# Opportunistic pathogens relative to physicochemical factors in water storage tanks

S. N. Al-Bahry, A. E. Elshafie, R. Victor, I. Y. Mahmoud and J. A. Al-Hinai

## ABSTRACT

Household water in Oman, as well as in other countries in the region, is stored in tanks placed on house roofs that can be subjected to physicochemical factors which can promote microbial growth, including pathogens and opportunistic pathogens which pose health risks. Water samples were collected from 30 houses in a heavily populated suburb of Muscat. The tanks used were either glass reinforced plastic (GRP), polyethylene or galvanised iron (GI). Heterotrophic bacteria, coliforms, faecal coliforms and iron sulphur bacteria varied significantly in the three tanks. Yeast and mould count showed significant variations. Isolation of *Aeromonas* spp., fluorogenic and pathogenic *Pseudomonas*, *Pasteurella*, *Salmonella*, *Serratia* and *Tatumella*, and *Yersinia* and *Legionella* in biofilms varied in the three tanks. The fungi isolates in the three tanks were *Penicillium*, *Cladosporium* and *Aspergillus*. Nephelometric turbidity unit, threshold odour number and free chlorine varied significantly in the three tanks. True colour unit values did not show a significant difference; however, GRP tanks had algae, autotrophic and pigmented microorganisms. In addition, GI tanks had sediments and corrosion. The results of this investigation are important to evaluate the status of the present household water tanks in countries with high annual temperatures, which may affect public health.

**Key words** | house storage tanks, opportunistic pathogens, physicochemical, water

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## INTRODUCTION

Microbial growth in distribution systems and storage tanks is well documented (Cook *et al.* 2001). The universal occurrence of heterotrophic bacterial biofilms in water storage tanks is an indication that the environment is capable of supporting a diverse microbial population including pathogens (Carter *et al.* 2000).

Desalinated sea water is the main source of domestic water for the residential areas of Muscat, Oman, similar to the other Arabian Gulf countries. The desalinated water is distributed through a network system from the main public water stations to the houses. All the water storage tanks have breather holes. The desalinated water is frequently inspected for its quality according to Omani Drinking Water Standards (Omani Standard number 8/2006). However, distributed water may be subjected to contamination during the process of distribution.

Today, most residential water storage tanks in Oman and elsewhere in the Arabian Gulf region are made from plastic material, whereas 20 years ago the majority of tanks were made from galvanised iron (GI). However, GI tanks are still used in some areas. Plastic tanks are available in two types: polyethylene (PE) and glass-fibre reinforced plastic (GRP). The reason for the replacement is that plastic tanks are about one-third the weight of steel tanks with as much or greater strength and also GI tanks are frequently subject to rust and corrosion.

Plastic tanks are manufactured in Oman, with no specific regulations controlling the quality of their production or post-production standards. The Omani Standard number 8/2006 (2006) for drinking water does not include criteria for water quality in household storage tanks. However, total and faecal coliforms are used as quality indicators for drinking

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water in general. Moreover, public awareness is lacking with regard to drinking water stored in household tanks.

Little is known about the presence of microorganisms, as well as the physicochemical factors that promote their growth in domestic storage tanks in the Arabian Gulf region. Drinking water contamination is frequently related to poor sanitation and hygiene which may be caused by abiotic factors which support microbial growth and cause deterioration in water quality (Pruss *et al.* 2002; Egorov *et al.* 2003; Khan *et al.* 2007). Millions of people around the world suffer from gastrointestinal illnesses attributable to polluted drinking water (WHO 2002).

The aims of this investigation are to compare the microbial populations in relation to physicochemical characteristics in the three household storage tanks (GRP, PE and GI) and to evaluate the corresponding drinking water quality. This will be achieved by determining the densities of heterotrophic microbial populations in the water in relation to its physicochemical characteristics, examining surface-adherent biofilms, and relating the physical conditions in the tanks to health issues.

## METHODS

### Study area

The study site was selected in North Al-Hail, a densely populated suburb in the city of Muscat, Oman, where the three main types of storage tank are used by the residents (Figure 1). The tank capacities ranged between 1,135.6 and 1,514 L. The study consisted of both high and low income inhabitants. However, the GI tanks were mostly used by the low income residents.

In Muscat, the upper range of ambient temperatures is 35–49 °C between April and October, and the lower range is 16–35 °C between mid-November and March.

### Collection of samples

A total of 30 houses were randomly selected based on the type of holding tank, with 10 houses for each type. The procedures for sample collection and handling were applied as described by *Standard Methods* (APHA/AWWA/WEF 1998). Samples were collected aseptically in sterile 500 mL glass containers. Sampling was carried out once every two weeks. All samples were analysed immediately after collection. Sodium thiosulphate pentahydrate ($N_2S_2O_3 \cdot 5H_2O$) (SIGMA, USA) concentration at 100 mg $L^{-1}$ was added to sample containers for water collection as a dechlorinating agent to prevent bactericidal activity (WHO 1995, *Guidelines*, Vol. I). A chelating agent was added to each sample collected from the GI tanks to prevent the oligodynamic activity of heavy metals. Di-sodium salt of phenylenediaminetetracetic acid (EDTA) was added at a concentration of 372 mg $L^{-1}$ with pH adjusted to 6.5 (ISO 1994). The majority of GI tanks examined in the sampling area were partially or totally corroded.

### Condition of the household storage tanks and health related issues

The water tanks of 30 houses were surveyed. This involved the age of tanks, number of roof tanks, size of tanks, types of household pipe, washing frequency, presence of algae and autotrophic microorganisms, and detection of colour and odour. Ten houses for each type of water storage tank were selected. The questions posed to residents are listed in Table 1. During the first visit to each house, the materials in the tanks (i.e. paints and coating materials) were recorded.

Owing to the scarcity of water, residents are forced to use storage tanks. The effect of storage of water on health has not been investigated. Moreover, little is known about the behaviour of microorganisms in storage tanks, especially in temperate regions. Until it reaches a consumer tap, drinking water is exposed to several types of contamination. Incidences of gastrointestinal illness were recorded at the time of sample collection. A questionnaire related to water quality and health issues was distributed to the inhabitants of the 30 surveyed houses in the study area. The questions are listed in Table 2.

