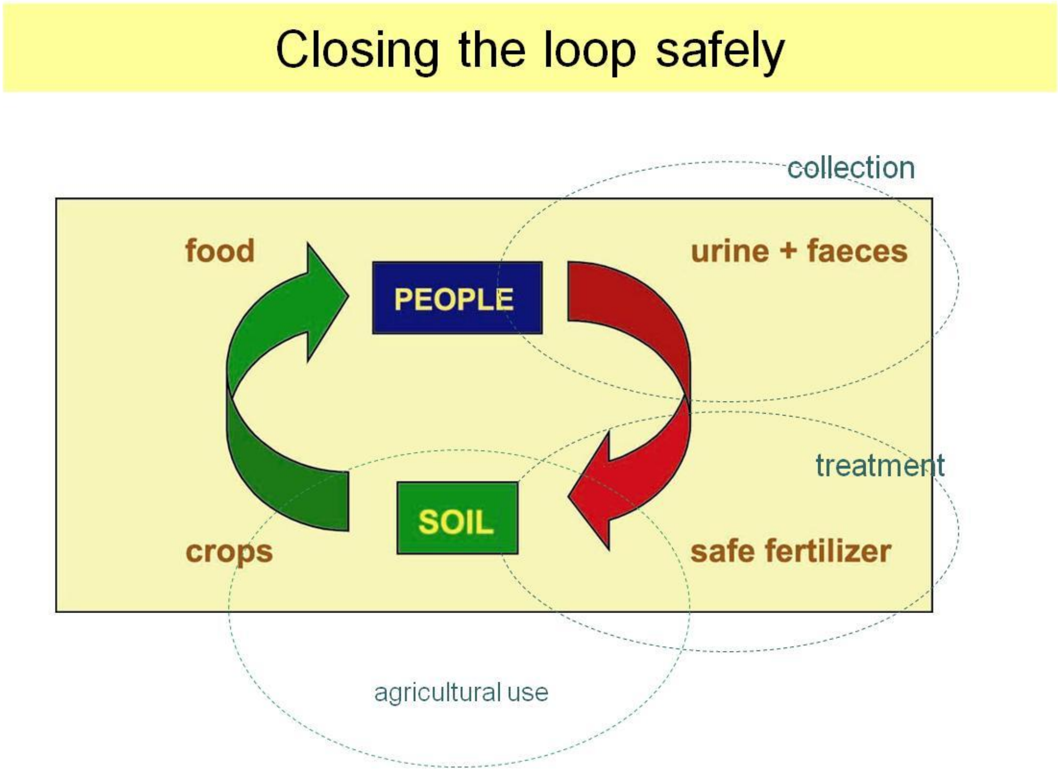
3.3 Pathogen reduction

How persistent are pathogens in the environment?

How can we prevent exposure and disease transmission in sanitation systems?

Learning objective: To know and be familiar with the behaviour of pathogens in the environment, including the effects of treatment and the potential of minimising disease transmission by other barriers, especially in relation to agricultural use of excreta.

Pathogens are by nature present in all flows in sanitation systems. To protect water sources and our close environment, collection and treatment of the various waste streams like greywater, urine and faeces is necessary. When the waste fractions are used – or ”reused” – as a resource in agriculture, new transmission routes for pathogens may be introduced. To minimize the risk for disease transmission treatment and other barriers that prevent humans and animals from exposure to pathogens need to be included in the management of the system. This module deals with potential reduction and survival of pathogens in the environment and what type of barriers that can be used to decrease health risks in sanitation systems where the waste fractions are aimed for agricultural use. The module is built on discussion of scientific theories with the purpose to give an understanding of the possibilities to utilize different barriers to reduce health risks. More practical information on how to treat excreta is included in chapter 4 and in module 3.4 and 3.5 further descriptions of how barriers are used in a systematic way in guidelines for reuse of excreta are included.



By closing the loop of nutrients, that is using, or reusing urine and faeces in agriculture, we can further improve health by increasing the food production and improving the nutritional status, which also makes people less susceptible to infectious diseases. However, it needs to be done safely which includes safe collection of the excreta, treatment (before use) and proper use of the products. Thus, the purpose is to close the loop – but when it comes to pathogenic microorganisms the loops representing transmission routes need to be broken!

Proper and optimal use of excreta and greywater depending on other aspects, such as utilization of nutrients and choice of crop is covered in chapter 4.

