TAMPERE POLYTECHNIC UNIVERSITY OF APPLIED SCIENCES
Environmental Engineering

Final Thesis

Hanna Launokorpi

BIOLOGICAL CONTAMINATION OF BARLEY AND CARROTS BY PATHOGENS IN SOIL FERTILISED WITH ANTHROPOGENIC NUTRIENTS

Supervisor Principle lecturer Marjukka Dyer
Commissioned by Tampere Polytechnic University of Applied Sciences
Tampere 2007

TAMPERE POLYTECHNIC UNIVERSITY OF APPLIED SCIENCES
Environmental Engineering

Launokorpi Hanna
Biological contamination of barley and carrots by pathogens in soil fertilised with anthropogenic nutrients

Final Thesis
56 pages, 7 appendices
Supervisor
Principle Lecturer Marjukka Dyer
Commissioned by
Tampere Polytechnic University of Applied Sciences
May 2007
Keywords
Salmonella, coliform bacteria, barley, carrot, septic tank sludge, STS, urine, faeces, composted faeces

ABSTRACT

The aim of this research was to study the possible occurrence and transfer of nutrients, heavy metals and pathogens due to the use of septic tank sludge, urine and composted faeces as fertilizer for barley and carrot crops. Commercial fertilizers of Kevätviljan Y3 (barley) and Puutarhan kevät (carrot) were used as a baseline. The study was accomplished as a greenhouse experiment in the premises of the Tampere Polytechnic University of Applied Sciences. This part of the research concentrated on the qualitative detection of *Salmonella* and a quantative detection of coliform bacteria in the soil - and more importantly, in the plant products. Methods used were based on instructions given on the compact salmonella (SL) detection plates and the Compact coliform (CF) detection plates with a modified method of standards SFS 3950 and SFS 4447. The results were compared among others to the European Commission regulation 2073/2005 on microbiological criteria for foodstuff, which states that for pre-cut fruits and vegetables, salmonella should be absent and the satisfactory amount of *Escherichia coli* should be under 100 cfu/g. Salmonella was absent in the fertilizers, and thus its presence would be impossible to find in the plant products. Coliform bacteria was detected during the study, but generally it decreased within time. Barley grains from the first urine duplicate and first composted faeces duplicate were totally free of coliform bacteria. Also the other results from barley grains were acceptable in hygienic terms, being less than 1 cfu per gram. The carrots grown in commercial fertilizer and composted faeces treatments were within the satisfactory limits. The small size of carrots from urine and septic tank sludge treatments did not allow complete handling, nevertheless, the amount of colony forming units was considered to be within the acceptable limit of 100-1000 cfu/g. Results from this research indicate that the use of excreta as fertilizer is not dangerous in terms of coliform bacteria. However, one indicator is not enough for stating the overall safety, and more thorough research is needed.

TAMPEREEN AMMATTIKORKEAKOULU
Environmental Engineering

Launokorpi Hanna	Ohran ja porkkanoiden biologinen kontaminaatio antropogeenisilla ravinteilla lannoitetussa maassa
Tutkintotyö	56 sivua + 7 liitesivua
Työn valvoja	Yliopettaja Marjukka Dyer
Työn teettäjä	Tampereen ammattikorkeakoulu (TAMK)
Toukokuu 2007	
Hakusanat	salmonella, koliformiset bakteerit, biologinen kontaminaatio, ohra, porkkana, sakokaivoliete, saostuskaivoliete, virtsa, ulosteet, kompostoidut ulosteet

TIIVISTELMÄ

Tutkimuksen tarkoituksena oli selvittää raskasmetallien, ravinteiden sekä patogeenien esiintyminen ja siirtyminen lannoitteena käytettävistä sakokaivolietteestä, virtsasta sekä kompostikäymäläjätteestä ohraan ja porkkanaan. Vertauskohtana käytettiin teollisuuslannoitteita Kevätviljan Y3:a (ohra) sekä Puutarhan kevättä (porkkana). Sadon kasvatus tapahtui kasvihuoneessa Tampereen Ammattikorkeakoulun tiloissa. Tässä osiossa keskityttiin tutkimaan *Salmonellan* kvalitatiivista sekä koliformisten bakteerien kvantitatiivista esiintymistä kasvatusalustoissa sekä erityisesti kasvatetuissa lopputuotteissa. Menetelminä käytettiin kasvatusalustojen mukana tullutta ohjeistusta sekä koliformisten bakteereiden osalta muunneltuja standardeja SFS 3950 ja SFS 4447. Tuloksia vertailtiin muun muassa Euroopan Komission asetukseen 2073/2005 ruoka-aineiden mikrobiologisista vaatimuksista. Pilkotuista vihanneksista ei saa löytyä *Salmonellaa*. Hyvän elintarvikehygienia ylläpitämiseksi *Escherichia colin* esiintyminen tulisi rajoittua alle 100 pmy/g. Tutkimus osoitti, että lannoitteissa ei ollut *Salmonellaa*, ja siten sen esiintyminen kasvituotteissa tutkimuksen edetessä oli mahdotonta. Koliformisten bakteereiden määrä laski yleisesti ajan kanssa. Virtsan ja käymäläjätteen ensimmäisessä rinnakkaisessa alustassa kasvatetun ohran jyvistä ei löytynyt lainkaan koliformisia bakteereita. Myös muiden alustojen jyvien tulos oli alle 1 pmy/g. Teollisuuslannoitteessa ja käymäläjätteessä kasvaneiden porkkanoiden baktereeripitoisuus oli alle 100 pmy/g. Pienen kokonsa, ja siitä seuranneen vaikean käsittelyn takia virtsassa ja sakokaivolietteessä kasvatettujen porkkanoiden bakteerien esiintyminen on välttävä. Tutkimuksen tulokset osoittavat, että kyseisten lannoitteiden käyttö ei ole vaarallista. On kuitenkin huomioitava, että yhtä indikaattoria käyttäneen tutkimuksen tulokset eivät ole kokonaisvaltaisia, ja juuri siksi lisätutkimuksia tullaan tarvitsemaan.

FOREWORD

This project was done for the Tampere Polytechnic University of Applied Sciences. It was the first research project in which I have participated, and the outcome was interesting and educative from both a personal and scientific point of view.

There are many people, to whom I am most grateful. First, thanks go to Marjukka Dyer and Eeva-Liisa Viskari for giving me the opportunity to take part in this research, and for all the help you gave me. In addition, I would like to thank co-students Ari Laukkanen and Sisli Piisilä, project engineer Seija Haapamäki, laboratory specialist Marja-Liisa Laaksonen, and William Dyer. To all the above, your help and assistance were worth their weight in gold.

Kasnäs, May 2007

Hanna Launokorpi

LIST OF ABBREVIATIONS

BPW	Buffered Pep-tone Water
CF	Composted faeces
CFU	Colony forming unit
EU	European Union
EC	European Commission
ISO	International Organization for Standardization
K	Potassium
MDG	Millennium Development Goal
MTT	Agrifood Research Finland, Maa- ja elintarviketeollisuuden tutkimuskeskus
N	Nitrogen
P	Phosphorus
STS	Septic Tank Sludge; residual sludge from septic tanks and other similar installations for the treatment of sewage
SUB	Substrate
SYKE	Finnish Environment Institute, Suomen Ympäristökeskus
WHO	World Health Organization

TABLE OF CONTENTS

1. INTRODUCTION

The value of excreta in modern society is insignificant. /24/ It is usually considered as waste, rather than a resource or a useful opportunity. In past times, excreta was widely used as a fertilizer in fields due to its nutrient-rich content. /7/ The main reason for use decline is the health risk it can pose to the environment, especially to the people working in the fields and those consuming the food products. /7/ Earlier, a problem also rose due to the accumulation of heavy metals in the soil, especially from city waste-water. In addition, the attitude of the public regarding the use of human faeces as a fertilizer is negative. /6/

Nevertheless, there are several national and international bodies that support the safe use of excreta as a fertilizer. Legislation of the European Union, the Finnish Government, as well as the United Nations Millennium Development Goals, speak for the more effective use of waste and resources.

This research, accomplished in an indoor greenhouse situated in the Tampere Polytechnic University of Applied Sciences examines heavy metal concentrations, nutrient contents, and possible health risks in cultivating crops (barley and carrots) in a growing medium fertilized with urine, composted faeces (CF) and septic tank sludge (STS). This specific study concentrates on investigating the health risks originating from pathogens, by using coliform bacteria and *Salmonella* as indicators of the hygienic level of the fertilizer, soil, and most importantly, the food product.

General information regarding the research topic is dealt with in the second chapter. The third chapter covers legal issues related to micro-organisms of excreta and foodstuff. The fourth chapter concentrates on the method and progress of the growing experiment. Laboratory working methods are dealt with in the fifth chapter, and finally, results and conclusions are discussed in the sixth and seventh chapter.

2. GENERAL INFORMATION

On average, an adult person annually produces approximately 500 kg of urine and 50 kg of faeces. Of that, 5,7 kg is nitrogen, 0,6 kg is phosphorus and 1,2 kg is potassium. /19/ The nutrient content of excreta is dependent on the amount and quality of food eaten. If the food is not nutrient-rich, neither is the excreta. Children especially, need nutrients for growth, but for adults, the energy gained is the most important thing. In examining the death rates of children in developing countries, it is estimated that malnutrition is the cause of approximately 50% of such deaths. /7/ That only reinforces the understanding that nutrients should be used in a more efficient way.

In Finland, approximately 23 % of the population, or 1,1 million people, live in a region not belonging to a municipal wastewater network. /30/ Often, wastewater is lead to a body of water as a direct effluent. As a consequence, the nutrient load created by those 1,1 million people is heavier than that produced by the 4 million people living inside the wastewater treatment network. /30/ In order to improve the situation, the Finnish Council of State developed a regulation (2003/542) for the treatment of the household wastewater outside a municipal network. According to this, wastewater must be treated also in rural areas in order to reduce the discharges to water bodies, and to reduce degradation of the environment. /28/ There are already several technical solutions available, some of which are very sustainable in terms of using only small amount of water, if at all. In recent years, the amount of treated wastewater as well as excreta, from using dry toilets will increase. Other more advantageous applications to use nutrients in excreta are being developed

In many countries, nutrients that the excreta contain are recycled. For instance in East-Asia, the use of excreta as fertilizer is common. /6/ No agricultural productivity problems have been encountered, although some health problems have occurred. /19/ In most cases, the most significant health risks are a result of improper handling during the different phases - the excreta used is untreated, the crops are eaten raw and unwashed, and the levels of personal hygiene are low. This latter aspect plays an important role, whenever disease is in question. In

earlier times in the Nordic countries, it was a normal habit to mix human excreta with animal manure, and to fertilize fields with the mixture. /6/ It is a historical fact that when the transition to water closets started taking place around the year 1900, many opposed the trend, arguing that agriculture was about to loose a source of fertilization. /19/ Stricter regulations and concern about possible health risks have brought about more negative attitudes to such use as fertilizer. Nevertheless, some 60% of sewage sludge is still used in agriculture and for urban landscaping. /31/

In 2000 the United Nations General Assembly set the Millennium Development Goals, and the first and seventh article are related to the safe use of excreta as fertilizer. The first target states “Eradicate extreme poverty and hunger”. In practice this means that the proportion of people, who subsist with the equivalent of only one dollar a day and that proportion of people, who suffer from hunger, should be halved by the year 2015. /27/ The seventh goal “Ensure environmental stability”, encourages countries to adopt the principles of sustainable development in all their policies. In addition, the goal includes the target of halving the proportion of people who lack sustainable access to safe drinking water and safe sanitation. /27/

According to the World Health Organization, 63 % of the fertilizers sold annually end up in developing countries. The total amount sold equals to approximately 130 million tons of fertilizers, of which 78 million tons is nitrogen. /7/ For example, six billion people, irregardless that many are children, would produce 34,2 million tons of nitrogen within their excreta. For subsistence farmers in poor communities where resources are scarce, the use of excreta as fertilizer would increase food production maintaining soil fertility, as well as the benefits of recycling organic matter and nutrients more efficiently. /7, 1/ Obviously, it is one of the few fertilizers that is available free of charge in every society. /19/ By using excreta, the use of artificial fertilizers is reduced, and negative impacts on soil, air and water is reduced. Environmental impacts created by agriculture and its side products are not only a problem of third world countries, but also of the developed nations. For instance the Baltic Sea suffers significantly from eutrophication due to an exceedingly great nutrient load from agriculture and industry. /9/

Several studies have been made regarding the use of excreta as fertilizer. There are an almost overwhelming number of issues which need to be taken into consideration at the same time in order to get an overall picture. Studies should be concentrated on pathogens, heavy metals and nutrients. In the case of pathogens, several different indicators should be studied. The Ministry of Agriculture and Forestry in Finland studied the hygienic quality of sludge from different treatment plants in 2002, in a research project called *Sewage Sludge and Sludge Products for Agricultural Use – a Study on Hygienic Quality*. /5/ They used several indicator bacteria, among others *Salmonella* and *Escherichia coli*. Both types of pathogens were found in all the raw sludge samples taken from 22 different treatment plants. However, after certain treatment, for instance in-vessel composting, salmonella was not found. /5/ In general, the abundant occurrence of coliform bacteria usually indicates insufficient composting. /1/ The study also found out that *Giardia* and *Crystosporidium* were the most resistant pathogens. Agrifood Research Finland made the study *Waste Composts as Fertilizers in Field Cultivation – Biological and Chemical Effects*. The research examined municipal waste composts (bio-waste and bio-waste-sewage sludge composts) and composted manure used in organic agriculture. The results confirmed that there were no harmful effects to the hygienic conditions of soil, and to a crop of potato and barley. However, they also point out that since the transfer of microbes from soil to vegetables is possible, the microbiological criteria for foodstuff should be high in order to maintain safety. /1/

Heinonen-Tanski et al researched outdoor cucumber cultivation fertilized with separated urine. Commercial fertilizers were used as a control. Results showed that there were no coliforms present in the cucumbers, though the urine used had not been preserved before utilization. There also were no negative features regarding taste. /20/

There is a considerable difference between both the nutrient and pathogen content of different excreta. Urine contains approximately 90 % of N, 50-65% of P and 50-80% of K that is excreted by humans. /19/ In addition, urine contains very little heavy metals and it is considered almost pathogen free, while faeces contain several pathogens. /31/ Urine also contains water, which is an important asset

especially in arid areas. That adds to the belief that urine is generally considered to be a more valuable source of nutrients.