### Microbiological analyses

Sample bottles and related glassware were cleaned with detergent followed by soaking in hypochlorite solution containing 0.1% free chlorine for 1 h and rinsed several times

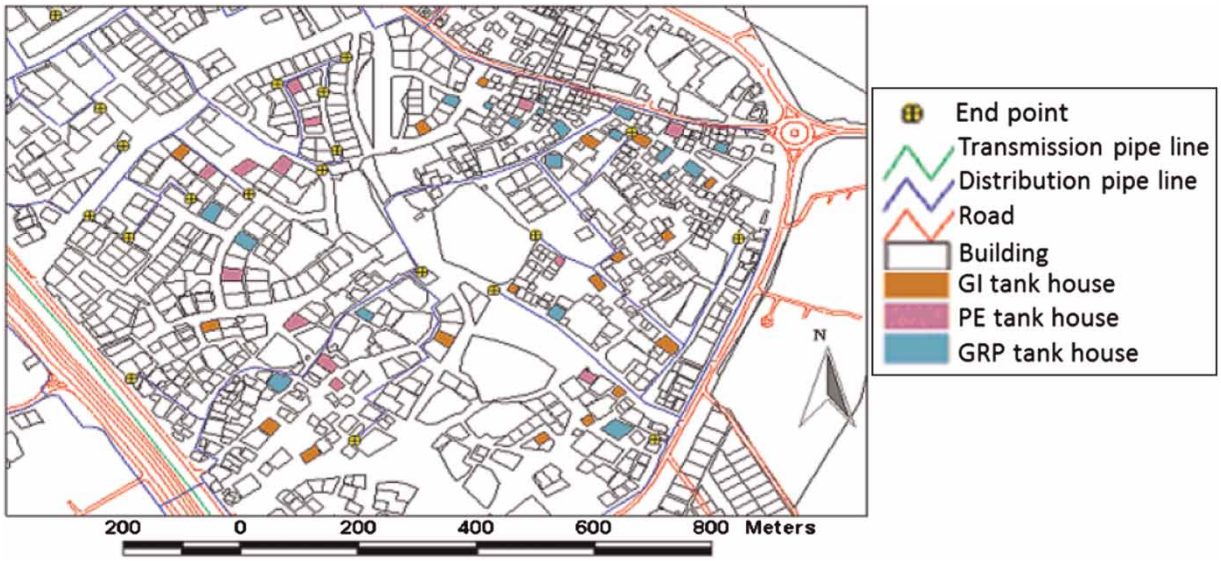


**Figure 1** | Town-planning map of North Al-Hail illustrating the houses that were supplied by the main line in the study area.

with distilled water. A final rinse was done using the chlorine-demand free water. All glassware was sterilised in a hot air oven at 170 °C for 1 h.

The microbiological analysis of water samples was undertaken for heterotrophic enumeration and isolation of target pathogenic bacteria. Enumeration of microbial populations recorded: total heterotrophic plate count (THPC), total yeast and moulds (TYM), total coliform bacilli (TC), total iron and sulphur bacteria (TIS), total pigmented bacteria (TPB), and algae and autotrophic microorganisms (Collins *et al.* 1995; British Standard BS 6068 1995).

Total coliforms and faecal coliforms were enumerated by the membrane filter (MF) technique (Augoustinos *et al.* 1993; Aulicino & Orsini 1996). Total iron TIS bacteria were estimated using microscopic examination of samples filtered

**Table 1** | Survey of physical conditions of water systems in the study area from 30 houses

| | Type of tank material | | |
|---|---|---|---|
| **Aspects of survey** | **GRP, % ($n = 10$)** | **PE, % ($n = 10$)** | **GI, % ($n = 10$)** |
| High inhabitant number (more than 10 individuals per house) | 40 | 50 | 100 |
| Age of tank (more than10 years) | 0 | 0 | 100 |
| Number of roof tanks (more than 1 tank) | 20 | 40 | 0 |
| Size (300–500 L) | 100 | 100 | 100 |
| Galvanised household pipes | 60 | 20 | 100 |
| PVC household pipes | 40 | 80 | 0 |
| Washing frequency (range of 7–12 months) | 66 | 50 | 0 |
| Presence of cracks or other defects | 90 | 10 | 100 |
| Presence of algae and autotrophic microorganisms | 100 | 0 | 10 |
| Detection of colour, corrosion and odour problems by consumers | 100 | 10 | 90 |

**Table 2** | Survey of health issues on water quality from inhabitants of 30 houses in the study area

| | Type of tank material | | |
|---|---|---|---|
| **Aspects of survey** | **GRP, % ($n = 10$)** | **PE, % ($n = 10$)** | **GI, % ($n = 10$)** |
| Individuals experienced gastrointestinal and other water-related illnesses | 13.3 | 6.7 | 26.6 |
| Tap water contains some chemical pollutants | 40 | 8.9 | 17.8 |
| Increased algal and autotrophic microorganisms during summer | 40 | 0 | 11.1 |
| Tap water was rated as very good or good by consumers | 4.4 | 6.7 | 8.9 |
| Seeking for alternatives was motivated by aesthetic concerns | 22 | 20 | 0.0 |

through a MF with a pore size of 0.45 ± 0.02 μm and a diameter of 47 mm (Millipore, Bedford, UK) (ASTM 1992).

Biofilm deposits and swab samples from the inner surface of the tanks, shower heads and inner tap faucets were taken for determining the presence of the following pathogenic bacteria: *Aeromonas* spp., *Pasteurella* spp., fluorogenic pseudomonads, pathogenic *Pseudomonas*, *Salmonella* spp., *Yersinia* spp. and *Legionella* spp. Methods of isolation and recovery were according to *Standard Methods* (APHA/AWWA/WEF 1998). Identification of bacteria was achieved as described by AOAC (2000). Isolation of *Legionella* spp. was carried out using the methods of Barrow & Feltham (1993) and of Kusnetsov *et al.* (1994) in buffered charcoal yeast extract (BCYE).

### Physicochemical factors

Distilled and deionised water was used for physicochemical analysis (British Standard BS 6068 1995). All glassware was cleaned with detergent followed by 0.1 N $HNO_3$ and then rinsed thoroughly with deionised water. The measurement of free and total chlorine concentrations was undertaken using the HACH free and total chlorine test kit (HACH Company, USA). The detection range was to be from 0.0 to 0.7 mg $L^{-1}$ for the free chlorine and from 0.0 to 3.5 mg $L^{-1}$ for the total chlorine. Colour was examined using the Lovibond drinking water test kit (Lovibond, USA) and was measured in Hazen units with a detection range of 5 to 70 units. The water pH was measured during sampling using a pH meter (Orion, USA). Measurement of turbidity in nephelometric turbidity units (NTU) was done according to World Health Organization (WHO 1997) *Guidelines* (Vol. 3) and *Standard Methods* (APHA/AWWA/WEF 1998).