Transmission of infectious disease during reuse

- Mexico, untreated wastewater: 33% higher risk of diarrhoeal diseases (Cifuentes et al. 1998)
- Israel (kibbutz), partially treated stabilization pond effluent: twofold excess risk of enteric disease in 0-4 year-old age group (Fattal et al. 1986)
- No recorded incidents associated with ”appropriately treated” wastewater (Cooper & Olivieri 1998)
- National Research Council (NRC, USA, 2000) evaluated 23 studies: no proof for either risk or non-risk for reuse of sewage sludge
- Risk assessments a valuable tool

Then, how high are the risks when using excreta or other waste products in agriculture? How hygienic risks are perceived varies, between regions of the world and between individuals and it is not possible to give exact answers. The relation between use of waste products and possible enteric disease is difficult to establish by epidemiological methods, but some studies have been done. The examples listed here gave the following conclusions:

In Mexico, children from households that irrigated with untreated wastewater had a higher prevalence of diarrhoeal disease. Compared to children that lived in areas where untreated wastewater NOT was utilised (”rainfall villages”), the risk for diarrhoeal disease was 33% higher (http://pdfserve.informaworld.com/216153_731211568_713671506.pdf). The use of partially treated wastewater in Israel also resulted in increased risk of enteric disease among children. In the study from 1986 a twofold excess risk was reported. With more advanced treated wastewater, no incidents have however been reported according to a previous summary. Regarding use of sewage sludge, the National Research Council in the USA evaluated a number of studies where individuals were exposed to sludge and concluded that no proof for either risk or non-risk could be defined. The NRC further stated the risks need to be further evaluated.

Since excreta and other waste products always contain pathogens, there is a potential risk for disease transmission in a system where the products are handled and reused. These risks can however be managed by limiting the exposure as is explored further on in this chapter.

As a complement to epidemiological method, risk assessments may be a valuable tool that is further presented in module 3.4 and 3.5.

Parameters affecting microbial survival in the environment

Temperature	Low temperature prolong survival. Inactivation - >40°C, treatment processes 55-65°C.
pH	Neutral pH (7) beneficial. Inactivation - highly acidic or alkaline conditions.
Moisture	Mositure (e.g. in soil) favours the survival. Inactivation – drying.
Solar radiation/ UV-light	Inactivation – by natural solar radiation or UV-lamps.
Other microorganisms	Longer survival in sterile material. Inactivation – competition and predation.
Ammonia	Often affects microorganisms negatively. Inactivation – ammonia produced at high pH.
Nutrients	Needed for growth of bacteria. Inactivation – lack of nutrients.
Other factors	Oxygen availability, chemical compounds.

How the pathogens survive in the environment is crucial for the risk for further transmission. This table lists some of the parameters important for survival of microorganisms.

Temperature – Even if human pathogens are adapted to the body temperature of 37°C, they can be favoured by other temperatures in the environment. A lower temperature (but above 0°C) generally prolongs survival. Inactivation is faster the higher the temperature is, and for effective treatment in e.g. composts, thermophilic temperatures (>50°C) are wanted, and sometimes required in legislation. (Bacterial spores require 120°C, sterilisation, to be killed)

pH – most pathogenic microorganisms are adapted to a neutral pH (7) and can potentially be killed by a (significantly) higher (alkaline) or lower (acidic) pH.

Moisture – living organisms requires moisture for ther survival so drying material (like soil or faeces) will have a negative effect on the pathogens. (Some life stages of parasitic protozoa and helminthes can be quite resistant to drying, e.g. Ascaris requires <5% humidity).

Solar radiation and UV-light is also a natural factor that can kill pathogens. It is also used technically in water treatment systems.

Microorganisms will affect each other by predation and competition and the survival of a pathogen in sterile water will survive longer than if there are other organisms around.

Ammonia is a factor that can have an important role in treating waste such as sewage sludge or faeces. The ammonia generated at high pH by treatment with lime or urea (see chapter 4) can kill pathogens.

Nutrients – the availability can have an effect on the survival of bacteria since this group of organisms can grow in the environment, i.e. bacteria can also starve and be inactivated (killed or in a dormant spore stage). Other factors such as oxygen or different chemical compounds can affect pathogens negatively.

Inactivation of microorganisms
- How can we kill pathogens?

- Eventual die-off outside the body
- Persistence varies depending on type
- Bacteria may grow in the environment
- Helminth eggs require latency period
- Natural conditions will affect inactivation
  - temperature, moisture, competing microflora, etc.
- Alter the conditions to increase the rate
  - temperature, pH, moisture, etc.

But difficult to state exact *time-parameter* limits for elimination of each (all) pathogens

So what will happen to the pathogens after excretion? Well, since they are not as fit for a life outside the host there will eventually be a die-off in the environment. How long the inactivation will take depends on type of microorganisms; the all have varying sensitivity to environmental factors. Bacteria can under favourable conditions actually grow, but that is not the case for the other groups. Some helminth eggs require a latency period e.g. in soil before being infective. Inactivation will depend on the conditions where some of the most important parameters are temperature and moisture (see next slide). It is possible to alter these conditions to increase the inactivation rate but it is difficult to state the exact time it will take for a ”total” inactivation at a specific condition. The inactivation is crucial in treatment of excreta and other organic wastes.

