The effect of container-biofilm on the microbiological quality of water used from plastic household containers

P. Jagals, C. Jagals and T. C. Bokako

ABSTRACT

Studies in Southern Africa have shown that even when microbiologically safe water is supplied to developing communities at communal standpipes, contamination by high numbers of pathogenic microorganisms may occur during the processes of fetching water from the supply source and storage during use at home, rendering such waters unsafe for human consumption. This study investigated the occurrence of biofilm in PVC storage containers as one possible reason for this deterioration, using heterotrophic bacteria and total coliform counts as well as turbidity as indicators. A second objective was to determine whether biofilm in water-storage containers could contribute to hazardous microbiological contamination indicated by *Escherichia coli* and *Clostridium perfringens*. Results indicated that increased microbiological contamination is associated with biofilm. The biofilm harbours heterotrophic bacteria, total coliforms and *C. perfringens*. *E. coli* could not be associated directly with the levels of biofilm in containers but rather appears to be introduced intermittently from the ambient domestic environment. When dislodged with the biofilm, these bacteria contributed substantially to the deterioration of the microbiological quality of supplied water stored in plastic containers.

Key words | biofilm, *Clostridium perfringens*, *E. coli*, total coliforms, turbidity, water-storage containers

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INTRODUCTION

The microbiological quality of water that people use significantly influences their health (Haas *et al.* 1999). Water intended for human consumption should therefore be safe, implying the absence of harmful microorganisms (WHO 1997). Southern African studies have shown that safe water supplied to developing-world communities at communal stand-pipes becomes microbiologically contaminated during the processes of fetching water in containers over the distance between home and the supply source, as well as storing and using it at home. This rendered the water microbiologically unsafe, especially for drinking (Daniels *et al.* 1990; Genthe & Seager 1996). There appear to be several probable causes for the quality deterioration. Amongst these are unclean containers, unhygienic domestic water-handling practices and natural contamination from the ambient domestic environment into containers (Jagals *et al.* 1999; Theron, 2000; Nala, 2001).

To illustrate unclean containers to community members during a water-hygiene project, Jagals *et al.* (1997) used a swabbing method to dislodge what appeared to be contaminant build-up adhering to inner walls of plastic (PVC) water-storage containers. Inner surfaces of metal containers appeared not to be as conducive to such contaminant build-up because the containers are largely wide mouthed and therefore cleaned more often. Bacterial analyses of these substances found substantial numbers of heterotrophic bacteria, indicating that the contaminants could be biological film similar to that reported in water-distribution pipelines by Kastl & Fisher (1997). Biofilms form when bacteria adhere to surfaces in aqueous environments and excrete slimy, glue-like substances that

anchor them to all kinds of material such as metals, plastics and soil particles. Once anchored to a surface, biofilm-related microorganisms may cause a variety of detrimental reactions that affect the water quality, depending on the environmental conditions (Camper 2000; Geesey 2001). Schaule & Flemming (1997) reported the occurrence of pathogens within biofilm in piped-water distribution systems. Jones & Bradshaw (1996) reported total coliform indicator bacteria re-growth in biofilm.

Since waters stored in containers in domestic environments are far more subject to environmental influences such as contamination by nutrients than water in enclosed pipe distribution systems, it is reasonable to assume that biofilm-like substances could also build up in containers (especially in those not regularly cleaned). It is therefore likely that the contaminants could contribute to hazardous microbiological contamination of container-stored drinking water, especially if particles from the film should become dislodged into, and ingested with, the water.

To gain an understanding of whether contaminant build-up on container inner walls could be likened to biofilm typically found in enclosed water distribution systems, and also the extent to which this could affect the health-related microbiological and aesthetic water quality in plastic containers, the objectives for the work that led to this paper were twofold. One was to report on whether contaminant build-up in storage containers could be biological (biofilm), thereby indicating probable sustenance and growth of bacteria on the inner walls of containers. Second was to determine the effect of the biofilm on the health-related microbiological quality of the water in the containers.

METHODOLOGY

The study was done in a suburb of Botshabelo, a low socio-economic level urban development in the Free State Province of South Africa. The city has a full supply-water reticulation system, which supplies treated chlorinated water to the community largely by means of public standpipes. The suburb had approximately 3,100 households of which 150 households were randomly selected and water samples taken from only the plastic containers in each selected household.