Odour was measured using the threshold odour number (TON) scale as described by *Standard Methods* (APHA/AWWA/WEF 1998). The odour test was statistically analysed using a Kruskal–Wallis test after ranking the data (TON). Five people were involved in collecting samples in odour-free glass bottles with glass closures and the tests were completed within 4 h of collection. To ensure reliable threshold measurements, odour-free glassware were obtained by cleaning with non-odourous soap (Labolene, Glaxo Ltd., India) and 0.1 N $HNO_3$ followed by rinsing with MilliQ water. The quality of the water was verified before every test run and ensured to give odourless results. Calibration of tester's response was done using known concentrations of n-butanol and artificial lemon flavour simultaneously.

The measurements of TDO, TDS, EC and salinity were done using field-equipped electrodes (Orion, USA).

### Statistical procedures

A randomised sampling design was used with 30 houses in the sampling area selected. Sample sites were chosen using a table of random digits as given in Fisher and Yates Tables. Data analysis was undertaken with Statistical Package for Social Science. Square root mode was used for data transformation. One-way analysis of variance was run to compare means. A *post hoc* multiple comparison test (Tukey's test) was run to examine the differences between all possible pairs of means. Control samples were collected from the main distribution station throughout the sampling period from different locations across the distribution system (Clarke 1994).

## RESULTS

### Condition of the household storage tanks

The data of age of tanks, number of roof tanks, size of tanks, types of household pipe, washing frequency, presence of algae and autotrophic microorganisms, and detection of colour and odour are summarised in Tables 1 and 2. Tanks which were 10 years old or more were all GI tanks with 100% having GI pipe networks. Algae and autotrophs occurred predominantly in GRP tanks followed by GI tanks, with most of odour and colour problems being reported in GRP and GI tanks. The most frequent washing of the tanks by the household owners occurred in GRP and PE and none from GI tanks. All GI tanks showed different degrees of corrosion.

It was observed that several manufacturers of GRP tanks used hazardous materials such as lead chromate which was the main source of contamination. One possible source of lead contamination was the pigment used in the painting of the inner surface of the tanks to prevent algal growth.

## Health risk perception of the inhabitants

Based on the data obtained from the interviews during the field survey (Table 2), it is apparent that various segments of the public have different perspectives of water quality problems. In addition, several inhabitants complained of gastrointestinal and related illnesses.

It is apparent from Table 2, relative to the health issues, that most gastrointestinal (GIT) illnesses are related to water tank types and the piping used. The most frequent correlation with GIT illness was GI tanks, followed by GRP and the least by PE. The frequency of the gastrointestinal illnesses may be related to the types of tank and pipe used. For example, the occurrence of colour, corrosion and odour was frequently found in households using GI pipes with GRP and GI tanks. Inhabitants who used such systems had high numbers of cases of gastroenteritis. In addition, algal and autotrophic microorganism growth, especially in GRP tanks and to a lesser extent in GI tanks, may contribute to gastroenteritis (Tables 1 and 2).

## Physicochemical factors of drinking water

Free chlorine residual, turbidity (NTU), odour (TON) and colour (TCU) values were compared according to the Omani Standard number 8/2006 (2006) for the physicochemical qualities of drinking water. For free chlorine residual, the highest value was detected in PE and GI tanks at a significant level ($P < 0.05$) over GRP samples. Total chlorine was undetectable in any of the three tank types. The turbidity (NTU) values of the water samples from GI tanks were significantly higher ($P < 0.05$) over GRP and PE, while GRP and PE were not significantly different from one another. There was a significant difference in TON values between the three tank types ($P < 0.05$). The maximum TON values were recorded from the GRP tanks. There was no significant difference between the values of water colour in the three tank types, but the highest value was detected in GI tanks (Table 3).

The pH values in all samples ranged from 6.8 to 7.6 with no variations between the tanks.

## Counts of the microbial populations in the tested tanks

All samples contained non-coliform, non-spore forming heterotrophic bacteria while the THPC count was found

**Table 3** | Physicochemical characteristics (mean ± SE) of water samples from GRP, PE and GI tanks with the limits of reference standard.

| Type of storage tank | Free chlorine (mg $L^{-1}$) | Turbidity (NTU) | TON | Colour (TCU) |
|---|---|---|---|---|
| OS. 8/2006 (MCLs) | 0.1–0.4 | 0–5 | 3 | 15 |
| GRP ($n = 10$) | $0.1 \pm 0.0^{b}$ | $3.2 \pm 0.0^{a}$ | $5.0 \pm 0.0^{a}$ | $8.5 \pm 0.8^{a}$ |
| PE ($n = 10$) | $0.2 \pm 0.0^{a}$ | $2.2 \pm 0.0^{a}$ | $1.0 \pm 0.0^{b}$ | $7.5 \pm 0.8^{a}$ |
| GI ($n = 10$) | $0.2 \pm 0.0^{a}$ | $5.7 \pm 0.0^{b}$ | $3.0 \pm 0.0^{c}$ | $9.5 \pm 1.1^{a}$ |

Values with different letters are significantly different at the $P < 0.05$ level. $n$ = number of replications per treatment; NTU, nephelometric turbidity unit; TON, threshold odour number; TCU, true colour unit; OS, Omani Standard; MCLs, maximum contaminant levels.

to be significantly higher ($P < 0.05$) in samples collected from GI tanks (Figure 2). All the samples contained different members of the coliform group. The total count (TC) of coliforms was significantly higher ($P < 0.0001$) in samples collected from GRP tanks followed by the GI tanks and the values from the GI tanks were significantly higher than from the PE tanks ($P < 0.05$) (Figure 3). In all samples, *Klebsiella pneumonia*, *Enterobacter cloacae*, *E. sakazakii*, *Serratia marcescens*, *S. fonticola*, *Citrobacter freundii* and *C. koseri* were found to be the predominant coliforms in all tank types with higher percentages in GRP when compared with PE and GI tanks. *Tatumella ptyseos* was isolated from GRP (6.1%) and GI (6.6%) tanks. *Escherichia vulneris* was found in GRP only at 8.4% (Figure 4).

