation 65–80% of the nitrogen was recovered as crystalline or adsorbed ammonium. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Struvite; Clinoptilolite; Wollastonite; Ion exchange; Adsorption; Zeolite; Ammonia; Nutrient recovery

1. Introduction

About 94% of the nitrogen, phosphorus and potassium in toilet wastewater emanates from the urine, together with abundant micronutrients in balanced concentrations (Wolgast, 1993). New ideas for eco-cycling of nutrients from waste water have been proposed based on urine separation, in order to maximise the recovery and recycling of nutrients and to reduce eutrophication in freshwater and coastal ecosystems. Urine separating toilets have been developed and installed in several eco-villages around the world, including different parts of Sweden.

Life cycle comparisons of conventional and urine-separation wastewater systems show that urine-separation systems have many advantages related to emissions and nutrient recovery (Bengtsson et al., 1997). Exergy (energy efficiency) analyses of sewage treatment systems show that phosphorous and nitrogen-recycling efficiency is highest if urine separation is used (Hellström and Kärrman, 1997). However, life cycle assessment-studies of urine-separation systems also show that the storage, transport and spreading of large amounts of urine, presents serious obstacles to system efficiency (Jenssen and Etner, 1996; Larsen and Gujer, 1996; Höglund et al., 1998). Large volumes of urine are needed to fertilise typical farmland, leading to high transport costs. Another problem of urine-separation systems is the loss of nitrogen by ammonia evaporation during storage and spreading (Hanaeus et al., 1996). Furthermore in many countries the spreading of liquid urine fertiliser is restricted to certain periods of the year.

We believe that many of the problems connected with urine separation could be met by transforming the nutrients in the urine into solid minerals. Handling and storage could be substantially improved, the volume would be dramatically reduced compared with liquid urine, loss of nitrogen into the atmosphere would be eliminated, a high level of hygiene could be maintained and spreading on arable land could be much more flexible in terms of time and dosage.

This paper presents the results of a pilot study of nutrient recovery from human urine by struvite crystallisation with further ammonia recovery using zeolites or wollastonite.

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2. Crystallisation and adsorption of nutrients from human urine

Human urine is a complex aqueous solution containing sodium chloride (NaCl) and urea ($CO(NH_2)_2$) as dominant compounds but also potassium (K), calcium (Ca), sulfate (SO_4) and phosphate (PO_4) in low concentrations. One of the most important compounds that can precipitate from human urine is the mineral struvite, $Mg(NH_4)(PO_4) \cdot 6H_2O$, formed by reaction of magnesium with ammonical phosphate solutions and usually found in deposits of guano or animal dung. Human urine contains an excess of ammonium relative to phosphate, but is deficient in magnesium. The normal pH of human urine is between 5.6 and 6.8 and most of the phosphate is present as $H_2PO_4^-$ or HPO_4^{2-}. With the addition of MgO the pH increases, shifting the phosphate equilibrium toward PO_4^{3-} and magnesium is provided for struvite crystallisation. Struvite precipitation may be a method to remove phosphate but if it happens spontaneously it can also cause operational problems in sewage systems. Struvite formation from mixed waste water has been studied by e.g., Liberti et al. (1986), Sen and Randall (1988), Mohajit et al. (1989), Maqeda et al. (1994), Momberg and Oellermann (1992), Pérez Rodríguez et al. (1992), Wrigley et al. (1992a,b) and Battistoni et al. (1997). The potential of struvite crystallisation for direct nutrient recovery from human urine has to our knowledge not been investigated before.

In fresh urine ammonium (NH_4^+) is formed from the decomposition of urea (Hanaeus et al., 1996). This offers possibilities for nitrogen recovery by ammonium uptake to specific adsorbents such as zeolite or wollastonite minerals. The ion exchange properties of natural zeolites especially clinoptilolite $[R(Na,K)_6(Al_6Si_{30}O_{72}) \cdot 20H_2O]$ are well documented (c.f. Breck, 1974; Jorgensen et al., 1976; Gottardi and Galli, 1985; Tomazovic et al., 1996). The use of wollastonite for removing heavy metals from aqueous solutions was investigated by e.g. Sharma et al. (1990) and Lifvergren (1997). The use of zeolite or wollastonite as adsorbents for ammonium recovery from human urine has not been investigated before.

3. Methods

Synthetic human urine containing 11 solutes, in concentrations equivalent to the daily average urine of normal healthy men was prepared according to conventional urological methods (Griffith et al., 1976a,b) for use in the experiments. Synthetic urine is free of pyrophosphates, organic macromolecules (matrix) and unspecified substances that could enhance or inhibit the nutrient recovery. In addition struvite precipitation was also tested using fresh human urine from five healthy, young to middle-aged people. For studies of NH_4 adsorption a NH_4Cl solution was prepared with concentrations similar to synthetic human urine without and with urea hydrolysis; 0.02 and 1 M, respectively. All solutions were stored at room temperature.

The chemical compositions of the adsorbents are presented in Table 1. The adsorbent minerals were not pretreated or activated in any way, just crushed and sieved to specific grain size fractions. Deionised water was used for all dilution, washing and preparation. Ammonium measurements were made using a Metrohm calibrated ammonia-selective electrode, complemented with Kjeldahl nitrogen analysis.

3.1. Struvite crystallization

Laboratory experiments were based on dephosphatisation procedures, using 98% pure MgO. First, different amounts of MgO were added to 20 ml samples of synthetic urine in 50 ml test tubes. The pH was measured continuously and MgO additions were adjusted to give pH values ranging from approximately 6.5–10. The tests were made at room temperature (20°C). Additional experiments were done using fresh, human urine. The MgO added was more than sufficient to supply the

Table 1
Chemical composition of adsorbent minerals

Component	%
Clinoptilolite, Mad, Hungary	
SiO_2	62.43
Al_2O_3	10.95
MoO_3	0.11
FeO	0.12
MgO	0.79
CaO	3.17
Na_2O	0.13
K_2O	2.74
Total[a]	80.44
Wollastonite, Hulta East, Sweden	
SiO_2	63.16
Al_2O_3	4.20
Fe_2O_3	1.12
MnO	0.26
CaO	3.12
Na_2O	0.12
K_2O	2.62
P_2O_5	0.01
Total[a]	74.61
Zeolite-mix, Mad, Hungary	
SiO_2	68.48
Al_2O_3	12.48
FeO	0.05
CaO	3.46
Na_2O	0.02
K_2O	3.27
MgO	1.12
TiO_2	0.08
Total[a]	88.90

[a] Does not include water.

stoichiometric ratio, Mg:P; 1.71:2.21, for urine containing 0.5 g P/l and to raise the pH to about 10. The precipitates were filtered, dried at 40°C and weighed. Electron microscope, energy dispersive X-ray analysis (EDS) and X-ray diffraction (XRD) analyses were made of the precipitates.

The combination of struvite precipitation with further ammonium uptake by clinoptilolite and wollastonite was also investigated.

3.2. Batch adsorption experiments

At constant room temperature (20°C) 25 ml solution, synthetic human urine or NH_4Cl solution, was used together with 0.5 g of adsorbent mineral for each experiment. Stirring at 200–250 rpm established contact between adsorbent and solution. pH and ammonium were measured during each experiment, after which the liquid was separated from the adsorbent with a vacuum filter. This process was repeated with the following variables:

1. three different mineral adsorbents: clinoptilolite, wollastonite and mixed zeolite,
2. two different concentrations for NH_4Cl and synthetic human urine,
3. four different grain sizes for each mineral,
4. a series of contact times between adsorbents and solutions from 0.5 to 60 min for each adsorbent, grain size and solution type.

4. Results and discussion

4.1. Struvite crystallisation

The initial test-tube experiments using synthetic urine resulted in the rapid formation of small crystals which settled on the tube wall. Precipitation of a white sediment occurred immediately and after shaking for 30–50 min, needle-shaped crystals became visible on the tube walls and bottom. After filtration and de-watering of the precipitate a fine crystalline white powder was obtained. Similar results were obtained using human urine.

Electron microscope studies of the precipitate revealed small (up to 1 mm long) crystals of struvite, see Fig. 1. Quantitative EDS analyses of both the crystals and the fine white precipitate gave the results in Table 2. XRD analysis in Table 3 confirmed that struvite was a major constituent in both the crystalline and the fine material. Calculations based on the mass and composition of the precipitates indicated that all phosphorous, 20–60% of potassium and 3–5% of sodium in the original solutions was settled as shown in Table 4.

It can be concluded from the experiments that struvite was formed from both human and synthetic urine by adding MgO to achieve the approximate proportion of 1.71:2.21 magnesium to phosphorous. It is also clear that potassium as well as calcium was included in the precipitate. Natural struvite does not have a pure end-member composition as shown in Table 2. Small amounts of Ca (Mn and Fe) may substitute for Mg and K may substitute for NH_4. A more correct formula for struvite is $[(Ca,Mg)(K,NH_4)(PO_4)\cdot 6H_2O]$. Together with struvite, other minerals, such as montgomeryite $[Ca_4Al_5(PO_4)_6(OH)_5\cdot 11H_2O]$, $[Ca_4MgAl_4(PO_4)_6(OH)_4\cdot$

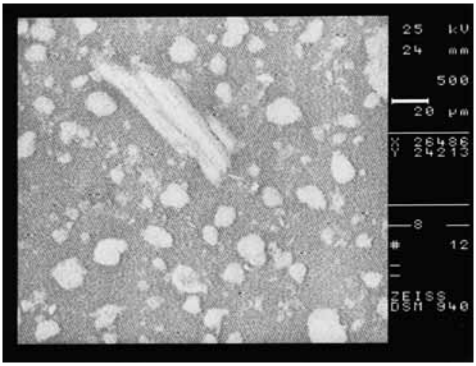

Fig. 1. SEM photo of struvite crystal (×1000).

Table 2
EDS quantitative analyses of amorphous precipitate and settled crystal structures

Compound	%
Crystal structure	
Synthetic urine (normal conc.)	
MgO	28.30
P_2O_5	57.79
SO_3	0.63
K_2O	7.63
CaO	0.09
Total[a]	94.48
Human urine (normal conc.)	
MgO	35.34
P_2O_5	47.66
SO_3	0.44
K_2O	10.50
CaO	0.67
Total	94.58
Amorphous structure	
Human urine (normal conc.)	
MgO	38.14
P_2O_5	41.76
SO_3	2.83
K_2O	7.27
CaO	6.50
Total	96.50

[a] Does not include water.

Table 3
XRD analysis results for 'struvite' composition. Crystallization made in synthetic human urine. Struvite, montgomeryite and brucite as main components

Detected compound	Standard d (Å)	Standard (I)	Detected d (Å)	Detected (I)	Counts
Struvite	4.2570	100	4.2540	100	746
	5.6010	60	5.6.30	24	178
$NH_4MgPO_4{\cdot}6H_2O$	2.9190	55	2.9199	11	79
	2.6900	50	2.6896	10	77
Lattice: Orthorhombic	2.6600	45	2.6602	11	79
Mol. Weight: 245.41	5.9050	40	5.9020	41	307
Volume (CD):477.58	2.8020	35	2.8020	9	66
Montgomeryite	12.0000	100	Outside	–	–
	5.1100	65	5.1121	100	181
$Ca_4MgAl_4(PO_4)_6(OH)_4{\cdot}12H_2O$	2.8950	45	2.8948	47	46
	2.5800	30	2.5793	6	102
Lattice: Monoclinic	2.8740	25	2.8744	13	23
Mol.Weight: 1146.59	2.6150	25	2.6154	56	102
Volume (CD):1502.79	3.1330	20	3.1340	61	111
Brucite	2.3650	100	2.3649	100	316
	4.7700	90	4.7680	91	285
$Mg(OH)_2$	1.7940	55	1.7940	95	300
	1.5730	35	Outside	–	–
Lattice: Hexagonal	1.4940	18	Outside	–	–
Mol. Weight: 58.32	1.3730	16	Outside	–	–
Volume (CD): 40-50	1.3100	12	Outside	–	–

Table 4
Results of MgO addition to synthetic human urine. Grams MgO added to 25 ml urine. Percentage uptake from solution

MgO (g)	pH	Precipitate (g)	Uptake from solution		
			P (%)	K (%)	Ca (%)
0.04	8.55	0.1186	100	22	2
0.06	9.1	0.1476	100	28	2.5
0.12	9.55	0.1820	100	35	3
0.16	10.2	0.2208	100	42	4
0.24	10.4	0.3325	100	64	5.6

$12H_2O$], brucite [$Mg(OH)_2$] or epsomite [$MgSO_4 \cdot 7H_2O$] may form depending on the amounts of Ca and metals available. The crystallisation process thus gives a much more complete precipitation of nutrients than suggested by the formation of struvite alone.

4.2. Ammonium uptake

The results from ammonium uptake by clinoptilolite, wollastonite and mixed zeolite are presented in Figs. 2–4.

Ammonium uptake was related to grain size, ion concentration and contact time. Higher adsorption at smaller grain sizes was shown at both low and high ammonium contents. This illustrates the obvious relationship between the amounts of adsorbent and the ammonium content.

The ammonium adsorption was stabilised after a few minutes and showed good results with 70–80% uptake from low-concentration solutions for the clinoptilolite, about 50% for the wollastonite and 50–60% for the mixed zeolite.

4.3. Combined crystallisation and adsorption

A series of experiments with a combination of struvite crystallisation and ammonia adsorption was made using cliniptilolite and wollastonite as adsorbents. 0.06g MgO and 0.5g of each adsorbent separately (grain size 0.125–0.250 mm) was added to 25 ml of synthetic urine. Results for contact times of 5 and 10 min are shown in Table 5. In experiments 1 and 2 the adsorbent was added to the separated supernatant after struvite precipitation whereas in experiments 3 and 4 the adsorbent was mixed simultaneously with the MgO. The results show that 67–80% of the nitrogen was taken up by struvite and clinoptilolite and 64–75% by struvite and wollastonite. The addition of MgO increased the pH to 9.0–9.5, which leads to the decomposition of urea and formation of ammonium ions. At pH 9.0–9.1 between 65 and 95% of the urea decomposes, depending mainly on storage time

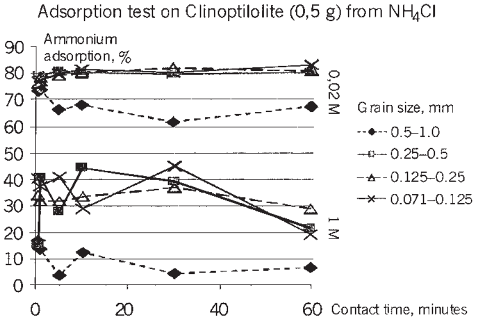

Fig. 2. Ammonium adsorption (concentration change in %) on clinoptilolite, from 0.02 and 1 M NH_4Cl.

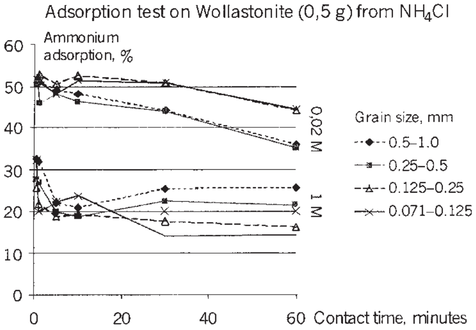

Fig. 3. Ammonium adsorption (concentration change in %) on Wollastonite, from 0.02 and 1 M NH_4Cl.

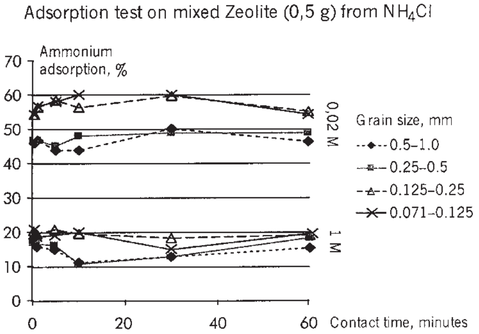

Fig. 4. Ammonium adsorption (concentration change in %) on mixed zeolite, from 0.02 and 1 M NH_4Cl.

Table 5
Results for nitrogen fixation as ammonia in combined struvite precipitation and adsorption experiments (% nitrogen fixed)

Exp. no	Contact time	Struvite +Clinoptilolite % uptake	Struvite +Wollastonite % uptake
1	5 min	67	64
2	10 min	75	64
3	5 min	79	74
4	10 min	80	75

(Hellström, 1998). Thus the MgO-addition and struvite precipitation would facilitate the nitrogen (ammonium) uptake by the adsorbent.

5. Conclusions

It proved possible to capture the majority of nutrients contained in urine in the form of solid minerals. By adding small amounts of MgO, struvite was obtained and identified as a main component together with montgomeryite, newberyite, brucite and epsonite in the precipitated crystalline mixture.

Natural zeolites, especially clinoptilolite, as well as natural wollastonite showed excellent ammonium adsorbent qualities in contact with human urine. In combination with struvite crystallisation, most of the phosphorous and potassium and 65–80% of the nitrogen could be recovered. The mixture of struvite and natural mineral adsorbent has good nutrient qualities and can be used as soil conditioner. Ammonia-clinoptilolite mixed with apatite is a well-known slow release fertiliser. Struvite and ammonia-clinoptilolite may have the same qualities.

Simple, effective, easy manageable and economically viable eco-technology is much in demand all over the world. We believe that the recovery of nutrients from separated urine reported here could play a role in the urban sanitary system of the future, which will almost certainly be much more diverse than it is today, with many different technologies at different places.

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III

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LABORATORY STUDIES ON RECOVERY OF N AND P FROM HUMAN URINE THROUGH STRUVITE CRYSTALLISATION AND ZEOLITE ADSORPTION

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ABSTRACT

Struvite $[Mg (K, NH_4)(PO_4) \cdot 6H_2O]$ crystallisation and adsorption to zeolite have been proposed as a method for nutrient recovery from human urine collected with urine separating toilets. The aim of the present study was to optimise the use of MgO (to precipitate struvite) and zeolite (to adsorb ammonia) in this process. The experiments were performed with fresh urine, to which various amounts of MgO and zeolite were added. After repeated stirring and settling for 3 days the supernatant was analysed for pH, total-N, total-P and acute toxicity for *Daphnia magna* (24- and 48-h EC50). The results show that addition of MgO reduced P and addition of zeolite reduced N in the supernatant, as expected. The required concentration of MgO added was less than expected from the stoichiometric Mg-P-ratio for struvite. In combination with zeolite the requirement for MgO was reduced even further. Zeolite was effective in reducing total-N, but because of its interaction with MgO the effect of zeolite on N as well as P reduction was rather complicated. The optimal combination of MgO and zeolite for combined N and P recovery was found to be around 0.5 MgO per litre urine and 15 g zeolite per litre urine. These additions reduced supernatant P from about 1300 mg l^{-1} to 10 mg l^{-1} and N from 8000 mg l^{-1} to 1000 mg l^{-1}. The 24-h EC50 for *D. magna* was not significantly affected by these additions. The expected recovery potentials for P and N by addition of MgO and zeolite are, thus, about 99% for P and 90% for N. However, these figures need to be verified, and pilot plant experiments at a science centre with urine separation wastewater treatment (www.universeum.se) are in progress.

Keywords: Struvite, zeolite, ammonia, phosphate, nutrient recovery

INTRODUCTION

Urine separating toilets based on new ideas of eco-cycling of nutrients from wastewater have been developed, installed and evaluated during the past decade in Sweden [1-9]. In order to achieve sustainable development it is necessary to maximise the recovery and recycling of nutrients and to strongly reduce eutrophication in freshwater and coastal ecosystems. About 80 % of the nitrogen, 50 % of the phosphorus and 60 % of the potassium from domestic wastewater emanate from the urine, together with several micronutrients in balanced concentrations [1-3]. Urine is also a body fluid, which normally lacks hazardous chemical compounds or heavy metals [1-4]. Urine separating systems are very promising regarding resource recovery compared with conventional systems [5, 6]. Human urine can also be used as a liquid fertilizer [7-9]. However, obstacles for using urine separation technology are still storage, transport and spreading of large amounts of urine [10-12]. Another problem with urine separation systems is the loss of nitrogen through ammonia evaporation during storage and spreading. However, this problem can be reduced through better management and agricultural practice [8, 13].

Human urine is an aqueous solution containing sodium chloride and urea as major constituents, and with calcium, potassium, magnesium, sulphate and phosphate as minor constituents. The chemical composition of fresh human urine is given by Altman and Dittmer [14]. One of the most important compounds that can precipitate from human urine is struvite $[Mg (K, NH_4) (PO_4) \cdot 6H_2O]$, which is formed by reaction of magnesium with phosphate in the presence of ammonium. Upon storage of urine ammonium is formed through hydrolysis of urea [13, 15]. Human urine contains an excess of ammonium relative to phosphate, but it is deficient in magnesium. At a pH between 4.8 and 6.6 most of the phosphate in urine is present as $H_2PO_4^-$ and HPO_4^{2-}. With MgO addition pH increases and the phosphate equilibrium is shifted toward PO_4^{3-}, and magnesium is also provided for struvite precipitation.

Struvite formation from mixed wastewater has been studied earlier, because struvite scaling has been a problem in conventional wastewater treatment plants [16-18]. However, in recent years the attitude towards struvite has changed to a more resource focusing view of struvite recycling possibilities in the wastewater treatment side-streams [19-28]. Struvite is also known as a slow-release fertilizer, but the cost to produce it from traditional raw materials used in the fertilizer industry turned out to be excessive [29-31]. The potential for struvite recovery from human urine is opening up a new possibility for system efficiency solutions regarding source-separating technology to reduce N and P in domestic wastewater and to provide a much cheaper source of raw material for struvite production.

Nitrogen recovery by ammonium uptake to specific adsorbents such as zeolite can complete this method making a highly efficient nitrogen recovery possible. The ion exchange properties of natural zeolites, especially of the NH_4^+ specific clinoptilolite, are well documented [32-36]. Use of clinoptilolite in wastewater treatment is also well known [37-42]. Recycling and reuse of ammonium-loaded zeolites as slow-release fertilizers has been studied and is well documented in the field of agricultural research, so called 'zeo-agriculture' [43].