Water samples (in sterile 900-ml Whirlpacks®) were collected weekly over a period of 12 months. A total of 48 samples of the municipal supply and 150 sample pairs from containers were taken. The samples were transported to the laboratory in cooler bags (<10°C) and analysed within 6 h of collection.

The approach was to test the water source, in this case at the communal standpipes constantly supplying treated, chlorinated water to the study community, for the presence of various indicator bacteria groups. To investigate changes in water quality, these organism numbers were then compared with the numbers of the same indicators that occurred in the containers after the households had collected the water from the supply.

Container-stored water was carefully decanted into a sterile Whirlpack® from a household container that had been left standing undisturbed for several hours. This represented an 'undisturbed' state of water (UWS) in the container assuming minimal suspended particles from the contaminant build-up in the liquid.

After taking the UWS, the inner sidewalls of the same container were scrubbed with sterile long-handle brushes to dislodge contaminant build-up and suspend it in the container water. Care was taken to minimise introduction of any substance from the outside environment. For instance, the analysts avoided touching the water or creating excessive floating dust in the dwelling during the operation. Sampling was also not done on dusty or windy days.

The container was then swirled to suspend the loosened material in the container-water content. A follow-up water sample was then immediately taken from the mixed suspension (mixed-suspension container water, MCW).

The data from each sample pair (UWS/MCW) were statistically compared for any changes in the indicator levels.

Water quality indicators

Turbidity, heterotrophic bacteria counts and total coliforms (TC) were used as indicators of contaminant

build-up (biofilm) in containers (SAWQG 1996; Grabow 1996; Ashbolt *et al.* 2001). Turbidity measurements were used to test for changes in the water clarity caused by suspended particulate matter in the container water (Standard Methods 1998). A HACH 2100 turbidity meter was used and the measurements recorded as nephelometric turbidity units (NTUs). Heterotrophic bacteria colonies (referred to as heterotrophic plate counts, HPC) indicated changes occurring in the general microbiological water quality of sampled water before and after dislodging. HPC were determined by a spread-plate method with glucose yeast agar, incubated aerobically at 35°C for 48 h (Standard Methods 1998). All visible colonies on the plates were counted as bacterial colony forming units (CFUs) per 1 ml. Total coliforms (TC) indicated bacterial re-growth potential of the water in containers. TC bacteria were grown on Chromocult® Coliform Agar, which is used for the simultaneous detection of coliforms and *Escherichia coli* in the same water samples (Merck 1996). The membrane filtration (MF) technique was used and membranes placed in triplicate on 90-mm petri dishes before being incubated aerobically at 35–37°C for 24 h. Colonies that appeared in various shades of salmon to red were counted as TC and the numbers were expressed as CFUs per 100 ml.

To determine whether the biofilm could play a role in hazardous microbiological contamination, indicators of potential health effects (Aucamp & Vivier 1990; Payment & Franco 1993; SAWQG 1996; Grabow 1996; WRC 1998; Ashbolt *et al.* 2001) were used. *E. coli* indicated faecal pollution. These were grown on Chromocult® Coliform Agar using the same membrane filtration technique used for total coliforms. Colonies that appeared in various shades of dark blue to violet were counted as *E. coli* (Merck, 1996). The numbers were expressed as CFUs per 100 ml. *Clostridium perfringens* (CP) indicated the persistence of resistant microorganisms such as protozoan parasites and viruses. CP was detected in triplicate on 90-mm petri dishes after sample pasteurisation (at 70°C for 8 min) using the MF technique and supplemented Perfringens Agar (Oxoid 1990) and the plates were incubated anaerobically at 37°C for 48 h. Oxoid® gas generating kits, producing atmospheres of 95% hydrogen and 5% carbon dioxide, were used. Colonies that appeared as partially or fully discoloured dark brown to black were counted. These were expressed as CFUs per 100 ml.

Colony verifications were done for TC, *E. coli* and CP. Between 12 and 40% of all the colonies cultured on the various media were randomly selected. API® 20E (an identification system for *Enterobacteriaceae* and other Gram-negative rods) was used for the verification of TC and *E. coli* colonies. Presumptive CP colonies were verified by the Rapid ID® 32A-identification system for anaerobes.