The highest frequency of TIS bacteria was found in both GRP and GI tanks, while in PE values were significantly

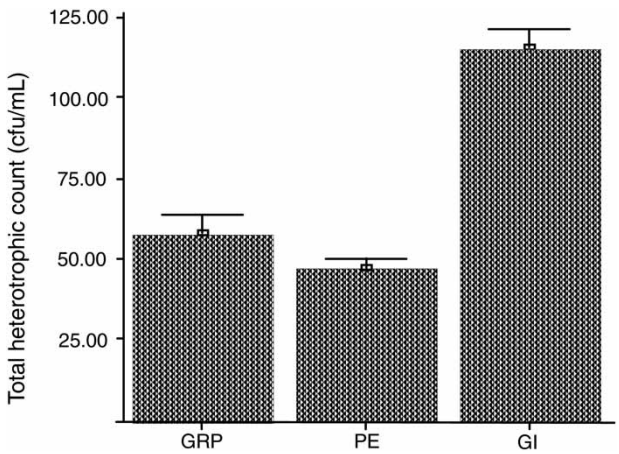


**Figure 2** | THPC of water samples in GRP, PE and GI tanks ($n = 40$ replications/treatment).

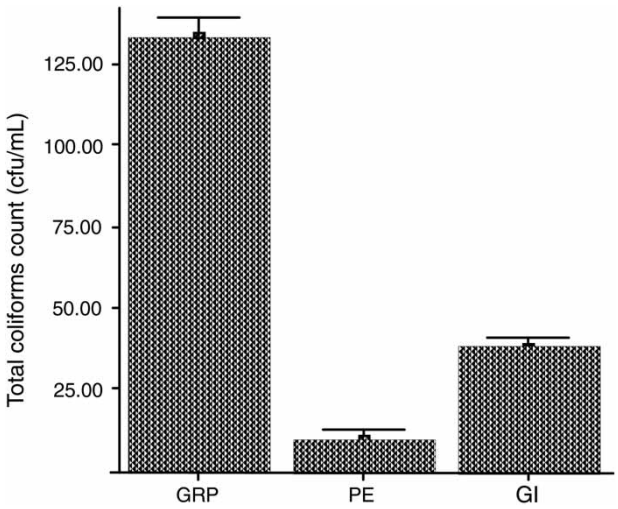


**Figure 3** | Total coliform count of GRP, PE and GI tanks ($n = 40$ replications/treatment).

lower ($P < 0.05$) (Figure 5). All water samples were found to contain TPB but in different frequencies. The count was highly significant in the PE and the GI tanks over the GRP tanks ($P < 0.0001$) (Figure 6).

Yeast and moulds (TYM) were detected in all tested samples with the highest count being from the GRP tanks where *Penicillium* spp., *Cladosporium* spp. and *Aspergillus* spp. were in significantly higher numbers than in the other two tanks ($P < 0.0001$) (Figure 7).

## Characterisation of surface-adherent biofilms in GRP, PE and GI tanks

*Aeromonas* spp., fluorogenic and pathogenic *Pseudomonas* spp., *Pasteurella* spp., *Salmonella* spp., *Yersinia* spp. and *Legionella* spp. were isolated from swab samples in the inner surface of the tanks, shower heads and inner tap faucets. GRP tanks supported the growth of target microorganisms (Figure 8). The highest frequency of *Aeromonas* was detected in GI tanks (77.5%) compared with the frequencies of isolation in GRP (26.3%) and PE (29.6%) tanks. Two species were isolated: *A. sobria* and *A. hydrophila*. No *Pasteurella* spp. was isolated from PE tanks but they were isolated from GRP. The frequency of isolation was 17.2% in GRP tanks and 23.4% in GI tanks. Two species were identified, *P. haemolytica* and *P. pneumotropica*. Fluorogenic *Pseudomonas* were isolated from all tanks with the highest recovery from PE tanks of 97.5%, followed by GRP, 46.1%, and GI tanks, 7.2%. Pathogenic *Pseudomonas* (*P. pseudomallei*) was isolated in low frequencies in all tanks. No *Salmonella* was isolated from PE tanks. In GI and GRP tanks two species of *Salmonella* were identified (*S. typhimurium* and *S. arizonae*). *Y. enterocolitica* and *Y. pseudotuberculosis* were isolated from GRP and PE tanks.

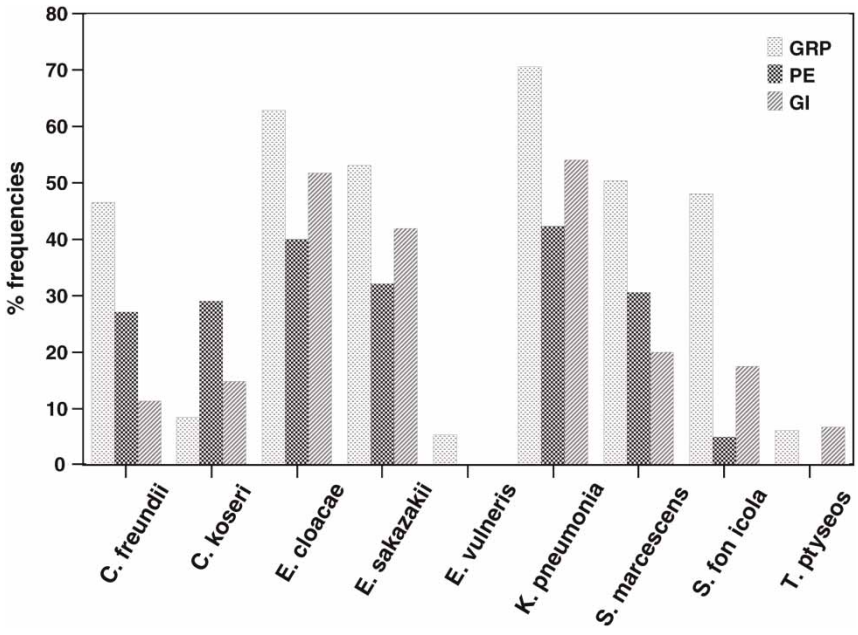


**Figure 4** | Coliforms and their percentage frequencies of isolation in the three tanks; Ent = *Enterobacter*; Esc = *Escherichia*.

