QUALITY OF RAINWATER AND RECLAIMED WATER USED IN BUILDINGS AND SELECTION OF APPROPRIATE INDICATORS

Šárka Bobková, Dana Baudišová, František Kožíšek, Hana Jeligová, Petr Pumann
Department of Water Hygiene, Centre for Health and the Environment, National Institute of Public Health, Prague, Czech Republic

SUMMARY

Objectives: The use of alternative water sources such as rainwater or greywater (i.e., wastewater excluding water from toilets) for non-potable purposes may save water but, on the other hand, can also pose health risks to users. The main health risks come from microorganisms (such as bacteria, viruses, fungi, and protozoa). This work aims to analyse especially microbiological quality of rainwater and greywater used inside buildings in detail and to expand the existing knowledge about the potential health risks associated with these alternative water sources. It also considers methodological problems during *E. coli* and coliform bacteria detection. The final objective is to discuss requirements and appropriate indicators for monitoring recycled water quality.

Methods: We examined 30 buildings with non-potable water systems in the Czech Republic and analysed a total of 137 samples of rainwater and 120 samples of greywater. From these 30 buildings, eleven, 5 of which used rainwater and 6 of which used greywater, were sampled regularly for 1–2 years for basic chemical parameters, various faecal indicators, *C. perfringens*, *Legionella* spp. and *P. aeruginosa*. Occasionally, samples were analysed also for the presence of environmental mycobacteria, amoebas, viruses, and selected pathogens.

Results: Nearly three quarters of rainwater samples contained the faecal indicators *E. coli* or enterococci, or both, and in samples from several buildings also *Clostridium perfringens* was repeatedly detected. Untreated and treated rainwater were in respect to microbiological quality similar, suggesting that treatment processes were not very efficient. In greywater samples, beside faecal indicators, also *P. aeruginosa* and thermotolerant amoebas were repeatedly detected. Treatment technologies used for greywater were more efficient than those for rainwater systems.

Conclusion: Based on the results we evaluated appropriate indicators for monitoring recycled water quality and drafted the first Czech regulation for non-potable water.

Key words: non-potable water, rainwater, reclaimed water, health risk, microbiological quality, hygienic requirements

Address for correspondence: Š. Bobková, Department of Water Hygiene, Centre for Health and the Environment, National Institute of Public Health, Šrobárova 48/49, 100 00 Prague 10, Czech Republic. E-mail: sarka.bobkova@szu.cz

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INTRODUCTION

The Czech Republic (CR) is a country that generally does not suffer from water scarcity (except for a few regions). However, like in other European countries there is general support for the use of alternative water sources including indoor use. The main alternative sources for non-potable use are rainwater and greywater (i.e., household wastewater excluding water from toilets). However, even though these alternative sources have been used in plenty of buildings worldwide, only some countries have set rules and limits for the reused water quality. In other countries, including the CR, parameters for reused water quality and other relating safety requirements are still being discussed. The discussions are not only about the choice of relevant parameters (microbiological, biological, chemical, physical) and their acceptable limits, including relevant laboratory methods best suited for this kind of matrices, but also about the requirements for treatment technologies.

In the CR, the use of alternative water sources is mentioned in all strategic documents, and according to Act No. 183/2006 Coll. and its implementing decree No. 501/2006 Coll. from 2007, all new constructed buildings are obliged to consider also the rainwater management by either rainwater accumulation and reuse or its retention on the property or discharge by regulated drain. The use of alternative water sources is also financially supported by government subsidies – for example the programme "Rainwater" of the Ministry of Environment and the State Environmental Fund of the Czech Republic (SEFCR). The data of SEFCR and our investigations show that nowadays thousands of such systems exist in family houses and approximately hundreds in commercial and public buildings in the CR, including schools, shopping centres, hotels, restaurants, and residential buildings with more than four apartments. This number is continuously increasing. Most of the buildings use rainwater, and the rest use treated greywater. The primary usage for both of these sources inside buildings is toilet flushing. Besides that, both sources are sometimes used also for cleaning, laundry and irrigation inside or outside the houses. Cleaned rainwater is also used for personal hygiene or as filling water for swimming pools in some family houses.

The main health risk associated with rainwater and reclaimed water is due to the presence of microorganisms. The relevant bacterial, viral and protozoan pathogens for each type of matrix (rainwater and greywater) have already been summarized in

several papers (1–3). The most common pathogens found in rainwater and greywater are those of faecal origin, originating from birds and small mammals in rainwater and from humans in greywater. In a minority of cases, pathogens can also come from human skin, animal fur and feathers, or the respiratory tract. Typical faecal bacterial pathogens found in rainwater and greywater include *Campylobacter* spp., *Shigella* spp., enteropathogenic *E. coli*, perhaps *Salmonella* spp. Typical faecal viral pathogens found in rainwater and greywater are Enterovirus, Norovirus, Adenovirus, Rotavirus, hepatitis A virus, and in rainwater Hantavirus. Typical faecal protozoan pathogens found in rainwater and greywater include *Giardia* and *Cryptosporidium*. However, since direct detection of pathogens is expensive, laborious and time-consuming, their presence is routinely sought indirectly via detecting faecal indicators. Faecal indicators represent a group of microorganisms naturally inhabiting gastrointestinal tract of warm-blooded animals including humans entering the environment through faeces. Thus, their presence in water signifies faecal contamination and therefore possible presence of enteric pathogens listed above or others. We have used three most common faecal indicators currently used for water quality control: *E. coli*, intestinal enterococci, and total coliforms (4). Some standards regulating the safety of reclaimed water use detection of faecal coliforms instead of *E. coli* (5, 6). Another faecal indicator is *Clostridium perfringens*, gram-positive bacterium that can form spores and therefore survive for a longer time in the environment. Thus, it appears to be an indicator of pollution that might be spatially or timewise distant. Moreover, since the *Clostridium* spores are relatively resistant to disinfection and are similar in size as protozoan cysts, the presence of *Clostridium perfringens* in treated water signifies deficiencies in the treatment processes. In addition to the aforementioned enteric pathogens, rainwater and greywater may also contain bacterial groups including several opportunistic pathogens that do not come from the gastrointestinal tract. These groups cannot be monitored via faecal indicators and include environmental mycobacteria (sometimes also referred to as non-tuberculosis or atypical mycobacteria), *Legionella* spp., *Pseudomonas* genus, and the genus *Staphylococcus*. *Legionella* spp. has its optimal growth temperature between 25–45 °C and survives in water systems as part of biofilm or inside amoebas or other protozoa. These groups have considerable medical importance associated with hospital-acquired infections, and *Legionella pneumophila* is also a common cause of community-acquired infections. The danger of *Pseudomonas,* specifically *P. aeruginosa*, also comes from its multidrug resistance (7).