Data were entered in Microsoft Excel® XP spreadsheets and analysed for central values and 95th percentiles. SigmaStat® Version 2.0 (1997) calculated and tested for sample sizes and statistically significant differences between data sets using non-parametrical tests for log-normal data to accommodate excessive variance (Helsel & Hirsch 1995). Significant differences between unequal data sets were tested for using ANOVA on Ranks (Kruskal–Wallis) and Rank Sum Testing (Mann–Whitney). Signed Rank Tests (Wilcoxon) were used for UWS/MCW data pairs. SigmaPlot® Version 8.00 (2002) plotted the data in graphs. In Figures 1 and 2, vertical bold and dashed lines indicate Target Water Quality Guidelines (TWQG) suggested in the South African Water Quality Guidelines (SAWQG 1996) as well as in the World Health Organisation (1997) *Guidelines for drinking water quality*.

RESULTS AND DISCUSSION

Indicators of contaminant build-up (biofilm) in containers (Figure 1)

Comparison of the turbidity measurements showed that the median turbidity value in the supply water was 0.62 NTU, which was significantly ($P = 0.022$) lower than the median NTU of water from the UWS samples (median 0.81). The turbidity levels for the MCW samples (median 1.87) were significantly higher than those in the UWS and in the municipal supply ($P \leq 0.001$). This means that the supply water became contaminated from filling containers at the supply point and storage as well as handling in the

