# Microbiological investigations of rainwater and graywater collected for toilet flushing

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**Abstract** Seven Danish rainwater systems were investigated with respect to the microbial water quality. The general microbiological quality (total numbers of bacteria (AODC)), and heterotrophic plate counts on R2A and Plate Count Agar in the toilets supplied with rainwater were approximately the same as in the reference toilets supplied with drinking water. However, in 12 of the 27 analysed samples one or more pathogens were observed (*Aeromonas sp., Pseudomonas aeruginosa, Legionella non-pneumophila, Campylobacter jejuni, Mycobacterium avium,* and *Cryptosporidium sp.)*. These pathogens were not found in any of the reference toilets (32 toilets). This means that the use of rainwater introduced new, potentially pathogenic micro-organisms into the households which would normally not occur in toilets supplied with water from waterworks. Furthermore, four graywater systems were investigated where water from the shower and hand wash basin was reused. The graywater systems gave more problems in terms of bad smell and substantially higher numbers of *E. coli* and Enterococcus in some toilet bowls supplied with graywater.
**Keywords** Bacteria; graywater; hygienic; microorganisms; rainwater; reuse

## Introduction

Growing interests in society for saving drinking water resources have led to different attempts to use rainwater for toilet flushing and clothes washing and for using gray water for toilet flushing. Thus numerous rainwater collection systems have been established in Denmark during recent years, and in Germany more than 100,000 decentralised rainwater storage tanks for service water purposes have been installed during the last 10 years (Herrmann and Schmida, 1999).

First of all, the increasing cost of drinking water is one of the major motivations for water savings in general, and furthermore, e.g. in Germany, there is a tendency in the municipalities to split up the charges for urban drainage into a consumption-dependent amount for wastewater and an impervious surface-area-dependent amount for stormwater, which is a permanent financial incentive to disconnect the roofs from the sewers (Herrmann and Schmida, 1999). In Denmark the water consumption in each house is metered and this metering constitutes the basis of the charge for drinking water as well as for wastewater treatment. In some municipalities the wastewater charge has been waived, at least for a period, to stimulate installation of rainwater collection systems. Another motivation for installing rainwater collection systems may be the increasing awareness of the importance of preventing over-exploitation of water resources. If rainwater was collected from all suitable Danish roofs for use for toilet flushing and washing of clothes, it could replace approximately 7% of the present drinking water production (Mikkelsen *et al.*, 1999). Thirdly, collection and reuse of rainwater also reflects the general desire for sustainable development where recycling is an important cornerstone.

Installations for rainwater collection from roofs, storage and distribution of rainwater may constitute a risk for contamination of the public drinking water supply systems by back siphonage, leakages and incorrect installations with cross-connections to the drinking water system. If such contamination should occur, and if the rainwater contains biological agents, constituting a health threat to the consumers of the drinking water, this threat may

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not be limited to the people living in the house with the reuse installation, since the population in the whole area supplied with water from the contaminated drinking water supply system may be affected.

The contamination risk is especially problematic in areas where the distributed drinking water contains no disinfection residual as is the case in e.g. areas in Holland, or especially in Denmark, where less than 1% of the distributed drinking water is disinfected.



The microbial quality of the water can be investigated in terms of the total numbers of bacteria, presence of indicator organisms or specific pathogens. So far, only a few investigations have considered the microbial quality of collected rainwater (e.g. Holländer *et al.*,1996; Lorch, 1996), and they mainly investigated indicator organisms, and a few specific pathogens. However, with our limited knowledge of the microbial populations in collected and stored rainwater, and graywater treated for reuse, other specific pathogens may be important, and there may be substantial variations between different locations, depending on climatic conditions and the type of wild life (e.g. birds, cats or foxes), which may come in contact with the collection surface.

The purpose of the present investigation was to investigate the microbial quality of water collected for reuse. The microbial quality was investigated in terms of the total microbial population, indicator organisms and a range of specific pathogens. Seven rainwater collection systems and fourgray water reuse systems were investigated.

## Methods

### Investigated systems

*Rainwater systems*. Seven different Danish rainwater collection systems were investigated where rain was collected from roofs; and in a few cases also from other types of collection surfaces, e.g. pavements. The samples were collected from the rainwater storage tank and from the toilet bowl. Toilets supplied with water from waterworks served as references, and in four sampling rounds water was collected from 8 different reference toilets in the same building, and the samples were mixed. Two to four samples were collected from each system during the period from October 1996 to January 1997, and in May 1997.