Estimated survival times

for microorganisms in faeces, sludge, soil and on crop (according to Faechem[a] 1983 and Kowan[b] 1985, in EPA 1999), in days if not other stated

Microorganism	Faeces and sludge[a] 20-30°C	Soil[a] 20-30°C	Soil[b] absolute max[c]/ normal max	Crop[a] 20-30°C	Crop[b] absolute max[c]/ normal max
Bacteria			1 år/2 månader		6 months /1 month
Faecal coliforms	<90 normally <50	<70 normally <20		<30 normally <15	
Salmonella	<60 normally <30	<70 normally <20		<30 normally <15	
Virus	<100 normally <20	<100 normally <20	1 year/3 months	<60 normally <15	2 months /1 month
Protozoa[d] (Amoeba)	<30 normally <15	<20 normally <10	10/2	<10 normally <2	5/2
Helminths (egg)	several months	several months	7 year/2 år	<60 normally <30	5 months /1 month

[c] Absolute maximum times for survival are possible during unusaual conditions, such as at constant low temperature or at externely protected conditions.

[d] Data is missing for Giardia and Cryptosporidium. Their cysts and oocysts, respectively, probably survive longer than wat is stated here for protozoa.

Earlier literature often states survival times of indicator bacteria and pathogens as ”total inactivation”. This table summarizes some commonly used references and illustrates the variation between different groups of organisms. It is possible to see that helminthes often have a long survival compared to the other microorganisms. Even if bacteria like Salmonella is stated to have a survival of days it is possible to find studies that found these bacteria in soil after a number of years!

For a full inactivation of pathogens in soil, sludge or faeces a long time (years) is needed. Even if a 100% removal is not necessary or expected, treatment methods are used to speed up the inactivation of pathogens. No treatment except sterilization however kills all the organisms. A zero risk is therefore not obtained, and can thus not be the goal for systems with use of organic waste as fertilizers. It should also be added that in commonly used laboratory methods to detect and enumerate microorganisms, it is not possible to measure a total inactivation. There is a limitation in the reduction that can be measured before and after a time period has passed (e.g. during storage of faeces) and there is always a detection limit for the method used, that is you don´t analyse 0 organisms, but rather < (less than) 1 or 10 per ml or per gram (numbers given as examples). Thus a sample cannot be said to contain 0 organisms, and consequently a total inactivation cannot be achieved in practice, only in theory.

Inactivation of microorganisms in faeces

Organism to be modelled	4°C/low temp range	20°C/high temp range
E.coli*	T_{90} = 70-100 days	T_{90} = 15-35 days
Enterococci*	T_{90} = 100-200 days	Same as 4°C
Bacteriophages	T_{90} = 20-200 days	T_{90} = 10-100 days
Salmonella*	T_{90} = 10-50 days	
EHEC*	T_{90} = 10-30 days	Same as 4°C
Rotavirus	conservative model – no reduction T_{90} = 100-300 days	T_{90} = 20-100 days
Giardia	T_{90} = 15-100 days	T_{90} = 5-50 days
Cryptosporidium	T_{90} = 30-200 days	T_{90} = 20-120 days
Ascaris	T_{90} = 100-400 days	T_{90} = 50-200 days

*Possible growth not taken into consideration (Arnbjerg-Nielsen et al. 2005)

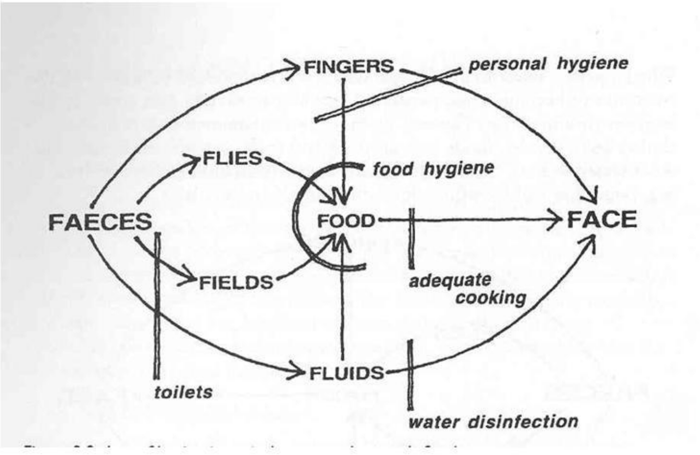
To more specifically describe pathogen inactivation (and estimate risks), so called T90-values are often used. That is the time required for inactivation of 90% (or 1 $\log_{10}$) of the microorganisms. The survival times given in this table build on a literature survey of survival experiments performed in faeces and other similar material as manure and sludge. The studies of inactivation in faeces are few and other studies needed to be considered in order to estimate the T90-values (that later was used in a risk assessment- see module 3.5). As can be seen, the results from different studies vary considerably and the variation between different organisms is large. In chapter 4, newer results from inactivation studies are presented to give a more practical view on how to treat excreta and you can also find explanations on how inactivation can be calculated and expressed, especially in relation to temperature.

Barriers

- To prevent and decrease disease transmission
  - Reduction of pathogens
  - Hindering actual exposure to the pathogen-containing material
- In analogy with different steps in e.g. drinking water treatment
- Health protection measures (WHO terminology)
  - Technical, behavioural, medical, etc.