2.1. PATHOGENS IN EXCRETA

There are several differences in both pathogen and nutrient contents of excreta (see chapter 2). Viruses are in general longer-lasting than the gram-negative indicator bacteria, which are studied here. /7/ However, human viruses are incapable of multiplying outside human cells. /5/ According to the Finnish Ministry of Agriculture and Forestry, it is recommended to store STS, CF and/or urine for a period not less than six months before use as fertilizer, in order to destroy all possible pathogens. /8/ The inactivation of pathogens is in general more rapid in soil or in the crop surface than in the stored excreta. /7/ In addition, sunshine and UV-radiation damages the bacteria, which speeds up destruction. /31/ Therefore the World Health Organization advises a waiting period of one month between the application of excreta to the field and the harvesting. /7/ The amount of pathogens can be greatly reduced by proper handling. Apart from storage, attention should be paid to the general hygienic level, both in the fields and in kitchens.

2.1.1. Pathogens In Urine

According to WHO, the use of source-separated urine in temperate climates poses no risks to health. When urine is in the bladder of a healthy person, it is usually sterile. When excreted, it takes bacteria from the urinary tract. Normally, fresh urine contains <10 000 bacteria per milliliter. The bacteria, however, is usually natural, and non-pathogenic, therefore source-separated urine is normally considered free of pathogens. /7/

Nevertheless one problem, especially in tropical climates, is presented by the possible cross-contamination with faecal matter. A study was conducted for source-separated urine in a large-scale collection system, and the cross-contamination from faecal matter was estimated to be within the range of 1,6-18,5 mg of faeces

per liter of urine. /7/ Thereafter preventing even the smallest mixture of urine and faeces can be crucial in terms of health.

Several factors affect the inactivation of pathogens in urine. Both the high concentration of the liquid, which can be attained by using less water, and acidic pH, kill pathogens such as coliform bacteria and salmonella effectively in urine. /31/ For instance WHO recorded that if the dilution of urine was 10-fold, the persistence of *E. coli* and *Salmonella* was increased. Other factors contributing to inactivation are the amount of ammonia present, and passage of time. /7/ The nutritional benefit of urine is due to its especially high content of nitrogen, which also acts as one of the limiting factors of plant growth. /19/ Thus urine is considered to be more valuable source of nutrients than faeces.

2.1.2. Pathogens In Composted Faeces

Faeces contain several different kinds of bacteria, of which in particular *Salmonella*, *Campylobacter* and *E. coli* (EHEC) are of great importance to human health world-wide. Basically, exposure to untreated faeces is always risky due to the high amount of pathogens. Within time, pathogens in faecal material will be inactivated. The organism type as well as the storage conditions, affects this die-off, and the pH, moisture and biological competition will also play their part in the process. Re-growth of pathogenic bacteria can however, take place if the moisture condition is reduced, for instance by irrigation or by mixing with moist soil. /7/

2.1.3. Pathogens In Septic Tank Sludge

The septic tank sludge (STS) that is collected in tanks, into which it flows from the toilets, sinks and showers, can be also called the primary effluent. /25/ It consists of a mixture of the gray-water, faecal matter and urine. The composition compared to sewage sludge is however different, because STS contains only household wastewater, not industrial. Thus it possibly contains all the same pathogens as faeces do.

2.2. THE PATHOGENS STUDIED

As discussed earlier, several pathogens can be present in excreta. For practical reasons, two pathogenic indicators, coliform bacteria and salmonella, were used in this research for the identification of the possible fecal contamination of soil and foodstuff. Both are gram-negative, belonging to the family *Enterobacteriaceae*, a group of bacteria found not only in water, waste-water and soil, but also in the intestinal tract of humans and animals. /26/ Table 1 presents some epidemiological data for Salmonella and EHEC, which is one of the strains of *E. coli*. /7/

Table 1. Example of different epidemiological data for Salmonella and EHEC. /7/

Pathogen	Incidence (/100 000 people)	Under-reporting	Morbidity (%)	Excretion (/gram faeces)	Duration (days)	ID_{50}
Salmonella	42-58	3,2	6-80	10^{4-8}	26-51	23 600
EHEC	0,8-1,4	4,5-8,3	76-89	10^{2-3}	5-12	1 120

Faecal pathogens mainly cause symptoms like diarrhoea, vomiting, stomach cramps and fever. /3, 7/ In children, elderly people and those who suffer from immunodeficiency, such a disease can be fatal. One also has to take into consideration that if a person becomes infected, he will excrete pathogens for many days and in very high numbers. /7/ Eventually, they end up in the excreta collection tank, which is stored and later used for fertilizing purposes.

In order to facilitate the good quality of food products, it is essential to carefully examine the possible health effects posed by improper usage of excreta fertilizer. The European Commission has devised regulations regarding the limits of specific bacteria found in foodstuff and waters, but additional standards and criteria are still needed.

2.2.1. Coliform Bacteria

Coliform bacteria is the common term for bacteria living in the intestinal tract of humans and other warm-blooded animals. /17, 18/ In one day, a human produces approximately 100-400 billion coliform bacteria. They are not necessarily

pathogenic, but if they are found in water, this usually indicates contamination from excreta, and therefore pathogenic coliform bacteria can also be present. /3/ An important feature of faecal bacteria is their ability to act as hosts for pathogenic viruses such as polio and hepatitis. Faecal coliforms (mainly *Escherichia coli*) and total coliforms are the two main groups of coliform bacteria. The total coliform group includes fecal coliforms and mainly the species of genera *Citrobacter*, *Enterobacter*, *Escherichia* and *Klebsiella*. /17/ One of the most significant coliforms is *E. coli* of EHEC-O157:H7, whose epidemiological data has been presented earlier in Table 1.

2.2.2. Salmonella

There are only two known species of Salmonella found in the world, *S. enterica* and *S. bongori*. They can be further divided into different subspecies and then again, into thousands of serotypes. In contrast to coliform bacteria, *Salmonella sp*. does not belong to the natural microbes of the intestinal tract of warm-blooded animals. /3/

Salmonellosis, the disease caused by any of salmonella bacterium, is usually transmitted by contaminated water and foodstuff. /3, 26/ The most common salmonellosis is caused by the *S. enterica*'s subspecies *enterica*. Generally known in Finland, but rarely occurring salmonellosis, are typhoid and paratyphoid fever. /3/ There are annually some 2500 – 3000 salmonella infections suffered by the Finnish population. Approximately 80% of these are contracted abroad. /26/ Typical symptoms include nausea, abdominal cramps, diarrhea, fever and headache. /3/ Symptoms are individual however, and not all cases of salmonellosis are recorded. /26/ Any mammal, bird or reptile can be asymptomatic carriers of salmonella, but in Finland, salmonella is not often found in animals. /5/ In principle it is possible that salmonella bacteria originating from animals can contaminate field plants. Another possibility for similar transmission is when field are fertilized with excreta from a person infected with salmonella. Seeds can also be infected if the crops were irrigated with contaminated water. /3/

3. LEGISLATION

The use of excreta as fertilizer is in certain instances, both promoted and restricted by legislation. There are several health and environmental perspectives, which need to be taken into consideration when using STS, CF and urine in the fields. First of all, field workers are subjected to illnesses due to the possible presence of pathogens, and secondly, those who consume the products can become infected. In addition, heavy metals and other harmful substances from fertilizers can accumulate in the soil and cause environmental degradation. In other words, pathogens can be present at every phase of the production, from cultivation to digestion. For that reason, legislation is specifically divided into numerous sections. The legal issues slightly differ according to the type of excreta in question, as well as according to the species cultivated and the time of the year. Besides such legislation, there are important international recommendations such as “Guidelines for the Safe Use of Wastewater, Excreta and Gray-water” published by the World Health Organisation.

3.1. LEGISLATION REGARDING FERTILIZERS

Excreta is considered as waste both in Finland and the EU. The safe use of excreta as fertilizer is closely related to the objective of the *Finnish Environmental Protection Act* of 2000/86, which is, according to Section one intended:

1) to prevent the pollution of the environment and to repair and reduce damage caused by pollution;
2) to safeguard a healthy, pleasant ecologically diverse and sustainable environment;
3) to prevent the generation and the harmful effects of waste;
4) to improve and integrate assessment of the impact of activities that pollute the environment;
5) to improve citizens' opportunities to influence decisions concerning the environment;
6) to promote sustainable use of natural resources; and
7) to combat climate change and otherwise support sustainable development. /16/

In addition, the Finnish *Waste Law* of 1993/1072 and EU council directive 1999/31/EC on *Waste Sites*, stipulate also that the prevention, recycling and recovery of waste should be encouraged as well as the reuse of the material and energy that the waste contains. /13, 15/

Furthermore, the EU Council Directive 1986/278/EEC on *the Protection of the Environment, and in Particular of the Soil, when Sewage Sludge Is Used in Agriculture* encourages the use of sewage sludge in agriculture, but at the same time regulates it in order to prevent the harmful effects to the environment and especially to the soil. /11/

In sparsely populated areas, excreta (including STS) is considered as waste that needs to be handled in accordance to the Finnish waste law. It is stated that the municipality in question is responsible for the transportation, handling and reuse of waste or in giving instructions for others to carry out. /21/ According to the Ministry of Agriculture and Forestry of Finland, the municipality is responsible to provide direction regarding household's usage of excreta as fertilizer for gardens. /8/

The Finnish Government has made a decision (1994/282) in the section of waste laws regarding the use of sludge in agriculture which set limits for the concentration of heavy metals in the sludge. Furthermore, STS cannot be used in fields without proper handling, and it can be only used for fields of grain crops, oil plants, sugar beets and nonfood-production plants. /29/ According to the Ministry of Agriculture and Forestry of Finland, agricultural use of CF and urine are comparable in terms of treatment to the use of STS. If the fertilizers (STS, CF, source-separated urine), have originated from the farm's own reservoirs, they need to be treated with various methods, such as lime stabilization, thermophile digestion, composting, or some other method in order to diminish significantly the amount of pathogens, odours and health- and environmental risks. /21, 25, 29/

3.2. THE MICROBIOLOGICAL CRITERIA

Microbes are well known for their ability to transfer from soil to certain vegetables. /1/ Documentation has been prepared recording various disease outbreaks caused by contaminated vegetables. Especially vulnerable are communities which lack immunity. /7/ In order to maintain a good hygienic level of foodstuff, it is most essential to design suitable international microbiological criteria. /1/

There are limits for the amount of coliform bacteria allowable in for instance, drinking and swimming water. These are both suitable waters for making comparisons, since fields are often irrigated with either prepared drinking water, or freshwater from lakes. The Finnish and European Union legislation regarding the microbiological quality of drinking and swimming water can be seen in Table 2.

Table 2. Microbiological quality of drinking and swimming water according to Finnish and EU legislation. /12, 14, 22, 23/

	Finnish regulation 19th May 2000/461 and 22nd Jan 1999/41		**EU regulation Directives 2006/7/EC and 1998/83/EC**	
(cfu/100 ml)	**Drinking water**	**Swimming water**	**Drinking water**	**Swimming water**
Escherichia coli	0		0	900 (inland); 500 (sea and river deltas)
Coliform bacteria (at 22°C)	0	<10 000	0	-
Fecal coliform bacteria	-	<500	-	-

The European Commission regulation 2073/2005 concerning the microbiological criteria for foodstuff provides limits for the amount of bacteria, fungus, etc found in different categories of food. For pre-cut fruits and vegetables (compare carrot) of 25 grams, salmonella should be absent. The limits for *E. coli* vary: under 100 pmy/g = satisfactory; 100 pmy/g - 1000 pmy/g = acceptable; and over 1000 pmy/g = unsatisfactory. /10/ Though this research did not use *E. coli* as an indicator

pathogen, the allowed limits provide an idea about its presence. The limits can be also seen in Table 3.

Table 3. Microbiological quality of foodstuff. /10/

	Micro-organism	**Limits**
Pre-cut fruit (25 g)	Salmonella	Absent
	E. coli	< 100 pmy/g (satisfactory)
		100 – 1 000 pmy/g (acceptable)
		> 1 000 pmy/g (unsatisfactory)

4. THE GROWING EXPERIMENT AT TAMK

4.1. MODEL PLANTS

Barley (*Hordeum vulgare var. Scarlett*) and carrot (*Daucus carota var. Napoli FI*) were used as model plants in the experiment, which commenced in November 2005 and ended in February 2006. Carrot seeds were fungicide treated with thiram, iprodiome and metalaxyl.

4.2. THE TIMETABLE OF THE EXPERIMENT

The preparations for the growth experiment were started in the fall of 2005, as was the preparation of substrates (mid October). The experiment itself was initiated on November 8th 2005 when sowing was completed, ending February 20th 2006 when the carrots were harvested. The timetable for the experiment and all routines carried out is shown in Table 4.

Figure 1. The Greenhouse inside Tampere Polytechnic.

Table 4. The timetable of the experiment.