The collection areas of the systems ranged from 200 $m^2$ to more than 5,000 $m^2$. The volumes of the collection tanks were between 4.2 and 115 $m^3$, and they were either buried or located in the basement. The rainwater systems supplied houses with 6 to 140 apartments. All the systems were relatively new, typically less than 2 to 3 years old.

*Graywater systems*. The investigation also included four graywater plants where graywater from showers and wash hand basins were collected, rinsed, UV-radiated and used for toilet flushing. Reference samples were collected from the cistern and toilet bowl from nine toilets supplied with water from waterworks.

### Analysis

The rainwater samples were investigated by standard methods for AODC (Acridine Orange Direct Counts), heterotrophic plate counts (HPC) on plate count agar (PCA) at 37°C, HPC on $R_2A$ at 21°C, yeast and microfungi and *Aeromonas, Pseudomonas aeruginosa, E. coli, Legionella, Campylobacter jejuni, Mycobacterium avium, Cryptosporidium* and *Giardia*. Additionally pH, temperature, non-volatile organic carbon (NVOC), smell, and appearance were measured. A questionnaire was distributed among the users.

The graywater samples were analysed for HPC on Kings Agar B at 21°C, *E. coli*, and Enterococcus, and for COD, pH, and temperature.

## Results

### Physico-chemical measurements

The average temperature in the rainwater systems storage tanks was 10.7°C (5.3–13.9°C). In the rainwater flushed toilet bowls the average temperature was 15.8° (12.6–18.0°C), and in the reference toilets the average temperature was slightly higher, 17.4° (12.1–23.1°C). pH was nearly the same in the three types of samples, with average values between 7.5 and 7.8. The content of organic matter, measured as NVOC, was the lowest in the storage tanks, on average 2.4 mg C/L (ranging between 1.0 and 4.6 mg C/L).

For the toilets which were flushed with rainwater, inconveniences in terms of smell or coatings in the toilet bowls were only registered by the users in a few cases.



### General microbial parameters

The total numbers of bacteria (AODC) ranged between $0.03 \times 10^6$ cells/mL and $5.8 \times 10^6$ cells/mL (Table 1), the heterotrophic plate count (HPC) on $R_2A$-agar at 21°C ranged between $<0.01 \times 10^6$ cells/mL and $2.1 \times 10^6$ cells/mL and the heterotrophic plate count at

**Table 1** Microbial quality of samples from rainwater storage tanks, from toilet bowls in rainwater flushed toilet and toilet bowls in reference toilets, flushed with drinking water

| | Rainwater | | Drinking water |
|---|---|---|---|
| | Storage tank | Toilet bowl | Reference toilets |
| Total numbers (AODC) $\times 10^3$ /mL | | | |
| Min.–max. value | 81–5,800 | 26–4,600 | 200–620 |
| Investigated samples | 5 | 4 | 4 |
| HPC $R_2A \times 10^3$/mL | | | |
| Min.–max. value | 0.38–2,300 | 63–1,530 | 29–520 |
| Investigated samples | 13 | 7 | 5 |
| HPC 37°C $\times 10^3$/mL | | | |
| Min.–max. value | 0.013–11 | 2.6–62 | 13–130 |
| Investigated samples | 14 | 7 | 5 |
| Yeast cells per mL | | | |
| Min.–max. value | 0.02–84 | <0.01–100 | 1–150 |
| Investigated samples | 13 | 7 | 5 |
| Microfungi per mL | | | |
| Min.–max. value | 0.06–26 | 0.13–11 | 0.3–140 |
| Investigated samples | 14 | 7 | 5 |

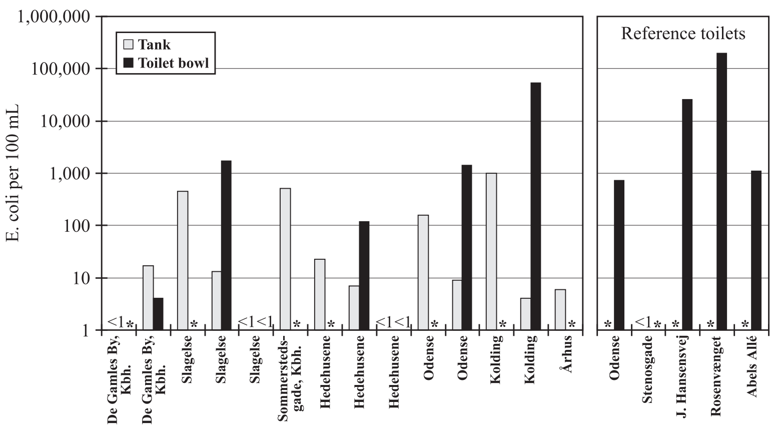


**Figure 1** Number of *E. coli* in samples from different rainwater collection systems in different sampling rounds. The samples were collected from the storage tanks, from toilet bowls in rainwater flushed toilets and toilet bowls in reference toilets, flushed with drinking water. Please note logarithmic scale at the y-axis. *: not measured