We believe that transforming the nutrients in human urine into solid minerals as struvite and ammonium-clinoptilolite may have several advantages. Our first pilot study regarding nutrient recovery from human urine by struvite crystallisation and ammonium adsorption on zeolite showed some promising results [44]. The aim of the present study was to optimise recovery of P and N from urine through struvite precipitation (by MgO addition) and NH_4^+ adsorption (by zeolite addition). Toxicological tests with *Daphnia magna* were also made in order to evaluate the potential use of the remaining supernatant in wastewater aquaculture. The next step in these studies is to test the technique at a pilot plant at Universeum, Sweden (see: www.universeum.se), which is based on wastewater treatment by aquaculture system.

METHODS

Fresh human urine was collected from 10-15 healthy, young to middle-aged people and used within 24 h for the experiments.

Two experimental tests were made on two occasions.

- In the first test larger amounts of MgO and zeolite were added to samples of urine. This test will be called the 'high concentration test'. The fresh human urine collected for this test was morning-urine with a high phosphorus concentration and with initial pH of 6.2.
- In the second test smaller amounts of MgO and zeolite were added to samples of urine. This test will be called the 'low concentration test'. The fresh human urine collected for this experiment had an initial pH of 6.7 and a lower phosphorus content, because it was collected during daytime. Daytime-urine is more dilute and has a lower phosphorus concentration than morning-urine [45]. The design of this second test was based on the results from the high concentration test. In the second test also acute toxicity tests with *Daphnia magna* were made on the supernatants.

Precipitation and Adsorption Tests

The experiments with struvite precipitation were combined with additional ammonium uptake by zeolite and were made in order to study:

- dephosphatisation using different amounts of MgO for struvite precipitation, and
- mineral adsorption using different amounts of zeolite for improved nitrogen removal.

The experiments were made at room temperature (20°C) and all dilutions, dishing and preparations of stock solutions were made with double deionised (MilliQ) water.

A sample of 100 ml human urine was used for each treatment with the following treatments:

1. For the high concentration test, additions corresponding to 0.5 - 3 g MgO and 30 - 960 g zeolite per litre urine.
1. For the low concentration test, additions corresponding to 0.005 - 0.5 g MgO and 7.5 - 120 g zeolite per litre urine.

The beakers with the test solutions were covered with watch glasses and left for 72 h with a short manual stirring once daily. The pH was measured at the start and at the end of this period.

The batch of zeolite, that was used, has high clinoptilolite content (originating from Mád, Tokay hills, Hungary) and a grain size of 1.2-2 mm, and it was used without pre-treatment or previous activation. The chemical composition of this batch has been analysed earlier [44].

After 72 h the supernatant was decanted and analysed as described below.

Chemical Analysis

The supernatants were analysed for total-P and total-N using a DR4000 spectrophotometer with relevant Hach reagents according to the Hach manual (Hach Company, USA, 1997). Changes in pH during the experiments were measured by a calibrated Metrohm 605 pH-meter.

Toxicity Tests

Toxicity tests with *Daphnia magna* were made on the supernatants from the low concentration test according to ISO 6341-1982. Neonates for testing (6-24 h old) were obtained from our laboratory culture. The dilution water for culture and toxicity tests had a hardness of 250 mg l^{-1} as $CaCO_3$ and a pH 8.1, and the dilution water was prepared from double deionised water (MilliQ) and ISO 6341- 1982 stock solutions. Ambient conditions were 20°C and photoperiod of 16 h light and 8 h darkness. All exposures were made in Petri-dishes (id 10 cm) with 50 ml test solutions and 20 daphnids, using a 0.5 dilution factor. Dissolved oxygen and pH was recorded at the start and after terminating exposure. Immobility was recorded after 24 h and 48 h and the fifty percent effect concentration (EC50) was determined by probit analysis or moving coverage angle [46]. Pure dilution water was used as positive controls and a reference toxicant test with potassium dichromate was made, in parallel with all toxicity tests.

Statistical Analysis

Using a statistical software package (Crunch version 4, Crunch, Software Corp., Oakland, CA, USA) all obtained data was statistically treated for analysis of variance (one-way ANOVA) and for Spearman rank correlations (two-tailed).

RESULTS

pH Changes During the Experiments

pH measurements are presented in Figure 1 (a and b). A significant increase in pH occurred when the amount the MgO added was about 0.5g l^{-1} or higher in the high concentration test (Figure 1a). However, also zeolite added in high amounts together with MgO had a significant pH-decreasing effect (Table 1) and caused a higher tot-N uptake (Figure 3a).

No significant changes in pH in the low concentration tests were seen as a result of addition of zeolite, but marginal pH changes resulted from addition of small amounts of MgO (Figure 1b, first columns in each group). This conclusion is also supported by the analysis of variance (Table 2) and the Spearman rank correlation test (Table 3). When zeolite alone

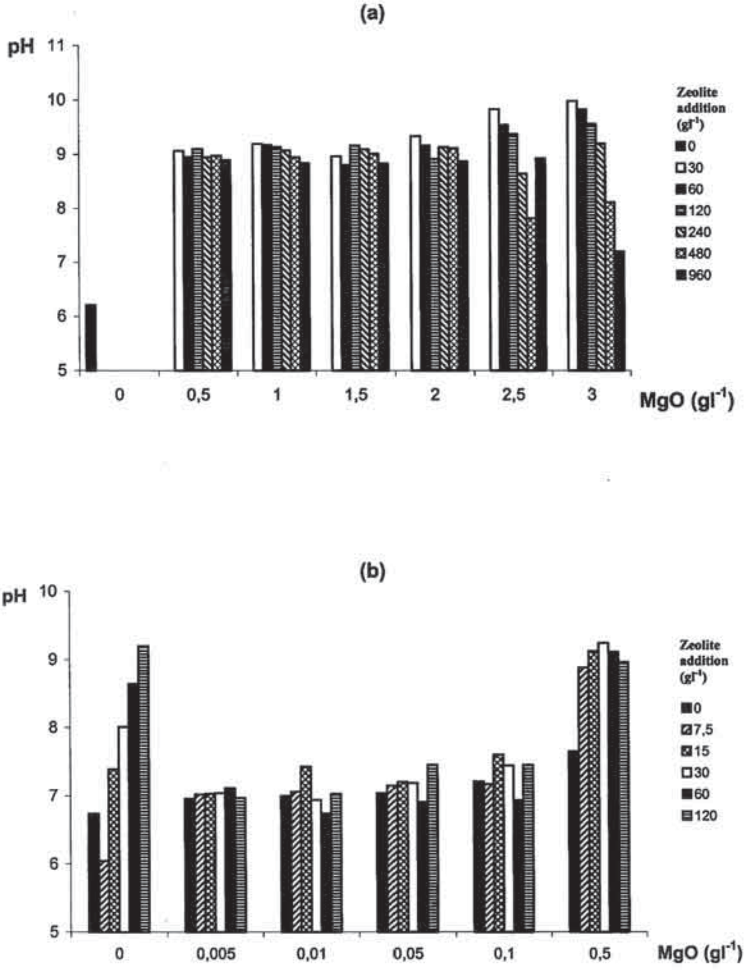
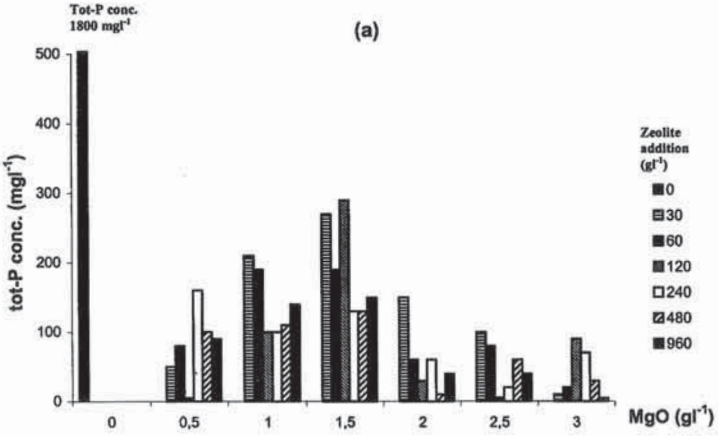


Figure 1. Supernatant pH in high (a) and in low (b) concentration tests.

Table 1. The results of the analysis of variance (ANOVA) for supernatants in high concentration tests.

Dependent variable	Source	DF	SS(U)	MSS	F	P
pH	Between subjects	35	9.118			
	Zeolite	5	3.202	0.640	2.735	0.042
	MgO	5	0.063	0.0126	0.054	0.998
Tot-P	Between subjects	35	189000.0			
	Zeolite	5	13966.6	2793.3	1.125	0.379
	MgO	5	112966.6	22593.3	9.100	0.000
Tot-N	Between subjects	35	54750000.0			
	Zeolite	5	26916666.6	5383333.3	5.261	0.002
	MgO	5	2250000.0	450000.0	0.440	0.816



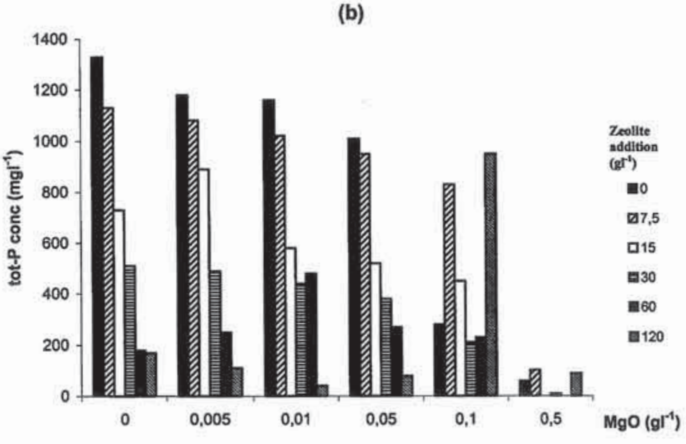


Figure 2. Total P in supernatants in high (a) and in low (b) concentration tests.

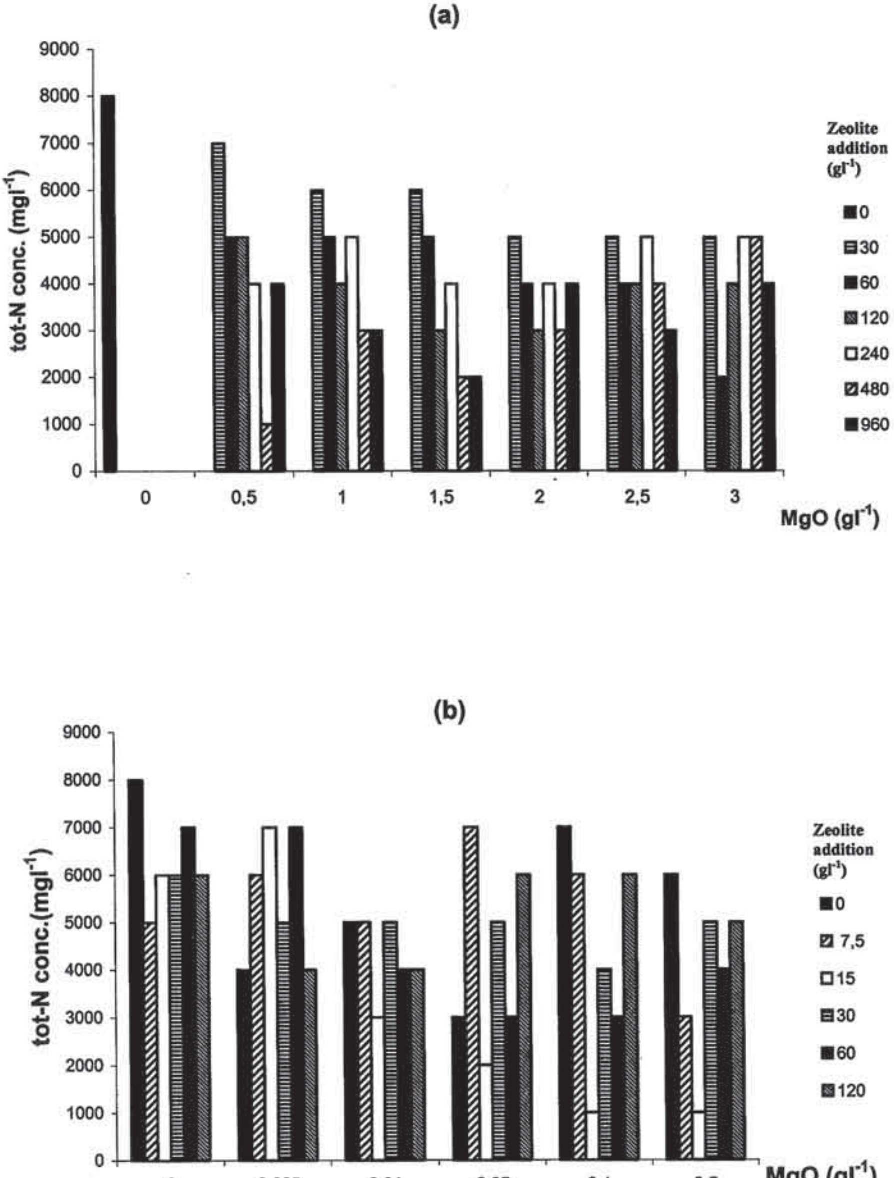


Figure 3. Total N in supernatants in high (a) and in low (b) concentration tests.

Table 2. The results of the analysis of variance (ANOVA) for supernatants in low concentration tests.

Dependent variable	Source	DF	SS(U)	MSS	F	P
pH	Between subjects	35	23.795			
	Zeolite	5	2.400	0.480	1.705	0.170
	MgO	5	14.355	2.871	10.196	0.000
Tot-P	Between subjects	35	5928880.0			
	Zeolite	5	2407833.3	481566.6	6.586	0.000
	MgO	5	1693000.0	338600.0	4.631	0.004
Tot-N	Between subjects	35	103000000.0			
	Zeolite	5	18666666.6	3733333.3	1.547	0.211
	MgO	5	24000000.0	4800000.0	1.989	0.115
EC50-24h	Between subjects	35	1713.839			
	Zeolite	5	663.155	132.630	8.240	0.000
	MgO	5	648.284	129.656	8.055	0.000
EC50-48h	Between subjects	35	459.877			
	Zeolite	5	67.693	13.538	1.338	0.282
	MgO	5	140.566	28.113	2.779	0.040

Table 3. Spearman rank correlation coefficients (r) and two-tailed p values for supernatants in low concentration tests (N= 35).

	Zeolite	MgO	pH	Tot-P	Tot-N	EC50-24h	EC50-48h
Zeolite	1.000	- 0.0630	0.1545	- 0.5462	- 0.0044	- 0.3235	- 0.4443
	0.000	0.7192	0.3757	0.0007	0.9800	0.0580	0.0075
MgO		1.000	0.4132	- 0.4646	- 0.3504	- 0.2899	- 0.2076
	-	0.000	0.0136	0.0049	0.0390	0.0912	0.2314
pH			1.000	- 0.5408	0.1005	- 0.6160	- 0.5052
	-	-	0.000	0.0008	0.5657	0.0001	0.0020
Tot-P				1.000	0.1567	0.4244	0.6427
	-	-	-	0.000	0.3686	0.0110	0.0000
Tot-N					1.000	0.0094	0.1451
	-	-	-	-	0.000	0.9574	0.4056
EC50-24h						1.000	0.6394
	-	-	-	-	-	0.000	0.0000
EC50-48h							1.000
	-	-	-	-	-	-	0.000

was added to the urine samples (Figure 1b, first group of columns) the tested amounts of zeolite significantly influenced the sample pH by increasing it from 6.7 to 9.2. The toxicity test also indicated a higher immobility for *Daphnia magna* in samples containing zeolite in urine without addition of MgO (Figures 4 and 5, first group of columns).

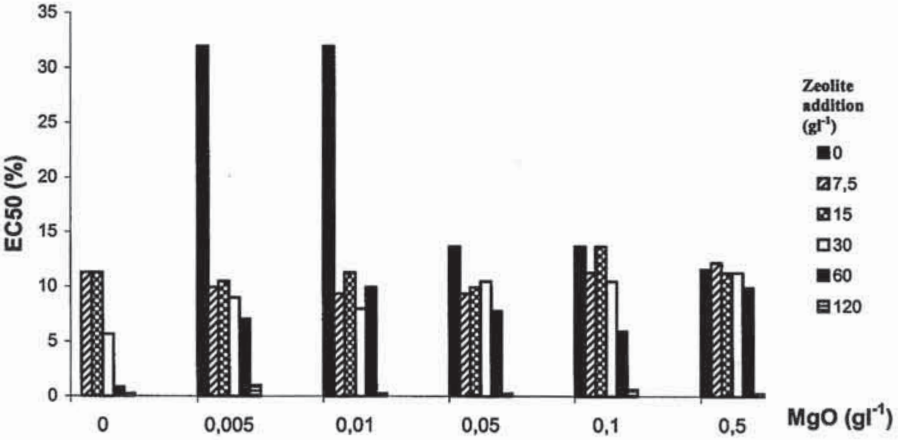


Figure 4. Toxicity (EC50-24h) for supernatants in low concentration tests.

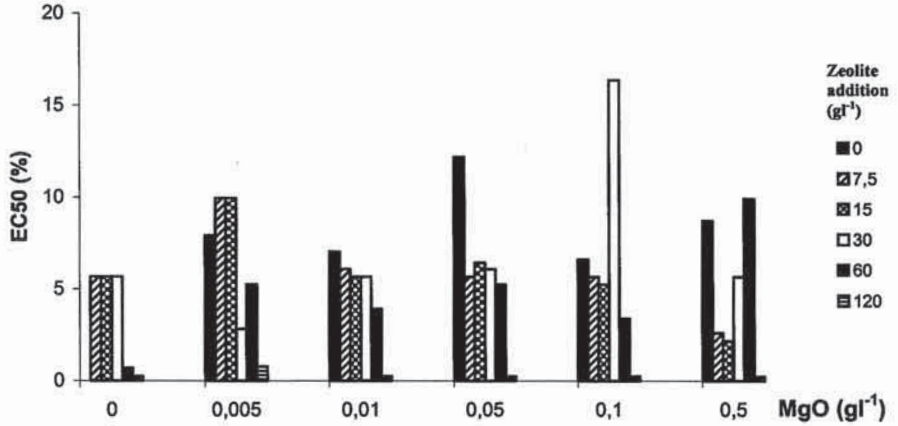


Figure 5. Toxicity (EC50-48h) for supernatants in low concentration tests.

Total-P reduction

The results on P in supernatants are presented in Figure 2 (a and b). The total-P concentration in urine decreased with increased addition of MgO in the high concentration test when the stoichiometric ratio for struvite Mg:P of 1.71:2.21 was reached for urine containing 1.8 g P per litre (ca. 2 g MgO per litre urine). The struvite precipitation process is assumed to dominate the phosphorus reduction at a constant pH around 9.0-9.2 (Figure 2a). The precipitation in this case was due to MgO addition. After a complete struvite precipitation process the supernatant pH showed a slight decrease. This decrease was observed in the samples with the highest zeolite content, where the nitrogen concentration was reduced both by struvite precipitation and zeolite adsorption.

In the low concentration test, where the stoichiometric ratio of Mg:P of 1.71:2.21 for maximal struvite precipitation was not satisfied, increasing amounts of added zeolite had a significant impact also on the phosphorus reduction (Table 2 and Figure 2b). In this experiment the pH was too low for maximal struvite recovery, and the phosphate removal was probably due to the impact from both zeolite and MgO. However, in the low concentration tests other processes can occur besides struvite formation at pH 7-9, like formation of montgomeryite, brucite or epsomite, as our earlier experiments have shown [44].

Total-N Reduction

The results on total-N reduction are presented in Figure 3 (a and b). In the high concentration test the nitrogen reduction was controlled by the zeolite (Table 1).

Depending on the added amount of the natural zeolite and MgO the nitrogen recovery varied in the low concentration test, but the total-N reduction cannot be ascribed to either MgO or zeolite, and statistically their influence was not significant (Table 2, analysis of variance, and Table 3, Spearman rank correlations).

Toxicity of Supernatants

The toxicity of the supernatants in the low concentration test to *Daphnia magna* (EC50 % after 24 and 48 h) is presented in Figures 4 and 5. Addition of small amounts of MgO contributed to a moderate increase in pH of the supernatant, with an ammonium-ammonia equilibrium change. When the nitrogen removal was low an increased toxicity to *D. magna* would be expected. However, an increase of the addition of both zeolite and MgO also increased the pH in supernatant, which resulted in an overall increase in toxicity for *D. magna* (Table 2). Zeolite was also tested separately in dilution water, and the result shows that zeolite itself is not toxic for *D. magna* (not presented here).

DISCUSSION

Total-P Recovery

After the struvite precipitation was completed the solution pH showed a slight decrease in the high concentration tests. As our previous results have shown, natural struvite does not have a pure end-member composition when it is precipitated from a complex solution like urine. Together with struvite, other minerals, such as epsomite [$MgSO_4 \cdot 7H_2O$], brucite ($Mg(OH)_2$] and montgomeryite [$Ca_4MgAl_4(PO_4)(OH)_4 \cdot 12H_2O$] may form depending on the amounts of other di- or trivalent metal cations available [44]. Newberyite [$MgHPO_4 \cdot 3H_2O$] was not found because of the very low probability for human urine of being supersaturated with respect to newberyite at higher pH than 5.3 [47]. Taking into account the interference by many other ions occurring in urine and the high ammonia activity due to urea decomposition, the conditions for struvite precipitation are easily reached, and not of newberyite [48].

When MgO and zeolite were added in low concentrations the phosphorus uptake is a result of the impact from both MgO and zeolite. When pH is higher (over 8.5) the precipitation with MgO is more likely. At lower pH values the impact from the ion exchanger is dominating the process. Zeolites can adsorb considerable amounts of phosphates (reported P adsorption capacity ca. 2.15 g kg^{-1}), to their hydrous oxides of Al structural sites [49, 50]. Therefore, the recovery of P is a result of both MgO and zeolite addition.