Date	Time		Action	End
	Weeks	Days		
12.10.2005	-4	-27	Mixing peat and lime	12.10.2005
04.11.2005	-1	-4	Mixing peat and sand	04.11.2005
07.11.2005			Salmonella, urine, STS, CF and blank (mixture of peat, sand, lime) samples	
08.11.2005	0	0	Growing experiment	20.02.2006
08.11.2005	0	0	Sowing	
09.11.2005	0	1	Coliform bacteria, soil samples	10.11.2005
16.11.2005			Salmonella, peat, sand, barley & carrot seed samples	
23.11.2005	0	1	Coliform bacteria, soil samples	24.11.2005
25.11.2005	2	17	Singling	28.11.2005
09.12.2005	5	31	Light-dark sequence 19/5	
13.12.2005	6	35	Greenhouse door left ajar	08.01.2006
08.01.2006	9	70	Greenhouse door closed	
17.01.2006	11	71	Sampling 30 spikes of barley	
19.01.2006	11	73	Coliform bacteria, barley grain samples	
20.01.2006	11	74	Barley harvested	
24.01.2006	13	87	Coliform bacteria, soil samples	25.01.2006
09.02.2006	14	94	Carrots picked	10.02.2006
17.02.2006	15	101	Coliform bacteria, carrot samples	

4.3. THE GREENHOUSE

The Greenhouse was built indoors in the process hall of Tampere Polytechnic University of Applied Sciences. The size of the greenhouse was (W*L*H) 2.3*5*2.5 m. The temperature was controlled by a fan cooler equipped with a condensation tank. No additional heating was needed due to the indoor location and heat supplied by lamps. The experiment was to simulate as closely as possible the field conditions of the month of June in Finland. Daylight and temperature maximums were set to match these requirements. The lights used were six 400 W high-pressure sodium lamps. Their luminous intensity was 10000 lx at the level of the substrates. The light–dark sequence was controlled with a timer with a sequence of 20/4 hours, and after 5 weeks, this was changed to 19/5 hours to correspond to the shortening days. The floor was covered with 50 mm thick Styrofoam slabs (expanded polystyrene) in order to avoid a drop in temperature as the result of possible cold drafts.

4.4. CARE-TAKING OF THE GREENHOUSE AND THE GROWTH CONDITIONS

Irrigation was achieved by use of a watering can and a bottle for carrots. Underground irrigation was also used from time to time. Growing crates were irrigated several times a week with no predetermined amounts or schedules, rather, the amounts and irrigation schedules were adjusted by monitoring moisture of substrates. If excess moisture was noticed, irrigation was diminished, and if water appeared in under drains, irrigation was suspended until substrate was dry enough to continue irrigation.

Fungal growth appeared in several substrates due to excess irrigation. Fungi were eliminated by diminishing or suspending irrigation for these substrates until they dried enough.

Five brandling worms (*Eisenia fetida*) were used per crate to loosen substrate soil composition.

Due to the heat produced by lights, the plants quickly increased in length, but not in strength. To add strength, watered down growth regulator was sprayed twice on the barley and carrots were mulched.

As the plants grew, lodging occurred due to stem weakness. For barley, a supportive netting was set by heaving the seedlings through it. Netting hindered the further lodging of barley. Mulching of carrots did strengthen stems, but lodging was resisted for only for a short time. When lodging in the carrot stems were noted again, a watering bottle was used to irrigate, and this was concentrated between seedling rows.

The fan cooler thermostat was set at the beginning of the growing experiment to 17 ˚C. This did not achieve the desired level, so the thermostats optimum temperature was dropped first to 15 ˚C and then to 13 ˚C. Excess heat was a major problem in the growing experiment, and the greenhouse was ventilated in December by means of opening the greenhouse door during measurements. During week 6, it was decided to keep the greenhouse door open until the temperature cooled down. Three weeks later however, the door was once again kept shut since it was unclear if ventilation was affecting the temperature levels.

4.5. THE MEASUREMENTS

The temperature was measured at least once during workdays (Monday to Friday). The temperature was measured with three different meters; digital centigrade thermometer; analogous centigrade thermometer and substrate centigrade thermometer. The digital centigrade thermometer showed not only the current temperature but also the last measurement of minimum and maximum temperatures last taken.

Air humidity was measured with two gauges, Vaisala digital RH meter and analogous RH hair meter. All measurements and other notes were written in a greenhouse diary, which also included the amount of water used for irrigation. Information concerning the highest and lowest measured values of temperature and moisture in the greenhouse during the growing experiment are found in Table 5.

Table 5. Highest and lowest measures of temperature and moisture from greenhouse in each category.

	T_{wall} (°C)	$T_{digital}$ (°C)	T_{max} (°C)	T_{min} (°C)	$T_{substrate}$ (°C)	RH (%)	RH_{hair} (%)
Lowest Value	20	19,7	22,2	12,5	16,7	21,8	27
Highest Value	26	29,5	30,3	28,9	29,8	75,2	95

4.5.1. Temperature

Highest temperature during the growing experiment, 30.3˚C, was measured in December 9th 2005 at 9 AM. Lowest temperature, 12.5˚C, was measured in December 22nd at 4 PM. The temperature variation can be seen in Figure 2.

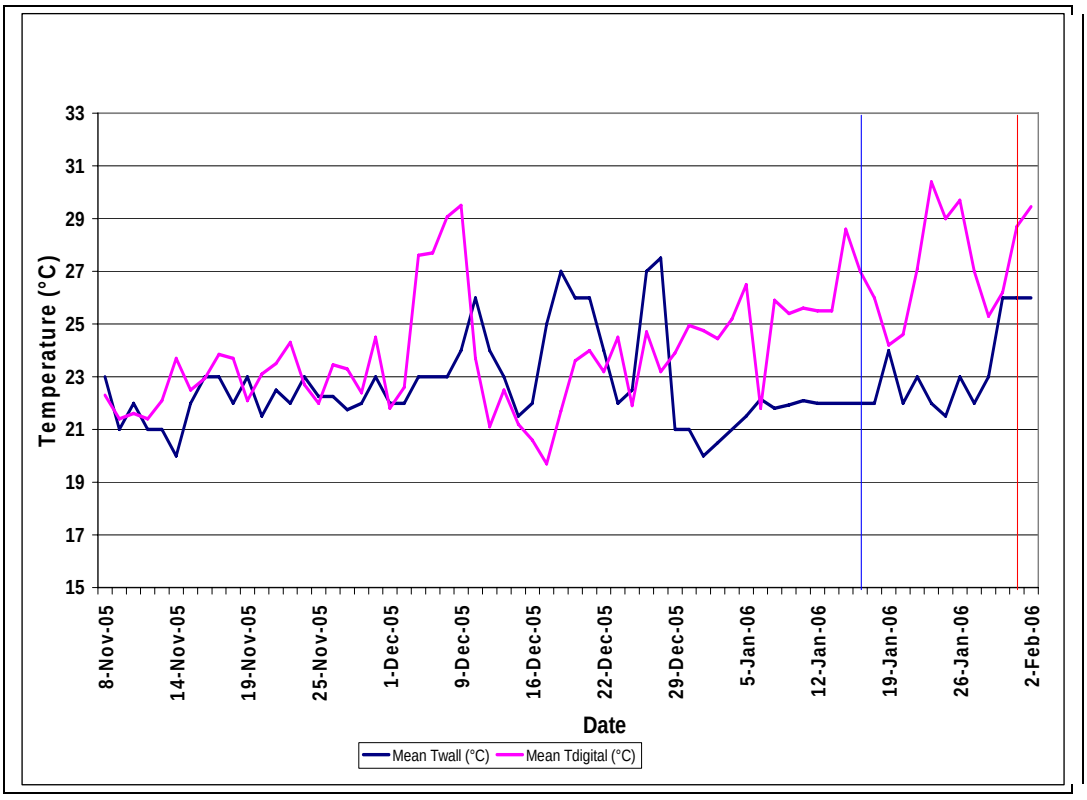


Figure 2. Greenhouse temperature variation during growing experiment.

4.5.2. Relative Humidity

Highest relative humidity during the growing experiment, 75.2 % was measured December 30 2005 at 3:35 PM. Lowest RH, 21.8 % was measured January 20 2006, the day the growing experiment ended.

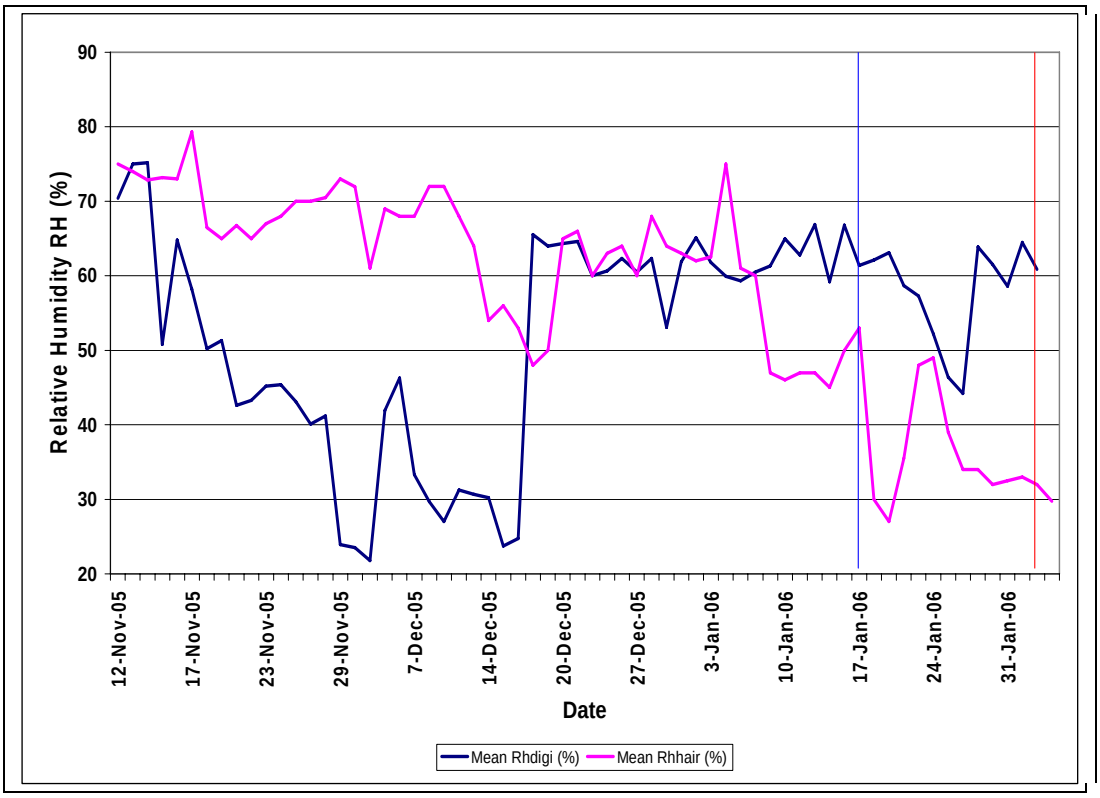


Figure 3. Greenhouse relative humidity (RH) variation during growing experiment.

Greenhouse diary with detailed report of actions can be found in Appendix 1.

4.6. THE SUBSTRATES

The substrate was made by mixing 2 m^3 of Biolan unfertilised horticultural peat, pH 3.5, density 65 g l^{-1}, particle size >35 mm, country of origin Finland, 6 kg of lime was added per m^3 to adjust pH, and 0.66 m^3 of sand (particle size 2–6 mm). Lime was added outdoors on a tarpaulin to the peat 4 weeks before starting the growing experiment to stabilise the pH of acidic peat. Mixing was done manually by spreading the lime over the peat and turning it over several times with spades. The mixed peat was left outside under the tarp. Three weeks later 0.66 m^3 of sand

was mixed to the peat to improve the substrate's aeration properties with the same method mentioned above.

Figure 4. Mixing substrate and lime outside.

Plastic crates, made from HDPE, size (W*L*H) 0.26*0.76*0.25 m were used as growth crates. The volume of one crate was 0.1064 m^3. Plastic drainage pipes, 80 mm diameter PP plastic, were placed at two corners of each crate to ensure adequate aeration of the substrate and to control possible excessive irrigation of substrates. A 50 mm thick layer of LECA gravel was added to the bottom of the crates for aeration and lower drain purposes. Different substrates were added on top of the LECA gravel and packed so that every crate was filled to a point 10 mm below the rim. It was expected that the substrate would become more compact, a result of water, and its own weight. There were a total of 16 crates in the greenhouse, 8 for carrots and 8 for barley. Two parallel treatments of both barley and carrot were used: commercial fertilizers, Kevätviljan Y3 -fertilizer for barley; Puutarhan kevät -fertilizer for carrot; 2 for separated urine; 2 for composted human faeces, collected from private households and 2 for STS, collected from private

households in municipality of Kangasala. The placement of the crates is in Figure 5.

Barley Fertilizer I	Barley Fertilizer II	Barley Faeces I	Barley Faeces II	Barley Urine I	Barley Urine II	Barley STS I	Barley STS II
							Door
Carrot Fertilizer I	Carrot Fertilizer II	Carrot Faeces I	Carrot Faeces II	Carrot Urine I	Carrot Urine II	Carrot STS I	Carrot STS II

Figure 5. Placement of the crates in the greenhouse.

Figure 6. Greenhouse with cooling unit and substrates ready for sowing.

The amounts of fertilizers were determined based on the recommendations of the manufacturers of commercial fertilizers. The recommendation for Kevätviljan Y3 fertilizer is 500 kg/100,000 m^2 and for Puutarhan kevät -fertilizer 8 kg/100 m^2. The nitrogen content was used as a determining factor in calculations for other fertilizers.

The amount of nitrogen in Kevätviljan Y3 -fertilizer is 20 % and in Puutarhan kevät fertilizer 8 %. The nutrient concentrations in percentage of weight are presented in Table 6.

Table 6. Nutrient concentrations of the fertilizers by percentage of weight.