To prevent disease transmission we can talk about barriers. One example can be different treatment steps for drinking water, e.g. filtration and disinfection. For waste products we can also talk about treatment as one barrier and other measures to limit exposures to individuals as other barriers. Barriers thus aim to reduce the exposure to pathogens, and thus decrease the risk for infection. The exposure is decreased either by an actual reduction of pathogens in the material (the human waste, the organic fertilizer or the crop) or by actually hindering people (and animals) to come in contact with the material. A wider definition used by the WHO is health protection measures that also include aspects such as chemotherapy and immunization, and health and hygiene promotion to decrease the risk for infection.

Barriers required to prevent the spread of pathogens



(Esrey et al. 1998)

Returning to the F-diagram presented in module 3.2, barriers to prevent the spread of pathogens from faeces include the following:

Toilets – the use of **toilets** to collect the **faeces** is a large improvement compared to the practice of open defecation, that may occur in **fields** (where **food** is produced) and nearby streams, contaminating **fluids** (water).

Water disinfection – is practiced in all large scale water production but can also be applied in the small scale. However, it is not easy to find a chemical or a filter that removes all pathogens that can be used easily. To boil unclean water before drinking it is therefore a common practice. It is important that water is stored in a safe way so that further contamination is avoided.

Personal hygiene – to wash hands is a simple measure to improve the health situation if water is available. It prevents the transfer of pathogens from **faeces** or the environment to the **food** or directly to your **face** (mouth, nose, eyes). Some pathogens are sensitive to alcohol gel whereas others are not and general handwashing, preferably with soap, is more effective.

Adequate cooking – by heating the food it is possible to kill pathogens present in the food. If a toxin has been produced by bacteria, it is however not possible to remove it by heating the food. This type of contamination and disease, caused either by infection or a toxic reaction, is generally referred to as food poisoning. Cooking also has little or no impact on the concentrations of toxic chemicals that might be present.

In practice, some communities rely on a barrier late in the food chain. For example, in China, it is an old tradition to use faecal material in crop production. It has then been the normal practice to heat all vegetables before they are consumed, i.e. they are not consumed raw as in many other countries. (Still there is/has been a high prevalence of helminth infections in (parts of) the population.)

The ideal situation is to apply a set of barriers, since no barriers are safe on their own, i.e. a failure in one barrier may occur and thus relying on more than one barrier will add in safety (see also module 3.4).

Treatment as a barrier

Treatment as a barrier
A combination of barriers to decrease exposure of humans to excreta should be applied in order to reduce risks for disease transmission in ecological sanitation systems. Treatment of the excreta is considered as a necessary step for the subsequent use as fertiliser on (agricultural) land.

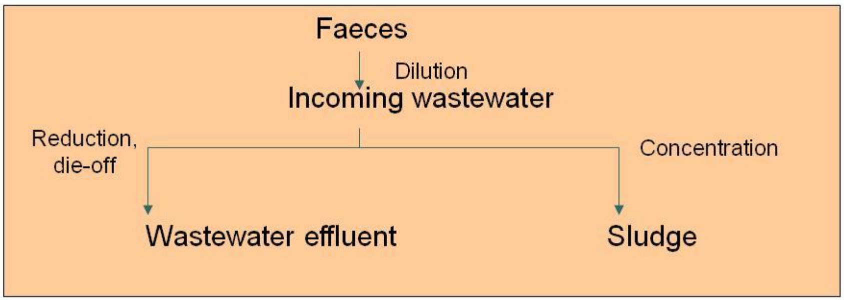
(EcoSanRes, 2004)

- The goal is to significantly reduce risks – zero risk is not possible
  - ”Minimise” risks (considering viable/practical/realistic measures)
  - Insignificant amounts of pathogens
  - No additional individuals diseased

So, as stated: To reduce the risks from using excreta a combination of barriers are recommended. We see treatment as a main barrier in sustainable sanitation systems. The goal is to significantly reduce the overall risk, and it can also be expressed as minimizing risks or decreasing the number of pathogens to insignificant levels or as that no additional cases of disease should occur from this practice. As previously described, a total inactivation of pathogens is not achievable and it is not viable to aim at a zero risk for a sanitation system.

Wastewater treatment

- Treatment steps - barriers
- Microorganisms generally reduced 70-99,99% in STP (Sweden)
- Not optimised for pathogen removal
- Generally no regulations on outgoing (treated) wastewater
- Disinfection efficient, but other problems
- Limit exposure from outlet important
- Sewage sludge – concentration of pathogens



