The number of viable/active *E. coli* cells (conventional surface water treatment plant with multi-barrier treatment system *vs.* groundwater and in distribution network) was measured and the effect of environmental parameters (temperature, chlorine, water residence time etc.) on the occurrence of *E. coli* was studied.
Biofilm collectors were installed in several spots within the surface drinking water treatment train in Riga Waterworks and in several spots within the water distribution network. *E. coli* was analyzed with both FISH (fluorescence in situ hybridization) and QRT-PCR/conventional PCR methods. Activity/viability detection methods were also employed in the combination with FISH. *E. coli* was present in the surface source water and in some of the sites within the drinking water distribution network albeit in small number (0.1-0.01% of total bacterial number). *E. coli* was present in the water distribution network even if water met EU water quality standards. *E. coli* number tended to increase with water residence time in distribution networks supplied with not treated groundwater.
During the sampling period the loads of *E. coli* through multi-barrier water treatment plant was minor (under detection limits), and even *E. coli* which was passing through water treatment train were not viable.

No source of intrusion and governing factors (temperature, biostability and residence time) were identified, although some possibilities, such as negative pressure, low water consumption and the proximity of the sewage pipes are discussed along with the further work, such as concentration of large water volumes from strategically selected sites, including the drinking water distribution network spots after repairs have been done and the sites with low water consumption.

Importance

The phenomenon of biofilm formation, or the attachment of microorganisms to the inner surfaces of the drinking water distribution system which has been shown to alter their physiology, has been well documented. Attached organisms were found to be generally more active in absorbing nutrients, as well as more resistant to the environmental stresses and disinfection. Biofilms in distribution systems may provide favorable conditions for some bacteria, such as opportunistic pathogens (e.g., *Legionella* spp., *Pseudomonas aeruginosa*, and *Mycobacterium avium*), to colonize it and may harbor pathogens, such as *Salmonella enterica*.
E. coli is often detected in the drinking water however the source of the contamination is not. The probability of detecting pollution according to monitoring program of Water Directive is rather low. Hence, traditional methodology for water sampling and analyses is not always able to ensure public safety regarding both (i) the strategy of the sampling and (ii) the choice of the detection method. The sampling strategy is limited to sampling water only, whereas most of the bacteria are attached to the inner surfaces of the pipes forming biofilms. This study was undertaken with the aim to investigate the effect of environmental parameters (temperature, chlorine,

water residence time *etc.*) on occurrence of *E. coli* and to determine the number of viable/active *E. coli* (conventional surface water treatment plant with multi-barrier treatment system *vs.* groundwater and in distribution network).

Approach

Biofilm collectors were placed in various sites within the surface water treatment train and in the drinking water distribution network. The coupons were removed, sonicated and analyzed for the presence and viability of *E. coli.* Both FISH and PCR (conventional and real-time) were used.

Result

The protocol of the analyses, optimized previously within the TECHNEAU project was now applied for testing in the field and the final version of this protocol is given in the report. During the sampling period the loads of *E. coli* through multi-barrier water treatment plant was minor (under the detection limit), and *E. coli* cells which were passing through the water treatment train were not viable. However it cannot be excluded that *E. coli* cells are brought into the distribution network either through a malfunction in the water treatment process or through the intrusion *via* pipes. The cells become trapped in the biofilm, where they remain in a metabolically active form, although they are not detected using the culture-based methods.

The number of *E. coli* tended to increase with water residence time in the distribution networks supplied with not treated groundwater but otherwise no correlation between physical and chemical parameters and the presence or absence of *E. coli* was found.

More information

E. coli was present in the water distribution network even if the water most of the time met EU water quality standards. Presence of even low number of *E. coli* cells in biofilm compromise water quality, thus more attention to on-line monitoring and probabilistic risk assessment is needed.

Future work will include not only the biofilm analyses but also the concentration of large water volumes to be subjected for the analyses, taken from strategically selected sites, including the drinking water distribution network places where repairs have just been done and the sites with low water consumption.

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