Total-N Recovery

During the 72 h experiment time, ca 60% of the nitrogen content in urine is expected to be hydrolysed [13-15] and available as ammonium (NH_4^+) for uptake by the mineral adsorbent, or for precipitation in the form of struvite. In the high concentration test the struvite precipitation will decrease the ammonium concentration and the zeolite will act as cation exchanger for the remaining ammonium uptake from the solution. It is known from earlier experiments that the NH_4^+-exchange capacity of natural zeolites is negatively affected by high NH_4^+ concentrations or by the presence of appreciable quantities of Mg^{2+} or Ca^{2+} ions in a solution [44]. In the low concentration test the ammonium recovery varied in a wide range possibly because of the competing processes between a continuously increasing amount of ammonium in the solution and the processes of phosphorus uptake, other than struvite precipitation, mentioned earlier.

Toxicity Test Evaluation

Previous studies of urine toxicity for *Daphnia magna* were made in our laboratory with the aim of studying human urine treatment in aquaculture systems [51]. It was fund that human urine, fresh or stored, is highly toxic for *Daphnia magna*. The solution had to be diluted to an initial urine concentration of 0.5 % in order to be used as an algal nutrient solution and culture medium for *D. magna*. The main causes for urine toxicity were identified as high pH in combination with high concentrations of ammonia-nitrogen (NH_3-N) [15, 52].

Urine treatment with MgO and zeolite in the low concentration test could confirm these results, with the following additional observations:

- Higher pH, higher ammonia concentrations and low ammonium removal contributed to an increased toxic effect for *D. magna*.
- Increased additions of zeolite increased the toxicity for *D. magna* in all dilution series. Part of this could be due to the increased pH values in the presence of NH_4^+-NH_3 equilibrium in the system.

CONCLUSIONS

Most of the phosphorus and nitrogen from urine can be recovered as solids through combined precipitation (by MgO addition) and adsorption (by zeolite addition).

Phosphorus is mainly recovered as struvite $[Mg\ (K, NH_4)(PO_4) \cdot 6H_2O]$, and MgO affects the precipitation. To some extent phosphorus recovery is also affected by zeolite addition, especially at low concentrations of MgO and zeolite. Nitrogen recovery was more complex and affected by both MgO and zeolite addition. The acute toxicity to *Daphnia magna* of the remaining supernatant solutions, after N and P recovery, was affected by both MgO and zeolite addition. High concentrations of zeolite increased the toxicity of the supernatant.

The recovery of both N and P from human urine was affected by the combination of MgO and zeolite, and maximum combined recovery of N and P occurred at added concentrations of 0.1 g l^{-1} of MgO and 15 g l^{-1} of zeolite. At these added concentrations, the toxicity of the supernatant to *Daphnia magna* was not altered, and the pH was around 7.5. The urine concentration of P was reduced from 1300 mgl^{-1} to 200 mgl^{-1} (6.5 fold), and the concentration of N was reduced from 8000 mgl^{-1} to 1000 mgl^{-1} (8 fold). Increasing the MgO addition could increase the recovery of P further. These recovery figures are preliminary because they are based on small-scale laboratory experiments, and they must be verified at a larger scale. Such experiments are in progress.

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IV

Sustainable utilisation of human urine in urban areas – practical experiences*

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Keywords

Aquaculture, ecological engineering, struvite, urine, zeolite, urine separation

Abstract

After several years of research on utilisation of nutrients in human urine an opportunity to build and use a greenhouse aquaculture demonstration plant appeared in the new science centre Universeum, in Göteborg, Sweden. The challenge for this project is to recycle all the nutrients in urine collected from the staff and the visiting public (about 500 000 visitors during the first year) and to demonstrate its value as nutrient with different eco-sanitation technologies on site. The nutrients are collected by the use of 26 urine-sorting toilets (estimated 100-300L urine per day, 50% diluted). Part of the urine is treated directly by the aquaculture food chain (algae-zooplankton-fish). Another part will be concentrated by chemical precipitation (struvite) and adsorption to minerals (zeolite). The main part of the urine is to be spread in agriculture. This multiple use of the source-separated urine favours a sustainable recycling of nutrients in urban areas, and it also demonstrates the usefulness of human urine as a nutrient source for the visitors. Furthermore, any eco-toxicological concern by its use can be directly investigated in the aquaculture food chain on site, opening possibilities for interesting research projects in the future. This paper will present a brief description of the site, the ongoing projects, and some practical experiences.

Introduction

The Universeum science centre

Universeum science centre located at Korsvägen in Göteborg, Sweden, was inaugurated in 2001 (Wallin, 2002). The science centre is an educational platform and an excellent forum to tempt youngsters (in ages between 7-19 years old) to develop a better understanding and a direct experiment of sciences by visiting this building (Ervik, 2003).

In the business plan for Universeum, demonstrations of science and technology from an ecological perspective was emphasised and the building was identified as a resource for communicating this perspective. Environmental ambitions were developed into system requirements. During the design process, a reference group of researchers gave advice on goal settings, system design and specific details (Wallin, 2002).

Ecological engineering and the wastewater system

Ecological engineering was defined in 1971 by Howard T. Odum as the management of nature for human use (Odum, 1971). The synthesis between ecology and technology (eco-technology) requires a combination of basic and applied research as well as interdisciplinary teams for its

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This paper has been peer reviewed by the symposium scientific committee proper application (Jensen et al, 1992; Mitch, 1991). Because of the complexity of nature a broad professional knowledge is required when creating ecological engineering systems.

After the Brundtland convention (1987), and environmental meetings (e.g. Rio, 1992) fundamental terms as "Agenda 21 ", "life cycle assessment" and "recycling", are stated and widely acknowledged, which has focused the practical local action on new technical developments towards local system for wastewater treatment. This has lifted environmental issues to a higher priority in decision-making processes, and also brought fundamental thinking on local scales to all levels in the society, people are involved. The future work with sustainable development in Sweden is following 15 environmental goals (http://www.environ.se). Two of these goals are "No eutrophication" and "A sea in balance with living coast and archipelago". These goals are especially important for urban areas producing enormous amounts of wastewater and discharging large amounts of nutrients to water recipients through agricultural runoff and wastewater treatment plants.

Figure 1: The Universeum exterior autumn 2001, view from the main entrance. Photo Zsofia Ban.

The discovery of nitrogen as a major cause of eutrophication, especially in the marine environment, has intensified the research on nitrogen removal from sewage water. Further demands on nutrient removal cause treatment plants to be rebuilt or extended (Mattsson, 1997). More than eighty percent of the nitrogen in sewage water from households originates from human urine (Adamsson, 1999). Therefore, source separating of urine could be a complement to decrease nitrogen discharge to estuaries.

Four aspects need to be considered for wastewater treatment methods (Jenssen et al 1992; Jonasson 1993):

- health aspects,
- recipient aspects,
- cost efficiency aspects and
- recycling aspects.

By using separating systems, the nutrients could be used as a resource directly after toilet disposal and appropriate hygienic stabilisation (i.e. storage) and thereby reduce the nitrogen load in incoming water to coastal sewage plants. The value of human urine as fertilizer or soil conditioner in agriculture is well known in Sweden (Jönsson et al., 2000; Johansson, 2000). However, its use can be controversial regarding different stages of urine management (storage, transports, spreading and overall aspects of hygiene). In recent years, research made at Göteborg University have shown that nutrient recovery in crystalline form to obtain a slow-release soil conditioner known as struvite [Mg (K, NH4)(PO_4) •6H2O] can be an ecologically and environmentally desirable way (Ban, 1998; Lind, *et al.*,2000). Human urine could also be used in an aquaculture approach (Adamsson, 2000). After several years of research on utilisation of the nutrients in human urine in a constructed food chain (Adamsson, 1999), the possibilities for demonstrating this technique became a reality at Universeum science centre.

The objectives of this paper are (1) to describe the technique of aquaculture and the initial research on crystallisation based on sorted human urine from Universeum, (2) to share some practical experiences from the operating toilet system at Universeum and (3) to discuss if new techniques like collecting of urine and crystallisation (struvite) could be a solution for sustainable utilisation of human urine in urban areas.

Methods

Collecting urine

The majority of the nutrients (N and P) are collected by the use of 26 urine-sorting toilets (system called "Dubletten", developed by Bibbi Innovation & Co AB). The front bowl is connected to a separated pipe, which collects urine into two storage tanks (volume of 6m^3 each). The back bowl is flushed to ordinary sewage system and the sewage plant for Göte borg city, with exception of 7 toilets on the personal floors. The faeces from these 7 toilets are connected to a sludge separator (3 chambers with a total volume of 12m^3) and this effluent could be used in the aquaculture or be transported to the ordinary sewage system.

The total urine volume collected per year is estimated by number of visitors and a dilution factor of 50% (about 1.2-1.5dl flushing volume according to the manufacturer of the toilet, see http://www.dubbletten.nu/english-presentation/WCdubbletteneng.htm).

Chemical and microbiological analysis have been made on the urine from the storage tanks by using a water-lifter (small plastic container on a wooden stick) directly placed into the manhole of the tanks, filling a glass bottle (1L), which were transported in a cool box (+4°C) directly to an accredited laboratory.

The aquaculture-principles (Figure 2)

The diluted urine is pumped [1] from storage tanks to a ble nder [2] (about 50L) in the water treatment centre (aquaculture). From this blender the solution flows by gravity to four cylinders (1m^3 each) which contain microscopic algae [3] (i.e. *Scenedesmus acuminatus*). Using photosynthesis, these algae assimilate the nutrients from the urine. The overflow of algae runs to each of four separated 1m^3 large concrete aquaria (replicates), were the algae are eaten by zooplankton, water fleas (*Daphnia magna*) [4]. The aquaria also contain a plastic foam (1m * 0,7m * 0.1m) with a pore size of about 0.1-10mm, which is aerated. The function of this is to act as a biofilter with an attached nitrification bacterial community. The water containing water fleas flows by gravity down to a 6 m^3 (water volume) large tank with small tropical fish (Guppy) [5]. These fishes fed on *Daphnia* sp. and are in turn harvested to become food for larger fish in some of the other aquaria in the building. The water runs down to a series of small pools [6] were water plants are cultivated. Not only these plants, but the roots of the rainforest trees are able to take up nutrients which still remain in the water. Then the water is

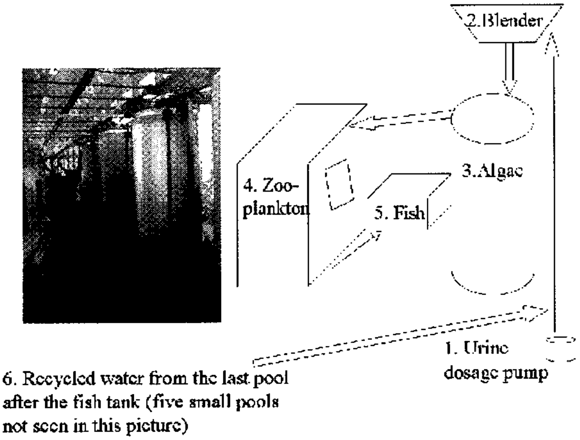

Figure 2: The aquaculture system at Universeum.

returned to the blender by a pump. In the blender, new urine is added. The water flows in a closed loop system, which is technically operated only by a dosage station for urine and the pump station in the final pool [6]. Biomass production of zooplankton has been recorded as well as pH, dissolved oxygen, total nitrogen, ammonia, nitrate and nitrite in order to monitor the nutrient flow and locate functional problems within the system during one month the first year (Barone, 2002). Urine from the storage tank in the aquaculture has also been sent for chemical and microbiological analyses.

The struvite process and mineral adsorption

Lind *et al.* (2000) showed that by addition of small amounts of magnesium oxide (MgO) to human urine most of the phosphorous (95-99%) and part of the nitrogen (20-50%) can be recovered as a precipitate. Crystalline struvite [Mg (K, NH4)(PO_4) •6H2O] was the major component of the precipitate, which also contained montgomeryite [$Ca_4MgAl_4(PO)_4(OH)_4 \cdot 12H_2O$], brucite [$Mg(OH)_2$] and epsonite [$MgSO_4 \cdot 7H_2O$]. In this way also 22-64% of K and 2-5.6% of Ca was recovered. Additional mineral adsorption steps improved the nitrogen recovery. Natural zeolites and wollastonite showed excellent adsorbent qualities in contact with ammonia solutions as well as in tests with human urine (Ban, 1998). When struvite crystallisation and mineral adsorption was combined 64-80% of the nitrogen (as ammonia) was recovered together with 95-99% phosphorus, (Lind *et al.*, 2000).

The experiments with Universeum urine were based on the combination of two steps:

a) *dephosphatisation* using different amounts of MgO for struvite crystallisation

b) *mineral adsorption* using different amounts of zeolite for improved nitrogen removal.

Human urine was collected from the urine tanks at Universeum and transported immediately in closed plastic cans to the laboratory for testing. The urine was directly used for the experiment, without freezing or further storage.

The mineral zeolite (with high clinoptilolite content originating from Mad, Hungary, and a grain size of 1,2-2mm) was used in its natural form. MgO and zeolite was added to the urine in a series of batch tests.

The contact time of the experiment was 72 h at room temperature (20°C), which is more than enough for both struvite formation and ammonium adsorption processes (Lind, 2000). A short, manual stirring was made once daily. After 72 hours the supernatant was decanted and analysed for total-P and total-N (Spectrophotometer DR 4000, Hach-methods 3036 and 2558). Figures 3 and 4 show the results of the experiments.

Results and discussion

Sorted human urine at Universeum

Collecting system

Since these systems are new, some technical problems were expected. Most of them were related to pipe dimensions and slopes and the design of the toilet seat. This has caused some additional work for the maintainers and cleaning staff at Universeum during the first year. Due to educated and interested staff at Universeum, these toilets have, in spite of their non-optimal design, been well maintained and are, in most cases, accepted and appreciated by the visitors. There are areas in the building that are more critical than others (i.e. more visited) like the entrance hall, where in high seasons, very frequent cleaning by staff is necessary. This was not always possible, which has lead to some complains from visitors of odours and blockages of the front bowl used for urine (paper jam, etc.).

Nutrients in collected urine:

Total N varied between 2 and 6g/L in collected urine (see Table 1 and Figure 3) and total P varied between 0.13 and 0.85g/L (see Table 1 and Figure 4).The reduction of *E.coli* in stored human urine occurs within a few days. Therefore, also other indicator organism should be used when evaluating hygienic risks from sorted human urine prior to its use as nutrient source (Jönsson, 2000; Höglund, 2001).

Table 1. Chemical and microbiological data for two samples of sorted human urine (about 50% diluted by flush water) at Universeum, Göteborg Sweden. Sample 1 was taken directly from the incoming water to the storage tanks. Sample 2 was taken when the urine enters the blending tank in the aquaculture. This means that the urine in sample 2 has been stored further for at least 1 week at 26 ± 2°C.

Parameters	Method	Unit	1	2
pH	SS 028122-2.Titro		9.2	8.7
BOD (7)	SS 029143-2	mg/l	1200	290
COD (Cr)	Hach	mg/l	2100	1200
Total Nitrogen	TRAACS	mg/l	2200	2700
Ammonium-N	TRAACS	mg/l	2300	2700
Total Phosphorous	TRAACS	mg/l	130	150
Potassium	ICP-AES	mg/l	840	930
Cadmium	ICP-MS	mg/l	< 0.0004	<0.0004
E. coli	SS 028166-1	cfu/100ml	>160000	<2
Heterotrophic bacteria, 20 C, 2d	SS 028171-1	cfu/ml	>300000	>300000
Coliform bacteria, 35 C	SS 028166-1	cfu/100ml	>160000	<2

The aquaculture

Research at a laboratory scale and in a small greenhouse system (Adamsson, 1999; Adamsson, 2000) showed that *Scenedesmus acuminatus* and *Daphnia magna* can grow and reproduce with human urine as nutrient source. Then the reduction of nutrients through the aquaculture system ranged 36-97% for nitrogen and 67-98% for phosphorous. The results from the first 18 months of operation of the aquaculture system at Universeum have shown that the critical step is the zooplankton production. This is mainly due to high temperature, low food quantity (lack of light for photosynthesis) and ammonia toxicity (Adamsson, not published; Barone, 2002). One aim is to increase the urine concentration (more nutrients) in the blender to produce more algae to improve *Daphnia* production. This must, however, be combined with acceptable pH value in the system to decrease the risk for ammonia toxicity to *Daphnia* (Adamsson, 1999). A complete evaluation of the production and reduction efficiency of this pilot scale system is so far unknown, although, a study based on one month performance of the plant (Barone,2002), indicated that the nitrogen concentration was reduced after the aquaculture steps, and the major part of nitrogen was in form of nitrate, indicating that both nitrification and nitrogen reduction was efficient.

The struvite process and mineral adsorption

The addition of MgO increased the initial pH value from 8.9 to 9.3 (data not presented here). Total-N and total-P reduction after addition of small amounts of MgO respective zeolite to human urine collected from the urine tanks is shown in Figure 3 and 4.

Nitrogen reduction:

a) The tot-N reduction from human urine is highly dependent of the amount of MgO added and of the stoichiometric conditions for *struvite* precipitation (molar ratio of 1:1:1 and weight ratio N:Mg:P of 1:1.7:2.2). Human urine contains an excess of ammonium relative to phosphate and with Mg added and a phosphate recovery of nearly 100% just a part of ammonia is recovered as struvite. Theoretically, the maximum part of the total N that can be captured in the struvite structure is 38% from Universeum urine.

b) The further nitrogen uptake by *zeolites* depends on the ion exchange property of the mineral, the amount used, the grain size and contact time (Lind, *et al.*,2000). The clinoptilolite type of zeolite used here has good ammonium absorbent capacity (Lind, *et al.*, 2000) but the struvite precipitation itself slightly reduces the end-pH of the urine tested and during the adsorption process to the zeolite a competition may occur between H-ions and NH_4-ions for the exchange sites in the mineral structure. The simultaneous precipitation and mineral adsorption processes are complex processes and a clear picture of the tot-N uptake due only to the zeolite was investigated earlier (Lind, *et al.*, 2000). A deeper discussion about the processes using concentrated urine is under publication elsewhere. However, an additional of 30-85% total N reduction (from remaining amounts after struvite precipitation) due to zeolite adsorption was found in this study (Figure 3).

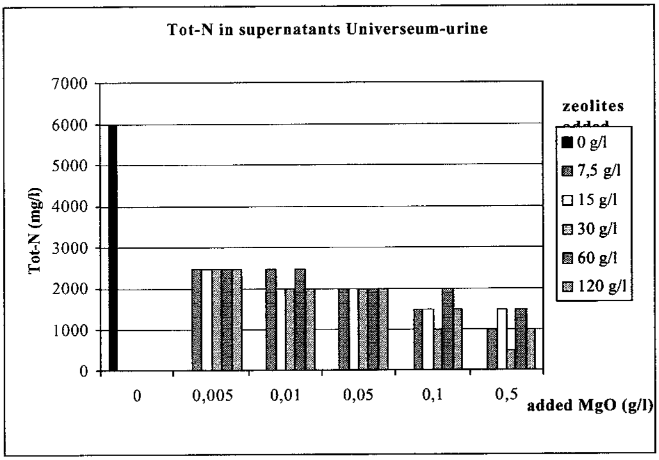

Figure 3: Total-N reduction in urine from Universeum by addition of MgO and zeolite.

Phosphorus reduction:

a) The stoichiometric ratio (molar weight based) Mg:P is 1.71: 2.21 for *struvite* precipitation for urine containing 0.85 g P/l. According to our experiments 0.5 g MgO per litre urine is sufficient to get a tot-P reduction of 95-98% in combination with zeolite, because the zeolite is a good phosphorus adsorbent.

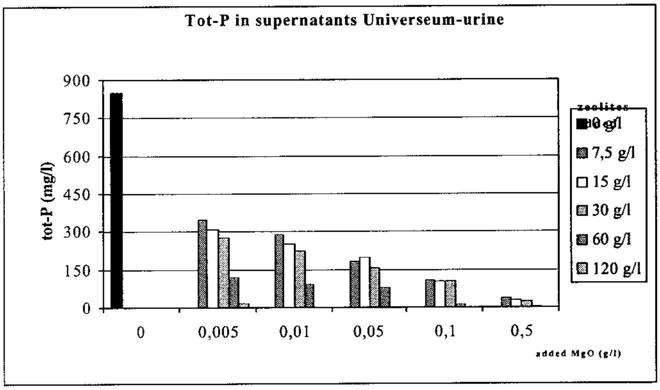

Figure 4: Total-P reduction in urine from Universeum by addition of MgO and zeolite.

b) The natural *clinoptilolite type of zeolite* has also a good tot-P adsorption capacity. When tested with human urine. This process is mainly due to the possibilities for chemisorption on the Ca-, Fe- or Al-oxide sites in the zeolite structure (under publication elsewhere).

Previous test results with combination of struvite precipitation and further ammonium uptake by zeolites have also demonstrated a 64-80% ammonium uptake together with a 98-100 % phosphorus uptake from human urine (Lind *et al.*, 2000). Process optimisation at Universeum is in progress.

Sustainability and future work

Wastewater is a resource and should, therefore, be treated as such. However, its use has important hygienic aspects that has to be considered. The aquaculture technique is perfect as a demonstration pilot plant and for educational purposes regarding understanding the scientific background behind eutrophication, and could also be used for research on bioaccumulation and biomagnification of different elements in the organisms living in aquatic environments. But the aquaculture technique as such has only a limited potential as a commonly used wastewater treatment technique, even if it can be used in combination with other techniques. The most realistic and interesting approach on utilising the nutrients in human urine in urban areas is, therefore, simple collection in storage tanks. The urine fraction must then be transported to farmers to close the loop between urban and rural areas for further use in agriculture. This transport could be environmentally improved by using the struvite process, and in this way decreasing the transport of water. Therefore we are looking forward to construct a pilot plant for mineralization of N and P from human urine at Universeum in the near future. The research is at experimental level, mostly at laboratory scale, and a holistic approach including a system evaluation is necessary.