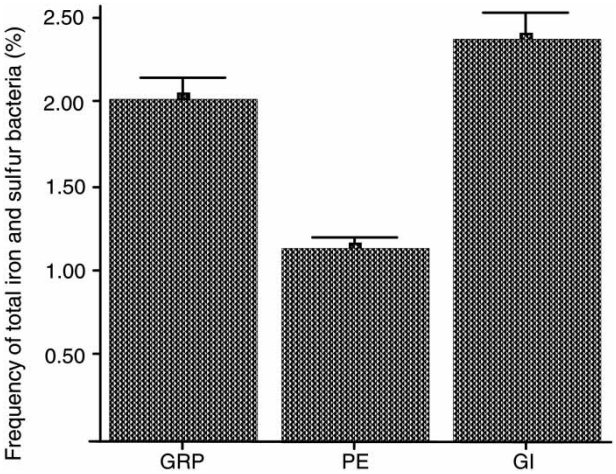


**Figure 5** | Frequency of detection of TIS bacteria in GRP, PE and GI tanks ($n = 10$ replications/treatment).

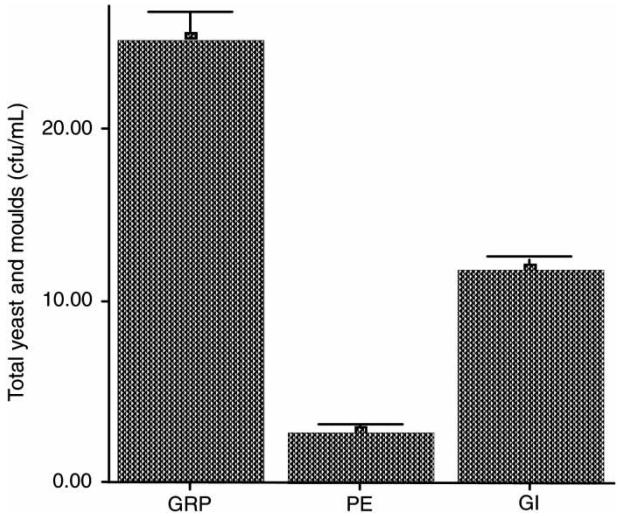


**Figure 7** | TC of yeast and moulds of GRP, PE and GI tanks ($n = 40$ replications/treatment).

## DISCUSSION

This study was designed to provide information about the microbial and physicochemical factors within drinking water storage tanks and their effects on public health. The present water storage system is commonly used throughout the Arabian Gulf region. Several studies have shown a health risk from drinking water stored in household tanks (Camper *et al.* 1998; Prévost *et al.* 1998; Momba *et al.* 2000; Simmons *et al.* 2001). The results in this study support the previous conclusion that drinking water quality from holding tank systems is affected by microbial regrowth facilitated by the physicochemical characteristics of the water.

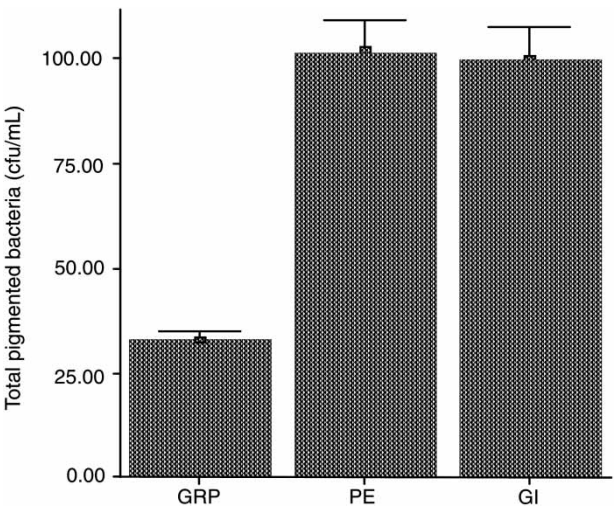


**Figure 6** | TC of pigmented bacteria in GRP, PE and GI tanks ($n = 40$ replications/treatment).

Biofilms are found in aquatic systems where different microbes can be trapped for long periods. In this study THPC, TC, TIS, TYM and the microbial isolates in biofilms varied between the three tanks. *Aeromonas, Legionella, Pasteurella*, fluorogenic *Pseudomonas*, pathogenic pseudomonads, *Salmonella, Serratia, Tatumella* and *Yersinia* were isolated from the three tanks at different frequencies. Other investigators isolated similar microbes from storage tanks (Van der Wende *et al.* 1989; Payment 1999; Nevondo & Cloete 1999). In those reports the microbes were isolated from biofilms and sediments found in water distribution systems (Allen *et al.* 1980; Martin *et al.* 1982). Most of the isolates are among the opportunistic pathogens that may pose health risks to immunocompromised individuals. For example, Göksay *et al.* (2008) and Venkatesh *et al.* (2006) found such bacteria to be an important source of cross-infection in hospitals. Some produce toxins which may cause mild inflammation to serious toxic shock (Venkatesh *et al.* 2006). A large number of potential pathogens may exist in water; however, in most cases they exist in small numbers and are unable to establish an infection (Calderon & Mood 1988, 1991).

The microorganisms which were found in GRP tanks were isolated at different frequencies. *Pseudomonas* (fluorogenic and pathogenic spp.) were frequently isolated.

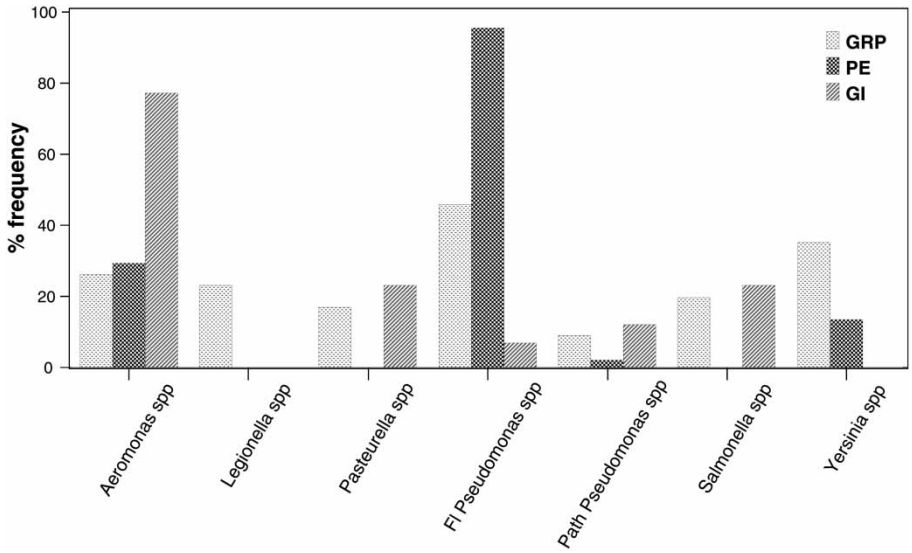


**Figure 8** | Composition of microbial biofilms in GRP, PE and GI tanks; Fl = fluorescent; Path = pathogenic.

Although *Legionella* spp. was less frequent, it has previously been reported to cause infection in immunocompromised patients (Van der Mee-Marquet *et al*. 2006).

In PE tanks, *Pseudomonas* spp. were isolated at high frequency, whereas enteric pathogens such as *Salmonella* and *Pasteurella* were undetectable. The absence of these pathogens correlated with low THPC numbers suggesting an antagonistic effect of *Pseudomonas* spp. However, there were high numbers of *Aeromonas* spp. in GI tanks, whereas fluorogenic *Pseudomonas* spp. were isolated at very low frequencies, and no *Yersinia* spp. were isolated from these tanks.