STP = Sewage treatment plant

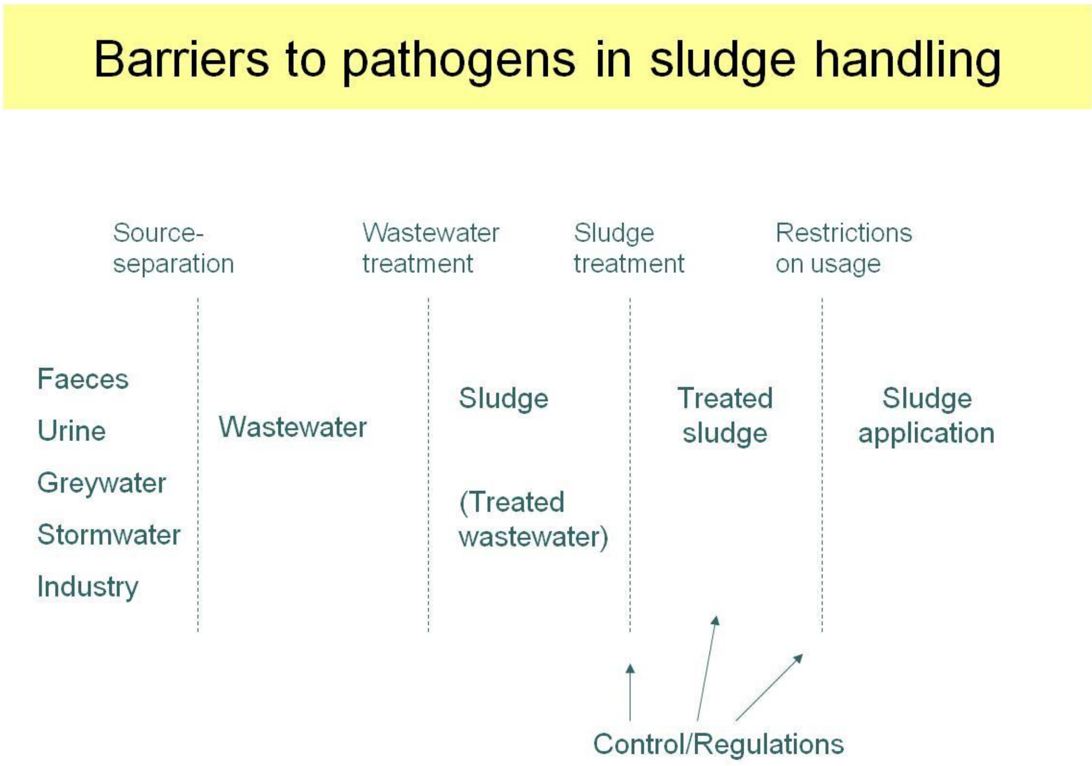
In wastewater treatment the different steps in the treatment plant can be seen as barriers. However, the treatment is not optimized for pathogen removal and basically has other primary functions such as reduction of solids and chemicals. It is only a final disinfection step that is aimed at pathogen reduction. In many countries disinfection is not included (if the wastewater is treated at all!) and therefore it is important to include a barrier in the disposal, that is preventing people and animals to come in contact with the outgoing wastewater by choosing a proper point of discharge. During such circumstances part of the pollution problem may also be solved by dilution that is for example discharge in the deep sea far from beaches or recreational areas.

Expected removal (log_{10}) of microorganisms in various wastewater treatments

Process	Bacteria	Helminths	Viruses	Cysts
Primary sedimintation				
Plain	0-1	0-2	0-1	0-1
Chemically assisted	1-2	1-3	0-1	0-1
Activated sludge	0-2	0-2	0-1	0-1
Biofiltration	0-2	0-2	0-1	0-1
Aerated lagoon	1-2	1-3	1-2	0-1
Oxidation ditch	1-2	0-2	1-2	0-1
Disinfection	2-6	0-1	0-4	0-3
Waste stabilization ponds	1-6	1-3	1-4	1-4
Effluent storage reservoirs	1-6	1-3	1-4	1-4

- Large variations, depend on organism, difficult to predict

This table shows approximate log_{10} removal of various types of microorganisms in different wastewater treatments. The removal will be due to both actual die-off but also removal by e.g. sedimentation processes. As can be seen, also low-tech systems such as waste stabilization ponds can result in high removal of pathogens. Treatment systems like this are further described in chapter 4 in relation to greywater treatment. Disinfection is often done by chlorination which is most efficient to reduce bacteria. Some cysts (or oocysts) like Cryptosporidium are however very persistent to chlorine.



Another example of barriers is in relation to sludge handling, where sewage sludge can be compared to other types of organic wastes. Treatment and restrictions for use is here seen as the main barriers to limit exposure of pathogens to humans and animals. It is also here we have the possibility to control or regulate the handling and use (further discussed in module 3.4).

Greywater treatment

- Treatment to remove grease, N, P, chemicals….and pathogens (see chapter 4)
- Treatment results - great variation
- Need dependent on use
- Specific risks related to use
  - Irrigation, subsurface
  - Treatment in ponds – limit exposure
  - Infiltration, drinking water
- Handling to avoid smell

Greywater contains a range of contaminants, among them pathogens as described in module 3.2. Treatment is needed, but the type of treatment is much dependent on constitution of the greywater and subsequent use. Initially handling and treatment must be conducted in order to avoid smell, which may be caused by anaerobic conditions. Results from studies of greywater treatment show a great variation and other barriers to prevent exposure is also needed. Specific risks are related to irrigation where irrigation methods are important barriers in combination with treatment, ponds where the treatment facility itself constitutes a risk both from a hygienic perspective and due to accidents (e.g. falling into the pond). If used for groundwater recharge and subsequent drinking water production, the infiltration process need to remove pathogens sufficiently, which is related to the specific field of hydrogeology. Greywater systems and treatment are widely covered in chapter 4.