Modern society will probably continue to have some large-scale wastewater plants that were constructed during the 70ths and forward for many years to come. This is not a question of either having large and efficient conventional systems or small scale, expensive, low functioning systems, to meet the future demand on sewage systems. This is about how to use the best technologies in combination to meet the areal restrains of large cities and to recycle materials in an as environmentally friendly way as possible to reach a sustainable society for coming generations, and also considering aspects of what is acceptable from the users point of view.

This multiple use of the source-separated urine from one facility demonstrates a possibility for a sustainable recycling of nutrients between urban and rural areas and it also demonstrates the usefulness of human urine as a nutrient source for the exhibition visitors. Furthermore, any eco-toxicological concern including endocrine disruption, can be investigated in the aquaculture food chain, opening possibilities for interesting research projects in the future.

The Universeum ecological wastewater system is a demonstrating plant and a research site in an educational environment that is visited by people of all ages and professions. It is a good starting point for discussions and debates on future sustainable utilisation of human urine and wastes in general in urban areas.

Conclusions

- Urine collection and the utilisation of its main nutrients (N and P) have been practiced for two years (2002-2003) at a science centre (Universeum) in Göteborg, Sweden.
- Problems relating to the function of urine-separating toilets and mineralization of the nutrients have been addressed and partly solved.
- Ecological recycling is demonstrated in an aqua cultural food chain (algae-zooplankton-fish).
- Mineralisation of N and P by precipitation as struvite and adsorption to zeolite have been tested at a laboratory scale with recoveries of 30-85% of the total nitrogen and 95-98% of the total phosphorous in the collected urine.

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V

Recovery of N and P from human urine by freezing, struvite precipitation and adsorption to zeolite and active carbon

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Abstract

The majority of the nutrients in domestic waste originate from human urine. This study deals with methods for recovery of N and P from urine. Results from a freezing-thawing method (FTM) together with struvite recovery and nitrogen adsorption on zeolite and active carbon (AC) are presented. Various amounts of MgO, zeolite and AC were added to samples of 100 ml urine. After 3 days the supernatants were analysed for pH, total-N, total-P and acute toxicity for *D. magna*. One set of samples was frozen and then thawed and the supernatants collected were tested as before. The FTM method concentrated 60% of the nutrients in 40% of the initial volume and significantly improved the N reduction and *D. magna* survival. The P recovery was 95-100 %, mainly as struvite. No significant effect of AC was found. Zeolite improved the P recovery and in some combinations of MgO also the N recovery.

Keywords: struvite, zeolite, activated carbon, freezing-thawing, nutrient recovery, adsorption, urine, acute toxicity

1. Introduction

Sustainable development in wastewater management includes recovery of nutrients to reduce eutrophication, but also ways to recycle the removed nutrients. About 80 % of the nitrogen, 50 % of the phosphorus and 60 % of the potassium from domestic wastewater emanate from urine (Wolgast, 1993; Sundberg, 1995; Jönsson et al., 1997). Urine separating systems are efficient for resource recovery compared with conventional systems (Bengtsson et al., 1997; Hellström and Kärrman, 1997; Hellström, et al., 2003). Human urine collected in these systems can also be used as a liquid fertiliser (Kirshmann and Pettersson, 1995; Kirshman, 1998; Jönsson et al., 2000). However, obstacles for using urine separation technology on a larger scale are still found in the storage, transport and spreading of large amounts of urine (Jenssen and Etnier, 1996; Larsen and Gujer, 1996; Höglund et al., 1998). Another problem with urine separation systems is the loss of nitrogen through ammonia evaporation during storage and spreading. This problem can be reduced through better management and agricultural practice (Haneaus et al., 1996; Jönsson et al., 2000).

Human urine is an aqueous solution containing sodium chloride and urea as major constituents, and with macronutrients such as calcium, potassium, magnesium, sulphate and phosphate are found as minor constituents. Urine normally lacks hazardous chemical compounds or heavy metals (Wolgast, 1993; Jönsson et al., 1997; Hellström, 1998). The chemical composition of fresh human urine was given by Altman and Dittmer (1974).

One of the most important compounds that can precipitate from human urine is struvite $[Mg\ (K, NH_4)\ (PO_4) * 6H_2O]$, which is formed by reaction of magnesium with

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phosphate in the presence of ammonium. Upon storage of urine ammonium is formed through hydrolysis of urea (Adamsson and Dave, 1996; Haneaus et al., 1996). Human urine contains an excess of ammonium relative to phosphate, but it is deficient in magnesium. At a pH between 4.8 and 6.6 most of the phosphate in urine is present as $H_2PO_4^-$ and HPO_4^{2-}. With MgO addition pH increases and the phosphate equilibrium is shifted toward PO_4^{3-}, while magnesium is provided for struvite precipitation. Struvite formation from mixed wastewater has been studied earlier (Mohajit et al., 1989; Maqueda et al., 1994). In recent years struvite recycling in wastewater treatment side-streams has proved to be an attractive option for phosphorus reuse as fertilizer (Booker et al., 1999; Liberti et al., 2001; Ueno and Fujii, 2001; Doyle and Parsons, 2002). Struvite is also known as a slow-release fertiliser, but the cost to produce it from traditional raw materials used in the fertiliser industry turned out to be excessive (Schuiling and Andrade, 1999). The potential for struvite recovery from human urine is opening a new possibility for efficient system solution regarding source-separating technology. In this way it is possible to reduce N and P in domestic wastewater and to provide a much cheaper source of raw material for struvite production.

Nitrogen recovery by ammonium uptake to specific adsorbents such as zeolites can complete this method, making additional nitrogen recovery possible. The ion exchange properties of natural zeolites, especially of the NH_4^+ specific clinoptilolite, are well documented and the use of clinoptilolite in wastewater treatment is well known (Jorgensen et al., 1976; Beler-Baykal et al., 1996; Beler-Baykal and Akca-Guven1997, Beler-Baykal 1998; Kithome et al., 1998; Rozic et al., 2000). Recycling and reuse of ammonium loaded zeolites as slow-release fertilizers have been studied, and the technique is well documented in the field of agricultural research, so called ‘zeo-agriculture’ (Pond and Mumpton, 1984). Our previous research on nutrient recovery from human urine by struvite crystallisation and ammonium adsorption on zeolite showed promising results (Lind et al., 2000; Bán and Dave, 2004).

Active carbon (henceforward AC) and zeolite as ammonia adsorbents have been compared in fish cultures and both adsorbents were found to be effective on ammonia reduction, but zeolite was considered cheaper and easier to manage (Emadi et al., 2001). Technologies using carbon based adsorbents (active carbon, fly ash, charcoal, etc.) are widely used in fields like: removal of phenolic acids from agro-industrial wastewater (Garcia-Aaraya et al., 2003), removal of synthetic organic chemicals (Matsui et al., 2003), removal of aquaculture therapeutants (Aitcheson et al., 2000), remediation of dyes from industrial wastewaters (Robinson et al., 2001) along with heavy metal removal from water and wastewater (Viragaghavan and Rao, 1991, 1998, Mituza et al., 2004). AC based filters are also used in urea and ammonia sorption in medical devices (Giordano et al., 1976; Lehmann et al., 1981).

However, little is known about the behaviour of carbon-based adsorbents in nutrient removal from domestic wastewater. The use of AC as adsorbent for total nitrogen recovery from human urine and spreading it as a fertilizer has not been investigated before. If it works, it can be a potential supplement to zeolite.

Freezing as a method in wastewater treatment has been investigated and evaluated previously (Lorain et al., 2000). The use of freezing is based on the fact that, when ice is crystallised from an aqueous solution, the ice crystal is essentially built up by pure water, leaving the solutes in the remaining liquid phase. Sludge dewatering methods have been developed and used, based on this technique (Halde, 1979; Hung et al., 1997; Martel, 2000; Örmeci and Vesilind, 2001; McMinn et al., 2003). Sludge treatment by freezing and thawing could also be a robust and cost saving method in cold climate regions (Hellström, 1998; Martel, 1998). Freeze concentration seems to be a very efficient method also for soluble pollutant removal from wastewater (van der Ham et al., 1998, 2004; Lorain et al., 2000; Gay et al., 2003). Our previous investigation into volume reduction and concentration of nutrients in human urine by freezing and thawing resulted in a nutrient concentration of 80% in 25% of the initial urine volume (Lind et al., 2001).

The aim of the present study was to optimise the recovery of P and N from urine by freezing-thawing in combination with struvite precipitation (by MgO addition) and nitrogen adsorption (by zeolite or AC). Toxicity tests with *Daphnia magna* were also made in order to evaluate the potential use of the remaining supernatant as dilution water in a wastewater treatment plant based on aquaculture (see www.universeum.se).

2. Method and material

Human urine was collected from the storage tank from Universeum (a building with a working urine separating sewage system) and transported in closed plastic cans to the laboratory for testing. The urine was stored in a refrigerator at +8 ^{0}C during 3 days before the experiments were started. This urine solution is diluted with about 50% tap water used for toilet flushing. Therefore, the concentrations of all solutes are only half of those found in directly collected human urine.

2.1. Precipitation and adsorption tests

The experiments for struvite precipitation by addition of MgO were combined with additional ammonium uptake by zeolite and AC. They were made in order to study:

- dephosphatisation using different amounts of MgO for struvite precipitation, and
- adsorption using different amounts of zeolite and AC for improved nitrogen removal.

Samples of 100 ml stored human urine were used for each batch with the following treatments:

1) 0.1; 0.5; 1.0 g MgO/l urine,
2) 7.5; 15; 30 g zeolite/l urine,
3) 7.5; 15; 30 g AC/l urine,
4) 0.1; 0.5; 1.0 g MgO/l and 7.5; 15; 30 g zeolite/l urine,
5) 0.1; 0.5; 1.0 g MgO/l and 7.5; 15; 30 g AC/l urine.

The same treatments were also made with samples of 100 ml of dilution water (SRW) for *Daphnia magna* culture, and these solutions were used as negative controls.

The whole experiment was duplicated and one set of the duplicates was used in freezing-thawing experiments. All dilutions, dishing and preparations of solutions were made with double deionised (MilliQ) water. The samples were kept in plastic beakers sealed with plastic covers. All the beakers were left for 72 h at room temperature (20^{o}C) with a short manual stirring once daily. pH was measured at the start and at the end of this period.

The mineral zeolite used has a high clinoptilolite content (originating from Mád, Tokay hills, Hungary) and a grain size of 1.2-2 mm, and it was used without pre-treatment or previous activation. Its chemical composition has been analysed earlier (Lind et al., 2000). The AC used was a high quality commercial charcoal (Chemviron, grain size 0,4-1.4 mm).

After 72 h the supernatants from one set of beakers were decanted and collected for chemical analysis and toxicity tests.

2.2. The freezing-thawing test

The other set of plastic beakers were transferred to a freezer box at -18^0C. After 24 h the frozen beakers were bottom-heated in a warm water bath (temperature ca 30-40^0C) for 5-10 minutes. Approximately 60% of the total volume was removed as the ice top (called supernatant) on each beaker and thawed and collected for chemical analysis and toxicity testing as described below. The precipitate and adsorbents (zeolite, AC) were left with the rest of the thawed solution.

2.3. Chemical analysis

The supernatants were analysed for total-P and total-N using a DR4000 spectrophotometer with relevant Hach reagents according to the Hach manual (Hach Company, USA, 1997). pH was measured by a calibrated Metrohm 605 pH-meter at the beginning and at the end of the experiments.

2.4. Toxicity tests

Toxicity tests with *Daphnia magna* were made on the supernatants according to ISO (1982). Neonates for testing (6-24 h old) were obtained from our laboratory culture. The dilution water for culture and toxicity tests had a hardness of 250 mg/l as $CaCO_3$ and pH 8.2. This dilution water (SRW) was prepared from double deionised water (MilliQ) and ISO (1982) stock solutions. Ambient conditions were 20.9-21.6^0C and a photoperiod of 16 h light and 8 h darkness. All exposures were made in six-well, plastic Nunc plates (multidish, Brand products), with 10 ml test solutions and 10 daphnids, using a 0.5 dilution factor. Immobility was recorded after 24 h and 48 h and the fifty percent effect concentration (EC50) was determined by probit analysis or moving coverage angle. Pure dilution water was used as negative controls, and a reference toxicant test with potassium dichromate was made, in parallel with all toxicity tests.

2.4. Statistical analysis

Using a statistical software package (Crunch version 4, Crunch, Software Corp., Oakland, CA, USA) all obtained data were statistically treated for analysis of variance (one-way ANOVA).

3. Results

The ANOVAs for both the MgO and zeolite experiment set (Table1) and the MgO and AC experiment set (Table 2) showed that MgO had the major effect on P reduction, while freezing had the major effect on N reduction and toxicity reduction.

3.1. Total-P reduction

The concentrations of total-P in supernatants are presented in the Figures 1 and 2 (missing columns means zero values).

Figure 1. Total-P in supernatants after zeolite treatment.

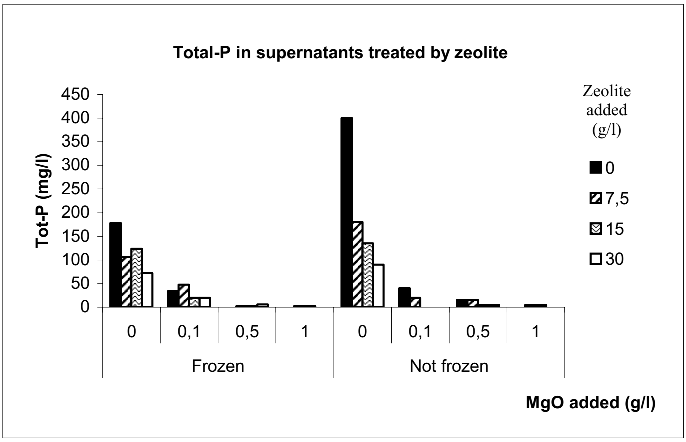
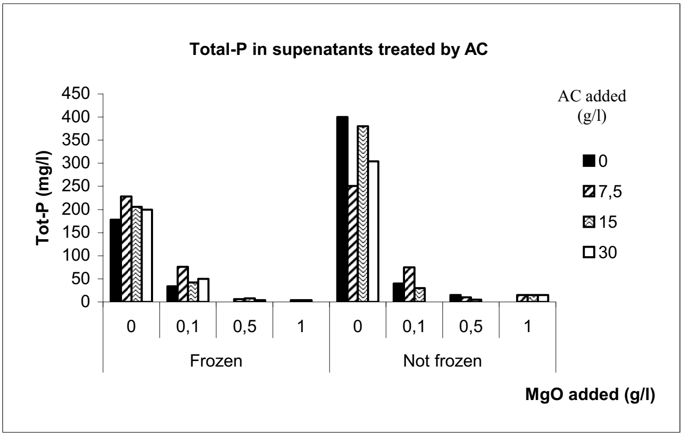


Figure 2. Total-P in supernatants after AC treatment.



The total-P concentration in urine decreased drastically with increased addition of MgO when the stoichiometric molar weight ratio for struvite (Mg:P of 1.71:2.21) was reached for collected urine containing 0.4 g P/l (using 0.5 mg MgO/l urine). The struvite precipitation process is assumed to dominate the phosphorus reduction at pH > 9.0 and in this experiment the pH values were between 9.2 and 9.5 (data not presented here). The precipitation was mainly due to the MgO addition, and phosphate removal was also affected by zeolite and freezing, especially when no MgO was added. It was observed in earlier experiments that the zeolite itself works as a phosphate adsorbent (Bán and Dave, 2004). No significant effect of AC on P removal was found in these experiments (Table 2 and Figure 2).

3.2. Total-N reduction

The reduction of N was mainly affected by freezing and to some extent also by MgO addition. However, zeolite addition had no significant overall effect (Table 1). The results on total-N reduction are presented in Figures 3 and 4.

Figure 3. Total-N in supernatants after zeolite treatment.

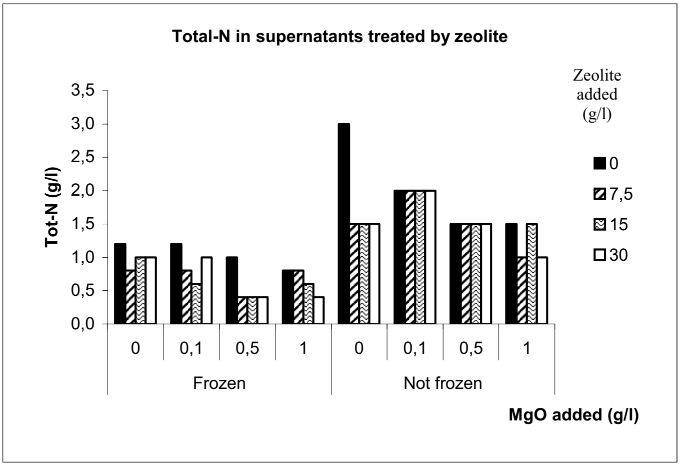
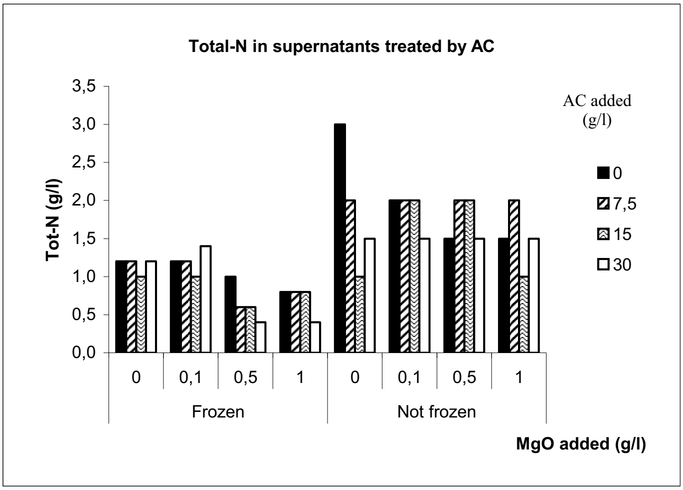


Figure 4. Total-N in supernatants after AC treatment.

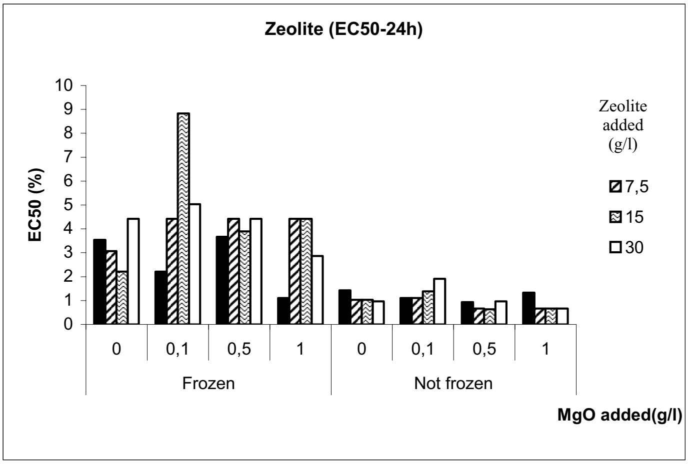


These results suggests that MgO, zeolite and AC reduced N in non-frozen samples, and both zeolite and Ac reduced N in frozen samples. The maximum reduction of N, from 3.0 g/l to 0.3g/l, was found in frozen supernatants with both MgO (0.5 g/l and 1.0 g/l) and zeolite or AC (all amounts) treatments.

3.3.Toxicity of supernatants

The toxicity of the supernatants to *Daphnia magna* (EC50 % after 24h and 48 h) is presented in Figures 5 and 6.

Figure 5. Toxicity of the supernatants to D.magna (EC50% 24h and 48h) after zeolite treatment.



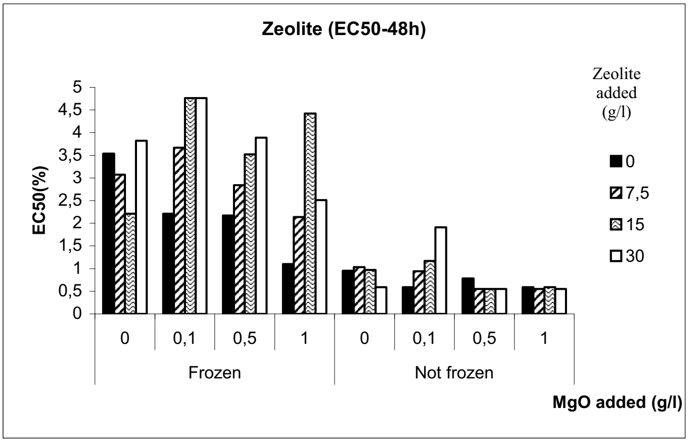
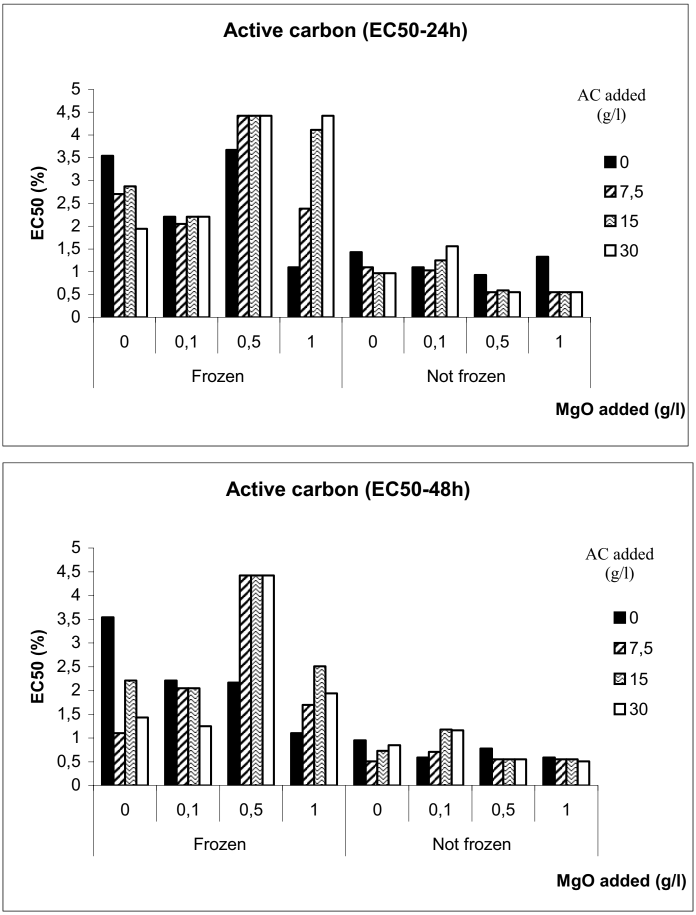


Figure 6. Toxicity of the supernatants to D.magna (EC50% 24h and 48h) after AC treatment.