37°C ranged between <0.01 × $10^6$ cells/mL and 0.68 × $10^6$ cells/mL. There was thus significant numbers of bacteria present, and in more than 70% of the cases (HPC on $R_2A$) or in all cases (HPC on PCA at 37°C) the numbers were higher in the toilet bowl than in the storage tank. Regarding the total numbers of bacteria (AODC) and the HPC on $R_2A$-agar at 21°C, there was a tendency to higher numbers in the rainwater flushed toilet bowls than in the reference toilets, whereas there was no clear difference for HPC at 37°C.

Yeast (0.02–100/mL) as well as microfungi (0.06–26/mL) were also found in the rainwater systems, showing that the systems are harbouring complex microbial communities. There was no consistent difference between the numbers of these organisms in the storage tanks and in the toilets or between the toilets flushed with rainwater or drinking water.



**Indicator organisms**

*E. coli* was observed in all the systems, and in most of the samples, with values ranging from <1 to 54,000 *E. coli* per 100 mL (Figure 1). In most cases the numbers of *E. coli* were higher in the sample from the toilet bowl than in the adjacent sample from the storage tank, which might indicate that the toilet flushing was insufficient to replace all the water in the toilet bowl after the toilet has been used.

*E. coli* was observed in 11 out of 14 samples collected from the storage tanks (4 to 990 *E. coli* per 100 mL) which indicated a faecal contamination, possibly from the collection area, and that there may be a risk for the occurrence of pathogens (e.g. vira, which were not analysed for). It cannot be deduced from these analyses whether such contamination comes from humans or animals.

There was no difference in the microbial quality in terms of *E. coli* in the toilet bowls when rainwater and drinking water flushed toilets were compared.

**Table 2** Overview of investigated pathogen microorganisms in samples from rainwater storage tanks, from toilet bowls in rainwater flushed toilets and toilet bowls in reference toilets, flushed with drinking water. Number of investigated samples are given in parenthesis

| | Rainwater | | Drinking water |
|---|---|---|---|
| | **Storage tank** | **Toilet bowl** | **Reference toilets** |
| *E. coli* per 100 mL | | | |
| Min.–max. value | 4–990 | 4–54,000 | <1–200,000 |
| Positive observations | 11 (14) | 5 ( 7) | 4 (5) |
| *Pseudomonas aeruginosa* per 100 mL | | | |
| Min.–max. value | <1–20 | <1–870 | – |
| Positive observations | 1 (14) | 2 (7) | 0 (5) |
| *Aeromonas sp*. per mL | | | |
| Min.–max. value | <10–30 | <10–4,400 | 10–8,800 |
| Positive observations | 2 (14) | 3 (7) | 5 (5) |
| *Legionella pneumophila* | | | |
| Positive observations | 0 (14) | 0 (7) | 0 (5) |
| *Legionella non-pneumophila* | | | |
| Min.–max. value | N.D. – Detected | Detected | N.D. – Detected |
| Positive observations | 5 (7) | 5 (5) | 1 (5) |
| *Campylobacter* | | | |
| Min.–max. value | N.D. – Detected | N.D. – Detected | N.D. |
| Positive observations | 2 (17) | 2 (10) | 0 (5) |
| *Mycobacterium avium* | | | |
| Min.–max. value | N.D. – Detected | N.D. | N.D. |
| Positive observations | 1 (14) | 0 (7) | 0 (1) |
| *Giardia* | | | |
| Positive observations | 0 (17) | 0 (10) | 0 (5) |
| *Cryptosporidium* per L | | | |
| Min.–max. value | N.D. – 50 | N.D. – 10 (20) | N.D. |
| Positive observations | 6 (17) | 1 (10) | 0 (5) |

#### Pathogens

*P. aeruginosa* was found in the rainwater systems in two samples from the toilet bowl (100–870/mL) and in one sample from a storage tank (30/mL). *P. aeruginosa* was not found in the reference toilet bowls (Table 2).

*Aeromonas sp.* was found in two samples (10–30/mL) from the storage tank in one system and in three samples from toilet bowls in different rainwater systems (16–4,400/mL). *Aeromonas sp.* was found in all mixed samples from the reference toilets (Table 2).