Treatment of faeces is as stated a crucial part of sanitation systems.

For dry systems mainly, it is valid to use the terminology of primary and secondary treatment (that however should not be mixed with the terminology used in large-scale wastewater treatment plants). The primary treatment occurs in the toilet and mostly depends on the toilet construction and habits of the users, e.g. if they add some material to cover the faeces. If for example desiccation or a pH-elevation occur, this ”treatment” can reduce risks in the subsequent handling of the faecal material.

The secondary treatment on the other hand should result in a significant reduction of pathogens. If considering dry collection of faeces or collection of blackwater in a tank this treatment is done after finished collection and can be performed off-site or on-site, which is often dependent on scale (and if the site is rural or urban). For example on the small scale the faecal material is further treated by (long-term) storage in one of the toilet chambers, and the other one is used for the next collection period. What methods that can be applied or if they need to be adapted will partly depend on the primary treatment.

Treatment of faeces

- Storage
  - Ambient conditions
- Biological methods
  - Composting (heat, microbial competition, pH-changes)
  - Anaerobic digestion (heat, microbial competition, pH-changes)
- Chemical treatment
  - Alkaline treatment
    - Ash, lime (pH-elevation and desiccation)
    - Urea (ammonia)
- Incineration

To give an overview of possible methods for treating faeces the following categorization can be made. In chapter 4, details and practical advice on how to perform the treatment is given.

Storage can be considered the simplest method of treatment. The material is preferably contained so that exposure is minimized and seepage is not transported from the material to the surroundings. Exposure should also be minimized by choosing a proper place for the storage and the material should be covered. Since storage is done at ambient conditions the reduction of pathogens will vary tremendously, and it is considered the least reliable method.

Biological treatment is composting and anaerobic digestion that both mainly rely on an increase in temperature to reduce pathogens, as described in chapter 4.

Chemical treatment involves the addition of a chemical, either from a ”natural” source such as wood ash or like pure urea. The increase in pH and production of ammonia that occurs in the controlled type of urea treatment (ammonia treatment) is effective in reducing pathogens. Lime treatment can also be performed on a larger scale, but is probably more common for sludge treatment, and can result in a significantly elevated pH (pH 12). The addition of ash on the other hand is generally used in the small scale, where some elevation in pH and desiccation results in pathogen inactivation. The mixing of chemicals with the faecal material is a crucial point.

Incineration of faeces is also possible but not commonly used since the material often is too moist (see chapter 4).

Urine diversion in dry sanitation systems

- Will result in (compared to mixing of faeces and urine):
  - Less smell
  - Less volume (slower filling up, less to handle)
  - Prevention of dispersal of pathogen-containing material (spilling, leaching)
  - Safer and easier handling and use of excreta (volume, treatment)

  → Less risk for disease transmission

- Urine diversion is recommended

By diverting urine from faeces in dry toilets several benefits can be obtained. There will generally be less smell, there will be smaller volumes to handle and the collection chamber will not fill up as quickly. Since the remaining material will be drier there will be less risk of spilling and leaching to groundwater and it can facilitate the further treatment. All this factors can contribute to a reduction of the risk for disease transmission. It is therefore possible to advocate for the implementation of urine diversion, and that is even if the urine is not reused.

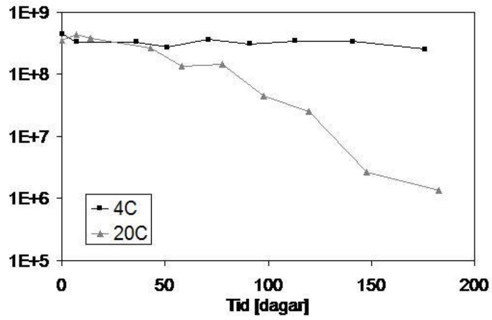
Diversion of urine can also have large benefits in pipe-bound and water-flush systems.

Survival of microorganisms in human urine

Organism group (ex.)		**Survival**
Bacteria (*Salmonella*, *E. coli*)	-	Short (T_{90} = days)
Protozoa (*Cryptosporidium*)	-	Average (T_{90} = ~1 month)
Virus (rotavirus, bacteriophage)	-	Long (no reduction at 4°C, T_{90} = ~ 1-2 months at 20°C)

Factors that increase die-off

- elevated pH (7 → 9, urea → ammonia)
- higher temperature
- lower dilution



If enteric pathogens end up in the urine by cross-contamination, the following question is what will happen with them and if they will imply a risk. Research on survival of pathogens in urine during storage has therefore been conducted.

Bacteria were inactivated within days. Protozoa, represented by *Cryptosporidium* had a T90 of one month whereas viruses were the most persistent organisms with no reduction at 4°C, and a T90 of 1-2 months at 20°C.

Factors that contribute or increase the inactivation is the pH that generally increase to around 9 after transport also through short pipes

The temperatures investigated corresponded to minimum and maximum temperatures in a Northern European climate. Since a higher temperature generally results in a faster inactivation it is likely that T90-values will be lower (shorter time needed) in tropical climates.