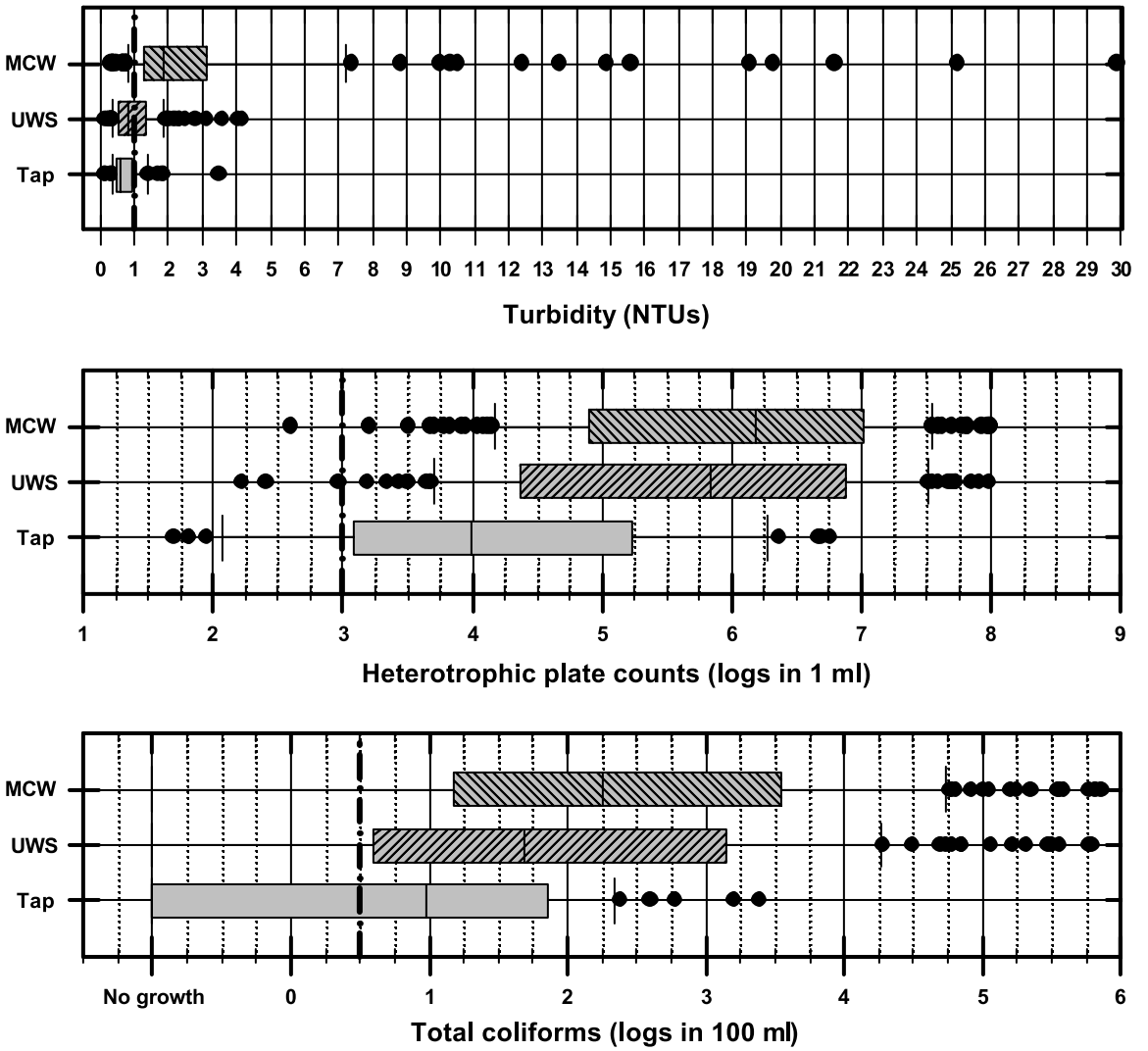

Figure 1 | Turbidity levels and numbers of heterotrophic bacteria and total coliforms associated with biofilm build-up in household water-storage containers.

dwelling. The further increase in turbidity after the dislodging operations indicated that particles had indeed built up on container inner walls.

The median turbidity for the tap water samples was well within the SAWQG (1996) target water quality range (TWQG) (indicated by the bold dashed line in each graph) of 0–1 NTU, which indicated no visible turbidity or adverse aesthetic effects (appearance, taste or odour) and no significant risks of associated transmission of infectious microorganisms. In other words no adverse health effects are expected as a result of the suspended matter that caused the turbidity. If, however, the NTU data for the tap water are compared with the water quality criteria at the 95th percentile, as suggested by the SAWQG, the NTU levels do exceed the TWQG of 0–1 NTU (1.82 NTU). This implies that, while no turbidity might be visible, a slight chance of adverse aesthetic effects and infectious disease transmission exists.

The turbidity levels in the undisturbed container water were significantly higher than those in the tap water. More than 30% of samples tested above 1 NTU, with a 95th percentile value of 2.3 NTU. This indicates that domestic

container-water handling leads to an increase in turbidity, causing a slight chance of adverse aesthetic effects and disease transmission. The increased levels in the MCW (median 1.9 NTU) meant that, at the 95th percentile of 13.85 NTU, there were severe aesthetic effects. Associated risk of disease due to infectious disease agents and chemicals adsorbed onto particulate matter (SAWQG, 1996) could be expected when contaminant particles were released into the container water.

The numbers of heterotrophic bacteria in the municipal water supply were significantly lower than those of the two container sample sets ($P<0.001$). The log-median of 3.9 and the 95th percentile (log 6.67) nevertheless indicated that the general microbiological quality of the supply water was not satisfactory, indicating contamination or definite re-growth in the distribution system as well as increased risk of infectious disease transmission. After-growth in distribution systems could provide harbourage for heterotrophic bacteria (Kastl & Fisher 1997). Another possibility is activities related to frequent pipe breaks, which periodically subject water in the distribution network to environmental pollution (Jagals *et al.* 1997, 1999). These excessive levels of bacteria were released from the distribution system into the containers during filling but the significantly higher HPC levels in the UWS suggested that even more HPC were introduced after filling, probably from further growth in the containers or from the environment.

There were no statistically significant differences between the numbers of UWS (log-median 5.84; log-95th percentile 7.68) and MCW (log-median 6.18; log-95th percentile 7.81) heterotrophic bacteria in the container water ($P=0.108$) when measured by means of rank and sum (Mann–Whitney Rank Sum Test). This is also apparent in Figure 1. However, when comparing the data of each sample pair before and after (Wilcoxon Signed Rank Test) the dislodging process, the UWS data were significantly lower than the MCW data ($P\leq0.001$). This means that the microbiological water quality deteriorated significantly each time contaminant build-up is dislodged, adding more heterotrophic bacteria to the high levels already introduced by other means as suggested earlier. This indicated that particles dislodged from container inner walls were likely to contain heterotrophic bacteria in greater numbers than the general undisturbed container water or the municipal supply. This suggests that after-growth similar to that occurring in enclosed distribution systems is also possible in drinking water container systems. From a risk perspective, the numbers of heterotrophic bacteria in all three data sets indicated an increased risk of infection to consumers by exceeding the TWQG of 1,000 organisms per 1ml (bold dashed line) proposed by the SAWQG (1996).