Toxicity was mainly affected by freezing (Table 1 and 2), which reduced toxicity (increased EC50). The effects of MgO and zeolite were more complex with minor but significant interactions with freezing (Table 1 and Figure 5), however, no significant effect of AC was found (Table 2 and Fig 6).

Table 1. The results of the analysis of variance (ANOVA) for supernatants after MgO and zeolite addition and the effect of freezing-thawing.

Dependent variable	Source	DF	SS(U)	MSS	F	P
pH	Between subjects	55	0.93982			
	Zeolite	3	0.05607	0.01869	5.4	0.0040
	MgO	3	0.43327	0.14442	41.4	0.0000
	Freeze-thaw	1	0.03846	0.03846	11.0	0.0023
	MgO*Zeolite	9	0.04143	0.00460	1.3	0.2639
	MgO*Freeze-thaw	3	0.05625	0.01875	5.4	0.0040
	Zeolite*Freeze-thaw	3	0.00179	0.00060	0.2	0.9153
Tot-P	Between subjects	55	527162			
	Zeolite	3	30031	10010	12.6	0.0000
	MgO	3	156260	52087	65.8	0.0000
	Freeze-thaw	1	1313	1313	1.7	0.2069
	MgO*Zeolite	9	55003	6111	7.7	0.0000
	MgO*Freeze-thaw	3	23469	7823	9.9	0.0001
	Zeolite*Freeze-thaw	3	2949	983	1.2	0.3105
Tot-N	Between subjects	55	16859825			
	Zeolite	3	522321	174107	1.6	0.2149
	MgO	3	1757404	585801	5.3	0.0044
	Freeze-thaw	1	6854711	6854711	61.9	0.0000
	MgO*Zeolite	9	262321	29147	0.3	0.9803
	MgO*Freeze-thaw	3	627679	209226	1.9	0.1508
	Zeolite*Freeze-thaw	3	72321	24107	0.2	0.8835
24h EC50	Between subjects	55	161			
	Zeolite	3	7	2.4	3.3	0.0338
	MgO	3	8	2.8	3.8	0.0188
	Freeze-thaw	1	92	92.4	126.3	0.0000
	MgO*Zeolite	9	16	1.8	2.5	0.0273
	MgO*Freeze-thaw	3	5	1.6	2.2	0.1051
	Zeolite*Freeze-thaw	3	6	2.1	2.9	0.0502
48h EC50	Between subjects	55	102			
	Zeolite	3	6	1.9	4.5	0.0092
	MgO	3	4	1.39	3.2	0.0366
	Freeze-thaw	1	57	57.1	131.3	0.0000
	MgO*Zeolite	9	7	0.8	1.9	0.0889
	MgO*Freeze-thaw	3	5	1.6	3.6	0.0239
	Zeolite*Freeze-thaw	3	4	1.2	2.9	0.0503

Table 2. The results of the analysis of variance (ANOVA) for supernatants after MgO and AC addition and the effect of freezing-thawing.

Dependent variable	Source	DF	SS(U)	MSS	F	P
pH	Between subjects	55	0.93982			
	AC	3	0.02482	0.00827	1.7	0.1792
	MgO	3	0.35971	0.11990	25.1	0.0000
	Freeze-thaw	1	0.06500	0.06500	13.7	0.0009
	MgO*AC	9	0.02268	0.00252	0.5	0.8435
	MgO*Freeze-thaw	3	0.05625	0.01875	3.9	0.0168
	AC*Freeze-thaw	3	0.00929	0.00310	0.6	0.5895
Tot-P	Between subjects	55	527162			
	AC	3	15425	5142	2.4	0.0832
	MgO	3	360747	129249	56.7	0.0000
	Freeze-thaw	1	4624	4624	2.2	0.1494
	MgO*AC	9	27410	3045	1.4	0.2133
	MgO*Freeze-thaw	3	23469	7823	3.7	0.0215
	AC*Freeze-thaw	3	1283	428	0.2	0.8945
Tot-N	Between subjects	55	16859821			
	AC	3	167634	55878	0.5	0.6826
	MgO	3	1841418	613806	5.5	0.0035
	Freeze-thaw	1	5469447	5469447	49.3	0.0000
	MgO*AC	9	540759	60084	0.5	0.8336
	MgO*Freeze-thaw	3	627679	209226	1.9	0.1514
	AC*Freeze-thaw	3	142634	47545	0.4	0.7341
24h EC50	Between subjects	55	161.1			
	AC	3	3.5	1.2	0.9	0.4482
	MgO	3	1.8	0.6	0.5	0.7052
	Freeze-thaw	1	60.3	60.3	47.0	0.0000
	MgO*AC	9	6.9	0.8	0.6	0.7910
	MgO*Freeze-thaw	3	4.9	1.6	1.3	0.3032
	AC*Freeze-thaw	3	1.3	0.4	0.3	0.7939
48h EC50	Between subjects	55	102.3			
	AC	3	2.8	0.9	1.5	0.2355
	MgO	3	6.1	2.0	3.3	0.0338
	Freeze-thaw	1	35.7	35.7	57.2	0.0000
	MgO*AC	9	6.5	0.7	1.2	0.3535
	MgO*Freeze-thaw	3	4.7	1.6	2.5	0.0760
	AC*Freeze-thaw	3	1.6	0.5	0.8	0.4740

4. Discussion

4.1. Total-P reduction

As our previous results have shown, struvite is the main precipitating compound from a complex solution like urine. Together with struvite, other minerals, such as epsomite [$MgSO_4{*}7H_2O$], brucite [$Mg(OH)_2$] and montgomeryite [$Ca_4MgAl_4(PO_4)(OH)_4{*}12H_2O$] may also form, depending on the amounts of other divalent or trivalent metal cations available, initial pH of the urine and the way of dewatering of the precipitate (Lind et al., 2000; Bán and Dave, 2004). Zeolite can also adsorb considerable amounts of phosphates to their hydrous oxides on Al structural sites (Vaughan, 1978; Wild et al., 1996). Sakadevan and Bavor (1998) reported a P adsorption capacity of 2.15 g/kg for clinoptilolite rich zeolites. Our tests also confirm that. In combination with MgO the recovery of P is a result of both MgO and zeolite addition. AC as phosphate adsorbent has not been investigated before, and consequently we cannot compare its efficiency in this case with other studies. However, AC might work as PO_4^{3-} adsorbent in our tests, due to its external surface and mesoporous texture (Díaz-Terán et al., 2001), but its structure and the chemical character may be affected by the carbon deposit from which the AC originates and the sort of pre-treatment (activation) this adsorbent went through (Leboda et al., 2003; San Miguel et al., 2003). However, the P reduction of AC was not significant in this study (Table 2).

4.2. Total-N reduction

During the 72 h experiment time ca 60% of the nitrogen content in urine is expected to be hydrolysed (Haneaus et al., 1996; Adamsson and Dave, 1996) and available as ammonium (NH_4^+) for uptake by the adsorbent, or for precipitation in the form of struvite. In our case the urine used in the experiments was collected from a storage tank (already stored for several days or weeks) and was further stored in the laboratory for 3 more days before the tests. Therefore, we expected a urea hydrolysis of over 80% in this case, even if it was not analysed. Struvite precipitation will decrease the ammonium concentration and the zeolite will act as cation exchanger for the remaining ammonium uptake from the solution. It is known from earlier experiments that the NH_4^+-exchange capacity of natural zeolites is negatively affected by high ion concentrations or by the presence of Mg^{2+} or Ca^{2+} in a solution (Vaughan, 1978). This may be the reason why the tot-N uptake in our case was not higher than 60%. To improve uptake higher amounts of zeolites can be used, but it is a matter of cost efficiency. Pre-treated zeolites are more efficient adsorbents and can also be used in much smaller amounts, but this is also a matter of cost for activation of zeolites (high temperature and high vacuum) as well as handling and management of this product.

AC as an adsorbent for ammonium and other nitrogen containing organic substances has been investigated before (Emadi et al., 2000, Li et al., 2003; Matsui et al., 2003), but not as an adsorbent used for such a high nitrogen concentrations as in urine. In our experiment AC turned out to have a comparable total-N adsorbent efficiency with the zeolite. However, zeolite is specialised for ammonium adsorption and AC seems to be a generalist in this case. During testing we observed a difference between these two materials. The solutions treated with AC had lost their original yellow colour and the typical urine odour, becoming colourless and almost odourless. These physical properties were not changed so much when zeolite was used. The question is if AC adsorbs the same type of nitrogen compound from urine as zeolites does. It is possible that AC adsorbs ammonium, but also larger organic molecules containing nitrogen, e.g. the pigment bilirubin from urine. However, this question was not investigated further in this study.

4.3.The effect of freezing

After freezing-thawing the solutes are concentrated in the remaining liquid phase, leaving the 'ice-supernatant' almost free from impurities. This significant reduction of ions was confirmed by the total-N and total-P analysis (Figures 1- 4) and by the ANOVAs (Table 1 and 2). The increased survival of *D. magna* in the toxicity tests made on the 'ice-supernatants' (Figure 5 and 6) shows the same positive effect. Approximately 60 % volume reduction alone could reduce the nutrient content in urine supernatant to 40% of its original value. In combination with precipitation and adsorption this method can radically improve the nutrient recovery from urine separating systems.

4.4. Toxicity test evaluation

Previous studies of urine toxicity for *Daphnia magna* were made in our laboratory with the aim of studying human urine treatment in aquaculture systems (Adamsson, 1999). It was found that human urine, fresh or stored, is highly toxic for *Daphnia magna*. The solution had to be diluted to an initial urine concentration of 0.5 % in order to be used as an algal nutrient solution and a culture medium for *D. magna*. The main causes for urine toxicity were identified as high pH in combination with high concentrations of ammonia-nitrogen (NH_3-N) (Adamsson and Dave, 1996; Adamsson et al., 1998; Bán and Dave, 2004).

In this study freezing was most effective for reducing toxicity. Addition of MgO increased toxicity (lowered EC50s), but addition of zeolite or AC counteracted this increase, especially in frozen solutions (Figure 5 and 6). Part of this could be due to the presence of ammonia-nitrogen from the NH_4^+- NH_3 equilibrium in the system due to the increased pH values.

Altogether, freezing-thawing alone or in proper combination with treatment with MgO and adsorbent can significantly decrease the toxicity for *D. magna,* mainly due to the high total-N removal from the supernatants.

5. Conclusions

The following conclusions regarding reduction of nutrients (N and P) from human urine and toxicity to *D. magna* can be drawn from this study:

- Freezing-thawing has an overall positive effect on nutrient reduction, especially for N, and reduction of toxicity for *D. magna*.
- Phosphorus is mainly recovered as struvite $[Mg\ (K, NH_4)(PO_4) * 6H_2O]$ and the added amount of MgO affects the precipitation. Additional phosphorus recovery is also affected by zeolite addition, but not by AC addition.
- Nitrogen reduction is more complex and is mainly affected by freezing, but is further reduced by both MgO and adsorbent addition. Both zeolite and AC acted as N adsorbents. However, they may act differently and affect different nitrogen compounds. AC reduced colour and smell.
- The acute toxicity of the supernatants to *Daphnia magna* was mainly affected by freezing. High concentrations of MgO increased the toxicity of the supernatant, but addition of zeolite or AC reduced it.

For further research a method based on freezing-thawing combined with nutrient recovery as solids can be a valuable step. Optimisation of the freezing and thawing step is necessary, as well as cost-benefit calculations in order to choose the best adsorbent.

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VI

Plant availability of nutrients recovered as solids from human urine tested on wheat, *Triticum aestivum* L.

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Abstract

Recycling of nutrients from domestic wastewater to growing plants is essential for a sustainable development. The majority of nutrients in domestic wastewater comes from the human urine. In a previous study N and P were recovered by freezing-thawing from human urine in combination with struvite precipitation and nitrogen adsorption on zeolite and activated carbon. In this study the recovered nutrients have been tested in pot trials with wheat, *Triticum aestivum* L., in a climate chamber during 21 days. Dry weight, plant growth morphology, total-P and total-N were analysed. The tests show a slow-release of nutrients from struvite and from N-adsorbents. The nitrogen concentrations used in all treatments were in the deficiency range for optimum yield for wheat. The results demonstrated the difficulty of nitrogen (plant available) recovery to solids from urine through precipitation and adsorption.

Keywords: struvite, zeolite, active carbon, nutrient recovery, wheat, fertilizer value, freezing-thawing

1. Introduction

Urine separating systems were developed in Sweden during the past decade, and they are very promising for recovery of nutrients compared with conventional systems (Bengtsson et al., 1997; Hellström and Kärrman, 1997; Hellström et al., 2003). Human urine collected in these systems can be used directly as a liquid fertiliser (Kirshmann and Pettersson, 1995; Kirshman, 1998; Jönsson et al., 2000). The fertilizer composition of the urine is similar to NPK (18:2:5) (Lindén, 1997; Simons and Clemens, 2003; Tidåker, 2003). However, obstacles for using urine separation technology at a larger scale are still not solved today, such as hygiene, storage, transport and spreading of large amounts of urine (Jenssen and Etnier, 1996; Larsen and Gujer, 1996; Höglund et al., 1998).

One of the most important compounds that can precipitate from human urine is struvite [$Mg\,(K, NH_4)\,(PO_4) * 6H_2O$]. In recent years struvite recycling in wastewater treatment side-streams has been proposed as an option for phosphorus reuse as fertilizer (Booker et al., 1999; Liberti et al., 2001; Ueno and Fujii, 2001; Doyle and Parsons, 2002). Struvite is also known as slow-release fertiliser, but the cost to produce it from traditional raw materials used in the fertiliser industry turned out to be excessive (Bridger et al., 1962; Ghosh et al., 1996; Schuiling and Andrade, 1999). The potential for struvite recovery from human urine is opening a new possibility for system efficient

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b) visiting scientist from Dongyu Environmental Engineering Co. LTD, Shenyang, Liaoning Province, People Republic of China solutions in relation with source-separating technology (Lind et al., 2000; Bán and Dave, 2004).

Nitrogen recovery by ammonium uptake to specific adsorbents such as zeolites can complete struvite precipitation making additional nitrogen recovery possible (Jorgensen et al., 1976; Beler-Baykal et al., 1996; Beler-Baykal and Acka-Gouven, 1997; Beler-Baykal, 1998; Rozic et al., 2000). Nitrogen recovery from human urine by struvite crystallisation and ammonium adsorption on zeolite showed promising results (Lind et al., 2000; Bán and Dave, 2004). Recycling and reuse of ammonium loaded zeolites as slow-release fertilisers have been studied, and the technique is well documented in the field of agricultural research, so called 'zeo-agriculture' (Pond and Mumpton, 1984).

Technologies using active carbon, fly ash, charcoal, etc. are widely used in fields like agro-industrial wastewater treatment (Garcia-Aaraya et al., 2003), aquaculture (Aitcheson et al., 2000). Active carbon (henceforward AC) as adsorbent for nitrogen recovery from human urine was tested by Ganrot et al.(2004).

Freeze concentration seems to be a very efficient method for soluble pollutant removal from wastewater (Halde, 1980; Lorain et al., 2001; Gay et al., 2003; van der Ham et al., 1998, 2004). Freezing-thawing of human urine alone (Lind et al., 2001) and in combination with struvite precipitation and mineral adsorption showed an overall positive effect for nutrient recovery from urine (Ganrot et al., 2004; Slivka, 2004).

The aim of the present study was to investigate the plant availability of the nutrients recovered from human urine by freezing in combination with struvite precipitation and nitrogen uptake to zeolite and AC. The batch samples with the nutrient-loaded materials were used in a 21 days long climate chamber pot trial for cultivation of *Triticum aestivum* L. (common spring wheat).

2. Method and material

The methods used for nutrient recovery from human urine are given in Ganrot et al. (2004). Briefly, various combinations of human urine and MgO, zeolite or activated carbon (AC) were mixed and allowed to settle. One set of samples were frozen and partially thawed. Then the supernatants were removed and analysed for total-N, total-P and tested for toxicity to *Daphnia magna* as described in Ganrot et al. (2004). The remaining precipitates and adsorbents from the tests using freezing-thawing were saved and used as fertilizers in this study.

2.1. Pot trials

The contents in the plastic beakers containing ca 40% of the total urine volume (after freezing-thawing) with struvite precipitate, materials added as adsorbents for were used as nutrient sources for cultivation of common spring wheat (*Triticum aestivum* L.). Plastic pots filled with washed quartz sand (grain size 0.5-1.2 mm, ca 0.4 kg per pot) were used as growth medium. The pots were placed inside the plastic beakers containing the nutrients in the bottom. Three pots with beakers containing 1 mg, 10 mg and 100 mg of NPK (14-4-21) placed at the bottom, and one beaker with no fertilizer addition were used as controls. The amounts of NPK were chosen to be comparable with the N and P amounts recovered in the 100 ml batch tests with human urine (as

described in Ganrot et al., 2004). The amounts of N used in the pot trials are in the nutrient deficiency range for optimum growth for *T. aestivum* L. (Wild, 1988; Marchner, 1999).
Deionised water was used for all washing and preparations and also for daily irrigation.

Four seeds of *T. aestivum L.* were sowed in each pot, and all pots were placed in a climate chamber with temperature 20^0C daytime and 16^0C night-time, humidity 75 % day RH and 60 % night RH, daylight 16 h PAR (750 mmol photons m^{-2} s^{-1}). After 5 days of sprouting they were culled to get an equal number of three plants in each pot. The climate chamber condition was: The plants were irrigated manually, when necessary.

After 21 days the plants were harvested, and the average heights of the shoots were recorded. Colour and development of leaf and stem, and development of the root system were also recorded. The whole plants were weighed before and after drying (in an oven at 70^0C for 3 days), and the plants fresh weight and dry weight were determined.

After harvest the remaining irrigation water was collected and diluted to 100 ml in each beaker. After one day at room temperature (20^0C) samples were collected from the liquid phase and analysed for total-N and total-P concentrations in solution (considered available for the plants) and the acute toxicity was determined for *D. magna*. To the rest of the liquid (48 ml in each beaker) with the struvite precipitate and adsorbents on the bottom, 2 ml 1N HCl was added to dissolve the struvite and analyse the liquid for tot-P and tot-N (considered not directly available fraction for the plants).
There are several routinely applied soil phosphorous extraction methods and their use differ among countries (Kvarnströn, 2001). We have chosen a strong acid for the dissolution of the struvite precipitate. Our intention was not to make fertilizing P recommendations for a given crop, but to evaluate the total-P pool, which remained insoluble after the pot trials, to be able to evaluate the struvite behaviour in the system.

2.2. Chemical analysis

The total-P and total-N concentrations were analysed using a DR4000 spectrophotometer with relevant Hach reagents according to the Hach manual (Hach Company, USA, 1997). pH was measured by a calibrated Metrohm 605 pH-meter at the beginning of the analysis and during struvite dissolution with HCl.

2.3. Toxicity tests

Toxicity tests with *Daphnia magna* were made on the samples of irrigation water before struvite dissolution, testing two dilutions: not diluted and 50% diluted, according to ISO (1982). Neonates for testing (6-24 h old) were obtained from our laboratory culture. The dilution water for culture and toxicity tests had a hardness of 250 mg/l as $CaCO_3$ and a pH 8.2. This dilution water was prepared from double deionised water (MilliQ) and ISO (1982) stock solutions. Ambient conditions were 22°C and photoperiod of 16 h light and 8 h darkness. All exposures were made in Petri-dishes with 50 ml test solution and 20 daphnids. Immobility was recorded after 24 h and 48 h. Dilution water was used as positive controls and a reference toxicant test with potassium dichromate was made, in parallel with all toxicity tests.

2.4. Statistical analysis

Data from total-P, total-N, average shoot height and average plant dry weight were statistically treated for analysis of variance (one-way ANOVA) and Spearman rank correlation using a statistical software package (Crunch version 4, Crunch, Software Corp., Oakland, CA, USA).

3. Results

The results are separated into those on crop growth and observations on crop condition, and those on nutrient availability.

The results from the ANOVAs are shown in Table 1 for pH, plant available (soluble) concentrations of N and P and plant growth responses (dry weight and plant shoot height), and for residual (not plant available) N and P in Table 2. Altogether the ANOVAs showed that additions of MgO (struvite precipitation) were much more important than additions of zeolite or AC. However, the addition of zeolite or AC modified the effect of MgO for both N and P availability as described below.

The growth responses (biomass and height) were limited by the lack of N, and no N was detected (< 1mg/l) in the irrigation water collected after harvest (after 21 days), and no toxicity (immobility after 24 and 48 h) for *Daphnia magna* was detected at any combination of MgO, zeolite and AC. However, some N remained in the insoluble solid phase (not plant available) as described below.

3.1. Crop growth

The average weight of the *T. aestivum L.* (on dry weight basis) after 21 days of growth is presented in Figures 1 and 2. The results are presented for the different treatments described previously (Ganrot et al., 2004).

Figure 1. Average dry weight of wheat after 21 days fertilized with struvite and urine-equilibrated zeolite.