In Höglund (2001) Evaluation of microbial health risks associated with the reuse of source-separated humna urine (http://urn.kb.se/resolve?urn=urn:nbn:se:kth:diva-3090), details on the inactivation of pathogens in urine is given.

Storage of urine

- The most appropriate treatment method (?)
- Other methods tried out in order to reduce the volume
  - Easier handling for agricultural use
- Storage with low air exchange (tight containers) best method to keep the nutrients in urine
- Only necessary in large-scale systems
- Existing guidelines in module 3.4

Storage is at present seen as the most viable method for treatment of urine. Other methods have been tried, but more for the reason to produce a fertilizer product that is easier to handle. However, if for example drying the urine most of the nitrogen is lost. Since it is a well balanced fertilizer it is considered most resource efficient to keep the urine as it is. Storage for hygienic reasons is only considered necessary in large-scale systems. On the household level the urine may be used directly, since the risk for disease transmission is considered low.

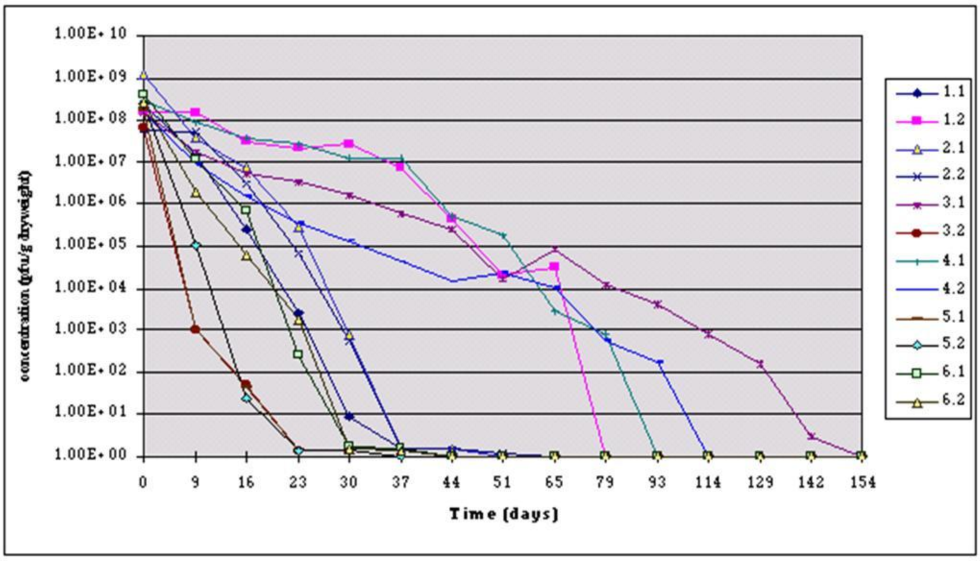
An example of a risk assessment for reuse of urine and existing guidelines are included in module 3.4. The agricultural use of urine is discussed in module 4.8.

Survival study –latrines in Vietnam
(Carlander & Westrell 1999)

- 12 double-vault latrines (different design)
- Ascaris and bacteriophage (model for virus) added to the material
- Study the effect of pH, temperature and moisture

In Vietnam, the double-vault dry latrine traditionally has been used in some rural areas. In a research study pathogen reduction was investigated by following the concentration of added Ascaris eggs and Salmonella bacteriophages (viruses that infect bacteria, in this case the Salmonella strain *salmonella typhimurium* 28B) during 6 months.

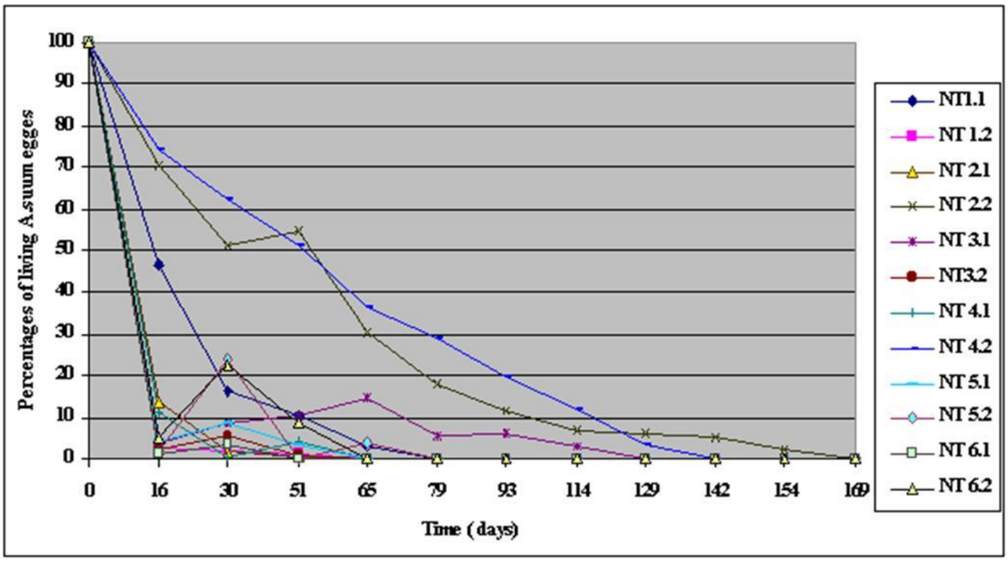
Reduction of *Salmonella typhimurium* phage 28B