The median for total coliform numbers (log-median 0.98) in the municipal supply was below the negligible risk limits of not more than 5 organisms per 100 ml proposed by the SAWQG (1996). Nevertheless, from Figure 1 it is evident that more than 50% of the samples contained TC in excess of this level with log 2.76 at the 95th percentile. This confirmed organic contamination of the distribution system indicated by the heterotrophic bacteria numbers. At the 95th percentile (UWS log 5.06 and MCW log 5.25), the TC numbers exceeded the TWQG of ≤100 (SAWQG 1996). This is indicative of post-treatment contamination or definite growth in water distribution systems (Jones & Bradshaw 1996) or, as in this instance, the containers. The significantly higher total coliform numbers in the dislodged particles sampled from the MCW ($P\leq0.001$) suggest biofilm formation on the sidewalls of the domestic water storage containers assessed during this study. Significant and increasing risks of infectious disease transmission were also indicated.

Indicators of hazardous microbiological contamination

The World Health Organisation (WHO) *Guidelines for drinking water quality* (1997) sets a TWQG of no detectable *E. coli* per 100 ml of sample. Figure 2 shows only intermittent *E. coli* occurrence with no statistically significant differences between any of the sample sets ($P<0.001$). This indicated occasional faecal pollution in the water. No *E. coli* were detected in the supply water at the 95th percentile. In the containers at the 95th percentile, however, *E. coli* occurred at log 1.7 and log 2.69 for UWS and MCW, respectively, which indicated risk of microbial infectious disease to consumers with occasional exposure (WHO, 1997).

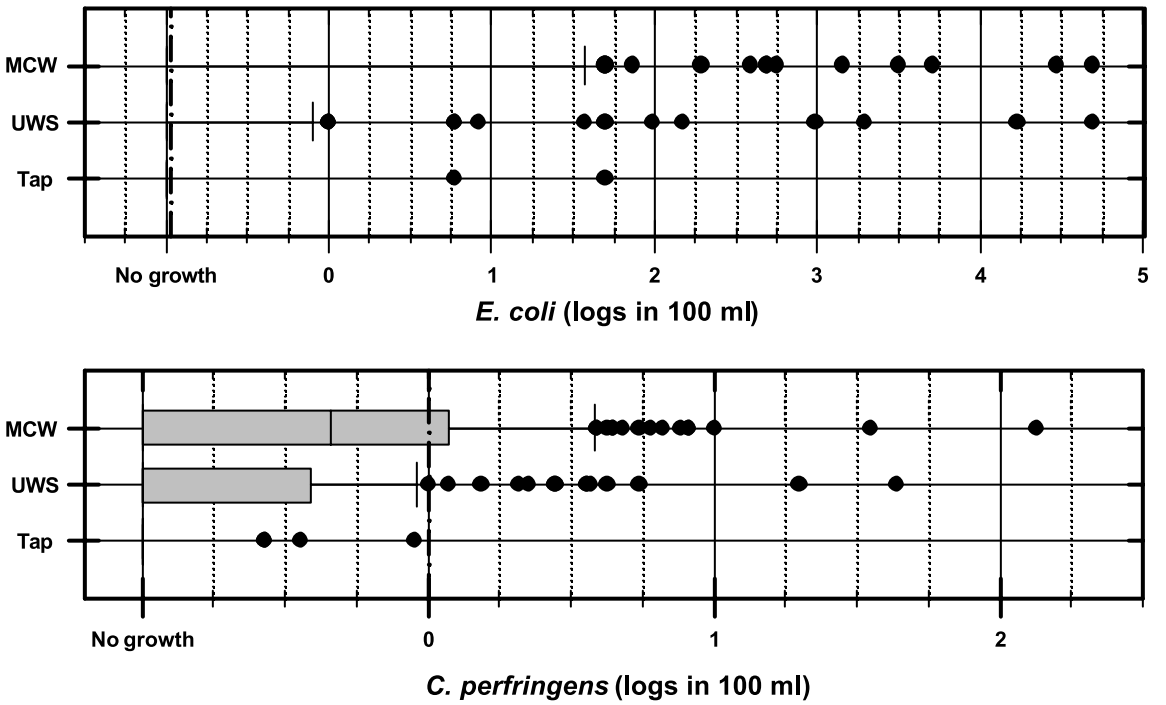

Figure 2 | Health-related microbiological indicators associated with biofilm build-up in household water-storage containers.