Nutrient	Puutarhan kevät	Kevätviljan Y3
Total Nitrogen (N)	8,00	20,00
Ammonium Nitrogen (NH_4N)	5,50	11,40
Nitrate Nitrogen (NO_3N)	N/A	8,60
Phosphorus (P)	2,50	3,00
Phosphorus, water soluble (P)	4,00	2,80
Potassium (K)	3,40	8,00
Magnesium (Mg)	14,00	0,50
Sulphur (S)	2,00	3,00
Boron (B)	8,00	0,02
Copper (Cu)	0,07	N/A
Iron (Fe)	0,05	N/A
Manganese (Mg)	0,35	N/A
Molybdenum (Mo)	0,01	N/A
Selenium (Se)	N/A	0,001
Zinc (Zn)	0,05	N/A

The area of a crate was 0.8 m *0.6 m = 0.48 m^2 and two crates were used for one treatment, making the total area for one treatment 0.96 m^2. The amount of Kevätviljan Y3 -fertilizer amount was calculated:

$$\frac{500kg}{100000m^2} = \frac{x}{0.96m^2}$$
$$\Leftrightarrow x = \frac{500kg * 0.96m^2}{10000m^2}$$
$$\Leftrightarrow x = 0.048kg \approx \underline{\underline{48g}}$$

The calculation for the amount of nitrogen was:

$$48g * 20\% = \underline{\underline{9.6g}}$$

The calculation for Kevätviljan Y3 -fertilizer was:

$$\frac{8kg}{100m^2} = \frac{x}{0.96m^2}$$
$$\Leftrightarrow x = \frac{8kg * 0.96m^2}{100m^2}$$
$$\Leftrightarrow x = 0.0768kg \approx \underline{\underline{76.8g}}$$

Calculation for nitrogen content was:

$$76.8g * 8\% = \underline{\underline{6.144g}}$$

A human produces 5.7 kg of nitrogen, 0.6 kg phosphorus and 1.2 kg of potassium yearly. This means approximately 500 kg of urine and 50 kg of faeces. 90 % of the nitrogen is secreted with urine and 10 % with faeces. When faeces are composted they are mixed with an equal amount of mixture compound bringing the total up to 100 kg. /19/

The nitrogen content calculation of faeces was:

$$\frac{5700g * 10\%}{100kg} = \underline{\underline{5.7g * kg^{-1}}}$$

The amount of composted faeces for barley was calculated as:

$$9.6g = x * 5.7g * kg^{-1}$$
$$\Leftrightarrow x = \frac{9.6g}{5.7g * kg^{-1}}$$
$$\Leftrightarrow x = \underline{\underline{1.684kg}}$$

The amount of composted faeces for carrot was calculated as:

$$6.144g = x * 5.7g\ kg^{-1}$$
$$\Leftrightarrow x = \frac{6.144g}{5.7g\ kg^{-1}}$$
$$\Leftrightarrow x = \underline{\underline{1.078kg}}$$

The nitrogen content of separated urine was calculated as:

$$\frac{5700g * 90\%}{500kg} = \underline{\underline{10.26g\ kg^{-1}}}$$

The amount of separated urine for barley was calculated as:

$$9.6g = x * 10.26g\ kg^{-1}$$
$$\Leftrightarrow x = \frac{9.6g}{10.26g\ kg^{-1}}$$
$$\Leftrightarrow x = \underline{\underline{0.936kg}}$$

The amount of separated urine for carrot was calculated as:

$$6.144g = x * 10.26g\ kg^{-1}$$
$$\Leftrightarrow x = \frac{6.144g}{10.26g\ kg^{-1}}$$
$$\Leftrightarrow x = \underline{\underline{0.599kg}}$$

According to Oksjoki (2004), the average amount of nitrogen in STS is 44 g l^{-1}. /2/

The amount of STS for barley was calculated as:

$$9.6g = x * 0{,}44g\ l^{-1}$$
$$\Leftrightarrow x = \frac{19{,}2g}{0{,}44g\ l^{-1}}$$
$$\Leftrightarrow x = \underline{\underline{21.8l}}$$

The amount of STS for carrot was calculated as:

$$6.144g = x * 0{,}44g\ l^{-1}$$
$$\Leftrightarrow x = \frac{6.144g}{0{,}44g\ l^{-1}}$$
$$\Leftrightarrow x = \underline{\underline{13.964l}}$$

Barley and carrot crates with commercial fertilizer treatment were filled up with arrant substrate without the addition of fertilizers. The fertilizers were added later alongside with the seeds. For barley and carrot fertilised with composted human faeces and STS, the substrates were mixed with a calculated amount of fertilizers before filling the crates. The mixing was made on a tarpaulin inside the process hall. For barley and carrot fertilised with separated urine, the crates were filled first with the substrate and afterwards the urine, mixed up with water was added. All crates were irrigated, except those fertilised with STS, in order to have the same moisture content in all crates. The amounts of fertilizers and water added are shown in Tables 7 and 8.

Table 7. Fertilizer type and amount and water added to barley crates.

BARLEY *Hordeum vulgare var. Scarlett*			
Crate	**Fertilizer**	**Amount/Crate**	**Water Added/ Crate**
Fertilizer I	Kevätviljan Y3	24 g	11 l
Fertilizer II			
Faeces I	Composted Faces	842 g	11 l
Faeces II			
Urine I	Separated Urine	468 g	up to 11 l
Urine II			
STS I	Septic Tank Sludge	11 l	None
STS II			

Table 8. Fertilizer type and amount and water added to barley crates.

CARROT *Daucus carota var. Napoli F1*			
Crate	**Fertilizer**	**Amount/Crate**	**Water Added/ Crate**
Fertilizer I	Puutarhan kevät	38,4 g	11 l
Fertilizer II			
Faeces I	Composted Faces	539 g	11 l
Faeces II			
Urine I	Separated Urine	300 g	up to 11 l
Urine II			
STS I	Septic Tank Sludge	7 l	4 l
STS II			

4.7. THE SOWING

The sowing was done on November 8th 2005. Barley was planted in 6 rows to a depth of approximately 10 mm, in all 8 substrates. The seeds were pressed into the substrate to ensure they stayed covered. The sowing was quite dense to encourage the maximum germination of seedlings. For commercial fertilizers, 7 rows were prepared between the sown seed and the fertilizer was added to an even depth of approximately 20 mm.

Carrots were planted in 5 rows to a depth of approximately 5 mm for the rest of the 8 substrates. Sowing was once again quite dense to ensure adequate seedling growth. For commercial fertilizer 6 rows were made between the sowed rows, then fertilizer was planted evenly to a depth of approximately 20 mm.

Figure 7. Sowed carrot seeds with fertilizers applied between rows of the planted seeds.

All substrates were compacted evenly by hand to ensure good seed and soil contact, and to avoid possible pooling of irrigation water.

4.8. THE THINNING PROCESS

Thinning was done two weeks after sowing; the carrot seedlings thinned by using tweezers, leaving some 6–7 seedlings per 10 cm, and barley was thinned in the same manner, so that 6 seedlings per 10 cm remained.

Figure 8. Singling barley.

4.9. THE CROP YIELD

In general, control substrates fertilised with artificial fertilizers grew fastest and produced the highest yield. All eight barley treatments starting from the forefront of the photograph figure 9 are: STS II, STS I, Urine II, Urine I, Compost II, Compost I, Fertilizer II and Fertilizer I. The six nearest plants are clearly shorter and have ripened earlier than two plant groups at the back, fertilized by commercial fertilizer treatments. These have longer stems, and have produced longer spikes.

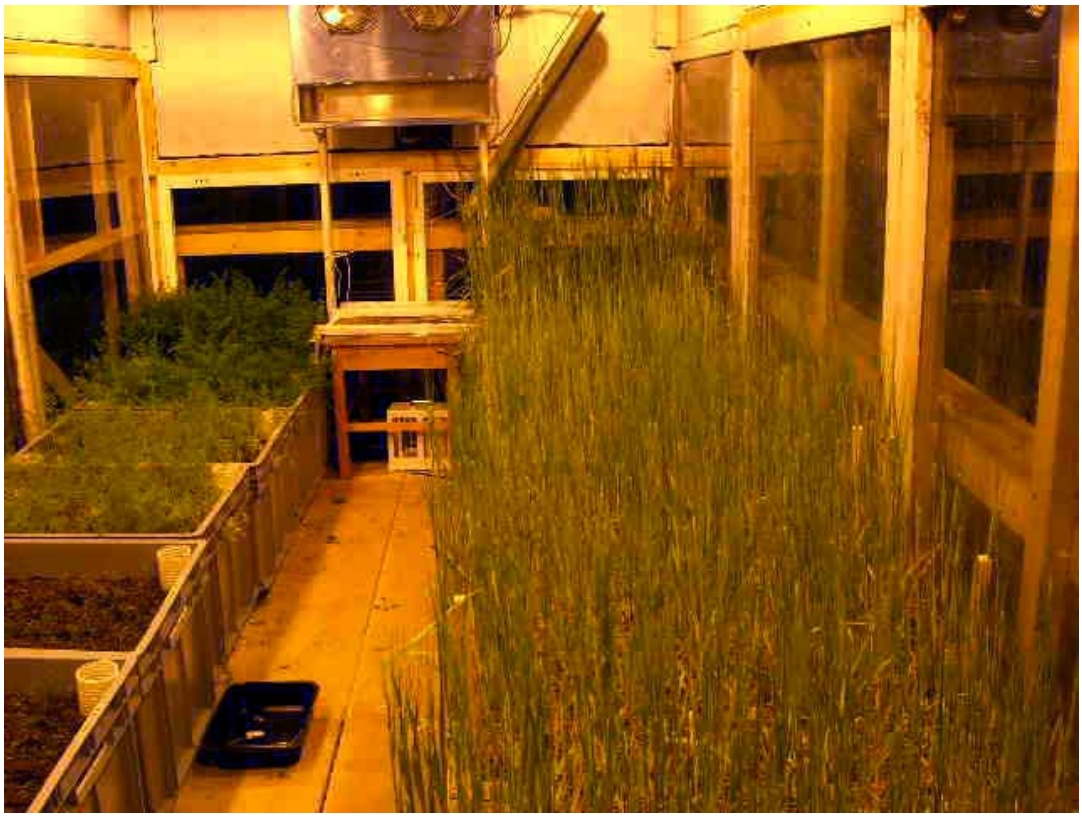


Figure 9. STS, urine and composted faeces treatments of barley in front. The clearly longer and greener plants with commercial fertilizer treatment are farthest back.

All 8 carrot treatments starting from nearest can be seen in figure 10: STS II, STS I, Urine II, Urine I, Compost II, Compost I, Fertilizer II and Fertilizer. The four closest ones, treated with STS and urine, did not grow large yields, and are visibly less robust. The next two, fertilised with compost, grew better and produced healthier looking tops, although slightly pale in colour. The last two treatments, using commercial fertilizer, produced healthy looking green tops.

Figure 10. STS and urine treatments of carrot are in the front. In the 4th container, stronger results can be seen from the composted faeces treatment, and furthest in the background, the most green and successful growth using a commercial fertilizer treatment.

The following yield reports were made by laboratory engineer Seija Haapamäki; a carrot yield report on February 2nd 2006, and a barley yield report on January 17th 2006.

4.9.1. The Carrot Yield

Carrot yield was best in control substrates using artificial fertilizers. The plan tops were the greenest and largest, although thin and limp.

Carrot tops in substrates fertilised with composted human faeces were the second largest but showed a clear difference to control substrates. Tops were firmer than with control substrates and thus no support was needed. Colour of tops was yellowish green.

Human urine fertilised substrates realized the poorest yield. Tops were stunted and coloured dark, reddish and lilac, and the length of these tops was only around a couple of centimetres. Growth was weak and completely stopped after the first couple of weeks of the growing experiment.

Human STS fertilised substrates developed a larger yield than those using human urine. Plant tops were a couple of centimetres longer than with human urine, but stunted compared to growth in plants fertilised with composted human faeces as seen in Figure 11. The colour of tops is more yellowish compared to those using composted human faeces, yet greener than plants fertilised with human urine. Human STS fertilised substrates tended achieve better growth during the last couple of weeks of the experiment.

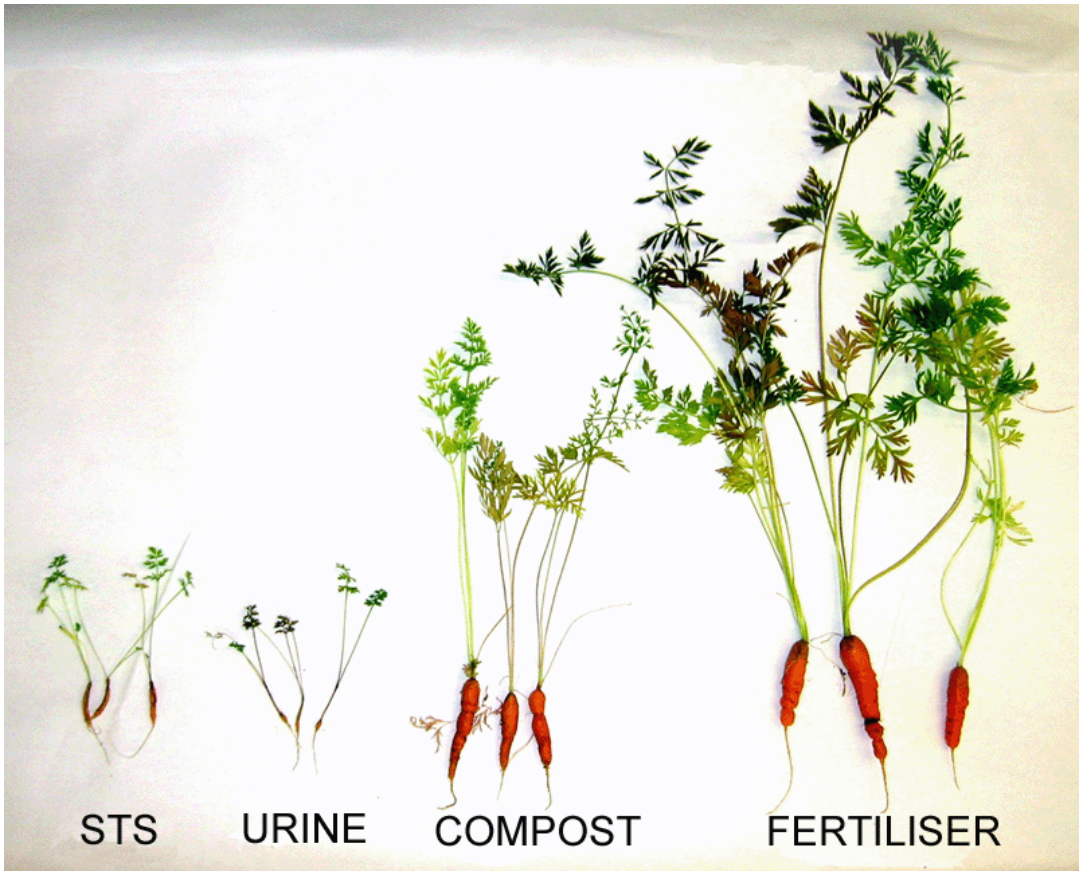


Figure 11. Carrots grown with STS, separated urine, composted faeces and commercial fertilizer.