(Carlander & Westrell 1999)

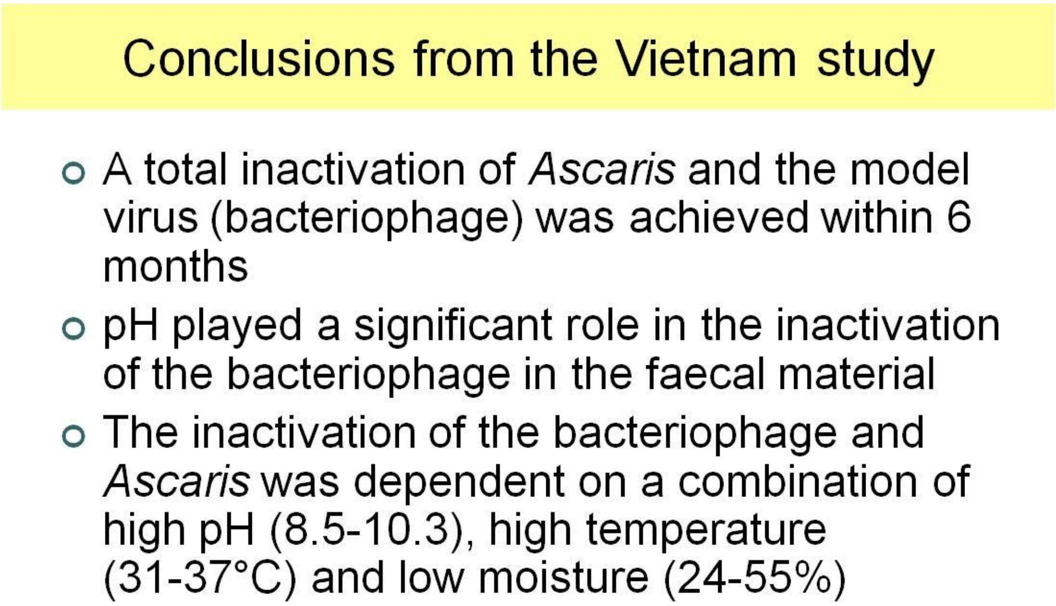
As can be seen the phages were reduced to below the detection level in about 1-2 months in some toilets, whereas they survived up to 6 months in one of the others.

Reduction of *Ascaris suum* eggs



(Carlander & Westrell 1999)

Looking at Ascaris, the situation was somewhat similar with more rapid inactivation in several of the toilet vaults, and prolonged survival in a few vaults.

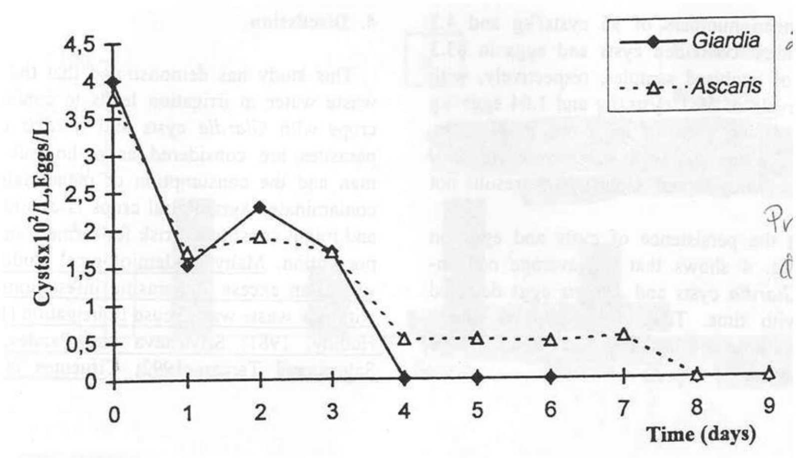


Three environmental parameters were measured in the study – pH, temperature and moisture. By statistical analysis it could be concluded that:

A total inactivation of *Ascaris* and the model virus (bacteriophage) was achieved within 6 months.

pH played a significant role in the inactivation of the bacteriophage in the faecal material and that the inactivation of the bacteriophage and *Ascaris* was dependent on a combination of high pH (8.5-10.3), high temperature (31-37°C) and low moisture (24-55%). It was thus not possible to separate the importance of the different factors on pathogen survival.

Inactivation on crops



Inactivation of Giardia and Ascaris on coriander leaves

Inactivation on crops is an important barrier for transmission of disease by food consumption. This example shows a quite rapid inactivation of *Giardia* (4 log reduction in 4 days) and of Ascaris (4 log reduction in 8 days) on coriander leaves.