*Campylobacter jejuni* was found twice in two rainwater systems. Two of the four positive samples were collected in the toilet bowl. However, *Campylobacter jejuni* was not found in any of the reference toilets.

*L. pneumophila* was investigated for in 21 samples, and *Giardia* was investigated for in 27 samples, but was not found. *Mycobacterium avium* was investigated for in 27 samples, and found once in a sample from a rainwater storage tank. *L. non-pneumophila* was found in 10 samples from 5 different rainwater systems and in one reference toilet. 12 samples were analysed.

*Cryptosporidium* was found in 4 rainwater systems, in 7 samples in total (up to 50 oocysts per L), in 6 sampling rounds (Table 2). *Cryptosporidium* was not found in any of the reference toilets.

#### Graywater systems

There were more inconveniences at the graywater plants, where there were several complaints regarding bad smell, and one plant was closed down because of the problems with smell. The investigated graywater treatment plants reduced the numbers of bacteria. However, very high numbers of *E. coli* (up to 510,000 per 100 mL) and Enterococcus (up to 570,000 per 100 mL) were observed in some toilet bowls supplied with graywater.

### Discussion

The general microbiological quality in the toilets supplied with rainwater were approximately the same as in the reference toilets, but in 12 of the 27 analysed samples one or more pathogens were observed. These pathogens were not found in any of the toilets flushed with water from waterworks (including more than 32 toilets). This means that the use of rainwater introduced micro-organisms into the households which do not usually occur in water from water-works.

A major investigation of 102 German rainwater storage tanks in systems used for toilet flushing, irrigation in gardens and for clothes washing was conducted by Holländer *et al.*, (1996). 1,600 water samples were investigated for HPC (at 20°C and 37°C), *E. coli*, coliforms, faecal entercoccus, *P. aeruginosa*, *Staphylococci*, *Yersiniae*, *Salmonellae*, *Shigellae*, *Legionellae* and yeasts. The investigation showed *P. aeruginosa* in 12% of the samples and *Salmonella* in one sample, but none of the other pathogens were found. Although Holländer *et al*. found no specific pathogens, most of the samples were contaminated to some extent since faecal indicators (*E. coli,* Enterococcus, or coliforms) were found in most of the samples. Although the precise frequency of these observations was not given, the median value was 1200 /mL for HPC at 20°C and 230 for HPC at 37°C. For *E. coli* the median value was 26 per 100 mL.

Another German investigation of 37 rainwater tanks (Lorch, 1996) showed thermotolerant coliforms in 77% of the samples.

For comparison, the present Danish investigation found *E. coli* in 11 out of 14 samples with a median value for the 14 samples of 245 per 100 mL. This indicated a higher degree of contamination in the investigated Danish rainwater systems.

In the tank samples, *Aeromonas spp.* and *P. aeruginosa* were only found in samples from one system (Kolding), which also collects rainwater from pavements and a parking lot. This indicated that rainwater collected from different surfaces may contain different types of microorganisms. However, the other parameters measured at this system (Kolding) did not differ significantly from the other systems.

None of the investigated rainwater samples met the Danish drinking water standards, and such comparisons may seem irrelevant since water in toilets is not considered to be drinking water. However, since no disinfection residual is present in Danish drinking water networks, rainwater with pathogens will, if introduced into the drinking water distribution system, increase the level of risk in the drinking water supply. Especially the potential for survival of such contaminants may be higher in Danish distribution systems than in distribution systems in countries where the water is routinely disinfected.

The lower microbial quality of the water in the toilets flushed with graywater, gave rise to concern. First of all, the treatment systems are much more complicated and rely on proper maintenance, which can be a problem. Secondly, such systems typically are installed in apartment buildings which means that graywater will be collected from many apartments including graywater from apartments with disease-carrying persons, who may excrete pathogenic agents (e.g. Hepatitis) which then will be mixed and distributed to all the other apartments connected to the system. In this way a graywater system theoretically constitutes a significant risk.

## Conclusion

The general microbiological quality in the toilets supplied with rainwater were approximately the same as in the reference toilets, but in 12 of the 27 analysed samples one or more pathogens were observed. These pathogens were not found in any of the toilets flushed with water from waterworks (including more than 32 toilets). This means that the use of rainwater introduced micro-organisms into the households which do not usually occur in water from water-works.

The graywater plants gave more problems in terms of bad smell and substantially higher numbers of *E. coli* and *Enterococcus* in some toilet bowls supplied with graywater.

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