5. THE METHODS

5.1. THE COLIFORM BACTERIA DETECTION

Coliforms were analysed by a modified method of standard SFS 3950 and SFS 4447, according to instructions given by the chromogenic Compact Dry CF plates (manufacturer HyServe/Germany; supplier VWR Finland), which were used for the quantitative detection of coliform bacteria (Appendix 3). The plates were chosen due to their simplicity and rapid results. The standard SFS 4447 is used in water sampling, but is also recommended for use in sludge sampling and in cases where samples contain a great deal of sediment. The dilutions were made based on the SFS 4447 according to the dilution water of SFS 3950. /4/ The samples were taken three times from the soil and at the experiment's end, once from foodstuff, i.e. carrots and barley grains.

5.1.1. The Preparation Work

The dilution water was prepared according to the standard SFS 3950 from the following substances:

I	Potassium dihydrogen phosphate	KH_2PO_4	0.0425 g
II	Magnesium sulphate	$MgSO_4 \cdot 7\ H_2O$	0.250 g
III	Sodium hydroxide	NaOH	0.008 g
	Distilled water	H_2O	1 l

Potassium dihydrogen phosphate-stock solutions (I):

42.5 grams of KH_2PO_4 was added to water to make a solution of one litre.

Magnesium sulphate-solution (II)

50.0 grams of $MgSO_4 \cdot 7\ H_2O$ was added to water to make one litre.

Sodium hydroxide-solution (III)

8 grams of NaOH was added to water to make one litre.

The same final dilution water was used in the forthcoming analysis. The solutions were kept in the refrigerator (for the sake of the cold and darkness both), and before using, they were well shaken and the amount needed was poured into a

beaker, which was then covered with para-film. This was done in order to let the solutions warm to room temperature without possible contamination.

From the three above mentioned solutions, dilution water was prepared one day prior to soil sampling by adding 1 ml of *I* and 5 ml of *II* into 990 ml of distilled water. The solution was mixed and thereafter 1 ml of *III* was added. The pH of the solution was measured with a pH-meter, in order to ensure that the pH is 7.0 ± 0.1. If this level was exceeded, a few drops of HCl were added, and in the opposite case, pH was raised with NaOH.

225 ml of final dilution was inserted by pipette into test bottles and 9 ml to test tubes. The solutions in the growing bottles represented a ratio of 1:10. The test tubes represented dilutions of 1:100, 1:1000 and 1:10000. Sterilization was done in an autoclave at 120 ºC for 15 minutes. Afterwards the test bottles and tubes were placed on a laboratory table were they remained until the start of cultivation which was usually the following day.

5.1.2. The Samples

Sampling was organised so that it was possible to accomplish the work goals within the workday.

5.1.2.1. Soil Hygiene Samples From the Substrates

Soil hygiene samples from the substrates were taken three times during the following dates:

1) 09.-10.11.2005
2) 22.-23.11.2005
3) 24.-25.01.2006

One composite soil sample was taken from each crate. In order to decrease the possible cross-contamination between different crates, it was decided to divide the sampling over a period of two days, on day one a sample from substrates' crate I

was taken, with crate II taken on day 2. Every sample consisted of soil taken from several random places of the substrate. The spoon used for sample taken was cleaned with 70% ethanol, and the same spoon was only used for one sample. After transferring the composite sample to the container (a clean plastic cup), mixing of the sample was done with a spoon. 25 grams of each soil sample was required, and these samples were weighed in the process hall where the greenhouse was also situated. Thereafter samples were transferred to the microbiological laboratory, which was only used for this part of the research.

5.1.2.2. Barley Samples

Barley samples were taken at the studies conclusion, when they were considered to be ripe enough for harvesting, on the date:

4) 19.01.2006

Barley grains were taken to the laboratory, and work continued in a fume cupboard, where the grains were crushed in a mortar which had been cleaned with 70% ethanol. Weight scales were brought to the laboratory in order to diminish possible contamination while transporting to and from the scales room.

5.1.2.3. Carrot Samples

Carrot samples were taken:

5) 17.02.2006

Carrots were taken to the laboratory and were pealed and crushed to some extent, and then weighed. Due to the small size of the carrots fertilized by STS and urine, peeling was not possible. Since the harvest from the two substrates was less than the 25 grams required, it was decided to take only one tenth of that amount, or 2.5 grams. Peeling and slicing was done with disposable knifes in order to avoid contamination. Due to the small amounts, growing bottles were not used and the first dilution was made in test tubes.

5.1.3. The Inoculation

The 25 grams of each sample (excepting carrot STS and urine, see above) were suspended in 225 ml of base solution. Homogenization was carried out manually for 1 minute under the fume cupboard. 1 ml of the suspension was transferred to two parallel plates and 1 ml was transferred to the test tube containing 9 ml of the buffer solution. The test tube contents were mixed using a test tube mixer for 1 minute and 1 ml was again transferred to two parallel plates. This was repeated until there were four dilutions of each soil sample (1:10, 1:100, 1:1000 and 1:10000). Plates were incubated at the temperature $+35 \pm 2°C$ for 18-24 hours.

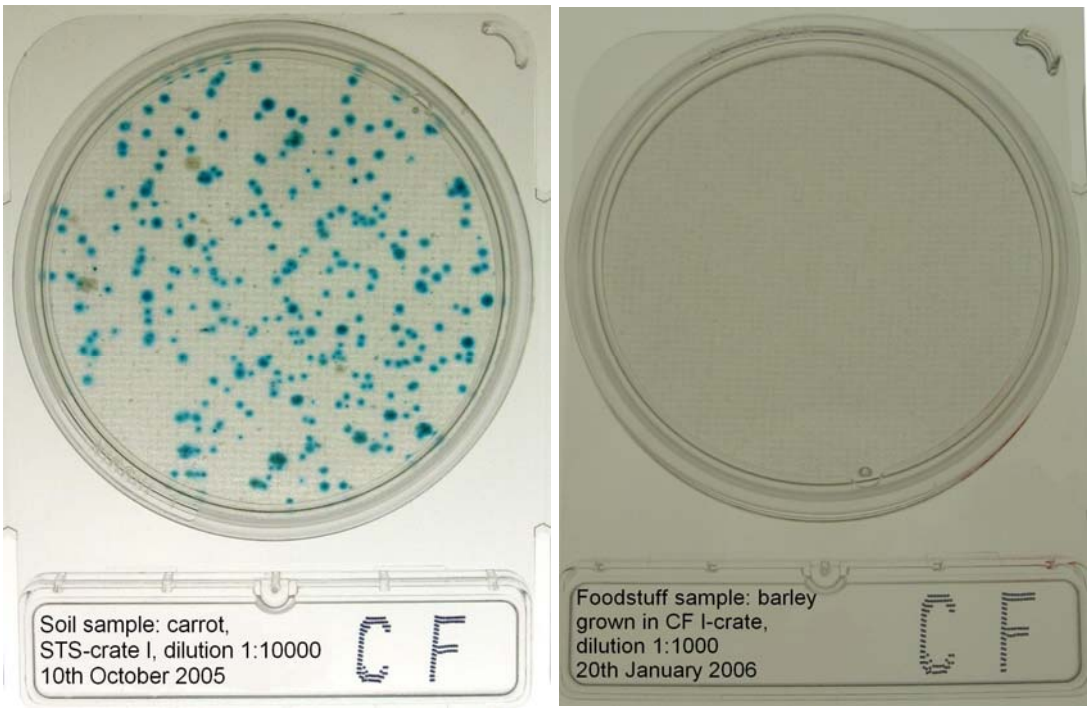


Figure 12. Coliform bacteria Dry compact plates.

5.1.4. The Interpretation

After incubation, the number of the blue/ blue green coloured colonies was counted with a digital counter pen, and occasionally, expired plates were used due to the absence of valid plates. Results compared to the parallel plates did not however significantly differ, and thus their usage on those few occasions was deemed to be acceptable.

Figure 13. Coliform bacteria colony counting.

5.2. THE METHODS FOR SALMONELLA DETECTION

Salmonella was detected according to instructions provided for the Compact SL plates (HyServe/Germany), which were used for the qualitative detection of salmonella (Appendix 4). The plates were chosen because of their simplicity and rapidity. Salmonella was first analysed from the various fertilizers, i.e. urine, STS, composted faeces, and a blank substrate, which was a mixture of peat, sand and lime. Later, samples from the soil mixture ingredients (i.e. peat and sand) and the seeds of barley and carrots were examined.

5.2.1. The Preparation Work

The broth was chosen to be sterilized buffered pep-tone water (BPW) according to the instructions given for Compact SL plates. The BPW for the enrichment was prepared according to instructions on the pep-tone jar. The distilled water was heated and the pep-tone was mixed in it using a magnetic mixer. Then 225 ml of

the liquid was poured into the growing bottles, which were sterilized in the autoclave.

5.2.2. The Samples

5.2.2.1. Fertilizer Samples

Fertilizer samples were taken on the 7th November 2006. Two samples were taken from urine, STS, composted faeces and the blank substrate (mixture of peat, sand and lime). The samples were weighed in the process hall and then taken to the laboratory in clean plastic cups.

5.2.2.2. Soil Mixture and Seed Samples

Peat and sand, which were the components of the soil mixture, and the seeds of carrots and barley, were examined on 16.11.2006. This was done in response to results realized in the fertilizer study. The peat and sand samples were taken from sacks, situated outside the building, and the peat sack was unopened at the time, while the sand bag had been previously opened, but carefully folded in order to prevent the entry of any unwanted material. Barley seeds were taken from their original plastic bag and carrots from an unopened bag. The samples were weighed in the process hall, from where they were taken to the microbiological laboratory for pre-enrichment. Prior to it, the seeds were crushed in mortars, which had been cleaned with 70% ethanol.

5.2.3. The Preparation of Specimen

Figure 14. Working under the fume cupboard.

Under the fume, 25 g of the sample was added to the buffered pep-tone water. The bottles were mixed manually for 1 minute, and before the bottles were put into the incubator, their caps were opened slightly, to make oxygen available to the culture. The pre-enrichment was done at the temperature of $+36 \pm 1°C$ for 20-24 hours. During both procedures, a blank pep-tone water was used to guarantee that contamination had not taken place.

5.2.3.1. Fertilizer Sample

From each fertilizer, two pre-enrichments were made, as there were two samples from each fertilizer.

5.2.3.2. Soil Mixture and Seed Sample

From the peat and sand, two pre-enriched cultures were made, and one culture was made from each of the barley and carrots samples, due to the improbability of finding salmonella in the seeds.

5.2.4. The Inoculation

The inoculation was done under the fume cupboard by transferring 0.1 ml of the enriched specimen into a Compact Dry SL plate. Thereafter 1 ml of sterilised water was added onto the other side of the plate. After that, these plates were taken to the incubator, which was set to a temperature of + 42°C, with an incubation period of 20-24 hours.

5.2.4.1.Fertilizer Sample

Eight inoculations were done from each pre-enriched fertilizer culture.

5.2.4.2.Soil Mixture and Seed Sample

Four inoculations were done from each pre-enriched culture of sand and peat. Eight inoculations were also carried out for the carrot culture, and for the barley culture, four inoculations were made.

5.2.5. The Interpretation

Interpreting the plates was difficult. According to the written instructions and microscope images, salmonella was present in all other types of fertilizers except urine I & II and peat II. Five of the most severely infected plates were forwarded for further examination to the research centre AnalyCen for independent examination in order to confirm the presence of salmonella. In any case, it was decided that further studies in the TAMK laboratory would be done from sand and

peat in order to estimate the probability of the bacteria originated from them. The same tests were made for seeds of barley and carrots. Some tests with expired Entero-tubes were also carried out, and a secondary inoculation from three of the plates (composted faeces, STS and substrate).