Most of the published studies concerning rainwater and greywater quality considered only limited number of sample types (samples from only few buildings) and/or limited number of microorganisms. Therefore, the novelty of this study is in the broader range of sample sources (samples from buildings of different size and types, with variety of treatment technologies) and the analyses of several types of potential pathogens in one study (bacteria, viruses, amoebas, etc.). The primary aim of this study was to evaluate the microbiological quality of rainwater and reclaimed water used inside buildings and to consider the efficiency of the treatment technologies used. Based on these evaluations, scientific literature and foreign regulations we identified relevant bacterial indicators for routine and preventive monitoring of recycled water quality. Additionally, we considered an approach based on technology efficiency evaluations as another means of regulating safety.

Table 1. *Summary of buildings using rainwater visited during this project*

Building type	Water source	End usage
Administrative building	RW – roof	Toilet flushing
Administrative building	RW – roof, terrace, also boring	Toilet flushing, irrigation
Car show and service	RW – roof	Car washing, cleaning, toilet flushing, irrigation
Company	RW – roof	Toilet flushing
Family house	RW – roof	Toilet flushing, laundry, filling water for swimming pool, irrigation
Hotel and café	RW – roof	Toilet flushing, irrigation
Company	RW – roof and near parking place	Toilet flushing, irrigation
Administrative building	RW – roof	Toilet flushing
Family house offering also commercial accommodation	RW – roof	Toilet flushing, cleaning, personal hygiene, laundry
Environmental (educational) centre	RW – roof	Toilet flushing, cleaning
Commercial centre	RW – roof	Toilet flushing
Hotel	RW – roof	Toilet flushing
Family house	RW – roof, others – well	Toilet flushing, cleaning, laundry, filling water for swimming pool
Family house*	RW – roof	Toilet flushing, cleaning, utility water in kitchen and bathroom (hand washing), inside irrigation
Elementary school *	RW – roof	Toilet flushing
Elementary school*	RW – roof	Toilet flushing
Elementary school*	RW – roof and near surfaces (streets)	Toilet flushing, irrigation
Family house*	RW – roof	Toilet flushing, cleaning

RW – rainwater
Buildings marked with asterisk were sampled repeatedly.

MATERIALS AND METHODS

Sampling Sites

Buildings that utilise rainwater and/or greywater inside the premises were sought out from various sources, as there is no central register of such facilities available in the CR. We gathered information from regional public health authorities, press articles and reviews, companies that specialized on rainwater and greywater treatment technologies, individuals who responded to our call for collaboration on specialized websites, Facebook, and questionnaires distributed by the SEFCR among subsidy beneficiaries. In total, we were able to find 85 buildings (both private and public) located throughout the Czech Republic. We excluded buildings that used recycled water solely for garden irrigation and car washing, those still under construction, and those that had treatment technology outside the building (root zone technology). Finally, we successfully contacted, visited and sampled water from 30 buildings (18 utilizing rainwater and 12 utilizing greywater). The types of buildings are described in Table 1 and Table 4 (and in detail in Table S1, Supplementary Materials). We analysed a total of 137 samples of rainwater (untreated, treated and at the point of use) and 120 samples of greywater (untreated, treated and at the point of use).

The research lasted for three years (2020–2022). In the first year, all 30 buildings were visited once and samples were taken. In the following two years, 11 out of the 30 buildings were sampled repeatedly, 5 using rainwater and 6 using greywater. All of the 11 buildings were sampled between three and six times. In 2022, two buildings using rainwater (a school and a family house) were visited and sampled every month (from February to November).

After inspecting the building and the treatment technology used, water samples were collected from at least three sites if possible: untreated water, treated water and water at the point of use. If more than one water source (i.e., greywater and also rainwater) was used or more than one type of final usage was applied, then samples from all of the sources and usage types were collected. Untreated water samples were collected from the accumulation tanks at a depth of approximately 30 cm below the water level. The homogeneity of water at the corresponding layer

Table 2. *Results of microbiological analyses of untreated rainwater samples collected from the accumulated tanks*

Microorganism tested	Total number of samples	Positive (%)	CFU or MPN/100 ml
E. coli	42	48	3.1–1,299.7
Intestinal enterococci	46	72	6–648
Thermotolerant coliforms	23	30	9–400
Clostridium perfringens	21	81	4–>300
Salmonella	5	0	Only qualitative
Campylobacter	12	0	Only qualitative
Environmental mycobacteria	8	13	20/1,000 ml*

Samples where CFU or MPN/100 ml was higher than 3 were considered positive.
*In case of environmental mycobacteria the results are reported from 1,000 ml and values above 3/1,000 ml were considered positive, therefore, for this microorganism the reported and considered value is related to 1,000 ml.

Table 3. *Results of chemical analyses of untreated rainwater samples (N = 46)*

Parameter	Median	Range
pH	7.2	5.6–8.2
Conductivity – without green roof (mS/m)	19.6	2.8–97.8
Conductivity – green roof building (mS/m)	168	107–224
Turbidity (NTU)	2.7	0.3–32.7
COD Mn (mg/L)	3.1	0.2–6.8
TOC (mg/L)	3.4	0.3–5.9

For conductivity only was n = 13 from green roofs and n = 33 without green roofs.

Table 4. *Summary of buildings using greywater or greywater and rainwater studied during this project*

Building type	Water source	End usage
Family house	GW – bathroom, partly kitchen	Irrigation also of eatable crop
Secondary school	GW – sinks in toilets and 1 shower, RW – roof and surrounding surfaces	Toilet flushing
Hotel and wellness	GW – wellness baths, showers	Toilet flushing, car washing, cleaning
Family house	GW – domestic waste water treatment plant, RW – roof	Toilet flushing, irrigation
Hotel	GW – swimming pool, wellness, RW – roof, others – well	Toilet flushing, occasionally irrigation and cleaning of surfaces
Residential building*	GW – showers and baths	Toilet flushing
Family house*	GW – showers and baths	Toilet flushing, laundry
Residential building*	GW – showers, baths, basins, washing machines	Toilet flushing
Hospital*	GW – rehabilitation baths, water from preparation of solution for haemodialysis	Toilet flushing
Commercial (shopping) centre*	GW – basins in customers toilets, RW – roof, upper parking lot	Toilet flushing
Environmental (educational) centre*	GW – showers, basins, washing machine, aquariums and terrariums	Toilet flushing

RW – rainwater; GW – greywater
Buildings marked with asterisk were sampled repeatedly.

below water level in the tanks was verified. Treated water was collected from tanks with treated water or another site where the treated water was stored. At the point of use, the samples were collected either from a tap of non-potable water, riser pipe or any possible point near the toilet (water tank for flushing, hose for water coming to the tank). All of the samples were collected as grab samples following the procedure described in EN ISO 19458, ISO 5667-1, and ISO 5667-3 norms. For microbiological analyses, they were collected into sterile glass or plastic containers with thiosulfate (if chlorine was used in the technology) or without thiosulfate (not disinfected samples, samples for virology). For chemical analyses, clean glass or plastic containers were used. Then they were transported in cooling boxes to the laboratory for further analyses or transported to collaborating laboratories for other specific analyses.