Similar to our study, Schwartz *et al*. (1998) in Germany reported variation of bacterial composition from biofilms in PE, PVC, GI and copper materials used in potable water systems. Synthetic materials (PE and PVC) were colonised very rapidly in significantly higher densities than iron and copper surfaces. They also found that leached materials used in construction and fabrication of pipes and storage tanks supported microbial regrowth and various microbial communities in the biofilms. Iron materials supported more diverse microbial communities than PVC materials, while PVC promoted more microbial regrowth (Camper 1996; Norton & LeChevallier 2000; Lehtolaa *et al*. 2004). Formation of the biofilm was slower in copper materials than in PVC products used for water storage. However, after 200 days, the microbial number did not show any difference between the two materials (Lehtola *et al.* 2004). In another study, the total microbial counts in biofilms from synthetic materials such as PVC materials declined due to water disinfection (Schwartz *et al.* 1998).

Our findings are in agreement with these studies. Although several different coliforms were isolated, *E. coli* was not detected from our three water tank types. By contrast, several other studies have reported the presence of *E. coli* in biofilms on the inner surfaces of water pipes and storage tanks (LeChevallier 1990; Van der Kooij 1997; Morin *et al*. 1999; US EPA 2001). An explanation for this discrepancy may be that coliform regrowth could be related to temperature, concentration of residual disinfectants, corrosion or material used in water distribution systems and water tanks (LeChevallier *et al*. 1996; Volk & LeChevallier 2000).

The physicochemical factors examined in this study are similar to those recommended to be monitored internationally (WHO 1997, *Guidelines, Vol. 3*; *Standard Methods* APHA/AWWA/WEF 1998). In this study, the highest value of NTU was significantly correlated with THPC,TIS bacteria, and pigmented bacteria, but was to a lesser extent correlated with the GI tanks. Egorov *et al*. (2003) reported that an increase in effluent water turbidity in drinking water or household usage was a major factor in causing

gastrointestinal illnesses. Therefore turbidity can be considered to be an important parameter for assessing water quality.

In this study, free chlorine was at low concentrations, while total chlorine was undetectable. Previously, it was reported that free and total chlorine residuals decreased rapidly with distance from the treatment plant (Al-Bahry *et al*. 2009). A significant inverse relationship between chlorine concentration and bacterial regrowth was also reported (Irvine *et al*. 2002; Al-Bahry *et al*. 2009). In this study, the low chlorine values obtained from the storage tanks may have had a significant influence in promoting microbial regrowth.

The tank capacities in this study ranged between 1,135.6 and 1,514 L. The larger the tank the higher the stagnation time and the higher the rate of deterioration of water quality. Coliforms were found more frequently in a large number of storage tanks used (LeChevallier *et al*. 1996). A prolonged stagnation of chlorinated water results in dissipation of the free chlorine, especially at elevated temperatures (Vasconcelos *et al*. 1997; Prévost *et al*. 1998; Ndiongue *et al*. 2005). Elevated temperatures are favourable for microbial regrowth during the summer months in the Arabian Gulf region. In this study, prolonged water stagnation time, elevated temperatures and low concentrations of chlorine favour the regrowth of heterotrophic bacteria, including opportunistic and potential pathogens. Similar results were reported by Morin *et al*. (1999) and Payment (1999).

The GI tanks were found to be corroded, and sediments of corrosion along with other contaminants were observed. Iron and sulphur bacteria were isolated from all tanks but concentrations were significantly higher in GI tanks and least in PE tanks. THPC was also high in GI tanks followed by the GRP tanks. Iron and sulphur bacteria were detected in the majority of houses with GRP and PE tanks fed by a network of GI pipes.

Swab samples from GI tanks and pipes from the corrosion sites were found to support bacterial communities similar to those recovered from the standard plate count agar. Reasoner (1990) reported that corrosion of cast iron occurred upon exposure to water containing a mixture of bacteria isolated from water distribution systems. Analysis of water and corroded samples showed that similar organisms were present in both. By comparison with PE and GRP tanks, GI tanks favoured microbial regrowth, which was detected in bulk water as THPC numbers. There was no clear difference in the tendency to support microorganism after-growth in GRP and PE storage tanks.

In addition to bio-corrosion, chemical corrosion cannot be ruled out by this study because elevated temperatures would have accelerated chemical reactions between sediments, disinfectants and the substrates in GI tanks and GI pipes. The corrosion rate is accelerated in the summer and consequently our results correlated with increased incidences of coliforms in water distribution systems (Volk & LeChevallier 2000).

Washing the tanks once every 7–12 months may not be sufficient to reduce microbial regrowth in the survey houses. Microbial regrowth was evident and sediments were observed in the majority of tanks. Because the tanks are not cleaned regularly, a potential hazard to human health may exist. In support of this, some individuals complained of diarrhoeal episodes. These episodes could be attributed to a combination of pathogens existing in water tanks as well as leached materials from corroded GI tanks and pipes. However, demonstration of this link would require further research to correlate the water storage system used and the frequency of related illnesses. Clearly, replacing corroded materials, frequent cleaning of sediments and thorough disinfection are crucial to minimise deterioration of the quality of drinking water in distribution and storage systems (Exner *et al*. 1987).

The TON and TYM values in this study were highest in GRP tanks. Most of the isolates were identified as *Aspergillus niger* and *Penicillium* spp. The GRP tanks also supported heavy algal growth and together fungi and algae are responsible for the biological source of odour and colour in water (Jardine *et al*. 1999). Even though algae in a freshwater environment are considered to be less toxic than cyanobacteria, the presence of both in freshwater storage tanks may have a significant effect on human health. Gastroenteritis and malabsorption on an airbase in the Philippines affected a large number of residents who suffered from diarrhoea, abdominal cramps and vomiting, which may be attributed to the presence of algae and microbes (Dean & Jones 1972). The presence of algae, cyanobacteria, bacteria and fungi in water tanks may be one of the factors contributing to gastrointestinal illnesses (Morin *et al*. 1999; Payment 1999; Volk & LeChevallier 2000; Oberholster & Botha 2007).

Leakage of organic compounds from the tank's material into the water may be a potential source of odour-causing substances. In GRP tanks, plastic fittings, organic coatings membranes, and epoxy re-lining products are progressively replacing traditional metallic materials. For different reasons (i.e. manufacturing defects), additives, solvents or monomers can leak into the drinking water (Rigal & Danjou 1997). Styrene is an integral solvent for the manufacture of GRP tanks. It was found that organic components such as styrene and chlorinated by-products are responsible for mechanisms which result in a high TON value (Rigal & Danjou 1997). The levels of such tank ingredients must meet current quality standards such as the Omani Standards number 8/2006 (2006) and those of the US Environmental Protection Agency (US EPA 2001). However, according to the International Agency for Research on Cancer (IARC), styrene has been proven as a human carcinogen and mutagen (Manolis *et al*. 1994).

Dark paint containing lead was used to coat the inner surfaces of GRP tanks to prevent sunlight penetration and inhibit algal growth. According to the recommendation of ASTM (1992) and WHO (1997) guidelines, all paints and related coating materials for drinking water should be lead free.