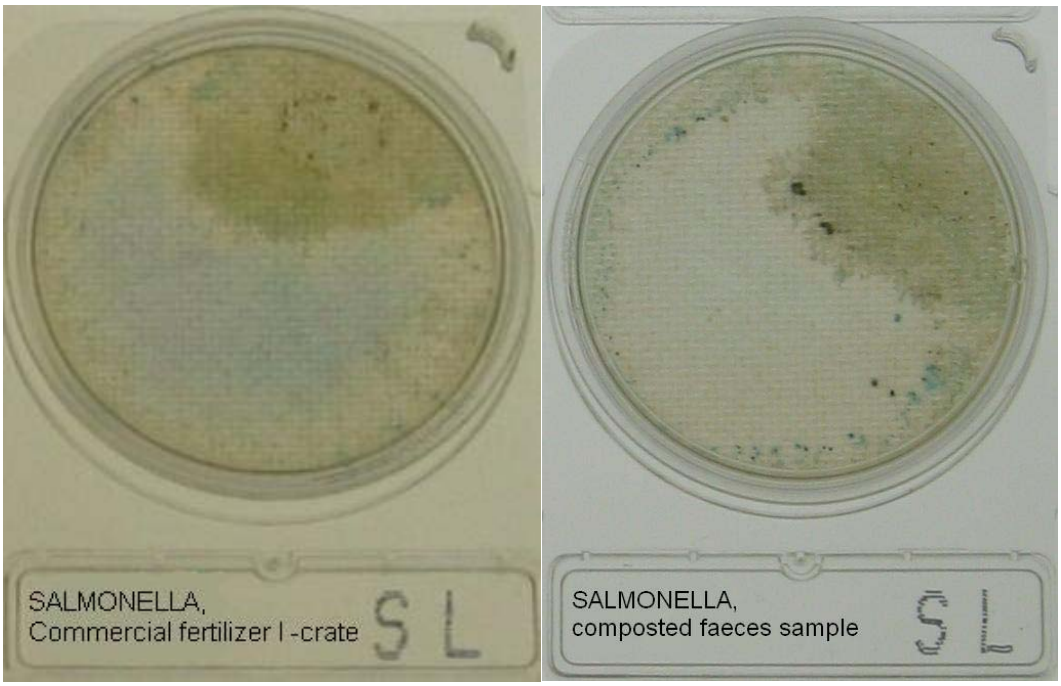


Figure 15. Salmonella growth plates.

6. RESULTS

6.1. THE CROP YIELD

The crop yield of carrots was best in substrate fertilized with the commercial fertilizer as can be seen from Figure 16.

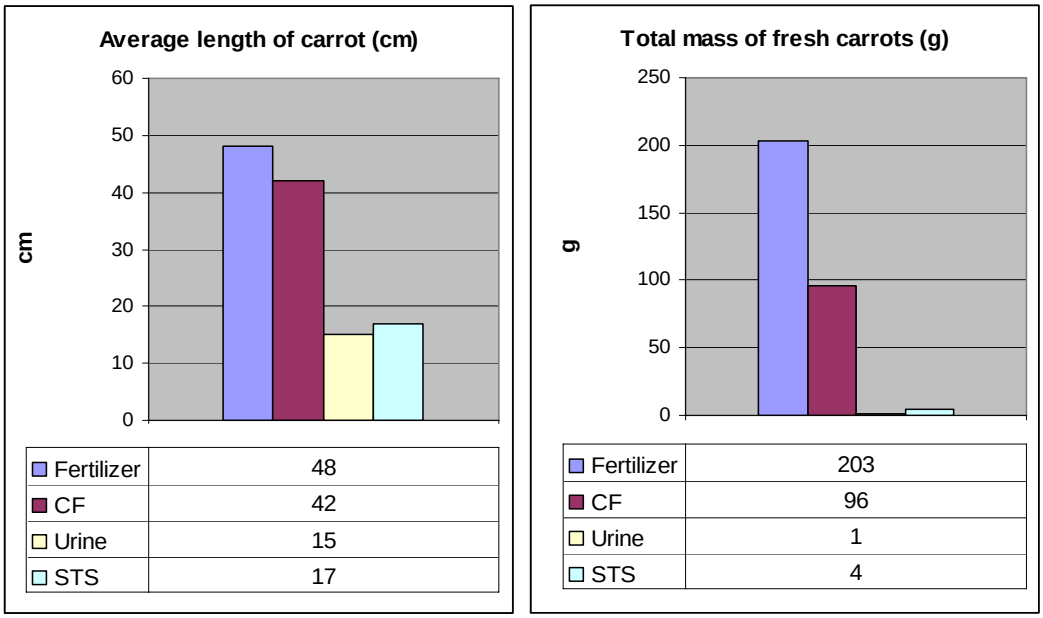


Figure 16. Physical features of carrots.

The second best result was from the use of composted faeces, with urine and STS fertilized substrates yielding the smallest crops

The best barley crop yield was also grown in the substrate of commercial fertilizer as indicated in Figure 17.

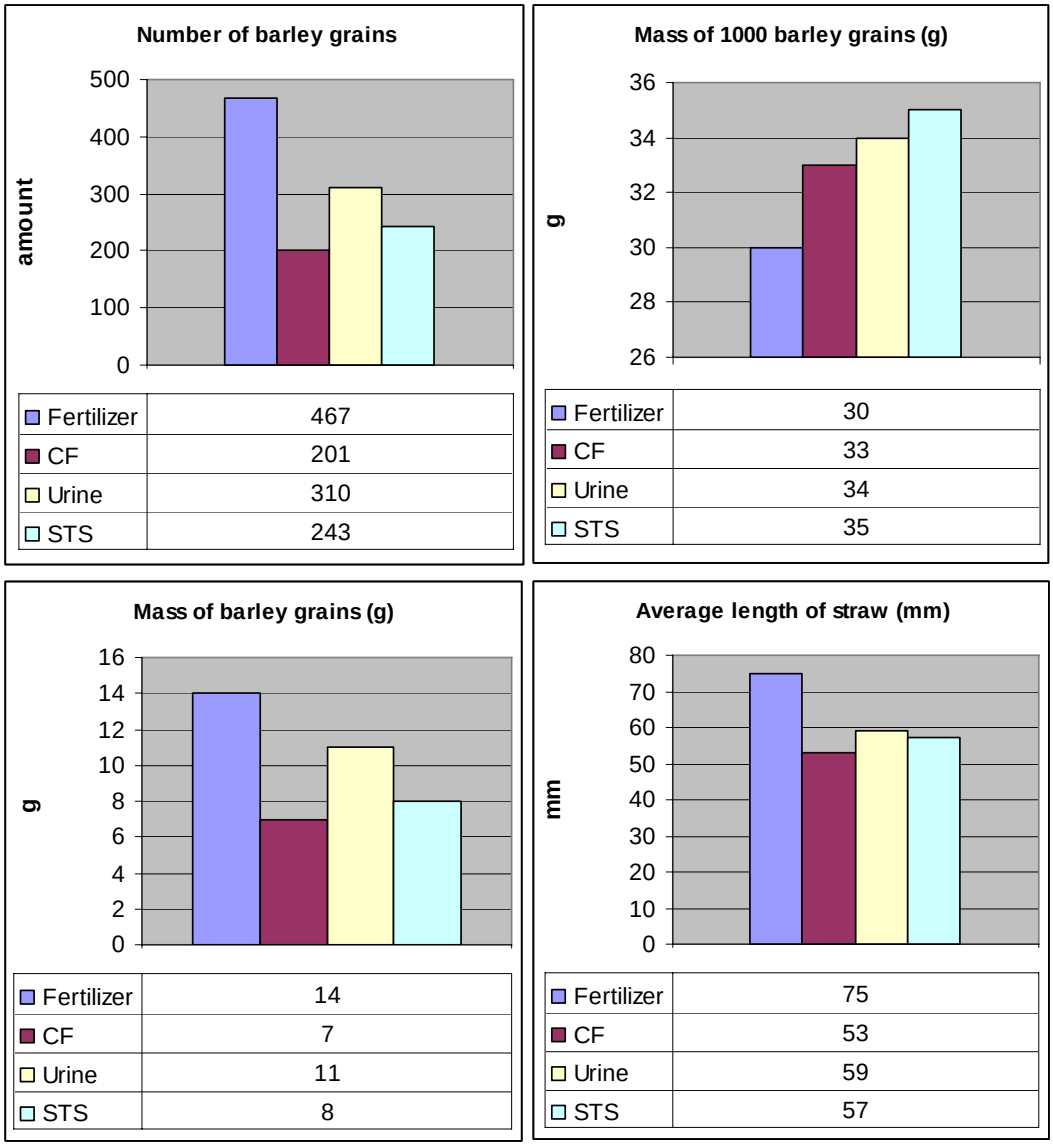


Figure 17. Physical features of barley.

6.2. COLIFORM BACTERIA

The results from the coliform bacteria detection are presented in Figures 18 and 19 as colony forming units (cfu) per 1 gram for each crate. The original result was expressed as cfu per 100 millilitres. However, in order to compare the results with the EC regulation of microbiological criteria for foodstuff, they must be converted into cfu per gram (cfu/g). /10/ That was done simply by dividing the result by 100 due to the relation between 100 ml and 1 g.

The control substrates were those treated with commercial fertilizers. In both barley and carrot crates, commercial fertilizers' crates were measured with the second greatest amounts of coliform bacteria almost consistently during the overall research. One reason might be that contamination took place during the mixing of sand and peat outside the building. In any case, fields will normally contain a small amount of coliform bacteria which originated from birds and warm-blooded animals.

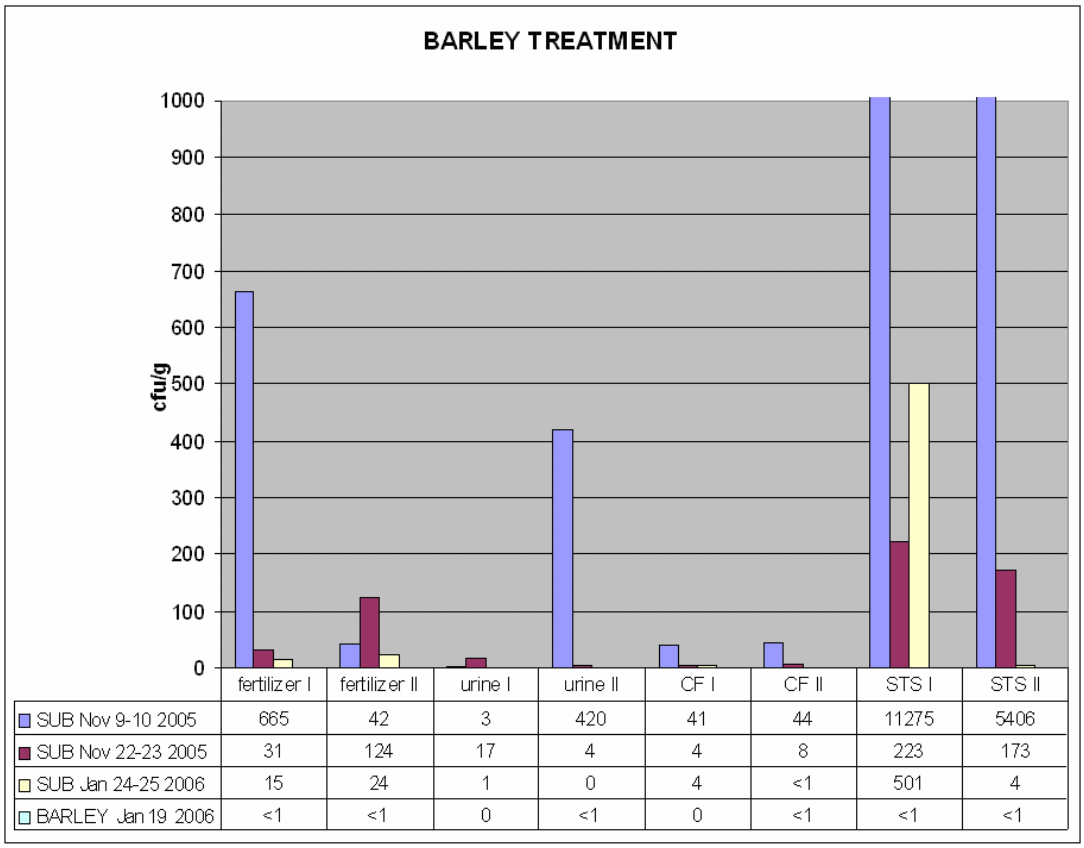
Some divergence between results were noted, however, the reasons for this can be rather easily explained. The amount of coliform bacteria at the start of the study was clearly highest in substrates fertilized with septic tank sludge, which was anticipated. The urine substrates were the cleanest in terms of coliform bacteria. In the barley substrate of barley, using urine II, contamination most likely took place, since the level of bacteria is already high compared to the duplicate and the carrot crates fertilized with urine. In addition to contamination, another reason for differences can be due to deficient homogenisation of the sample, resulting in varying amounts of bacteria in the different duplicates. In general, incomplete mixing of excreta in the substrate causes differences in the amount of bacteria in the same sample.

The most important goal of this research was to determine whether or not pathogens transfer from fertilizer to the end product, in this case, carrots and barley grains. Barley grains from substrates' urine I and composted faeces I were completely free of coliform bacteria, and in all the other barley substrates, less than one colony forming unit per one gram were found.

The results from the carrot substrates do however show a clear difference compared to barley grains, not only in the last sampling but also at the beginning, when it was found that soil samples from barley substrates contain a greater amount of colony forming units.