At the sampling site the following properties were measured: aesthetic quality (odour, colour), temperature, free and bound chlorine (if used in the technology).

Microbiological Analyses

The samples were processed either immediately upon arrival to the laboratory or within 24 hours from sampling. The laboratory analyses differed slightly between rainwater and greywater samples as well as between the first and second/third year of the study. During the pilot (first year) the following indicators were selected for both rainwater and greywater samples: total coliforms and *E. coli* using the standard EN ISO 9308-2 method (due to the abundant background microflora) and EN ISO 9308-1 (pilot analyses), thermotolerant coliforms according to the Czech technical standard (8), and intestinal enterococci according to ISO 7899-2. Total colony counts were performed according to ISO 6222. In the years 2021 and 2022 additional indicators were added. For rainwater samples: *C. perfringens* was performed according to the method described in Annex III of the Council Directive 98/83/EC, *Campylobacter* according to EN ISO 17995, and *Salmonella* spp. according to ISO 19250. For greywater samples, the following indicators were added: *P. aeruginosa* performed according to EN ISO 16266, *Legionella* performed according to EN ISO 11731 in both, our laboratory and in the National Reference Laboratory for Legionella, Public Health Institute, Ostrava; environmental mycobacteria were performed according to norms (9) in the Department of Bacteriology and Mycology, Public Health Institute, Ostrava; and thermotolerant amoebas were performed by cultivation methods and also microscopically, suspected amfizoic amoebas were confirmed by PCR at the Public Health Authority of the Slovak Republic. The detection of viruses by molecular biology methods (qPCR, RT-PCR) was performed at the Elisabeth Pharmacon Company and the analyses of total bacterial counts by fluorescence microscopy using DAPI staining were performed at the Water Research Institute, Brno.

Physical and Chemical Analyses

For both rainwater and greywater samples, turbidity was measured according to the standard method EN ISO 7027. Electric conductivity was measured according to EN 27 888 and COD-Mn was measured according to EN ISO 8467. Total suspended solids were measured according to EN 872.

RESULTS

Rainwater Quality in Buildings

The buildings that used rainwater and from which samples were analysed during this project are listed in Table 1 (also in Table S1, Supplementary Materials). For further repeated analyses in the second and third year, only five buildings were selected including three elementary schools and two family houses. In all of the buildings rainwater was collected from roofs, and in some of them, it was also collected from surrounding streets and pavements. Altogether 137 samples of rainwater (untreated, treated and at the point of use) were analysed.

Untreated Rainwater Quality

The primary microorganisms used to assess the microbiological quality of water are the so-called faecal indicators (total coliforms, *E. coli* and enterococci) and therefore they were among the indicators used during the initial pilot analyses (Table 2). If we consider samples with CFU or MPN/100 ml higher than 3 to be positive, then 48% of untreated rainwater samples were positive for *E. coli* and 72% were positive for enterococci. These findings reflect the fact that the number of enterococci in the gastrointestinal tract of animals is typically higher than that of *E. coli*, and also that enterococci can survive in the environment for a longer period (10).

In untreated rainwater tanks the number of *E. coli* ranged from 0 to 1,299.7 MPN/100 ml and the number of intestinal enterococci ranged from dozens to hundreds with the maximum of 648 CFU/100 ml.

The group of coliform bacteria is very heterogeneous and includes also strains that are not related to faeces. It was interesting to determine the percentage of *E. coli* among total coliforms. In most of the untreated rainwater samples the number was quite low (an average of 2%, which is of lower hygienic importance). Only in 3 out of 39 samples did the percentage range from 20–38%. All the three samples were from a family house (three samples from different days) with a green roof, which might attract birds and other animals more than other roof coverages. The number of enterococci was also high in these samples (between 122–576 CFU/100 ml). Regarding potential pathogens, neither *Campylobacter* nor *Salmonella* was detected in any of the samples. It should be mentioned that the negative result might be due to the low number of analysed samples (5 for *Salmonella*, 12 for *Campylobacter*). Similarly from members of environmental mycobacteria, only the genus *Mycobacterium gordonae*, which is often found in soil and tap water and is rarely implicated in diseases, was detected in one sample out of 8 samples of untreated water tested.

On the other hand, *Clostridium perfringens* was continuously and repeatedly detected in several buildings in concentrations ranging from 4 to more than 300 CFU/100 ml. It should be also noted that when *C. perfringens* was detected in our samples, it was often in similar quantities in untreated and treated water. This might implicate that the cleaning process was not very effective, even though there are currently no regulatory requirements for efficiency of treatment of rainwater for non-potable use.

The parameters that describe the basic physicochemical quality of water are pH and turbidity (Table 3). The pH of the collected rainwater ranged from 5.6 to 8.2 with the average of approximately 7. Turbidity depends also on the roof material, position of the building and the season (pollen in spring, soot in winter, etc.). In our samples turbidity ranged from 0.3 to 32.7 with a median of approximately 2.7. The considered buildings mainly collected rainwater from roofs (made of different materials) and in one case also from neighbouring streets. However, in buildings that were periodically sampled throughout the year we did not observe any seasonal variations in turbidity or pH.

The amount of inorganic substances in water can be estimated by measuring its conductivity and it was reported that conductivity of samples collected from the so-called green-roofs was generally higher than from other roof materials. In most of the analysed samples the conductivity ranged between 2.8 and 76 mS/m. However, in one of the family houses collecting rainwater from a green roof the conductivity was constantly very high (107–224 mS/m), corresponding to findings reported in literature (11).

The amount of organic matter in a sample can be characterized by measuring its total organic carbon (TOC) and its oxidability or chemical oxygen demand (COD). In our analyses, CODs in untreated and treated rainwater were similar (median 3.07 in untreated water and 2.27 in treated water). The TOC values (medians) were 3.35 mg/L in untreated water and 2.45 mg/L in treated water.

Rainwater Quality Changes During the Year

Periodic sampling of two buildings using rainwater allowed us to evaluate changes in water characteristics and quality throughout the year and to assess the efficiency of the treatment technologies used.