Pigmented bacterial isolates, algae and autotrophs varied in the three tanks examined in this study and there was no significant difference in colour measurement in the tested tanks, which met the Omani Standard number 8/2006 (2006), and those of the US Environmental Protection Agency (US EPA 2001) and WHO (1997). According to Ramsden (1999), high colour intensity at any point in the distribution system is caused by defective plumbing in the network.

The severe water shortage in Oman and the Arabian Gulf region led to the use of water storage tanks placed on house roofs. This system is ideal for conserving water. However, high temperatures in the Arabian Gulf area may contribute to chemical and physical deterioration and enhance microbial contamination as well as rust and paint leached from the water network system and into the drinking water.

This study suggests that physicochemical factors can promote microbial growth and have a great affect on water quality, which may impact on public health. A study of materials used in the manufacturing of storage tanks reveals the need for a high level of inspection by the relevant government authorities, including frequent quality tests in accordance with applicable international standards. Greater public awareness is needed regarding water quality issues and health risks. These recommendations are of particular importance to high risk groups such as infants, the elderly and immunocompromised individuals. In addition, appropriate monitoring criteria should be established which ensures the delivery of safe drinking water to the entire population which may be affected by this contamination.

## REFERENCES

Al-Bahry, S. N., Mahmoud, I. Y., Al-Khaifi, A., Elshafie, A. E. & Al-Harthy, A. 2009 Viability of multiple antibiotic resistant bacteria in distribution lines of treated sewage effluent used for irrigation. *Water Sci. Technol.* **60**, 2939–2948.

Allen, M. J., Taylor, R. H. & Geldreich, E. E. 1980 The occurrence of microorganisms in water main encrustations. *J. Am. Water Works. Assoc.* **72**, 614–625.

AOAC 2000 *Official Methods of Analysis of AOAC International*, 17th edition, Volume I. (W. Horwitz, ed.). AOAC Publications, Gaithersburg, Maryland.

ASTM (American Society for Testing and Materials) Standard 1992 *Paints, Related Coating and Aromatics*, Section 6. ASTM, Philadelphia.

Augoustinos, M. T., Grabow, N. A., Genthe, B. & Kfir, R. 1993 An improved membrane filtration method for enumeration of faecal coliforms and *E. coli* by a fluorogenic β-glucuronidase assay. *Water Sci. Technol.* **27**, 267–270.

Aulicino, F. A. & Orsini, P. 1996 Presence of biofilm in a drinking water system in Piemont. *L'igiene Moderna* **150**, 29–40.

Barrow, G. I. & Feltham, R. K. A. 1993 *Cowan and Steel's Manual for the Identification of Medical Bacteria*, 3rd edition. Cambridge University Press, Cambridge.

British Standard BS6068/1995 1995 *Water Quality: Specification and Method of Tests*. British Standards Institution (BSI), London.

Calderon, R. L. & Mood, E. W. 1988 *Bacterial Colonizing Point-of-Use, Granular Activated Carbon Filters and Their Relationship to Human Health*, CR-811904–01–0, US Environmental Protection Agency, Washington, DC.

Calderon, R. L. & Mood, E. W. 1991 *Bacterial Colonizing Point-of-Entry, Granular Activated Carbon Filters and Their Relationship to Human Health*, CR-813978–01–0, US Environmental Protection Agency, Washington, DC.

Camper, A. 1996 *Factors Limiting Microbial Growth in Distribution Systems: Laboratory and Pilot-Scale Experiments*. American Water Works Association Research Foundation, Denver, CO, pp. 1–121.

Camper, A. K., Warmecke, M., Jones, W. L. & McFeters, G. A. 1998 *Pathogens in Model Distribution System Biofilms*. AWWA Research Foundation, Montana.

Carter, J. T., Rice, E. W., Butchberger, S. G. & Lee, Y. 2000 Relationship between level of heterotrophic bacteria and water quality. *Water Res.* **34**, 1495–1502.

Clarke, G. M. 1994 *Statistics and Experimental Design*. Arnold, London.

Collins, C. H., Lyne, P. M. & Grange, J. M. 1995 *Collins and Lynes Microbiological Methods*. Butterworth-Heinemann Ltd, Oxford.

Cook, D., Newcombe, G. & Sztajnbok, P. 2001 The application of powdered activated carbon for MIB and geosmin removal: prediction PAC dose in four raw waters. *Water Res.* **35**, 1325–1333.

Dean, A. G. & Jones, T. C. 1972 Seasonal gastroenteritis and malabsorption at an American military base in the Philippines. *Am. J. Epidemiol.* **95**, 111–127.

Egorov, A. l., Naumova, E. N., Tereschenko, A. A., Kislitsin, V. A. & Ford, T. E. 2003 Daily variation in effluent water turbidity and diarrheal illness in a Russian City. *Int. J. Environ. Health Res.* **13**, 81–94.

Exner, M., Wegmann, U. & Haun, F. 1987 Infection control measures in dentistry. *Zahnarztl. Mitt.* **77**, 1841–1849.

Göksay, D., Çotuk, A. & Zeybek, Z. 2008 Microbial contamination of dental unit waterlines in Istanbul, Turkey. *Environ. Monit. Assess.* **147**, 265–269.

Irvine, K. N., Pettibone, G. W., Bako, S., Caruso, J., Fransina, R., Aures, G., Ork, J. & Bentivogli, D. 2002 Effect of lower chlorine dosage at Buff WWTP. *Clearwaters* **32**, 5.

ISO (International Organization for Standardization) 1994 *Technical Committee ISO/TC 147: Water Quality, Vol II, Chemical Methods*, 1st edition. ISO Central Secretariat Geneve, Switzerland.

Jardine, C. G., Gibson, N. & Hrudey, S. E. 1999 Detection of odour and health perception of drinking water. *Water Sci. Technol.* **40**, 91–98.

Khan, R., Philiphs, D., Fernando, D., Fowles, J. & Lea, R. 2007 Environmental health indicators in New Zealand. Drinking water: a case study. *Eco. Health* **4**, 63–71.

Kusnetsov, J. M., Jousimies-Somer, H. R., Nevalainen, A. I. & Martikainen, P. J. 1994 Isolation of *Legionella* from water samples using various culture methods. *J. Appl. Bacteriol.* **76**, 155–162.

LeChevallier, M. W. 1990 Coliform regrowth in drinking water: a review. *J. Am. Water Wks Assoc.* **82**, 74–86.

LeChevallier, M. W., Welch, N. J. & Smith, D. B. 1996 Full-scale studies of factors related to coliform regrowth in drinking water. *Appl. Environ. Microbiol.* **62**, 2201–2211.