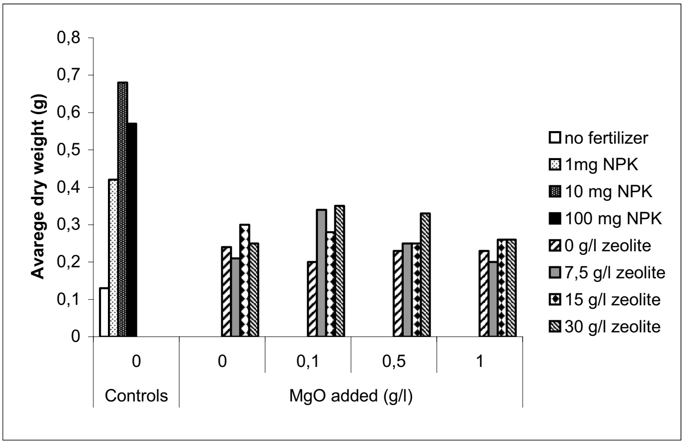
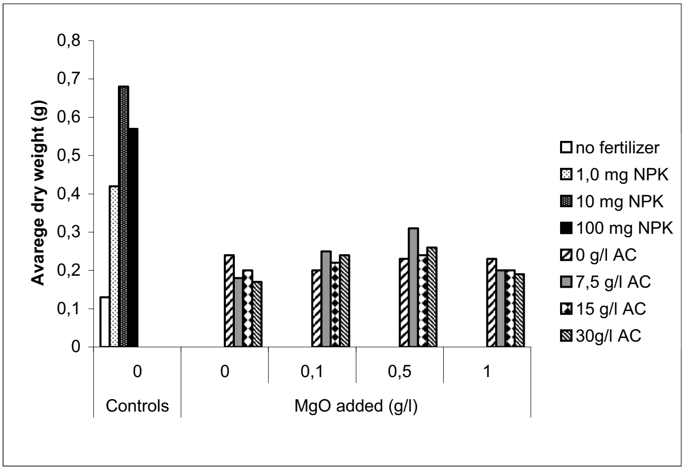


Figure 2. Average dry weight of wheat after 21 days fertilized with struvite and urine-equilibrated active carbon.



The average dry weight (DW) after 21 days was only half as much as that of the plants getting the optimum concentration of balanced NPK fertilizer as nutrient source (controls). This indicates a lower or slower nutrient availability from the recovered nutrients than from the highly soluble NPK used.

The average heights after 21 days are presented in Figures 3 and 4.

Figure 3. Average height of wheat after 21 days fertilized with struvite and urine-equilibrated zeolite.

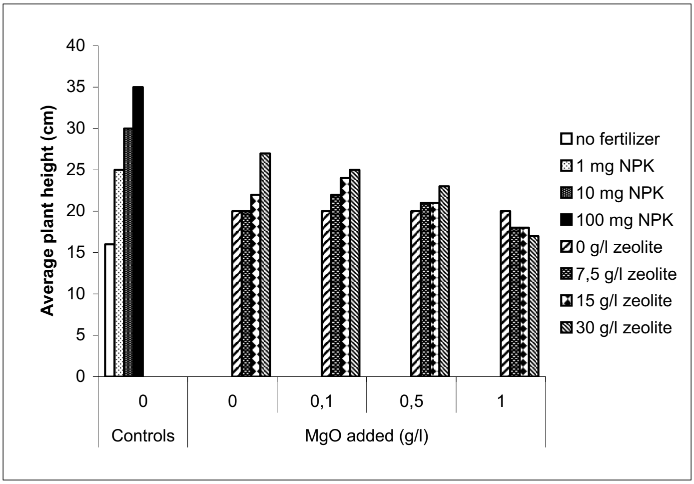
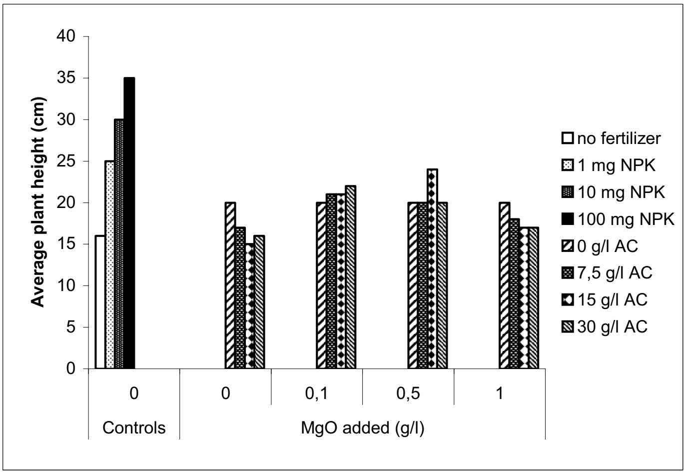


Figure 4. Average height of wheat after 21 days fertilized with struvite and urine-equilibrated active carbon.



The MgO addition (struvite) had a small but significant effect on plant height (Table 1). The overall plant height response to zeolite was not significant (Table 1), but it seems as growth was stimulated by zeolite at low additions of MgO (0, 0.1 and 0.5 mg/l) but inhibited at the highest addition of MgO (1.0 mg/l), as the Figure 3 shows. This conclusion was supported by Spearman rank correlation tests made separately for the different MgO additions. The root systems of the plants growing in the pots with high additions of MgO were also affected in a negative way with small roots with fine, short root hair structure.

3.2. Nutrient uptake

The availability of nutrients from struvite (the main P-source here) and from the nutrient-loaded materials (the additional N-source here) can be estimated if the remaining amounts of nutrients are measured after harvest. In the last irrigation water tot-P and tot-N was measured in both the liquid phase (available fraction for the plants) and in solid phase (not dissolved, not directly available fraction). After 21 days of growth the plants were in the early tillering stage of *T. aestivum* L. How much more nutrients that can be available from the solid phase later on was not tested in this study.

The plant available (water soluble) total-P contents in the last irrigation water are presented in the Figure 5 and 6. The P present in the last irrigation water in all treatements indicates that there was plant available PO_4^{3-}-P present for plants during 21 days of growth. The one-way ANOVA shows that P is affected by MgO addition (struvite formation), but that the adsorbents (zeolite and AC) had no effect (Table1).

The plant available total-N measurements showed zero total-N concentrations in the last irrigation water in all treatments, which indicates an efficient N-uptake by the plants, and it also shows that the experiment was conducted under an overall N deficiency for plants in all treatments and the negative controls. The positive controls with NPK demonstrated that ambient conditions (temperature, light, substrate and water), as well as the biological material (the quality of seeds and plants) were adequate. The N deficiency in the last irrigation water was also verified by the toxicity test made on *D. magna*, which showed 100% daphnid mobility and survival after both 24 h and 48 h in both the non-diluted and 50% diluted irrigation water (data not presented in tables and figures). The toxicity of urine containing irrigation water is known to be due to its ammonia content (Ganrot et al., 2004).

Figure 5. Total-P in the irrigation water collected after 21 days with struvite and urine-equilibrated zeolite as nutrient source for wheat.

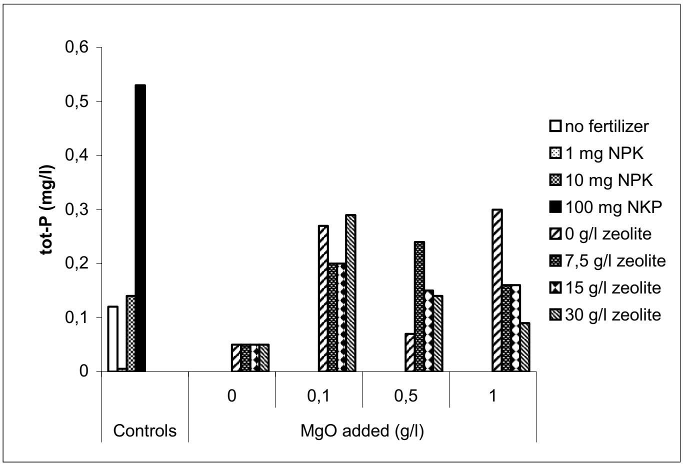
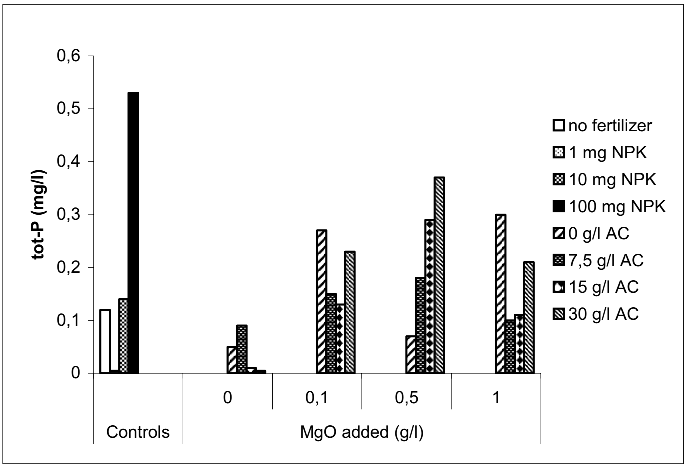
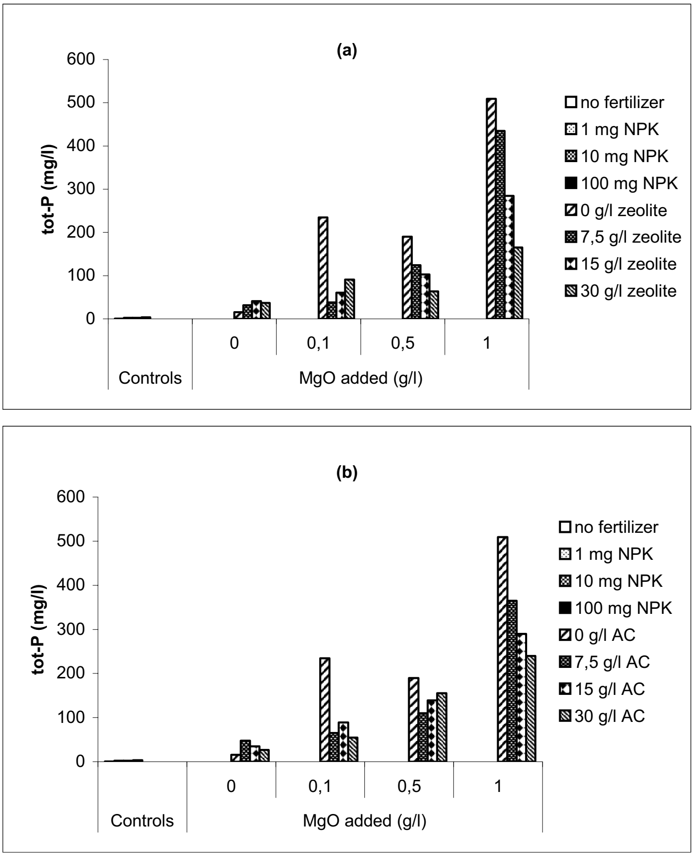


Figure 6. Total-P in the irrigation water collected after 21 days with struvite and urine-equilibrated active carbon as nutrient source for wheat.



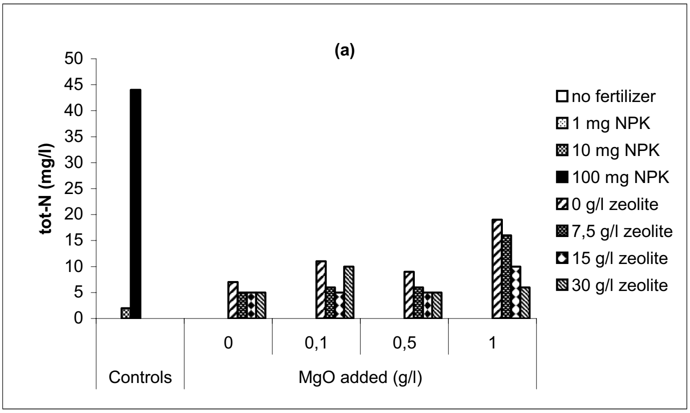
The not directly available amount of nutrients, which remained at the bottom of the beakers, were dissolved by addition of HCl after the pot trials, and their concentrations are presented in the Figure 7 (a and b) for total-P, and Figure 8 (a and b) for total-N.

Figure 7. Total-P remained in solid phase after 21 days with struvite and urine-equilibrated zeolite (a) and struvite and urine equilibrated active carbon (b) as nutrient sources.



The amounts of not directly available phosphorus was negligible for all concentrations of the NPK, and it increased in proportion to MgO addition indicating the low solubility of struvite (Table 2 and Figure 7). At most of the MgO doses the concentration of remaining N and P decreased (Figure 7 and 8). Thus, the non-available N-pool followed the same pattern as the non-available P-pool, indicating the slow-release of nutrients (N and P) from struvite (Table 2 and Figure 8).

Figure 8. Total-N remained in solid phase after 21 days with struvite and urine-equilibrated zeolite (a) and struvite and urine equilibrated active carbon (b) as nutrient sources.



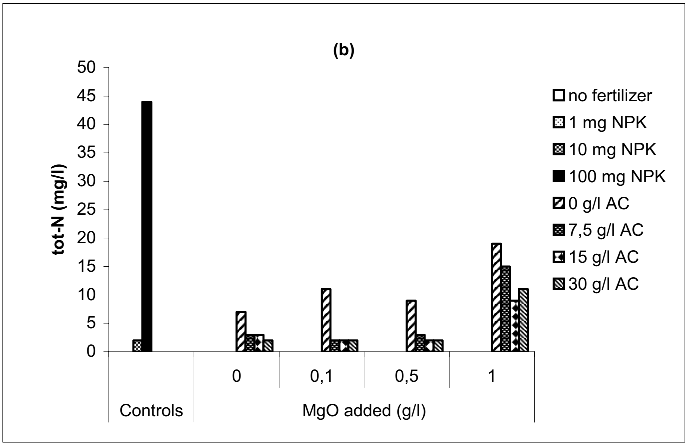


Table 1. The results of the analysis of variance (ANOVA) for pH, plant available nutrient content in the irrigation water after harvest, plant dry weight (DW) and shoot height (SH).

Dependent variable	Source	DF	SS(U)	MSS	F	P
pH	Between subjects	15	9.889			
	MgO	3	8.987	2.995	99.6	0.0000
	Zeolite	3	0.631	0.210	7.0	0.0099
	Between subjects	15	10.554			
	MgO	3	10.162	3.387	89.5	0.0000
	AC	3	0.052	0.017	0.5	0.7190
Tot-N	Not detected	x	x	x	x	x
Tot-P	Between subjects	15	0.120			
	MgO	3	0.080	0.025	5.4	0.0208
	Zeolite	3	0.003	0.001	0.2	0.8838
	Between subjects	15	0.181			
	MgO	3	0.085	0.028	3.1	0.0821
	AC	3	0.014	0.005	0.5	0.6854
DW	Between subjects	15	0.034			
	MgO	3	0.007	0.002	1.3	0.3282
	Zeolite	3	0.012	0.004	2.3	0.1510
	Between subjects	15	0.019			
	MgO	3	0.009	0.003	3.6	0.0602
	AC	3	0.001	0.0004	0.4	0.7464
SH	Between subjects	15	105.750			
	MgO	3	48.750	16.250	4.2	0.0406
	Zeolite	3	22.250	7.417	1.9	0.1968
	Between subjects	15	85.000			
	MgO	3	51.000	1.167	5.0	0.0258
	AC	3	3.500	3.389	0.3	0.7942

Table 2. The results of the analysis of variance (ANOVA) for not plant available nutrient content in the solid phase after harvest (pH=1.5 after 1 N HCl addition).

Dependent variable	Source	DF	SS(U)	MSS	F	P
Tot-P	Between subjects	15	328316.9			
	MgO	3	225131.7	75043.9	12.4	0.0015
	Zeolite	3	48609.5	16203.2	2.7	0.1106
	Between subjects	15	288509.9			
	MgO	3	222262.7	74087.6	20.2	0.0002
	AC	3	33193.8	11064.6	3.0	0.0869
Tot-N	Between subjects	15	269.8			
	MgO	3	127.3	42.4	5.3	0.0224
	Zeolite	3	70.3	23.4	2.9	0.0929
	Between subjects	15	439.8			
	MgO	3	271.3	90.4	38.3	0.0000
	AC	3	147.3	49.1	20.8	0.0002

4. Discussion

The balanced macronutrient demand (weight %) for cereals according to Ingestadt is: N = 100; P = 17; K = 80; Ca = 9; Mg = 9. The nutrient requirement for one wheat plant during approx. 30 days of optimal growth (with a dry weight of approx. 1,4 g) with a plant N-content of 2% (dry weight basis) is 80 mg N, 13 mg P, 64 mg K (calculated from Ingestadt and Stoy, 1982; Wild, 1988; Marchner, 1999). For the 3 plants/pot this is 240 mg N, 39 mg P and 192 mg K. During 21 days of growth the requirement should be approximately 160 mg N, 26 mg P and 128 mg K in our experiment. The initial amounts of nutrients recovered from 100 ml urine with different treatments were: 180-260 mg total-N/beaker and 17-40 mg PO_4-P/beaker (Ganrot et al., 2004). Thus, the experiment was designed with nutrient limiting conditions in order to detect differences among the treatments.

4.1. Crop growth

The biomass production of the plants having nutrient supply from the treated urine was only half as much as of the plants using balanced NPK fertilizer as nutrient source (controls). This indicates a lower nutrient availability from the recovered nutrients than from the highly soluble NPK used. Both struvite and zeolites are known to be slow release fertilizers (Bridger et al., 1962; Pond and Mumpton, 1984; Sikora et al., 1989; Ghosh et al., 1996). As such, they are not containing and releasing enough nutrients recovered from 100 ml urine for optimal plant growth during the first 3 weeks of intensive growth period for *T. aestivum* L. In the case of the pots having struvite and NH_4-zeolites as nutrient sources the average dry weights of the plants indicated a slightly higher biomass production than in those treated by struvite and N-loaded AC. This may be explained by a synergistic effect of the zeolite/struvite mixed mineral system on nutrient solubility.

Earlier research have shown that non-calcareous soils fertilized with different ammonium-zeolite/apatite mixtures enhanced P dissolution from the apatite (Lai and Eberl, 1986; Allen et al., 1993,1995) and increased the biomass production of greenhouse-grown sorghum-sudangrass (*Sorgum bicolour* L.) by a factor of 1.6 (Barbarick et al., 1990). Higher zeolite/apatite ratios gave higher P availability, because of a decrease in the soil pH, dissolution of apatite rock and more available NH_4^+ from zeolite in the plant-soil interactions during growth. The system is based on the principle that zeolites can sequester Ca^{2+} released by the dissolution of apatite, thereby leading to further dissolution of the apatite (Lai and Eberl, 1986). In our case a similar nutrient carrying system with zeolite/struvite mixture and high Mg^{2+} ion content and zeolite:struvite ratio (7:1 for 15 g/l zeolite used and 15:1 for 30 g/l zeolite used) may increase the nutrient availability as a result of the synergistic effect from the mineral system. In the case of AC, the nutrients are not recovered by cation exchange and chemisorption, but by a physical adsorption in the porous structure of the material. Desorption in the water microenvironment of root-substrate system with slight pH changes is easier in this case. However, no similar previous experiments or research results can confirm this.

The pH decreased during the pot trials from 10.0 to 8.0 on average (data not presented here), with the greatest decrease at high zeolite additions, where pH was between 7.6 and 8.0 after harvest. In the treatments with high MgO the pH decreased to 9.0 only after harvest, causing small and short root system development and a generally lower shoot height growth for the plants. The direct and indirect effects of high soil pH are well described in Marscher 1999.

4.2. Nutrient uptake

Apart from environmental factors like light, temperature and water, biomass production and healthy plant development is highly dependent on the quality and amount of nutrients applied. The former can be tested, different treatments compared in a climate chamber, if the other factors are optimally regulated for plants. In this study the amounts of N was found to be in the deficiency range for *T. aestivum* L. However, the pot trials were performed to estimate the behaviour of the nutrients recovered as solids from small amounts of human urine, regardless of optimal nutrient supply.

The NaCl content of fresh human urine is about 150 mM (calculated from Altman and Dittmer,1974). In our treatments the 50% diluted urine (NaCl concentration approx. 75 mM) was concentrated by freezing-thawing (60% of nutrients in 40% of volume). The plants growing in the system (except for the controls) were exposed to approx. 120 mM NaCl concentration. Plant performance may be affected by salinity-induced nutritional disorders (Hu et al., 1997; Grattan and Grieve, 1999). In our experiments a complex interaction between the slow-release fertilizers (struvite and zeolite or AC), the saline conditions from urine concentration, a high pH and a generally low N addition may take place, resulting in low dry weight and height for the plants compared with the controls.

Plant nutrient analysis was not performed. However, visual observations on plant development indicate some N and P deficiency symptoms on the plant leafs as yellow apex on old leaves and reddish stem on the plants treated only with struvite or only with AC. Plants treated with zeolites, struvite/zeolite mixture or struvite/AC mixture showed no such visual deficiency symptoms.

5. Conclusions

Pot trials with nutrients recovered from human urine on *T. aestivum* L. in a climate chamber gave the following results:

- The N recovered from 100 ml human urine as struvite and loaded zeolite or AC was not enough for wheat growth during 21 days of cultivation. The whole experiment was therefore made under N deficiency.
- The struvite/adsorbent mixtures showed better nutrient availability than the struvite alone, probably due to a synergistic effect between the struvite dissolution and zeolite ion exchange or AC release of nutrients.
- Struvite, struvite/zeolite mixture and struvite/AC mixture acted as slow-release fertilizers.

Further plant availability tests are necessary for optimisation and evaluation of struvite and N-loaded materials as multi-nutrient fertilizers recovered from human urine.

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VII

Fertilizer value of nutrients (N and P) recovered as solids from human urine compared to two commercial fertilizers and two liquid fertilizers in five substrates tested on spring wheat (*Triticum aestivum* L.)

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Abstract

Closing the nutrient loop between domestic wastewater streams and agriculture is essential in a sustainable society. Human urine is the main source of nutrients in domestic wastewater. N and P can easily be recovered from human urine as solids like struvite and in nutrient loaded minerals like zeolites. In this study the recovered nutrients have been tested in pot trials with spring wheat (*Triticum aestivum* L.) in five substrates when grown in climate chamber during 33 days. Four nutrient sources (two of them commercial fertilizers) were compared with the solids recovered from urine. Shoot and root biomass, shoot mineral status, and total-P and total-N in substrate solution were analysed. The tests showed that the solid recovered from urine is a good P source, fully comparable with the tested commercial fertilisers DAP (di-ammonium-phosphate) and CaP (mono-calcium-phosphate).