According to different studies, rainwater should be stored in tanks that are protected from light, frost and large temperature changes. Therefore, it is recommended to have the tanks situated either underground or in the cellars (souterrains) of buildings. The two buildings that were monitored for several months had their tanks located underground according to these recommendations. Figure 1 shows that even in these conditions the temperature in the tank fluctuated between around 5 °C in February and March and almost 20 °C in July and August. This might be explained by the fact that the temperature of rainwater is influenced not only by the surrounding atmosphere but also by the surfaces from which it is collected.

The microbiological indicators fluctuated throughout the year due to other factors (such as the amount of precipitation, etc.). However, while the number of *E. coli* fluctuated between zero and more than 1,000 MPN/100 ml, the number of *C. perfringens* was more constant and present in almost all of the samples during the year. This was obvious especially in the samples from elementary school (Fig. 1, left). This might be due to the fact that *C. perfringens* can form spores and therefore survive in the environment for a long time, while *E. coli* dies relatively quickly when outside the gastrointestinal tract.

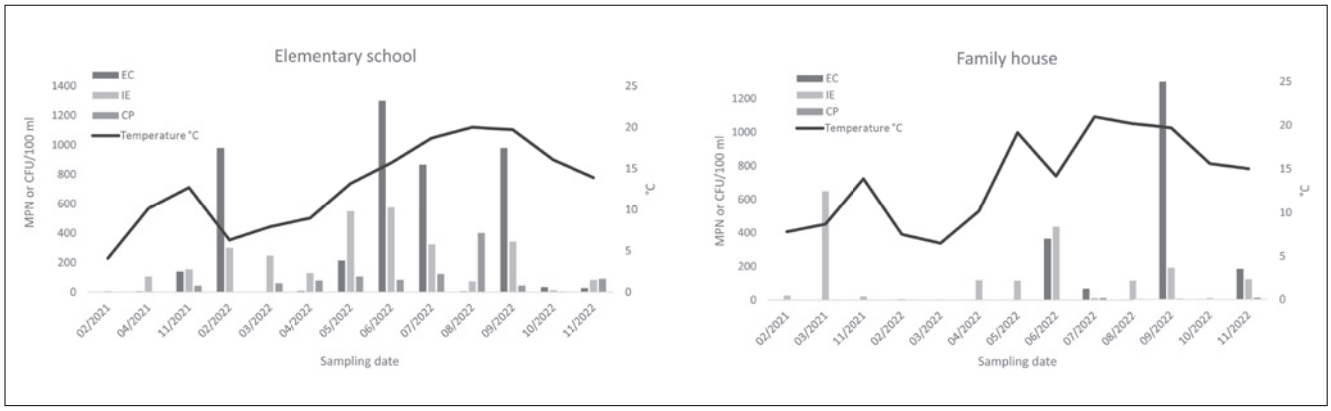

Fig. 1. *Fluctuation of microbiological indicators and water temperature throughout the year in the two periodically sampled buildings.*

EC – *E. coli*; IE – intestinal enterococci; CP – *C. perfringens*

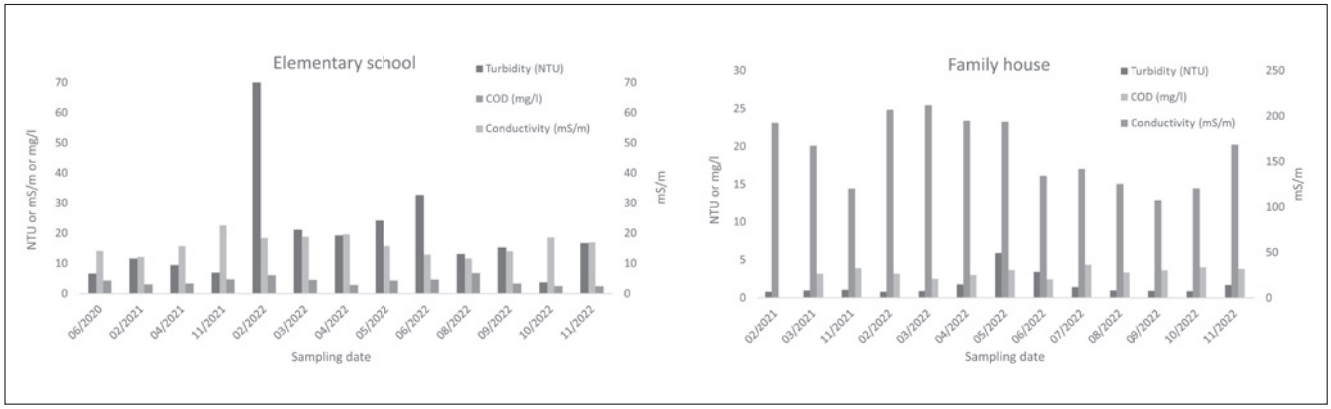

Fig. 2. *Fluctuation of chemical indicators in untreated rainwater throughout the year in the two periodically sampled buildings.*

The sample from elementary school from 02/2022 might have been influenced by the fact that there was lower water level in the accumulation tank than usually due to reparation of part of the technology few days before our sampling.

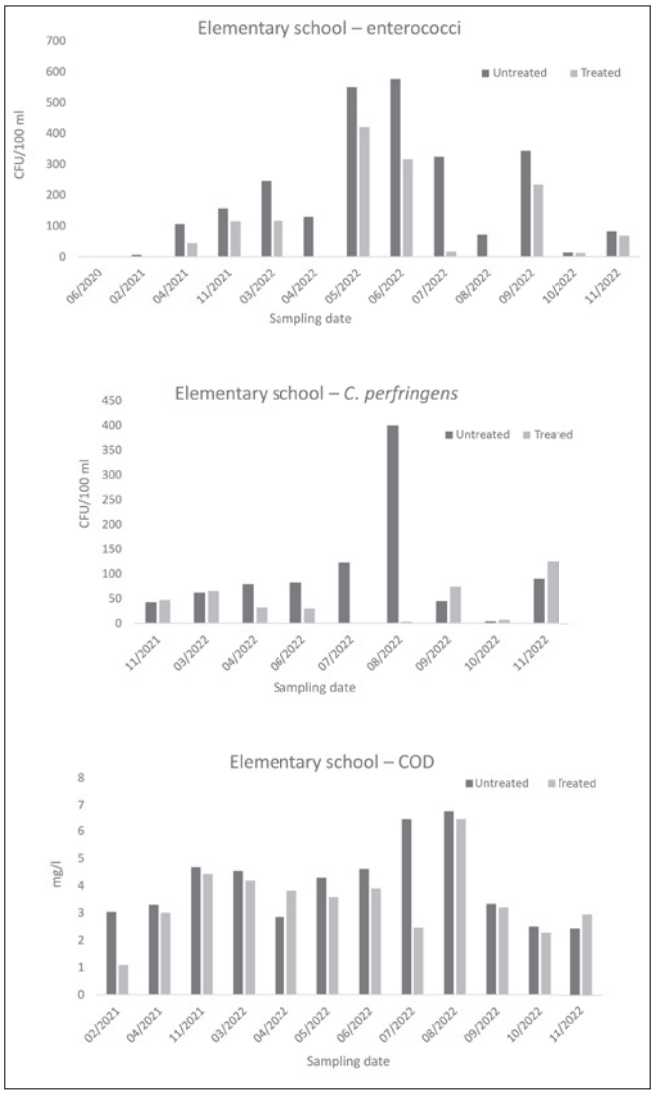

Fig. 3. *Numbers/concentrations of enterococci, C. perfringens and COD-Mn in the samples of untreated and treated rainwater from a school building.*