E. coli appeared not to be associated with the occurrence of the biofilm-indicating organisms HPC and TC in the water-storage containers ($P>0.05$) for both UWS and MCW. Poor personal hygiene practices during the use of water from containers and unhygienic domestic environments (Theron, 2000) could be the indirect cause of the introduction of *E. coli* from the ambient domestic environment.

All the water samples sporadically contained vegetative *C. perfringens* spores with the lowest detection in the municipal water supplies (none detected at the 95th percentile). Tap water, therefore, did not contribute to the significantly higher ($P<0.001$) CP numbers in the container water samples. The CP numbers for municipal water were well within the TWQG of 0 organisms per 100 ml, above which constitutes a slight risk of microbial infectious disease to consumers suggested by the Water Quality Criteria for South Africa proposed by Aucamp & Vivier (1990). While most of the data for UWS and MCW were also within the limit, spores did occasionally occur in numbers exceeding this at the 95th percentile. CP numbers were significantly higher ($P<0.001$) in the MCW (log-median 0, log-95th percentile 0.447) than in the UWS (log-median 0.337, log-95th percentile 0.781) indicating that the biofilm could readily contain and even accumulate CP spores. This implies that the biofilm could harbour pathogens as suggested by Schaule & Flemming (1997).

CONCLUSIONS

This study has shown that contaminant build-up on the inner sidewalls of plastic domestic water-storage containers contains microorganisms and can therefore be described as container-biofilm. These films break loose from the sides (especially during filling with no subsequent rinsing) and form particulate suspensions which harbour significant numbers of viable bacteria. The dislodging (scrubbing) technique simulated this process of biofilm being loosened during the normal filling and transporting

process. The increase in turbidity after scrubbing the inner sidewalls of water containers and suspending the loosened matter in the container water (MCW), supported this.

The suspended particles apparently harboured and possibly propagated potentially hazardous microbiological contaminants as suggested by the increase in numbers of *E. coli* and *C. perfringens* spores. The mixed suspension of container water and biofilm particles contained significantly higher levels of these bacteria than the undisturbed container water or water from the source tap. The intermittently occurring *E. coli* as opposed to the constantly occurring higher numbers of total coliforms and *C. perfringens*, indicated that *E. coli* could have been introduced from the ambient domestic environment or from handling of water with contaminated hands and utensils. *E. coli* were apparently not effectively supported by the biofilm and probably died off after a while in the container water.

While the microbiological quality of water supplied to the community generally did not comply with the microbiological limits in terms of guidelines, the *E. coli* and *C. perfringens* levels generally did. Biofilm in the containers contributed substantially to the deterioration of the quality of supplied water due to circumstances surrounding storage and handling of such water during collection and transportation as well as at the points of use.

RECOMMENDATIONS

Containers should be cleaned and disinfected thoroughly, preferably before each filling. Domestic water-handling practices associated with container-stored water also need to be improved. Containers should be effectively covered or closed between water extractions. Containers should generally be stored away from the floor or windows. Children, pets and other domestic animals should be denied access to water in containers. Water-users should wash hands before handling water (Pinfold 1990), especially when scooped out for drinking or food handling. In previous studies (Jagals *et al.* 1999), the methods of extracting water from containers were also associated with microbiological contamination of such water. In many instances, water was scooped from the containers with mugs kept uncovered as well as with unwashed hands touching the water (Theron 2000).

In areas where safe water is supplied to communities, the ultimate way to prevent this type of contamination is to shorten the tap-to-glass sequence by supplying people with water inside their houses. In many areas, this might not be feasible in the medium to long term as public services struggle to catch up on providing treated, piped water to people. In the meantime, other ways to increase consumer water-safety in areas where water is still being fetched from communal taps and stored at home, should be devised. For instance communities should be made aware of the essence of sound domestic water hygiene (including container hygiene). People should also be educated to apply sustainable, precautionary anti-contamination measures during water haulage, water handling and usage during storage at home.

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