Keywords: struvite, zeolite, DAP, CaP, urine, nutrient recovery, spring wheat, mineral status

1. Introduction

Urine separating systems have been developed worldwide during the past decade as a way to close the nutrient loop between domestic wastewater in human settlements and arable land (Bengtsson et al., 1997; Hellström and Kärrman, 1997; Hellström et al., 2003). Human urine collected in these systems can be used directly as a liquid fertiliser (Kirshmann and Pettersson, 1995; Kirshman, 1998; Jönsson et al., 2000). The urine has a content of N, P and K in the following weight-based proportions: 18:2:5 (Lindén, 1997; Simons and Clemens, 2003; Tidåker, 2003). The plant availability of N and P from urine is the same or better as that of artificial ammonium and phosphate fertilizers (Kirshmann and Pettersson, 1995; Richert Stintzing, et al., 2001).

One of the most important compounds that can be precipitated from human urine is struvite [$Mg (K, NH_4) (PO_4) * 6H_2O$]. In recent years struvite recycling in wastewater treatment side-streams has been proposed as an option for phosphorus reuse as fertilizer (Booker et al., 1999; Liberti et al., 2001; Ueno and Fujii, 2001; Doyle and Parsons, 2002). Struvite is also known as slow-release fertiliser, but the cost to produce it from traditional raw materials used in the fertiliser industry turned out to be excessive (Bridger et al., 1962; Ghosh et al., 1996; Schuiling and Andrade, 1999). The potential for struvite recovery from human urine is opening a new possibility for system efficient solutions in relation with source-separating technology (Lind et al., 2000; Bán and Dave, 2004, Ganrot et al., *submitted)*.

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Nitrogen recovery by ammonium uptake to specific adsorbents such as zeolites can be combined with struvite precipitation making additional nitrogen recovery possible (Jorgensen et al., 1976; Beler-Baykal et al., 1996; Beler-Baykal and Acka-Gouven, 1997; Beler-Baykal, 1998; Rozic et al., 2000). Nitrogen recovery from human urine by struvite crystallisation and ammonium adsorption on zeolite showed promising results (Lind et al., 2000; Bán and Dave, 2004; Ganrot et al., *submitted*). Recycling and reuse of ammonium loaded zeolites as slow-release fertilisers have been studied previously, and the technique is well documented in the field of agricultural research, so called 'zeo-agriculture' (Pond and Mumpton, 1984; Bish and Ming, 2001).

The aim of the present study was to investigate the fertilizer value of P and N recovered from human urine by struvite precipitation and nitrogen uptake to zeolite in test where spring wheat was grown in different substrates. A mix of struvite and zeolites was compared to two well-known commercial fertilizers acting similarly to struvite, liquid fertilizer with and without phosphorus and a fertilised commercial soil. Biomass, as well as the mineral nutrient composition of the cultivated plants was used as assessment parameters.

2. Materials and methods

2.1 Plant material and growing conditions

The value of different fertilizers combined with different soil substrates was tested by growing spring wheat (*Triticum aestivum* L.) from seed for a period of 33 days in plastic pots (0.3 l). The seeds were soaked in aeriated and distilled water at room temperature (~ 20 °C). Three seeds were sowed in each pot at a depth of (~1 cm). The pots were placed on carriages in a climate chamber with temperatures of 20 ^{0}C day-time (16h) and 16 ^{0}C night-time (8h). The relative humidity was 75 % at day-time and 60 % at night-time. Illumination was provided by a combination of Sylvania Fluorescent tubes (cool white, 215 W) and common electric bulbs (60 W) giving a light intensity at the plant level of 520 μmol m^{-2} s^{-1}. The carriages were daily rotated in the chamber to achieve similar climate conditions for all plants. The pots were thinned down to one plant per pot by removing the smallest plants after 5 days.

2.2 Treatments and preparation of nutrients and substrates

Five nutrient sources and 4 soil substrates were used plus a commercial nutrient enriched soil (K-soil) as a separate control. All together 21 treatments were used, each treatment having 15 pots for 3 biomass harvests giving 5 replicates per treatment and harvest. Three solid fertilizers; a struvite-zeolite mix with nutrients recovered from urine (SZM); di-ammonium hydrogen ortophosphate (DAP); mono-calcium-phosphate monohydrate (CaP) and two nutrient solutions; one with an optimal nutrient composition (OPT) and a corresponding solution without P (OPTnoP) were used as nutrient sources. The solid fertilizers were supplemented with a nutrient solution aiming at an optimal nutrient composition for wheat seedlings according to Ingestad and Stoy (1982). The stock nutrient solutions were prepared according to Ingestad and Stoy (1982), Ingestad and Lund (1986) and Ingestad (1987). The nutrient ratios demanded by the plants and the nutrient ratios in the different nutrient treatments are given in Table 1. A total balance of all macronutrients in the solid fertilizer treatments is not possible due to their original composition. However, in order to get comparable nutrients supplies in the different treatment, the aim was to obtain equal P and N contents except in OPTnoP The balance of K, Ca, Mg and S content is regarded as less important as long as there are excessive amounts of them.

The following substrates, commonly used in growing tests and having low amount of available N and P, were used; sand and dolomite mix (S+D); sand; peat and dolomite (S+P+D); sand and vermiculite (S+V) vermiculite (V) and soil (K-soil). The mixing ratios are given in Table 2. The origin and quality of most of the substrates and nutrient sources are described in Table 3. The recovery of the nutrients in SZM is described below and the nutrient solutions are prepared from laboratory chemicals of p.a. quality.

According to the amounts per pot given in Table 2 the fertilizers were mixed with a suitable amount of the substrate. The solid fertilizers were first added to the substrates in the following amounts per pot; 7.868 g SZM; 0.204 g DAP; 0.195 g CaP; 7.868 g SZM. The supplementary nutrient solution and the full nutrient solutions were then added to obtain the full combination of substrates and nutrient sources. The nutrient solutions were used to adjust the pH of the final substrates by adjusting the balance between HNO_3 and KNO_3 as N source. This is a reason why the N and P nutrient ratio varies a little between the nutrient treatments (Table 1). The water percolated through the final treatment substrates varied in conductivity from 480 to 700 $\mu S\ cm^{-1}$ and in pH from 5.3 to 7.0.

Deionised water was used for all washing and preparations and also for daily irrigation. The daily irrigation was made manually. Small volumes of deionised water were added in such a way that the water on the plates under the pots was re-adsorbed within 2 hours after each irrigation.

2.3. Nutrient recovery as solids from human urine

The SZM was made from human urine collected from the storage tank at the science centre Universeum in Göteborg that has functioning urine separating sewage system. The urine solution was diluted with about 50% tap water used for toilet flushing. Therefore, the concentrations of all solutes are approximately the half of those found in fresh human urine. The urine was transported in closed plastic cans to the laboratory and stored at room temperature (~20°C) one day before the processing was started.
The processing was started by adding 1 g MgO (for struvite precipitation) and 30 g zeolite (for additional nutrient uptake) per litre to a total volume of 30 litre diluted urine and left for 3 days at room temperature with a short manual stirring once daily. After that, the resulting solid phase was separated from the liquid phase through gravity filtration (paper filter Duni 250, Sweden) and the solids were left in room temperature to dry. The mineral zeolite used had high clinoptilolite content (originating from Mád, Tokay hills, Hungary) and a grain size of 1.2-2.0 mm, and it was used without any pre-treatment or previous activation. The zeolite chemical composition has been analysed earlier and is given by Lind, et al., 2000. In SZM, the major mass consists of the zeolite and 5.3 % struvite content empirically observed in earlier experiments (theoretical calculations gives 5.8%).

Table 1. The demand of nutrients and the amount of nutrient (g) per pot and the corresponding ratios in relation to N (weight ratio in %) that were used in the different treatments in the experiment. In addition an optimal amount of micronutrient was added to all treatment.

Macronutrients	**N**	**P**	**K**	**Ca**	**Mg**	**S**
Balanced nutrient demand ratio 1)	**100**	**16**	**80**		**5**	**9**
Maximal total nutrient demand for one plant 2)	0.340	0.054	0.272	0.017	0.017	0.031
SZM + supplementary nutrients 3)						
Nutrient supply from struvite	0.024	0.048	0.09	0.015	0.005	0.003
Nutrient supply from solution supplementary to struvite	0.311	0.005	0.249	0.16	0.087	0.03
Total amount of nutrients	0.335	0.053	0.339	0.175	0.092	0.033
Nutrient ratios	**100**	**16**	**101**	**52**	**27**	**10**
DAP+supplementary nutrients 4)						
Nutrient supply from DAP	0.043	0.048	none	None	none	None
Nutrient supply from solution complementary to DAP	0.31	0.005	0.249	0.189	0.114	0.03
Total amount of nutrients	0.353	0.053	0.249	0.189	0.114	0.030
Nutrient ratios	**100**	**15**	**71**	**54**	**32**	**8**
CaP+supplementary nutrients 4)						
Nutrient supply from Ca-P	none	0.048	none	0.03	none	none
Nutrient supply from solution complementary to Ca-P	0.34	0.006	0.252	0.18	0.108	0.03
Total amount of nutrients	0.340	0.054	0.252	0.210	0.108	0.030
Nutrient ratios	**100**	**16**	**74**	**62**	**32**	**9**
OPTimal nutrient solution						
Total amount of nutrients	0.346	0.055	0.254	0.160	0.096	0.030
Nutrient ratios	**100**	**16**	**73**	**46**	**28**	**9**
OPTnoP nutrient solution						
Total amount of nutrients	0.333	0.005	0.249	0.17	0.087	0.03
Nutrient ratios	**100**	**2**	**75**	**51**	**26**	**9**
K-soil 5))						
Total amount of nutrients	0.09	0.05	0.105	1.15	0.125	0.06
Nutrient ratios	**100**	**56**	**117**	**1278**	**139**	**67**

1) from Ingestad and Stoy (1982).
2) Calculated by assuming a maximal dry weight per plant at the end of the experiment of 8.5 g and a nitrogen content of 4 mg g^{-1}.
3) Calculated from the element analysis made on struvite precipitated from human urine and analysed by AnalyCen accred. Lab., Sweden, that resulted in the following macro nutrient composition (mg/g SZM); N (8.5-9.4); P (4.6-6.4); K (14-15); Ca (12-13); Mg 6.1-9.4). N was analysed as Kjeldahl and others with HNO_3 dissolution)
4) The nutrient content was calculated from the chemical composition declaration on the packages.
5) Each pot have a volume corresponding to 200 g K-soil

Table 2. The composition of the different substrates used in the experiment. Specification of the substrates is given in Table 1.

Substrate	**Substrate abbreviations**	**Proportions (weight based in % of total)**					**Nutrient amounts (g) per pot**
		S	**D**	**T**	**V**	**Soil**	
Sand + dolomite	(S+D)	98	2				400 + 7.0
Sand + peat + dolomite	(S+T+D)	65	3	32			200 + 100 + 10
Sand + vermiculite	(S+V)	67			33		200 + 100
Vermiculite	(V)				100		200
K-soil	K-soil					100	200

Table 3. Description and origin of the nutrients and substrates used in the experiment.

Product name[1)]	**Manufacturers or suppliers**	**Product description**
Washed Quartz Sand (Filtersand)	Rydbergs AB, Sweden	Grain size 0.8-1.2 mm
Vermiculite (Vermikulit)	Askania, Finland	Medium grain size (2-4 mm) Density 70-90 kg m^{-3}
Peat (Torvmull, naturell)	Econova Garden AB, Sweden	Sphagnum peat pH 3.5-4.5 Dry matter 80 kg m^{-3} Ash content < 5%
K-soil (K-jord)	Weibull Trädgård AB, Sweden	Nutrient enriched soil composed of: Peat 80% (volume) Clay 20 % (volume) pH 5.5-6.5 Dry matter 300 kg m^{-3} Nutrient content (g m^{-3}): N 180 Fe 10 P 100 Mn 3 K 210 B 0.5 Mg 250 Cu 1.0 S 120 Zn 1.1 Ca 2300 Mo 0.4
Dolomite (Effektkalk)	Weibull Trädgård AB, Sweden	Grain size 0-4 mm CaO 52 % Mg 12 %
DAP (di ammonium hydrogen ortophosphate)	BDH Lab. Supplies, England	132.05 g $mole^{-1}$
CaP (mono calcium phosphate monohydrate)	BDH Lab. Supplies, England	Bolifor ACP, Feed grade P 22.7 % Ca 16 % Mg 1 %

1) when applicable Swedish commercial names are given

2.4. Harvest and analysis of plant properties

Plants were harvested three times; after 11, 22 and 33 days. Each plant was separated into shoot and root and weighed before and after drying in a ventilated oven (70 ^{0}C) for 3 days. Average heights of the shoots were recorded at the last harvest by measuring the length from the base shoot base to the top of the uppermost leaf on each tiller. Colour and general appearance of shoot and the root system were also recorded.
The relative growth rate between the harvests at day 22 and day 33 was calculated according to the following equation:

$$R_G = \frac{(\ln(W_2) - \ln(W_1))}{t_2 - t_1}$$

where W_1 and W_2 are the plant dry weights on days t_1 and t_2, respectively. W_1 and W_2 are expressed in grams and the unit used for R_G is % day^{-1}.

For analysis of mineral contents, the dried shoots were cut into small pieces and a sub-sample was grinded in a ball-mill to a fine powder. The N concentration was determined using an elemental analyser (Model: EA 1108 CHNS-O, Fison, Italy) using 2,5-Bis-[5-tert.-butyl-bensoaxzol-2-yl]-thiophen (BBOT) as a standard. The content of P, K, Ca, Mg, Fe, Mn, Zn, Cu, Mo and Na were analysed at Analytica accredited laboratory, Sweden by EPA methods (200.7 ICP-AES and 200.8 ICP-SFMS).

2.5. Analysis of physical and chemical properties of the substrate and nutrients

After the last harvest the substrates were collected and extracted for analysis. A 10 g composite sample of each substrate was shaken with 100 ml deionised water during 24 hours, and then vacuum filtered (MUNKTELL Analytical filter paper 3). Conductivity, pH, total-N and total-P were measured in each filtrate from the substrates. pH was measured by a calibrated Metrohm 605 pH-meter (Herisau, Switzerland). Conductivity was measured with a calibrated Conductivity Meter CDM 3 (Radiometer Copenhagen). The total-N and total-P concentrations were analysed using a DR4000 spectrophotometer with relevant Hach reagents according to the Hach manual (Hach Company, USA, 1997). For analysis of the solid fertilizer from urine (SZM) a 2 replicate samples were sent to AnalyCen accredited laboratory, Sweden.

2.8. Statistical analysis

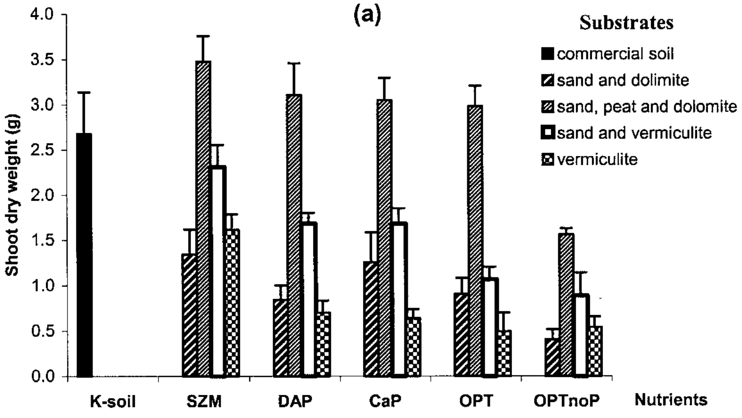
Data from substrate analysis, plant dry weights, shoot heights and shoot biomass component analysis were analysed statistically by analysis of variance (one-way ANOVA) and Spearman rank correlation test using a statistical software package (Crunch version 4, Crunch Software Corp., Oakland, Ca., USA).

3. Results

The results are separated into those on plant biomass and development, those on nutrient and substrate behaviour, and those on nutrient and substrate impact on mineral nutrient content in plant shoots.

3.1. Plant biomass and development

The average shoot and root dry weights of wheat plants after 33 days of growth are presented in Figure 1(a, b).



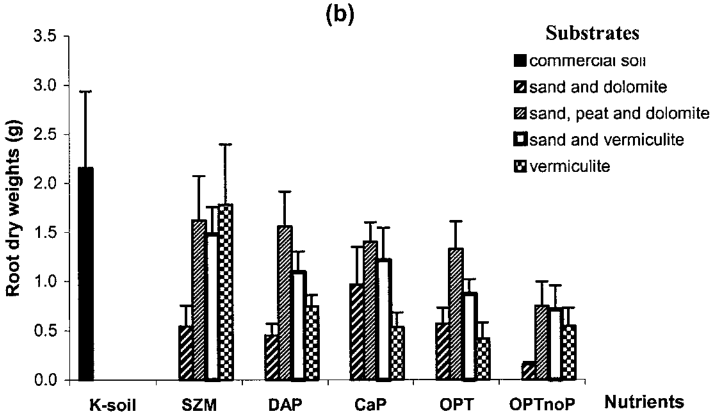


Figure 1(a, b). Average biomass (dry weight) of shoots (a) and roots (b) of 33 days old wheat plants grown in different substrates and nutrient sources. K-soil, commercial nutrient enriched soil; SZM, struvite-zeolite mix with nutrients recovered from urine; DAP, di-ammonium hydrogen ortophosphate; CaP, mono-calcium-phosphate monohydrate; OPT, nutrient solution with an optimal nutrient composition; (OPTnoP), nutrient solution with without P.

The biomass in the treatments with DAP, CaP, and OPT fertilizers was very similar when identical substrates were used. The dry weight was generally higher in the pots having SZM as P source. The lowest dry weight was found in the treatment with fertilizer without P

(OPTnoP). Plant biomass was significantly influenced by the substrate type (Table 4), and it was highest in the combined sand, peat and dolomite (S+P+D) substrate (Figure 1).

Table 4. The results of the analysis of variance (ANOVA) for the nutrient and substrate impact on the plant growth (root and shoot dry weights and shoot heights after 33 days of growth).

Dependent variable	Source of Variation	df	SS	MS	F	P-value
Plant (g)	Between treatments	19	36.498			
	Nutrient	4	9.896	2.474	8.40	0.0018
	Substrate	3	23.070	7.690	26.12	<0.0001
Shoot (g)	Between treatments	19	17.749			
	Nutrient	4	3.763	0.941	10.18	0.0008
	Substrate	3	12.877	4.292	46.44	<0.0001
Root (g)	Between treatments	19	4.195			
	Nutrient	4	1.460	0.365	4.49	0.0190
	Substrate	3	1.760	0.587	7.22	0.0050
Root:shoot ratio	Between treatments	19	0.935			
	Nutrient	4	0.005	0.001	0.07	0.9891
	Substrate	3	0.742	0.247	15.73	0.0002
Shoot height (mm)	Between treatments	19	625.800			
	Nutrient	4	115.800	28.950	5.04	0.0128
	Substrate	3	441.100	147.033	25.61	<0.0001

The average shoot heights after 33 days are presented in Figure 2.

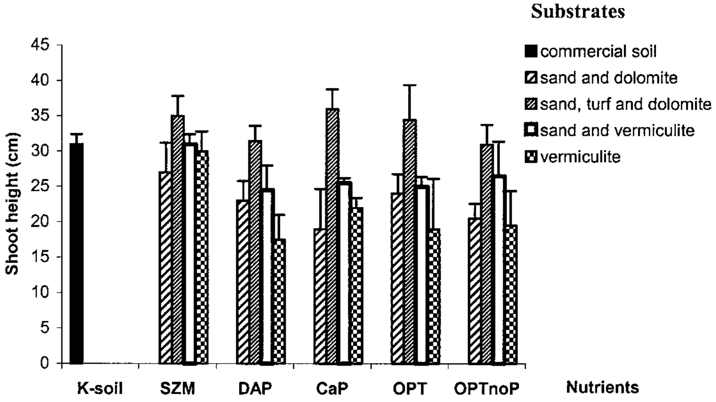


Figure 2. Average shoot heights (cm) of 33 days old wheat plants grown in different substrates and nutrient sources. For abbreviations see Figure 1(a, b).

Shoot height was mainly affected by the substrate (Table 4), but also to a certain extent by the nutrient source used. The root and shoot development is strongly correlated (Table 4). The root development is affected mainly by substrate type, but the nutrient source is also important (Table 4). The root/shoot ratios and the relative growth rate between 22 and 33 days of growth calculated for each treatment are presented in Table 5.

Table 5. Root/shoot rations of 33 days old wheat plants and relative growth rates between 22 and 33 days of growth for wheat plants. For abbreviations see Figure 1 (a, b) and Table 2.

Nutrient	Substrate	R/S ratio	R_G (% day^{-1})
K-soil	K-soil	0,8	17
SZM	S+D	0,4	15
	S+P+D	0,5	18
	S+V	0,7	18
	V	1,1	18
DAP	S+D	0,5	14
	S+P+D	0,5	19
	S+V	0,7	16
	V	1,0	14
CaP	S+D	0,8	16
	S+P+D	0,5	17
	S+V	0,7	16
	V	0,8	14
OPT	S+D	0,6	15
	S+P+D	0,4	18
	S+V	0,8	17
	V	0,9	13
OPTnoP	S+D	0,4	11
	S+P+D	0,5	15
	S+V	0,8	15
	V	1,0	13

The results show a similar plant development, when the same substrate was used with different fertilizers. The main impact on the root development is due to the substrate, and the nutrient source affected mainly the shoot development (Table 4).

3.2. Nutrient source and substrate behaviour

The plant available P and N was extracted with water from substrates after the final harvest (after 33 days) and the result is presented in Table 6.

Table 6. The pH, conductivity, plant available total-P and total-N in the substrate extracted with water after 33 days of wheat growth.
For abbreviations see Figure 1(a, b) and Table 2.