From the chemical indicators the most variable during the year was turbidity, while COD and conductivity stayed more or less stable during the whole year (Fig. 2). At the same time, the conductivity in the family house was always an order of magnitude higher than in the elementary school and in the previously monitored buildings (Fig. 2). This was caused by the green roof of the building as already mentioned above.

The periodic sampling of the two buildings enabled us to evaluate the efficiency of the treatment processes. Efficiency in water quality assessment is often indicated through the so-called $\log_{10}$ reduction targets (LRT). In our study, the concentrations of the measured indicators in the untreated water were too low to calculate the $\log_{10}$ reduction. Therefore we compared the absolute values of three selected indicators in untreated and treated water (Fig. 3 and 4), namely the quantities of enterococci, *C. perfringens* (in school building) and COD. We chose these microbiological indicators for this evaluation because they were present in most samples throughout the year and COD is one of the basic indicators considered during efficiency evaluation of the wastewater treatment technologies. It is clear from Figures 3 and 4 that the reduction of microbiological indicators was only partial, especially in the school building and that the COD-Mn values remained nearly unchanged between the untreated and treated water in both buildings.

Greywater Quality in Buildings

The buildings that used greywater and were surveyed and sampled during this project are listed in Table 4 (and also in Table S1, Supplementary Materials). For subsequent monitoring six buildings were selected, namely a family house, residential building, commercial centre, environmental centre, hotel, and a hospital. The sources of greywater included baths, showers and washbasins (including rehabilitation baths and whirlpools), and sometimes also washing machines. In one case it also included water from aquariums and in another case also part of the water from a kitchen sink.

Overall, 120 samples of greywater (untreated, treated and at the point of use) were analysed and the results of microbiological analyses are shown in Table 5.

Although greywater samples usually have lower total bacterial counts than rainwater, they have higher levels of *E. coli* due to their origin. We detected *E. coli* in 66% of the untreated greywater samples. If we exclude buildings using "clean source water" (i.e., highly disinfected water from wellness and rehabilitation baths) the percentage of untreated samples containing measurable amounts of *E. coli* (above 3 MPN/100 ml) was 80%. Intestinal enterococci were positive in 78% of all untreated samples and

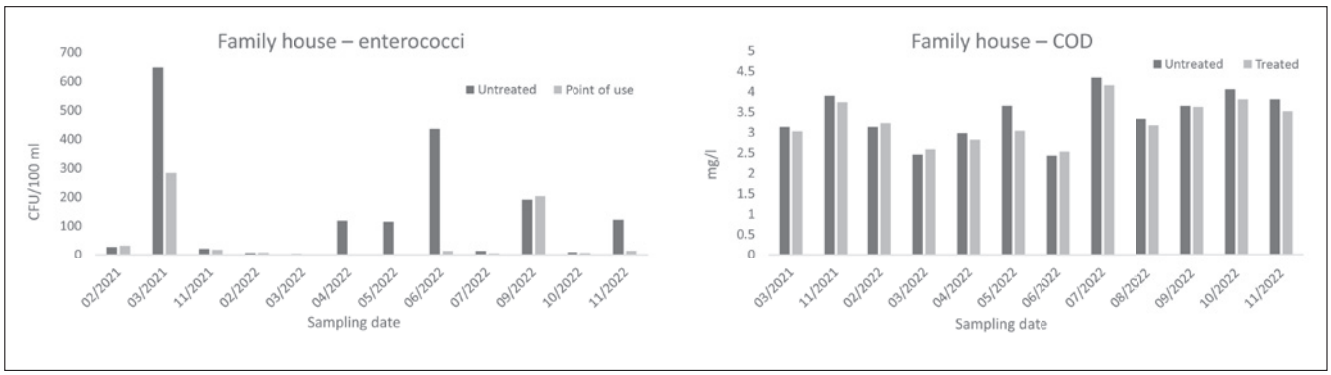

Fig. 4. *Numbers/concentrations of enterococci and COD-Mn in the samples of untreated rainwater and treated water from the point of use* from a family house.*

*Tap for non-potable water was used for cleaning floor.

Table 5. *Results of microbiological analyses of untreated and treated greywater samples*

	Untreated samples			Treated samples		
Microorganism tested	**Total number of samples (n)**	**Positive (%)**	**MPN or CFU/100 ml**	**Total number of samples (n)**	**Positive (%)**	**MPN or CFU/100 ml**
E. coli	32	66 (80)	4.1–>4,838.4	36	14	8.5–1,000
Intestinal enterococci	32	78 (88)	$3.2–4*10^4$	36	25	6.5–169.3
Thermotolerant coliforms	32	59	7–>6,000	36	17	110–1,000
P. aeruginosa	16	31	Only qualitatively	18	44	Only qualitatively
Legionella spp.	19	11	70–2,000	18	16	10–2,200
Environmental mycobacteria	9	50	Only qualitatively	9	56	Only qualitatively
Thermotolerant amoebas	5	100	Only qualitatively	6	100	Only qualitatively

Positive sample means MPN/CFU > 3.
The total number of treated samples is higher than untreated samples because in a few cases it was not possible to sample the untreated water due to technical problems. For the microorganisms *E. coli* and enterococci, the numbers in parentheses refer to values obtained from buildings excluding those using "clean water source, i.e., highly disinfected water from wellness and rehabilitation baths".

in 88% of the samples from "typical" greywater systems (i.e., without buildings using wellness and rehabilitation baths). The number of *E. coli* ranged from units to thousands of MPN/100 ml.