Lehtolaa, M. J., Miettinena, I. T., Keinänen, M. M., Kekkia, T. K., Laineb, O., Hirvonenc, A., Vartiainenb, T. & Martikainen, P. J. 2004 Microbiology, chemistry and biofilm development in a pilot drinking water distribution system with copper and plastic pipes. *Water Res.* **38**, 3769–3779.

Manolis, K., Gilles, F., Anderson, A., Bellander, T., Biocca, M., Coggon, D., Gennaro, V., Hutchings, S., Kolstad, H., Lundberg, I., Lynge, E., Partanen, T. & Saracci, R. 1994 Cancer mortality in a historical cohort study of workers exposed to styrene. *Scand. J. Work Environ. Health* **20**, 251–261.

Martin, R. S., Gates, W. H., Tobin, R. S., Grantham, D., Sumarah, R., Wolfe, P. & Forestall, P. 1982 Factors affecting coliform bacteria growth in distribution systems. *J. Am. Water Wks Assoc.* **74**, 34–36.

Momba, M. N. B., Kfir, R., Venter, S. N. & Cloete, T. E. 2000 Overview of biofilm formation in distribution systems and its impact on the deterioration of water quality. *Water SA* **26**, 59–66.

Morin, P., Gauthier, V., Saby, S. & Block, J. C. 1999 Bacterial resistance to chlorine through attachment to particles and pipe surfaces in drinking water distribution systems. In: *Biofilms in Aquatic Systems* (C. V. Keevil, A. Godfree, D. Holt & C. Dow, eds.). Royal Society of Chemistry, Cambridge, pp. 171–190.

Ndiongue, S., Huck, P. M. & Slawson, R. M. 2005 Effects of temperature and biodegradable organic matter on control of biofilms by free chlorine in a model drinking water distribution system. *Water Res.* **39**, 953–964.

Nevondo, T. S. & Cloete, T. E. 1999 Bacterial and chemical quality of water supply in the Dertig village settlement. *Water SA* **25**, 215–220.

Norton, C. D. & LeChevallier, M. W. 2000 A pilot study of bacteriological population changes through potable water treatment and distribution. *Appl. Environ. Microbiol.* **66**, 268–276.

Oberholster, P. J. & Botha, A. M. 2007 Use of PCR based technologies for risk assessment of a winter cyanobacterial bloom in lake Midmar, South Africa. *Afr. J. Biotechnol.* **6**, 1794–1805.

Omani Standard number 8/2006 2006 *Unbottled Drinking Water*. Ministry of Commerce and Industry, Directorate of Specifications and Measurements, Muscat, Oman.

Payment, P. 1999 Poor efficacy of residual chlorine disinfectant in drinking water to inactivate waterborne pathogens in distribution systems. *Can. J. Microbiol.* **45**, 709–715.

Prévost, M., Rompré, A., Coallier, J., Servais, P., Laurent, P., Clement, B. & Servais, P. 1998 Suspended bacterial biomass and activity in full-scale drinking water distribution systems: impact of water treatment. *Water Res.* **32**, 1393–1406.

Pruss, A., Kay, D., Fewtrell, L. & Bartram, J. 2002 Estimating the burden of disease from water sanitation and hygiene at a global level. *Environ. Health Perspect.* **110**, 537–542.

Ramsden, J. J. 1999 A sum-parameter sensor for water quality. *Water Res.* **33**, 1147–1150.

Reasoner, D. J. 1990 Monitoring heterotrophic bacteria in potable water. In: *Drinking Water Microbiology* (G. A. McFeters, ed.). Springer-Verlag, London, pp. 452–476.

Rigal, S. & Danjou, J. 1997 Tastes and odors in drinking water distribution system related to the use of synthetic materials.

In *5th International Symposium on Off-flavors in the Aquatic Environment*, Elsevier, UK.

Schwartz, T., Hoffmann, S. & Obst, U. 1998 Formation and bacterial composition of young natural biofilms obtained from public bank-filtered drinking water system. *Water Res.* **31**, 2787–2797.

Simmons, G., Hope, V., Lewis, G., Whitmore, J. & Gau, W. 2001 Contamination of potable roof-collected rainwater in Auckland, New Zealand. *Water Res.* **35**, 1518–1524.

American Public Health Association/American Water Works Association/Water Environment Federation (APHA/AWWA/WEF) 1998 *Standard Methods for the Examination of Water and Wastewater*, 20th edition. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC. United Book Press Inc., Baltimore. Maryland.

US EPA (United States Environmental Protection Agency) 2001 *Factoids: Drinking Water and Ground Water Statistics for 2000*. EPA 816-K-01–004, Office of Water, US Environmental Protection Agency, Washington, DC. Available from: www.epa.gov/safewater (accessed 28 March 2007).

Van der Kooij, D. 1997 Multiplication of coliforms at very low concentrations of substrates in tap water. In: *Coliforms and E. coli: Problem or Solution?* (D. Kay & C. Fricker, eds.). The Royal Society of Chemistry, Cambridge, UK, pp. 195–203.

Van der Mee-Marquet, N., Domilier, A., Arnault, L., Bloc, D., Laudat, P., Hartemann, P. & Quentin, R. 2006 *Legionella anisa*, a possible indicator of water contamination by *Legionella pneumophila*. *J. Clin. Microbiol.* 44, 56–59.

Van der Wende, E., Characklis, W. G. & Smith, D. B. 1989 Biofilms and bacterial drinking water quality. *Water Res.* **23**, 1313–1322.

Vasconcelos, J. J., Rossman, L. A., Grayman, W. M., Boulos, P. F. & Clark, R. M. 1997 Kinetics of chlorine decay. *J. Am. Water Works Assoc.* **89**, 54–65.

Venkatesh, V. K., Vidyashree, N. V., Velmurugan, Parameswarn, A. & Kandaswamy, D. 2006 Evaluation of bacterial contamination of dental unit water lines and the efficacy of a commercially available disinfectant. *J. Conserv. Dent.* **9**, 93–98.

Volk, C. J. & LeChevallier, M. W. 2000 Assessing biodegradable organic matter. *J. Am. Water Works Assoc.* **92**, 64–76.

WHO 1995 *Guidelines for Drinking-water Quality*, 2nd edition. Vol I: Recommendation, Eastern Mediterranean Regional Office, Regional Center for Environmental Health Activities (CEHA), Amman, Jordan.

WHO 1997 *Guidelines for Drinking-Water Quality*. Vol. III: Surveillance and Control of Community Supplies. Eastern Mediterranean Regional Office, Regional Center for Environmental Health Activities (CEHA), Amman, Jordan.

WHO 2002 *World Health Report 2002 Reducing Risk, Promoting Healthy Life*. World Health Organization, Geneva. Available from: http://www.who.int/whr/2002/en/index.html.

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