Nutrient	Substrate	pH	Conductivity (µS/cm)	Tot-P (mg/l)	Tot-N (mg/l)
K-soil	K-soil	7.8	120	5.5	0
SZM	S+D	8.0	270	12.5	20
	S+P+D	8.0	195	21.5	10
	S+V	8.0	275	20.0	20
	V	8.7	330	15.5	30
DAP	S+D	7.5	220	7.0	30
	S+P+D	7.4	100	8.0	10
	S+V	7.8	250	26.0	20
	V	8.0	435	23.0	30
CAP	S+D	7.7	140	9.0	10
	S+P+D	7.5	115	9.5	0
	S+V	8.0	175	18.0	10
	V	8.1	385	14.5	40
OPT	S+D	7.6	180	6.5	10
	S+P+D	7.6	100	5.5	0
	S+V	7.9	205	9.0	20
	V	8.0	450	10.0	40
OPTnoP	S+D	7.2	225	0.5	20
	S+P+D	7.3	340	0.5	30
	S+V	7.3	235	0.5	20
	V	7.7	410	2.5	40

After 33 days of growth the plants were in the late tillering stage. The total-P present in the extracted solution from all treatments indicates a sufficient plant available PO_4^{3-}-P for 33 days of growth. The total-P was extremely low in OPT noP treatment. The P availability is mainly affected by the nutrient, and to some extent also by the substrate type (Table 6 and Table 7). The plant available total-N was mainly affected by the substrate, and in the treatment with K-soil no total-N was detected in substrate solution (Table 6). Thus, the N availability at the end (33 days) was significantly affected by the substrate type (Table 7), but not affected by the nutrient source. Conductivity was also only affected by substrate, but not by nutrient source, while pH was equally affected by both substrate and nutrient source (Table 7).

Table 7. The results of the analysis of variance (ANOVA) for the nutrient and substrate impact on the wheat plant environment (pH, conductivity, total-N and total-P in substrate leachate after 33 days).

Dependent variable	Source	df	SS	MSS	F	P
pH	Between treatments	19	2.426			
	Nutrient	4	1.328	0.332	21.625	0.0000
	Substrate	3	0.9136	0.305	19.859	0.0001
Conductivity (µS/cm)	Between treatments	19	226163.750			
	Nutrient	4	21807.500	5451.875	1.446	0.2785
	Substrate	3	159123.750	53041.250	14.072	0.0003
Tot-P (mg/l)	Between treatments	19	1133.738			
	Nutrient	4	717.050	179.263	9.347	0.0011
	Substrate	3	186.538	62.179	3.242	0.0603
Tot-N (mg/l)	Between treatments	19	2895.000			
	Nutrient	4	370.000	92.500	1.563	0.2467
	Substrate	3	1815.000	605.000	10.225	0.0013

3.3. Impact of nutrient source and substrate on the plant nutrient status

The nutritional status of the spring wheat (measured in the above ground dry matter) for each treatment after 33 days of growth is presented in Table 8. The statistics made on this material is presented in Tables 9 and 10.

The mineral nutrients in the plant shoots were affected differently by the nutrient source and by the substrates. The N and Fe status was affected by the nutrient source, but not by the substrates. However, the N availability was mainly affected by the substrate, as described before (Table 7). The P, Mg, Mn and Na status was affected by both factors, while the Ca, Zn, Cu and Mo status and the micronutrient status is only affected by the substrate type (Table 9). Root development was influenced by the substrate, which indirectly affects the mineral balance in the shoot, as is the case for substrates containing vermiculite with high P and Mg content and resulting in lower Zn content in the plant shoot (Table 9 and Table 10). The nutrient status in the shoot was normal for all treatments, with some variations in the case of K, Mg, Zn, Cu and Mo, but never achieving toxic or deficiency levels (Table 8). The fertilizer affected the N, P, K and Na content, and the substrate affected Ca, Mn, Zn, Mo and Na status.

Table 8. The mineral nutrient content of the shoots of the wheat plants after 33 days of growth. For abbreviations see Figure 1 (a, b) and Table 2.

Nutrient	**Substrate**	**Mineral nutrient content (mg/g biomass dry weight)**										
		N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	Mo	Na
K-soil	K-soil	15.7	4.22	25.6	4.34	1.65	0.053	0.017	0.020	0.005	0.015	0.113
SZM	S+D	45.1	3.96	32.6	10.7	9.68	0.206	0.036	0.010	0.009	< 0.002	0.512
	S+T+D	43.6	5.80	43.6	5.47	5.56	0.119	0.157	0.026	0.005	< 0.002	0.240
	S+V	50.8	5.68	40.9	4.04	5.74	0.208	0.093	0.007	0.008	0.003	0.212
	V	48.4	11.4	46.0	3.81	7.24	0.420	0.135	0.007	0.006	0.004	0.158
DAP	S+D	54.1	6.79	38.1	10.6	8.54	0.413	0.070	0.011	0.012	< 0.002	0.186
	S+T+D	50.4	6.96	42.3	6.11	4.88	0.222	0.157	0.028	0.006	< 0.002	0.204
	S+V	49.2	10.1	45.4	4.60	6.96	0.271	0.151	0.007	0.011	0.004	0.127
	V	51.2	14.7	38.1	5.32	9.07	0.170	0.232	0.007	0.006	0.005	0.110
CaP	S+D	38.7	3.50	33.2	10.6	6.60	1.620	0.082	0.042	0.020	0.004	0.216
	S+T+D	38.9	4.87	38.0	4.19	3.56	0.396	0.173	0.026	0.005	0.003	0.212
	S+V	46.8	5.57	32.6	4.28	8.46	1.410	0.170	0.007	0.010	0.004	0.160
	V	46.1	11.7	41.0	4.58	8.52	0.648	0.219	0.006	0.006	0.005	0.137
OPT	S+D	44.1	3.31	35.8	10.6	7.58	0.453	0.075	0.010	0.013	< 0.002	0.253
	S+T+D	32.6	3.06	35.3	3.85	3.48	0.241	0.161	0.022	0.005	< 0.002	0.183
	S+V	49.1	6.25	43.1	4.72	7.48	0.721	0.239	0.007	0.013	0.005	0.129
	V	40.0	6.17	32.7	5.34	9.17	0.338	0.226	0.005	0.007	0.006	0.111
OPTnoP	S+D	38.9	0.97	36.4	11.3	5.76	0.330	0.061	0.013	0.007	< 0.002	0.210
	S+T+D	29.3	0.99	32.2	2.08	1.80	0.084	0.141	0.027	0.003	< 0.002	0.115
	S+V	30.1	0.87	33.1	3.08	3.32	0.099	0.104	0.007	0.006	0.004	0.093
	V	32.6	2.14	34.0	3.12	4.81	0.343	0.141	0.005	0.006	0.006	0.093
Nutrient* Sufficiency Range (mg/g)		40-50	4-7	32-40	2-5	1.5-5	0.03-0.1	0.04-0.5	0.02-0.07	0.005-0.03		

*Nutrient suffuciency range for spring wheat in tillering stage and in whole plant tissue (from Westfall et al., 1990)

Table 9. The results of the analysis of variance (ANOVA) for the nutrient and substrate impact on the elemental composition of the plant shoot biomass (mg/g dry weight).

Dependent variable	Source	df	SS	MSS	F	P
N	Between treatments	19	1065.170			
	Nutrient	4	745.930	186.483	11.090	0.0005
	Substrate	3	117.458	39.156	2.328	0.1262
P	Between treatments	19	274.286			
	Nutrient	4	152.926	38.231	14.824	0.0001
	Substrate	3	90.414	30.138	11.686	0.0007
K	Between treatments	19	408.272			
	Nutrient	4	150.522	37.631	2.106	0.1430
	Substrate	3	43.316	14.439	0.808	0.5134
Ca	Between treatments	19	172.018			
	Nutrient	4	6.579	1.645	2.189	0.1319
	Substrate	3	156.422	52.141	69.392	0.0000
Mg	Between treatments	19	96.121			
	Nutrient	4	31.677	7.919	6.243	0.0059
	Substrate	3	49.222	16.407	12.935	0.0005
Fe	Between treatments	19	3.133			
	Nutrient	4	1.819	0.455	6.428	0.0053
	Substrate	3	0.465	0.155	2.192	0.1419
Mn	Between treatments	19	0.068			
	Nutrient	4	0.015	0.004	4.851	0.0146
	Substrate	3	0.043	0.014	18.293	0.0001
Zn	Between treatments	19	0.0021			
	Nutrient	4	0.0002	0.00005	1.099	0.4011
	Substrate	3	0.0013	0.00043	8.941	0.0022
Cu	Between treatments	19	0.00029			
	Nutrient	4	0.00005	0.00001	2.225	0.1274
	Substrate	3	0.00016	0.00005	9.405	0.0018
Mo	Between treatments	19	0.00007			
	Nutrient	4	0.00001	0.00000	2.394	0.1084
	Substrate	3	0.00006	0.00002	30.483	0.0000
Na	Between treatments	19	0.1597			
	Nutrient	4	0.0534	0.0134	4.281	0.0222
	Substrate	3	0.0689	0.0230	7.362	0.0047

Table 10. Spearman rank correlation coefficients (r) and two-tailed p values for the nutrient and substrate impact on the plant growth and macro- and micronutrient status in the plant shoot biomass (N= 20).

	pH	Cond.	Tot-P Substr.	Tot-N Substr.	Root DW	Shoot DW	Shoot height	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu	Mo	Na
pH	1.000 0.000	0.4119 0.0712	0.7971 0.0000	0.2956 0.2058	0.1993 0.3997	0.0389 0.8706	-0.1283 0.5898	0.4207 0.0648	0.6327 0.0028	0.2925 0.2108	-0.0329 0.8906	0.5960 0.0055	0.2144 0.3639	0.3326 0.1519	-0.4722 0.0355	0.0958 0.6877	0.4411 0.0516	0.0115 0.9618
Cond.	-	1.000 0.000	0.1861 0.4320	0.9161 0.0000	-0.4485 0.0473	-0.5942 0.0057	-0.4915 0.0277	0.0673 0.7779	0.2287 0.3322	-0.0677 0.7766	-0.2426 0.3028	0.3332 0.1511	-0.2505 0.2868	0.0832 0.7274	-0.7013 0.0006	-0.0866 0.7167	0.5327 0.0156	-0.5666 0.0092
Tot-P Substr.	-	-	1.000 0.000	0.0151 0.9496	0.4054 0.0762	0.3337 0.1505	0.0170 0.9434	0.6217 0.0034	0.7435 0.0002	0.4734 0.7766	0.0920 0.3028	0.4874 0.0293	0.0467 0.8450	0.3521 0.1279	-0.1884 0.4264	0.0980 0.6810	0.2664 0.2563	0.1176 0.6216
Tot-N Substr.	-	-	-	1.000 0.000	-0.5506 0.0119	-0.6937 0.0007	-0.5636 0.0097	0.1348 0.5708	0.2935 0.2091	-0.0050 0.9832	-0.1795 0.4489	0.3569 0.1224	-0.0564 0.8133	0.0560 0.8144	-0.6754 0.0011	-0.0124 0.9587	0.5112 0.0212	-0.6151 0.0039
Root DW	-	-	-	-	1.000 0.000	0.8951 0.0000	0.7091 0.0005	0.1494 0.5297	0.2046 0.3869	0.4415 0.0531	-0.3405 0.1418	-0.4175 0.0670	-0.1181 0.6200	0.2126 0.3681	0.3905 0.0887	-0.2842 0.2247	-0.1777 0.4535	0.2340 0.3207
Shoot DW	-	-	-	-	-	1.000 0.000	0.8548 0.0000	0.0993 0.6771	0.0887 0.7099	0.2904 0.2141	-0.1898 0.4230	-0.4150 0.0688	-0.2195 0.3523	0.1008 0.6723	0.5739 0.0081	-0.3017 0.1961	-0.4486 0.0473	0.4242 0.0623
Shoot height	-	-	-	-	-	-	1.000 0.000	-0.1129 0.6355	-0.1415 0.5519	0.1679 0.4792	-0.3353 0.1484	-0.5538 0.0113	-0.3717 0.1066	0.0147 0.9510	0.4648 0.0389	-0.4989 0.0251	-0.5357 0.0149	0.3511 0.1290
N	-	-	-	-	-	-	-	1.000 0.000	0.8116 0.0000	0.6007 0.0051	0.3435 0.1381	0.5927 0.0059	0.1685 0.4777	0.1080 0.6503	-0.1482 0.5328	0.3564 0.1230	0.0447 0.8515	0.1166 0.6244
P	-	-	-	-	-	-	-	-	1.000 0.000	0.6757 0.0011	0.1506 0.5262	0.5759 0.0079	0.2120 0.3695	0.4560 0.0433	-0.2572 0.2736	0.0745 0.7549	0.2931 0.2098	-0.0993 0.6771
K	-	-	-	-	-	-	-	-	-	1.000 0.000	0.0739 0.7570	0.0451 0.8501	0.0971 0.6839	0.2108 0.3722	-0.0519 0.8278	-0.0252 0.9159	0.0152 0.9494	0.0768 0.7477
Ca	-	-	-	-	-	-	-	-	-	-	1.000 0.000	0.5708 0.0086	0.2289 0.3316	-0.3203 0.1686	0.3043 0.1920	0.4702 0.0364	-0.3644 0.1142	0.6034 0.0049
Mg	-	-	-	-	-	-	-	-	-	-	-	1.000 0.000	0.3835 0.0951	0.0534 0.8230	-0.3859 0.0929	0.5365 0.0147	0.2480 0.2918	0.1113 0.6403
Fe	-	-	-	-	-	-	-	-	-	-	-	-	1.000 0.000	0.1001 0.6746	-0.1046 0.6609	0.5786 0.0075	0.2838 0.2253	0.1587 0.5039
Mn	-	-	-	-	-	-	-	-	-	-	-	-	-	1.000 0.000	-0.3184 0.1713	-0.3287 0.1571	0.5753 0.0080	-0.4682 0.0373
Zn	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.000 0.000	-0.1701 0.4733	-0.8030 0.0000	0.6407 0.0023
Cu	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.000 0.000	0.1420 0.5504	0.1897 0.4231
Mo	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.000 0.000	-0.7085 0.0005
Na	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.000 0.000

4. Discussion

4.1. Plant biomass and development

The biomass production of the plants having their nutrient recovered from (SZM) was similar to or higher than those receiving commercial fertilizers (DAP and CaP), when identical substrates were used. This indicates that the nutrient availability from SZM is as good as that from the other solid fertilizers. A slightly higher biomass obtained in pots having SZM as fertilizer may be explained by the difficulties to calculate a very exact nutrient addition (especially for N and other macronutrients from urine). Another explanation may be a better P and N availability from SZM compared to DAP and CaP, due to the synergistic effect of the zeolite/struvite mixed mineral system on nutrient solubility. The nutritional status of the shoots do indicate that there is less nutrient available for the plants in the CaP and the OPT treatment as well, however not in the DAP. That means that there also are other factors than nutrient availability that contributes to the differences in biomass between treatments. However, the fact that the nutrient status of the plants from the SZM treatment were among the highest could be explained by earlier research showing that non-calcareous soils fertilized with different ammonium-zeolite/apatite mixtures enhanced P dissolution from the apatite (Lai and Eberl, 1986; Allen et al., 1993, 1995) and increased the biomass production of greenhouse-grown sorghum-sudangrass (*Sorgum bicolour* L.) by a factor of 1.6 (Barbarick et al., 1990). Higher zeolite/apatite ratios have resulted in increased P availability, because of a decrease in the soil pH, dissolution of apatite rock and more available NH_4^+ from zeolite in the plant-soil interactions during growth. The system is based on the principle that zeolites can sequester Ca^{2+} released by the dissolution of apatite, thereby leading to further dissolution of the apatite (Lai and Eberl, 1986). In our study a similar nutrient carrying system with zeolite/struvite mixture and high Mg^{2+} ion content and high zeolite:struvite ratio (15:1 for 30 g/l zeolite used) may increase the nutrient availability as a result of an interaction with the mineral system. The plant biomass production was lower in the pots receiving substrates containing vermiculite. A lower biomass production may be explained by the impact of this clay on the nutrient availability. Vermiculite is extensively used in greenhouses as root media, fertilizer or pesticide carrier or soil conditioner. It is well known for its high K and NH_4-fixation and high P adsorption on the hydroxyaluminium-interlayer in the vermiculite structure (Corey, 1990; McBride, 1994; Uttam and Katsuhiro, 1997; Scherer and Zhang, 1999; Chappell and Evangelou, 2000; Evangelou and Lumbanraja, 2002). In our case the nutrient addition was calculated to be sufficient for the plant, regardless of the substrate type. A higher retention of N and P in the substrate containing vermiculite can be the cause of lower biomass production. The pH and conductivity of the solutions extracted from the substrates with vermiculite was the most elevated in the experiment (pH 7.7-8.7 and conductivity 330-450 μS/cm). This indicates some difficulties for nutrient acquisition of the plants grown in vermiculite, although the final nutrient status in the plants was not lower than in the plants from several other substrates.

The lowest biomass production was obtained in the pots treated by the nutrient without P (OPTnoP). Severe P deficiency symptoms were also visible on these plants, like dark-green leaves and magenta coloured stems.

The relative growth rates (R_G) of the plants were similar to plants growing at optimised nutrient supply (OPT) in the case of the same substrate types, highest in the case of SZM fertilizer and lowest for the plants grown under P deficiency (Table 6).
A proper balance between root and shoot is of a great importance for a healthy plant development. The root/shoot ratio may differ depending on environmental conditions around

the plant and the type of cultivars (Shangguan, *et al.*, 2004). In early stages of wheat growth the root/shoot ratio is near 1.0, but at anthesis this can fall to 0.1 (Brar and Reynolds, 1996). For winter wheat tested in climate chamber at sufficient water and nutrient additions and with optimal environmental factors a root/shoot ratio of 0.5 was found to be optimal (Shangguan, *et al.*, 2004). A similar root/shoot ratio was found in this study in the treatments having sand, peat and dolomite (S+P+D) as substrate, which also had the highest biomass production. In the substrates containing vermiculite the root/shoot ratio was high (0.7-1.1) and the biomass production was the lower. High root/shoot may lead to reduced nutrient and water use, in spite of root redundancy (Shangguan, *et al.*, 2004).

4.2. Nutrient source and substrate behaviour

The availability of nutrients from the fertilizers and the impact of substrate can be estimated, if the water-soluble and plant available amounts of nutrients are measured in the extracted substrate after the last harvest. The solid fertilizers showed very similar qualities as P sources. Both DAP and CaP are known slow release fertilisers, and the SZM obtained from urine showed the same P source behaviour, with slightly better biomass production, probably due to the increased nutrient availability of the zeolite/struvite system, as described above. The water soluble, plant available P content in the extracted solutions indicated also a substrate impact in the case of vermiculite, probably due to its nutrient adsorption, as described above. The main N source for all treatments originates from the supplementary nutrient solutions added, containing 93% of NO_3-N as an easy available N source for plants (Ingestad and Lund 1986). The N was sufficient everywhere, except for K-soil, which did not receive supplementary nutrient solution. In a commercial soil the nutrient content must be sufficient and balanced for any type of cultivars and growth conditions (Table 3). In our case the P was sufficient, but the N was not enough for the 33 days long growth of one wheat plant (Table 1). In spite of a good biomass production (Figure 1), the plants in the pots with K-soil showed N deficiency symptoms, like light green leaves and stem, chlorotic old leaves and yellow apex on the youngest leaves. The high root/shoot ratio also indicates N deficiency, with increased root biomass and root surface area, and reduced N transportation to shoots. The plant N status was in the deficiency range (Table 8). This may be explained by the fact, that the N from 200 g K-soil was simply not sufficient for one plant during 33 days of growth.

4.3. Impact of nutrient source and substrate on the plant nutrient status

The primary macronutrient content (N, P, K) in the above ground dry matter is directly dependent of the fertilizer macronutrient supply, and the majority of treatments are in the sufficiency range (exceptions were discussed above). However, Ca was in excess in the substrates having dolomite for pH adjustment, but these levels were never on the toxic range for wheat. Higher Ca in the substrate may inhibit Fe, Zn and Mn uptake (Wild, 1988; Marchner, 1999). In our case, the plants grown in the substrate with sand and dolomite (S+D) showed the lowest Mn and highest Fe status (Table 8). This negative relationship between Fe and Mn in plant tissue is normal (Vitosh et al., 1995). The Mn plant contents in our treatments were in the sufficiency range. A high Fe content in plant tissue was found all over the experiment, but not in the toxic level for wheat. The lowest Fe tissue content was found in the case of the substrate with peat, sand and dolomite (S+P+D), which others also have observed (Vitosh, *et al.*, 1995). The lowest Zn status was found in plants grown in substrates with vermiculite. The Zn availability for Plants is influenced by the high substrate P level (Vitosh et al., 1995), and in our case this is related to substrates with vermiculite (Table 8). The Cu and Mo were in the sufficiency range in all treatments, and the spring wheat is not sensitive to the Na levels found in the tissue.

5. Conclusions

Measured values in the substrate solution extracted after the last harvest showed that pH was affected equally by nutrient source and substrate type, and the conductivity by the substrate type. The P availability was affected mainly by the nutrient source, but also by the substrate type. The N availability was affected only by the substrate.

The plant biomass production was affected by the nutrient source and the substrate type, root growth equally by nutrient and substrate, and shoot growth mainly by substrate type. Plant biomass was highest in the combined sand, peat and dolomite substrate and generally higher when P was supplied from the urine recovered nutrients (SZM).

The mineral nutrient status of the shoots was affected differently by nutrient source and substrate type. The N content was affected by the nutrient source, the P content by both the nutrient and substrate. In general, the shoot mineral status showed to be in the sufficiency range for all treatments, except for N in K-soil and P in the treatment without P addition.

These results have demonstrated that the nutrients recovered from urine as struvite/zeolite mineral (SZM) used in proper amounts acts as a good P source, fully comparable with DAP (di-ammonium-phosphate) and CaP (mono-calcium-phosphate) slow release commercial P-fertilizers. These fertilizers were also similar as P sources to the liquid fertilizer used in the experiments.

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