Regarding opportunistic pathogenic bacteria we often detected *P. aeruginosa*. Interestingly, in some buildings these bacteria were detected in treated water and at the point of use but were undetectable in the accumulated untreated water suggesting that these microorganisms multiplied in the system itself. Other plumbing-associated microorganisms are *Legionella* species. In our samples *Legionella* was repeatedly detected only in samples from one hotel where the source water also comes from wellness baths. In another building – a residential building – they were detected only in one out of 12 analysed samples. The number 12 corresponds to all of the samples collected from one building (i.e., untreated and treated water) while the numbers in Table 5 correspond to untreated and treated samples from all of the buildings visited. In all cases the identified species were non-pathogenic serogroups of *L. pneumophila* (sg 6 and 10) or less known *Legionella* species. It is known that *Legionella* can survive in the plumbing inside eucaryotic amoebas, and in some environments, they even require an intracellular environment for their multiplication (12). During this study the samples from three buildings (two residential buildings and one commercial centre) were repeatedly tested for the presence of amoebas. Different types of amoebas were present in all 14 tested samples of both untreated and treated greywater. In most cases there were saprophytic species, and only in one sample the amphizoic species *Acanthamoeba* was detected.

There are several studies regarding the presence of environmental mycobacteria in water distribution systems, however, only a few are connected to greywater (13). In our greywater samples we found *M. gordonae*, *M. fortuitum*, *M. scrofulaceum*, and *M. intracellulare* species. Of those the *M. fortuitum* and *M. intracellulare* can cause human infections, however, they are risky especially for immunocompromised individuals, patients after surgery or those having other lung disease.

In addition to bacteria, also enteric viruses have been detected in greywater. In this study, we analysed 14 samples for the presence of viruses from the group of Adenoviridae, Noroviridae, Rotaviridae and SARS-CoV-2. In one of the samples human Adenovirus was detected in concentrations $3.12*10^9$ GE (genome equivalents)/10 L and Adenovirus 40/41 was detected in concentrations of $2.89*10^9$ GE/10 L. This sample was from a residential building, and it is important to note that it was a sample of accumulated untreated water and that following the treatment process (in treated water and at the point of use) neither of the viruses was found.

The temperature of untreated greywater was on average higher than that of untreated rainwater (with a median of 20 °C, ranging from 10 °C in a tank of accumulated water collected only from basins and situated underground to 36 °C in water from rehabilitation baths). This higher temperature might support the growth of microorganisms in the plumbing system. However, we did not observe a strong correlation between higher temperature and the presence of faecal indicators, *Legionella* or *P. aeruginosa.* With regard to pH, the median pH of greywater samples was 7.4, ranging from 5.4 to 8.4.

Table 6. *Results of chemical analyses of untreated and treated greywater samples*

	Untreated samples			Treated samples		
Parameter	**Total number of samples**	**Median**	**Min–max**	**Total number of samples**	**Median**	**Min–max**
Temperature (°C)	32	20	10–36	36	18	2.8–28.7
pH	32	7.4	5.7–8.6	36	7	5.4–8.4
Turbidity (NTU)	32	16.5	0.23–413.7	36	2	0.3–46
Conductivity	32	62.3	5.6–184.5	36	58.9	5.9–188.8
TOC	20	17.6	1.16–173.5	23	3.2	0–13.6
Suspended solids	32	73.9	6–2,170	36	13.9	8.8–26.3

In some cases it was not possible to collect the untreated water due to technical problems, therefore, the number of analysed treated samples is slightly higher than that of untreated samples.

The mean conductivity was around 62 mS/m and in more than a half of the samples, the amount was the same in untreated and treated samples (Table 6). In eight out of 32 analyses, conductivity was higher in treated than in untreated samples. These were either samples from a hotel possessing a hybrid greywater and rainwater system or from a hospital where the source water is from wellness, rehabilitation baths and preparation of haemodialysis solution. On the other hand, turbidity was quite low even in the samples from the accumulation of untreated water (with a median of 16.5 NTU) and it decreased in most of the samples below 10 NTU in treated samples or samples from the point of use (Table 6). The amount of organic matter was monitored by measuring total organic carbon (TOC). TOC was in units to hundreds of mg/L and this parameter decreased to units or tens of mg/L in treated water samples.

Based on the results from repeatedly sampled buildings we were able to assess the efficiency of treatment technology by comparing the numbers of intestinal enterococci and TOC in untreated and treated samples. For these evaluations we only considered the technologies/buildings from which the number of enterococci and TOC in untreated samples were in measurable quantities. These included two residential buildings, a hotel, an environmental centre, and a commercial centre.

It is evident from Figures 5 and 6 and the data presented above, that the surveyed buildings and their tested technologies can be divided into those working perfectly (with a log reduction of viral indicators of up to 9 in residential building) and those, where at least in two samples the reduction of the studied indicator was less than 2 orders of magnitude (hotel, commercial centre, environmental centre). It should be mentioned here that the hotel possesses a hybrid system for rainwater and greywater treatment and the source water of environmental centre is heterogenous including showers, baths, washing machines, and aquariums and terrariums. The observed differences might be related to the technology design itself but according to our experience they are also related to the maintenance of the technology and proper operation. The operators sometimes save on disinfection (turn off UV lamp or dose hypochlorite only occasionally) or on the system's maintenance (such as changing membranes after a longer period than is recommended, or not washing filters regularly), which results in malfunctioning treatment.

Methodological Problems in Detection of *E. coli*

Both untreated rainwater and greywater samples are highly colonised with different microorganisms, which complicates

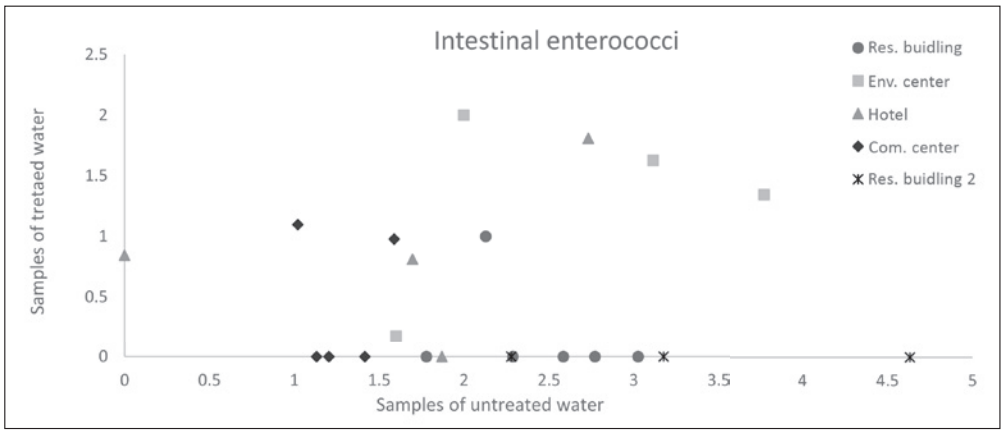

Fig. 5. *Log concentrations/numbers of intestinal enterococci in untreated and treated water samples from residential building, environmental centre, hotel and commercial centre.*