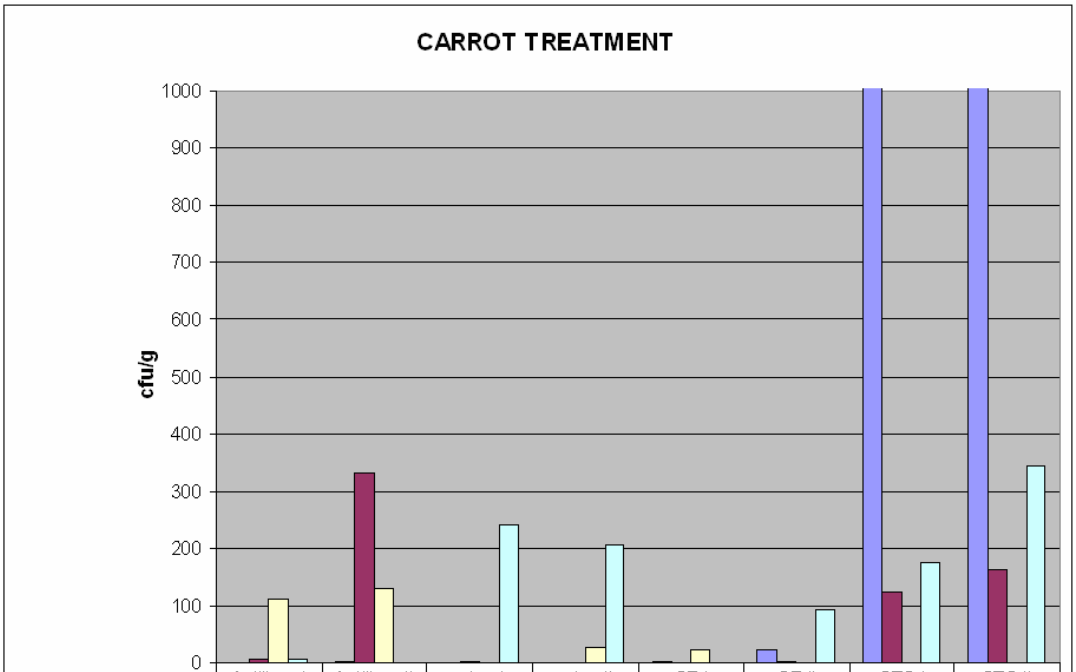
During the final sampling phase to detect coliform bacteria, it was necessary to peel the carrots in order to find out whether or not the bacteria had survived and

then transferred from the fertilizer to the foodstuff, however, due to the small size of urine and STS treated carrots, it was impossible to peel them properly. In Figure 19 one can clearly observe that incomplete handling has affected the amount of coliform bacteria present. The amount of colony forming units per gram in urine treatments is over 200 and in STS crates a clear rise can be noticed.



	fertilizer I	fertilizer II	urine I	urine II	CF I	CF II	STS I	STS II
SUB Nov 9-10 2005	665	42	3	420	41	44	11275	5406
SUB Nov 22-23 2005	31	124	17	4	4	8	223	173
SUB Jan 24-25 2006	15	24	1	0	4	<1	501	4
BARLEY Jan 19 2006	<1	<1	0	<1	0	<1	<1	<1

Figure 18. Coliform bacteria in the barley substrates and barley grains.



	fertilizer I	fertilizer II	urine I	urine II	CF I	CF II	STS I	STS II
SUB Nov 9-10 2005	<1	2	<1	1	2	23	21167	2730
SUB Nov 22-23 2005	7	332	2	<1	<1	2	123	162
SUB Jan 24-25 2006	111	130	<1	28	24	<1	1	1
CARROT Feb 17 2006	6	<1	241	206	<1	93	177	344

Figure 19. Coliform bacteria in the carrot substrates and carrots.

6.3. SALMONELLA

The plates from fertilizer samples where the most evident salmonella infection was detected were sent for further examination to the Tampere AnalyCen laboratory. They confirmed that there was no *Salmonella spp.* present in any of the five samples, though the plates' instructions clearly stated that there should have been (see Appendix 5). Thus a conclusion was made that salmonella was also absent in the other samples' plates.

7. CONCLUSIONS

7.1. PATHOGENS

The aim of this research was to determine whether or not coliform bacteria and *Salmonella* present in the different fertilizers could transfer to an end product such as carrot and barley grains, and therefore represent a health risk to the people

handling and consuming them. Since salmonella was not present in the fertilizers, its occurrence in the soil mixture would have been impossible. Coliform bacteria, on the other hand, was present often in great amounts, while it was also observed that it decreased in amount over time.

The study for barley grains indicated that the amount of coliform bacteria was well under the satisfactory limit of 100 cfu/g, with all grains resulting in less than 1 cfu/g. Similarly, the barley crop yield did not greatly vary depending upon the type of fertilizer. The conclusion of this study then, is that excreta used as a barley fertilizer does not pose any significant risks to human health.

The amount of coliform bacteria in the carrots tested using commercial fertilizer and composted faeces, stayed below the limit of 100 cfu/g. In the case of urine and septic tank sludge treated substrates, the amounts exceeded a satisfactory limit, but were nevertheless still within in the acceptable limit. This latter result however, is due to the small size of the carrots, which inhibited proper peeling and they were therefore exposed to soil contamination, and so the results cannot be considered completely reliable. This research therefore concludes that the use of composted faeces as a fertilizer for carrots does not pose any significant risk to human health.

7.2. OBSERVATIONS AND RECOMMENDATIONS FROM THE RESEARCH

Some few issues were not taken into consideration and this prevented the research in realizing all of the goals set. First of all, the absence of *Salmonella* in the excreta used meant that it had no use as a pathogen indicator, which somewhat diminished scope of the study. Secondly, the indicator bacteria should have been the species *Escherichia coli* for its presence in water clearly demonstrates faecal contamination, rather than using coliform bacteria which is less reliable. The occurrence of coliform bacteria indicates there might be faecal bacteria also present, but this is by no means certain.

There were problems with the temperature of the greenhouse. The goals was set to provide climatic conditions as close as possible to the Finnish growing season, but

on occasion, the temperature rose exceedingly high, with the result that the barley in particular, grew very high, but not with sturdy stems.

The level of nutrients, especially in urine and septic tank sludge treated carrots, was inadequate, and this yielded tiny carrots, whose pathogen concentrations were impossible to study and compare in the sense of ordinary size and use in a household kitchen.

For future studies, it is recommended to use the standards prepared by the European commission concerning regulations for microbiological criteria for foodstuff. The methods for studying *salmonella* and *E. coli* from foodstuff are clearly stated there.

This research was the first microbiological study made by the author of this paper. Guidance for the study was not always clear and as this was a learning experience partly based on trial and error, a period of familiarization before the study might have better prepared the way for the later research.

8. REFERENCES

Printed

1. HALINEN ARJA et al. *Jätekompostit lannoitteena peltoviljelyssä - biologiset ja kemialliset vaikutukset*. Maa- ja elintarviketalouden tutkimuskeskus. Jokioinen: MTT, 2006. ISBN 952-487-024-X.

2. OKSJOKI Oksjoki J. *Sakokaivolietteiden käsittely*. Ympäristö ja Terveys, 2004, 35 vsk., nro. 5, s. 32–33. ISSN 0358-3333

3. SALKINOJA-SALONEN, MIRJA (ed.). *Mikrobiologian perusteita*. Jyväskylä: Gummerus Kirjapaino Oy, 2002. ISBN 951-45-9502-5.

4. SUOMEN STANDARDISOIMISLIITTO. *Mikrobiologiset vesitutkimusmenetelmät 2003*. SFS-käsikirja 94. 3. ed. Helsinki: Kyriiri Oy, 2003. ISBN 952-5420-15-9.

5. VUORINEN ARJA (ed.) et al. *Sewage sludge and sludge products for agricultural use – a study on hygienic quality*. Maa- ja metsätalousministeriö, LIVAKE-2001-2002. Helsinki: 2003. ISBN 952-453-113-5.

6. WECKMAN, ANJA. Ihmisen ulosteet lannoitteina. Työtehoseuran monisteita 1/2000. Helsinki: Työtehoseura, 2000. ISBN 951-788-300-5.

7. WORLD HEALTH ORGANIZATION. *Guidelines for the safe use of wastewater, excreta and greywater, Volume 4: Excreta and greywater use in agriculture*. World Health Organization, 2006. ISBN 92-4-154685-9

Unprinted

8. SALMINEN PIRJO. Discussion about the use of anthropogenic nutrients as fertilizer in Finland. [phonecall.] 8.2.2007.

Electronic

9. BALTIC SEA PORTAL. *Eutrophication of the Baltic Sea* [online]. Finnish Marine Research Institute, 3.4.2007 [cited 12.2.2007]. Available from World Wide Web: http://www.itameriportaali.fi/en/tietoa/artikkelit/ihminen/en_GB/rehevoityminen_itameri/

10. COMMISSION REGULATION 2073/2005. *Microbiological criteria of foodstuff* [online]. Brussels: European Commission, 15.11.2005 [cited 11.2.2007]. Available from World Wide Web: http://eur-lex.europa.eu/LexUriServ/site/en/oj/2005/l_338/l_33820051222en00010026.pdf

11. COUNCIL DIRECTIVE 1986/278/EEC. *The protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture* [online]. Brussels: European Council, 1986 [Cited 2.2.2007]. Available from World Wide Web: http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/consleg/1986/L/01986L0278-20030605-en.pdf

12. COUNCIL DIRECTIVE 1998/83/EC. *The quality of water intended for human consumption* [online]. Brussels: European Council, 3.11.1998 [cited 10.2.2007]. Available from World Wide Web: http://europa.eu.int/eur-lex/pri/en/oj/dat/1998/l_330/l_33019981205en00320054.pdf

13. COUNCIL DIRECTIVE 1999/31/EC. *The landfill of waste* [online]. Luxembourg: European Council, 1999 [Cited 2.2.2007]. Available from World Wide Web: http://europa.eu.int/eur-lex/pri/en/oj/dat/1999/l_182/l_18219990716en00010019.pdf

14. COUNCIL DIRECTIVE 2006/7/EC. *The management of bathing water quality and repealing directive 73/160/EEC* [online]. [cited 10.2.2007] Available from World Wide Web: http://europa.eu.int/eur-lex/lex/LexUriServ/site/en/oj/2006/l_064/l_06420060304en00370051.pdf

15. EDUSKUNNAN PÄÄTÖS 3.12.1993/1072. *Jätelaki* [online]. [cited 3.2.2007] Available from World Wide Web: http://www.finlex.fi/fi/laki/ajantasa/1993/19931072?search%5Btype%5D=pika&search%5Bpika%5D=j%C3%A4te

16. EDUSKUNNAN PÄÄTÖS 4.2.2000/86. *Ympäristönsuojelulaki* [online]. [cited 5.2.2007]. Available from World Wide Web: http://www.finlex.fi/fi/laki/ajantasa/2000/20000086?search%5Btype%5D=pika&search%5Bpika%5D=ymp%C3%A4rist%C3%B6nsuojelulaki

17. FEACHEM, RICHARD G. et al. *Sanitation and Disease: Health Aspects of Excreta and Wastewater Management* [online]. World Bank studies in water supply and sanitation 3. Chicester: John Wiley & Sons, 1983 [cited 10.2.2007]. Available from World Wide Web: http://www-wds.worldbank.org/servlet/WDSContentServer/WDSP/IB/1999/12/23//000178830_98101911180473/Rendered/PDF/multi0page.pdf

18. FINNISH ENVIRONMENT INSTITUTE. *Environmental dictionary EnDic* [online]. [cited 15.3.2007]. Available from World Wide Web: http://mot.kielikone.fi/mot/endic/netmot.exe?UI=ened&height=165

19. HEINONEN-TANSKI H., VAN WIJK-SIJBESMA C. *Human excreta for plant production* [online]. Bioresource Technology 96 (2005) 403-411 [cited 3.5.2006]. Available from World Wide Web: http://www.sciencedirect.com

20. HEINONEN-TANSKI, HELVI et al. *Pure human urine is a good fertiliser for cucumbers* [online]. Bioresource Technology 98 (2006) 214-217 [cited 15.2.2007]. Available from World Wide Web: http://www.sciencedirect.com

21. MAA- JA METSÄTALOUSMINISTERIÖ. *Kuivakäymälätuotteiden hyödyntäminen ravinnekierrossa* [online]. 9.1.2007. [cited 7.2.2007]. Available from World Wide Web: http://www.huussi.net/pdf/MMM_lausunto_2007.pdf

22. SOSIAALI- JA TERVEYSMINISTERIÖN ASETUS 461/2000. *Talousveden laatuvaatimukset ja valvontatutkimukset* [online]. Helsinki: 19.5.2000. [cited 10.2.2007] Available from World Wide Web: http://www.finlex.fi/fi/laki/alkup/2000/20000461

23. SOSIAALI- JA TERVEYSMINISTERIÖN PÄÄTÖS 41/1999. *Yleisten uimarantojen veden laatuvaatimuksista ja valvontatutkimuksista annetun sosiaali- ja terveysministeriön päätöksen muuttamisesta* [online]. Helsinki: 21.1.1999 [cited 10.2.2007]. Available from World Wide Web: http://www.finlex.fi/fi/laki/alkup/1999/19990041?search%5Btype%5D=pika&search%5Bpika%5D

24. STOCKHOLM ENVIRONMENT INSTITUTE. Ecological sanitation – revised and enlarged edition. 2004. ISBN 91-88714-98-5. Available from World Wide Web: http://www.ecosanres.org/pdf_files/Ecological_Sanitation_2004.pdf

25. SUOMEN YMPÄRISTÖKESKUS. *Voiko sakokaivolietettä levittää pellolle?* [online]. Kujala-Räty, Katriina, 17.12.2004. [cited 15.11.2006] Available from World Wide Web: http://www.ymparisto.fi/default.asp?contentid=110015&lan=fi

26. TERVEYSKIRJASTO. *Enterobacteriaceae* [online]. Duodecim. Helsinki: Kustannus Oy Duodecim, 2007 [cited 12.2.2007]. Available from World Wide Web: http://www.terveysportti.fi/terveyskirjasto/tk.koti?p_artikkeli=ltt00716

27. UNITED NATIONS. *UN Millennium Development Goals* [online]. 2005 [cited 27.1.2006] Available from World Wide Web: http://www.un.org/millenniumgoals/goals.html

28. VALTIONEUVOSTON ASETUS 542/2003. *Talousjätevesien käsittely vesihuoltolaitosten viemäriverkostojen ulkopuolisilla alueilla* [online]. Helsinki: , Ympäristöministeriö, 11.6.2003 [cited 10.2.2007]. Available from World Wide Web: http://www.finlex.fi/fi/laki/alkup/2003/20030542

29. VALTIONEUVOSTON PÄÄTÖS 282/1994. *Valtioneuvoston päätös puhdistamolietteen käytöstä maanviljelyksessä* [online]. Helsinki: Valtioneuvosto, 1994 [cited 3.2.2007]. Available from World Wide Web: http://www.finlex.fi/fi/laki/alkup/1994/19940282

30. VARSINAIS-SUOMEN AGENDA 21. *Haja-asutuksen jätevesien käsittely* [online]. 20.6.2005 [cited 20.2.2007].Available from World Wide Web: http://www.vsagendatoimisto.fi/vesiensuojelu/jatevesien_kasittely/jatevesien_kasittely.htm

31. WECKMAN, ANJA. *Ravinteet käymälästä peltoon* [online]. Luopioinen: 16.7.2005 [cited 10.2.2007]. Available from World Wide Web: http://www.huussi.net/tietoa/pdf/Anja_Weckman.pdf

APPENDIXES

Appendix 1. Greenhouse Diary

The growing experiment began in early November. On November 19, 2005 the lights and fan cooler arrived and were installed to the greenhouse. The fan cooler had a condensation tank that had to be manually emptied daily, but later, condensation waters was lead to a 1000 l plastic tank so that it was not necessary to empty a tank in the middle of an experiment. The fan cooler turned on, and on that same day, five brandling worms (Eisenia fetida) per crate were added to loosen substrate soil composition. Watering of 1 l per crate was done on top of the crates. The fan cooler thermostat was set to 17 ˚C.