Res. building – residential building; env. centre – environmental centre; com. centre – commercial centre

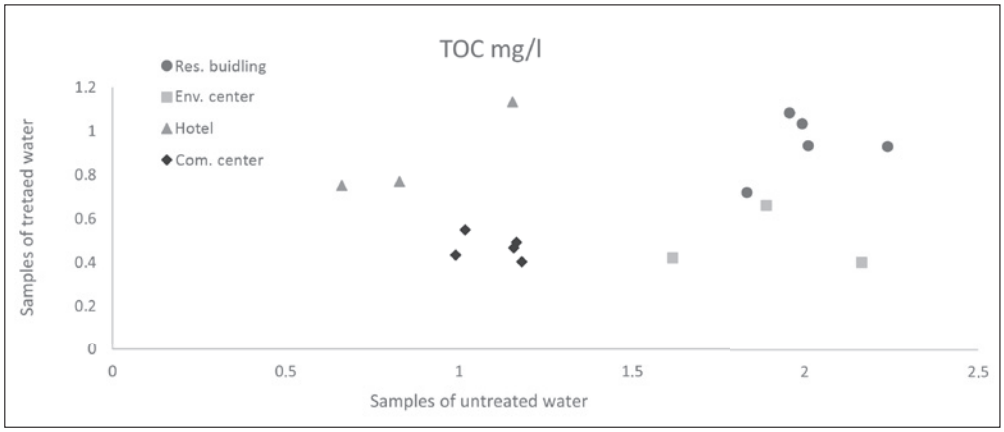

Fig. 6. *Log of TOC concentrations in untreated and treated water samples from residential building, environmental centre, hotel and commercial centre.*

Res. building – residential building; env. centre – environmental centre; com. centre – commercial centre

Table 7. *Results of DAPI, CC and CC/DAPI*

Samples		DAPI (n/ml)	CC 22 °C (CFU/ml)	CC/DAPI (%)
Rainwater samples				
Family house	Untreated water	1,093,400	15,000	1.37
	Treated water	946,600	2,400	0.25
School	Untreated water	1,217,800	135,000	11.09
	Treated water	743,600	75,000	10.09
Greywater samples				
Residential building 1	Untreated water	636,400	340,000	53.43
	Treated water	21,400	113	0.53
	At the point of use	55,600	103	0.19
Residential building 2	Untreated water	699,300	190,000	27.17
	Treated water	102,700	67,000	65.24
	At the point of use	58,100	75,000	100

DAPI – bacterial counts; CC – number of colony counts at 22 °C; CC/DAPI – percentage of cultivable bacteria among total bacterial counts

several microbiological analyses. Coliform bacteria and *E. coli* in drinking and bathing water are detected using ISO 9308-1 (cultivation on chromogenic cultivation media: Chromogenic Coliform Agar – CCA). However, this medium is not suitable for samples with high background microflora. During our analyses, *E. coli* (blue colonies) was very often overgrown by other coliforms and by *Aeromonas* on this medium (Fig. 7), making it hardly detectable among them. When the samples were diluted, *E. coli* was also diluted, leading to false negative results that did not correspond to reality. Although a miniaturized method according to EN ISO 9808-3 is available for detecting *E. coli* in surface and waste waters, it is not convenient due to its high detection limit (15 MPN/100 ml).

Therefore, during the pilot analyses we compared the results of *E. coli* detection using CCA medium and method according to ISO 9308-2 (Colilert Quanti-Tray, IDEXX, USA). Both of these methods are based on the detection of the enzymatic activity of ß-D-glucuronidase enzyme. In the case of CCA, samples are cultivated on solid medium and the results are expressed as CFU/100 ml. In the case of Colilert Quanti-Tray the cultivation is performed in liquid medium and results are expressed as most probable number (MPN)/100 ml. As shown in Figure 8 the use of the biochemical test Colilert Quanti-Tray enabled us to quantify *E. coli* more accurately (repeated tests) than by the cultivation on CCA medium. For this reason we recommended the use of Colilert Quanti-Tray or similar assay for the detection of *E. coli* in rainwater and greywater samples.

Total Bacterial Counts in Rainwater and Greywater Samples

It is known that rainwater and greywater samples are highly colonised with organotrophic microorganisms and also contain organic and inorganic substances. We analysed samples from two buildings that used rainwater and two buildings that used greywater in order to determine the total number of microorganisms present in these environments as well as the number of cultivable vs. total bacteria (Table 7). Although the number of tested samples is low (10 samples in total), it can be assumed that rainwater samples contained more bacterial counts (around 10^6/ml) than greywater samples ($6*10^5$/ml) and that the number of bacteria decreased during the treatment process. This decrease is more obvious with greywater than rainwater samples (Table 7) and it is in agreement with our data concerning the concentrations of faecal indicators in rainwater and greywater samples. It also reflects the fact that most of the rainwater systems we examined do not use any disinfection during the treatment process.

DISCUSSION

Both rainwater and greywater samples contain various microorganisms due to their origin, some of which might be pathogenic. Infections can occur through aerosol respiration, aerosol ingestion, hand-to-mouth transmission, or skin contact. If there is an accidental cross-connection between potable and non-potable water distribution systems, direct ingestion may also occur. Therefore, like drinking water, bathing water or other water for human use, the quality of rainwater and greywater used inside households should be controlled.

In this respect, faecal indicators, especially *E. coli*, have been used for many years to indicate water contamination and associated health risks. In our analyses, we found that nearly half (48%) of rainwater samples contained *E. coli* and 72% of samples contained intestinal enterococci. Similarly, Chidamba and Korsten reported that out of their 285 rainwater samples 44% contained *E. coli* and 58% contained *Enterococcus* spp. (14). Hamilton et al. collected data from 20 papers concerning rainwater quality in roof-harvested rainwater tanks and found that the number of *E. coli* positive samples ranged between 36–81% (2). In our analyses the presence of *E. coli* or intestinal enterococci did not correlate with rainwater relevant pathogens *Salmonella* and *Campylobacter* since neither *Campylobacter* nor *Salmonella* was detected in any of the samples. However, the negative result might be due to the low number of analysed samples (5 for *Salmonella*, 12 for *Campylobacter*). Another potential pathogens tested were environmental mycobacteria. A member of this group *M. gordonae* was found in one out of 8 analysed samples. In the literature *Salmonella* has been reported in 4–10% of roof rainwater samples from stormwater tanks and *Campylobacter* in 3–40.7% of roof harvested rainwater samples (2, 15). Other studies have reported the presence of *Mycobacterium avium* in 17.2% of samples and *Mycobacterium intracellulare* in 78.4% of rainwater samples from a total of 134 samples tested (2). It should be noted that some of the positive samples reported in the literature (especially those concerning pathogens) were detected by the PCR method, and therefore they might also include dead bacteria that would not be detected by the cultivation methods we used. Nevertheless, the reported presence of pathogenic bacteria in different studies of rainwater samples and also the presence of *E. coli* in nearly half of the samples analysed here and reported in the literature, support the current trend that rainwater, usually considered clean, should be monitored. Once again, *E. coli* seems to be a suitable indicator, which is also easily cultivable and fast-growing (results are known within 24 hours).