A disinfection lotion VirkonS 1% was used for shoe sole disinfection, since it was assumed that salmonellae and other bacteria might be found in some crates due to use of human faeces as a fertilizer. The seed rows were covered lightly after sowing and substrate compacted. Lights were turned at night manually. The first signs of barley germination were noted.

11 November 2005 a RH hair hygrometer, temperature graphic plotter and temperature min-max-meter were ordered. The anniversary clock was set for a light period of 20 hours and a dark period of 4 hour, and the cooling fan thermostat was set to the optimum 15 ˚C. Four Dyno boxes were place on the greenhouse floor and filled with water to provide air moisture. One litre of water was used per crate. It was noted that barley shoots growing in the artificial fertilizer control substrates were up to 1 cm long

12 November 2005 barley germinating in all crates, with the strongest growth again noted in artificial fertilizer control substrates. Dew drops had appeared at the ends of barley shoots.

13 November 2005 carrot substrates still not germinating.

14 November 2005, 2.5 l of water was used per crate for carrot substrates fertilised with STS, composted human faeces and urine.

15 November 2005 all carrot substrates had begun germinating. Barley was watered 3 l per crate; carrots 4.5 l per crate for substrates fertilised with urine and composted human faeces, 2 l per crate for artificial fertilizer control and STS substrate. Thermostat was re-set to 13 ˚C because despite earlier thermostat adjustments the greenhouse temperatures were over 20 ˚C and even the minimum temperature measured was over 16 ˚C.

17 November 2005 lodging was noted in the barley. Barley substrates were watered 2.5 l per crate. Thinning was done and carrot crates fertilised with urine and STS were watered. All barley substrates except for the artificial fertilizer control substrate were watered again, 2.5 l per crate. The minimum temperature had risen to over 20 ˚C.

18 November 2005 carrots were watered, 2 l per crate. One bottle of watered down growth regulator was sprayed onto barley and later on the same day all substrates were watered ,2 l per crate.

19 November 2005 it was noted barley was recovering from lodging.

21 November 2005 fungi or mould growth was noted in substrates Carrot STS II, Barley Y3 II, Barley Y3 I, Barley Compost I, Barley Compost II and Barley Urine I.

22 November 2005 carrot substrates were watered. Carrot stems were observed to to be very weak nor were barley stems very strong.

23 November 2005 fungi growth was noted also in substrate Carrot STS II.

24, 26 and 28 November 2005 all crates were watered. 26 November 2005 watering to carrots was done between rows of seedlings, due to their weakness, by use of bottle. Barley substrates fertilised with composted human faeces seemed more sturdy since they did not lodge while watering with a watering can.

Thinning of carrots carried out 24–25 November 2005, and for barley, 28 November 2005.

29 November 2005 barley had lodged in all crates. Carrot Compost seemed quite stout, Carrot Kevät lodged slightly. Urine and STS fertilised substrates were showing matching growth rates. A growth regulation spray was sprayed to barley.

30 November 2005 all crates were watered 5 l per crate. Barley rows were assorted preliminary for their supporting element. Netting is set to support barley by heaving the seedlings through it.

1 December 2005 carrots were again singled and also mulched, except for substrates fertilised with STS.

2 December 2005 carrot crates were watered 1.5 l per crate except for crates with STS fertilised substrates. Watering was done between the sapling rows with a bottle. After Carrot STS crates were mulched on the same day they were watered with the same amount.

3 December 2005 the mulching was noted to have a clear effect. Carrot saplings looked stronger. All crates were watered with a watering can 3 l per crate. Barley crates were quite dry which could be expected. Netting had hindered barley from further lodging. Carrot substrates were noted to be quite moist and barley substrates were noted to be compacted quite hard.

7 December 2005 all crates were watered 3 l per crate with a watering can. Carrot lodged again which showed it was still too weak for watering with a watering can. Barley substrates fertilised with composted human faeces looked yellow which might be a sign of some deficiency.

8 December 2005 all crates were watered 3 l per crate. Barley substrates showed a significant difference in top soil hardness compared to carrot substrates.

9 December 2005 temperature was noted to be on the sharp rise as e.g. 2 December 2005 the temperature was measured at 21.8˚C (T_{digi}) yet on 9 December 2005 it was 28.8˚C (T_{digi}). Light period was changed to a 19 h light period and 5 h without lights. The compressor's cooling pipe was insulated. All crates were watered 3 l per crate.

12 December 2005 the condense container overflowed due to blockage in the drain pipe. The greenhouse was aired for a couple of hours by leaving the greenhouse door open, in order to combat the rise in temperature. Additional watering was given to substrates Carrot STS 1 & 2 and Carrot Urine 1 & 2 because they seemed dry. Process hall ventilation intake was shut and extraction set on, in order to bring the process hall temperature under control. Greenhouse temperature was still 24 ˚C which was considered too high.

13 December 2005 the process hall seemed much cooler yet this did not have a notable effect on greenhouse temperature. It was decided to leave the greenhouse open except for during the time measurements are being taken, until a change in temperature was achieved.

14 December 2005 all crates were watered 2.5 l per crate, 16 December 2005 5 l per crate by underground irrigation. 17 December 2005 again 5 l per crate watering.

19 December 2005 crates Barley Y3 1 & 2, Barley Urine 1 & 2, Barley STS 1 & 2, Carrot Compost 2, Carrot Kevät 1 & 2 were dry from below. Door was closed as temperature had dropped a bit. Barley STS and Barley Urine were noted to be developing ears (sheaves).The condensing container was emptied before the Christmas holidays. 23 December 2005 all crates were watered 5 l per crate by underground irrigation. Additional watering was given to barley 2 l per crate, for carrot Kevät and carrot Compost 1.5 l per crate. Carrot Urine and Carrot STS were moist and thus left without watering.

27 December 2005 all barley crates were watered 2.5 l per crate. All substrates were noted to be in ear. Carrot Urine 1, Carrot STS 1 and STS 2 were noted to have fungi growth. Barley Compost was seen as the palest and the shortest of substrates. Carrot STS and Urine were on that date growing little or no roots and appeared to be stunted. Carrot urine was so moist that water could be seen in in the under drains. Battery changed for digital Vaisala RH meter. It was decided to keep the $T_{substrate}$ and Vaisala RH meters outside the greenhouse so that high level of air humidity could not damage them.

28 December 2005 all substrates appeared moist. In both Carrot Urine substrates water was found in the under drains. Thinning Carrot Compost and Carrot Kevät began.

30 December 2005 Barley Urine was watered 3 l per crate. Carrots were still moist and thus their moisture should be monitored for some time yet.

2 January 2006 all barley crates were watered 3 l per crate. Carrot Urine 2 was left without watering for the crate showed water in under drains. For other carrot substrates watering was 1.5 l per crate.

4 January 2006 all barley crates were watered 3 l per crate and carrot crates 1.5 l per crate.

8 January 2006 the greenhouse door was shut since temperatures had been rising again.The opened door was thought to be a possible reason for this.

9 January 2006 all crates were watered 5 l per crate.

12 January 2006 barley crates Y3 1 & 2 were noted to be clearly more lodged.

13 January 2006 all crates were watered 2 l per crate.

17 January 2006 the majority of barley was harvested. Some quantity of barley was left in all substrates to continue to grow so that Launokorpi could take samples for later microbial analysis.

18 January 2006 humidity was noted to have dropped severely due to barley harvest. 19 January 2006 the remainder of the barley was harvested. All carrot crates were watered 3 l per crate.

20 January 2006 carrots were harvested except for those left for Launokorpi to use for later sampling. The growing experiment had now concluded.

Appendix 2. Results.

SUB Nov 9-10 2005
CARROT

dilution	1:10/A	1:10/B	1:100/A	1:100/B	1:1000/A	1:1000/B	1:10000/A	1:10000/B
fertilizer I	90	60	300	0	0	0	0	0
fertilizer II	480	280	200	300	0	0	0	0
urine I	340	240	0	0	0	0	0	0
urine II	320	370	400	100	0	0	0	0
CF I	400	470	400	500	0	0	0	0
CF II	2170	1590	1100	200	2000	1000	10000	0
STS I					720000		2900000	2730000
STS II			66000	40000	333000	289000	350000	560000

SUB Nov 22-23 2005
CARROT

dilution	1:10/A	1:100/A	1:100/B	1:1000/A	1:1000/B	1:10000/A	1:10000/B
fertilizer I	1270	600	1200	1000	1000	0	0
fertilizer II	0	37000	14500	56000	45000	60000	20000
urine I	100	100	0	0	1000	0	0
urine II	30	0	500	0	0	0	0
CF I	120	0	100	0	0	0	0
CF II	100	400	100	0	1000	0	0
STS I	7500	11100		9000	6000	10000	30000
STS II	7400	15600	17200	16000	17000	40000	0

SUB Jan 24-25 2006
CARROT

dilution	1:10/A	1:10/B	1:100/A	1:100/B	1:1000/A	1:1000/B	1:10000/A	1:10000/B
fertilizer I	12390	12250	18100	20000	8000	8000	10000	0
fertilizer II	7520	8050	10500	11800	13000	13000	20000	20000
urine I	180	160	200	100	0	0	0	0
urine II	1140	1600	1700	2300	3000	3000	0	10000
CF I	1020	1000	1700	1100	2000	2000	0	10000
CF II	210	170	0	0	0	0	0	0
STS I	370	350	100	100	0	0	0	0
STS II	240	190	400	200	0	0	0	0

CARROT Feb 17 2006

dilution	1:10/A	1:10/B	1:100/A	1:100/B	1:1000/A	1:1000/B	1:10000/A	1:10000/B
fertilizer I	180	500	700	1300	1000	1000	0	0
fertilizer II	10	190	100	400	0	0	0	0
urine I	220	320	62000	52000	15000	13000	40000	10000
urine II	69900	69200	4800	5900	9000	6000	0	0
CF I	50	70	0	0	0	0	0	0
CF II	6300	4250	14500	14400	9000	16000	10000	0
STS I	13700	13170	14100	15600	23000	22000	10000	30000
STS II	4160	4370	13500	13200	45000	45000	80000	70000

SUB Nov 9-10 2005
BARLEY

dilution	1:10/A	1:10/B	1:100/A	1:100/B	1:1000/A	1:1000/B	1:10000/A	1:10000/B
fertilizer I	300	220	1500	1300	126000	143000	160000	100000
fertilizer II	430	450	0	4100	16000	13000	0	0
urine I	740	560	500	200	0	0	0	0
urine II	7840	7120	19100	14300	87000	101000	80000	20000
CF I	800	640	2400	2100	4000	3000	10000	10000
CF II	1500	1670	2600	3300	6000	20000	0	0
STS I	ovd	ovd	ovd	ovd	602000	828000	1640000	1440000
STS II	ovd	98800	103200	94400	736000	572000	1300000	880000

SUB Nov 22-23 2005
BARLEY

dilution	1:10/A	1:100/A	1:100/B	1:1000/A	1:1000/B	1:10000/A	1:10000/B
fertilizer I	2720	2700	2100	1000	3000	0	10000
fertilizer II	3190	9300	11100	5000	8000	30000	20000
urine I	430	400	300	1000	0	0	10000
urine II	470	200	100	1000	1000	0	0
CF I	610	500	600	0	1000	0	0
CF II	530	1300	1000	3000	0	0	0
STS I	8700	21100	19600	25000	22000	40000	20000
STS II	6020	19300	20000	21000	25000	10000	20000

SUB Jan 24-25 2006
BARLEY

dilution	1:10/A	1:10/B	1:100/A	1:100/B	1:1000/A	1:1000/B	1:10000/A	1:10000/B
fertilizer I	1780	1790	2500	2200	3000	1000	0	0
fertilizer II	380	280	700	100	2000	6000	10000	0
urine I	320	330	300	0	0	0	0	0
urine II	80	70	100	100	0	0	0	0
CF I	480	280	100	200	0	2000	0	0
CF II	20	30	0	0	0	0	0	0
STS I	16800	18400	53300	57900	73000	51000	50000	80000
STS II	700	670	600	600	1000	0	0	0

BARLEY Jan 19 2006

dilution	1:10/A	1:10/B	1:100/A	1:100/B	1:1000/A	1:1000/B	1:10000/A
fertilizer I	30	0	0	0	0	0	0
fertilizer II	20	10	100	0	0	0	0
urine I	0	0	0	0	0	0	0
urine II	20	10	0	0	0	0	0
CF I	0	0	0	0	0	0	0
CF II	20	10	0	0	0	0	0
STS I	10	10	0	0	0	0	0
STS II	10	30	0	0	0	0	0

Appendix 3. Instructions of Compact Dry CF plates.
Can be obtained by contacting hanna.launokorpi(a)gmail.com

Appendix 4. Instructions of Compact Dry SL plates.
Can be obtained by contacting hanna.launokorpi(a)gmail.com.

Appendix 5. Results from AnalyCen.
Can be obtained by contacting hanna.launokorpi(a)gmail.com.