In addition to the widely used *E. coli* and intestinal enterococci, there is another indicator that might be employed as an indicator of effectiveness of the treatment process. This indicator is *Clostridium perfringens*. During our analyses *Clostridium*

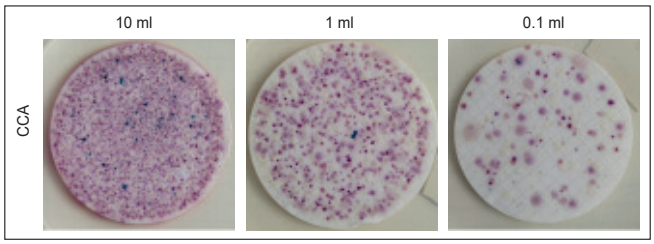

Fig. 7. *Cultivation of greywater samples on CCA agar.*
Red – coliforms; blue – *E. coli*

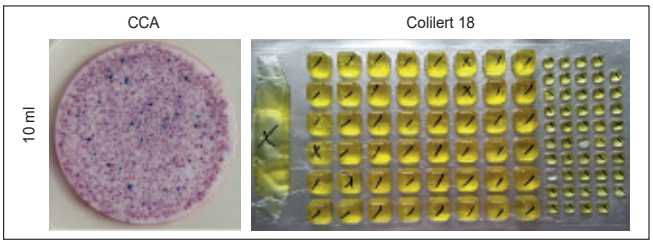

Fig. 8. *Comparison of E. coli detection and quantification on CCA medium and Colilert Quanti-Tray Assay (IDEXX, USA).*
On CCA medium presumptive coliforms grow as red colonies which should be afterword confirmed by oxidase test. *E. coli* grows as blue colonies. On Colilert, both coliforms and *E. coli* turn yellow, and where *E. coli* is present it also fluoresces. On CCA the number of *E. coli* was uncountable, on Colilert it was 6.3 MPN/10 ml, i.e., 63 MPN/100 ml.

perfringens was repeatedly detected in several buildings in concentrations ranging from units to more than 300 CFU/100 ml. In the study of Ahmed et al. it was reported that 46% out of their 100 samples contained *C. perfringens* in measurable quantities and the numbers were in the range of units to 200 CFU/100 ml (1). In our study it was often detected in similar quantities in untreated and treated water, which might raise questions about the effectiveness of the treatment processes. For the rainwater quality monitoring, we therefore suggest using *C. perfringens* beside *E. coli* as another indicator of water quality.

Regarding the greywater samples, *E. coli* was detected in 66% of the untreated greywater samples and intestinal enterococci were present in 78% of the untreated samples. The number of *E. coli* ranged from units to thousands of MPN/100 ml. Similar amounts of *E. coli* have also been reported in the literature for greywater samples (16, 17). However, the number of *E. coli* in bath water can be much higher (up to 10^6 CFU/100 ml) (18). In greywater the possible potential pathogenic bacteria considered in this study were *P. aeruginosa*, *Legionella* and members of environmental mycobacteria. All of these groups of bacteria may not dominantly originate from the source water itself but may multiply in the distribution system. This was supported by the fact that in some buildings *P. aeruginosa* was detected in treated water and at the point of use but was undetectable in the accumulated untreated water. Several studies have examined bacterial growth in plumbing and *P. aeruginosa* was often identified among the microorganisms present in the bacterial biofilm inside the plumbing (13, 19). *Legionella* species are often associated with warm water systems since their optimal growth temperature is above 25 °C. In our study they were repeatedly found in samples from one building, where the source water was from wellness baths which is quite warm. The fact that both of these microorganisms may multiply in the distribution system means that their presence cannot be monitored via general (faecal) indicators, and that they should be monitored directly. The question is whether both microorganisms are necessary or if the possible presence of one can indicate the presence of the other. *L. pneumophila* is particularly relevant in greywater systems due to the higher temperature of the source water, while *P. aeruginosa* is relevant in all types of systems in places where the number of other nutrient-competing organisms has depleted, and they can pass through membranes or colonize them. Each of the two microorganisms (*P. aeruginosa* and *L. pneumophila*) has different transmission modes. While *L. pneumophila* is mainly transmitted through inhalation, *P. aeruginosa* is transmitted mainly through direct contact or ingestion. Therefore, we suggest monitoring both these microorganisms, although not in all of water uses.

It has also been confirmed that viruses can enter greywater through wash-offs during showering and, therefore, they are additional candidates for water quality indicators. Bacterial faecal indicators do not always indicate viral faecal contamination, especially if insufficient disinfection is applied. However, analysing viruses in water is complicated and not feasible for routine monitoring. A better approach would be based on defined minimum requirements for virus removal from raw water. Treatment technology installed should comply with defined log reduction targets for bacteria, viruses and protozoans.

CONCLUSION

The survey and monitoring described above were part of a project aimed at drafting a national regulation for non-potable water, which has been missing in the Czech Republic so far. The recommendation for the Ministry of Health, which will issue the regulation, is primarily based on defined requirements of treatment efficiency to remove reference pathogens – log reduction targets for various sources of non-potable water – and on operational monitoring to ensure the desired and continuous function of treatment technologies. Each treatment technology should be assessed, tested and validated before entering the market. Verification monitoring should be based on three microbiological indicators (*E. coli, L. pneumophila,* and *P. aeruginosa* with a limit value/median of < 3 CFU/MPN/100 mL) and eight chemical indicators (pH, turbidity, NSS, TOC, odour, free chlorine, visible pollution, and temperature). However, not all three microbial indicators are applied to all water sources and end-use types of non-potable water. This seems to be a relevant modern approach to ensuring the safety of reclaimed water used in buildings (20) but also for irrigation (21, 22).

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Conflicts of Interest

None declared

Electronic Supplementary Material

This article contains supplementary material available at https://doi.org/10.21101/cejph.a7884.

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