Influence of disinfection processes on the microbial quality of potable groundwater in a laboratory-scale system model

Maggy N. B. Momba, T. E. Cloete, S. N. Venter and R. Kfir

ABSTRACT

Groundwater was used to evaluate the influence of the disinfection processes on the microbial quality of potable water distribution systems using laboratory-scale units. Coliform bacteria, heterotrophic plate count and total bacteria were used for the evaluation of the bactericidal effectiveness of each disinfectant. The microbial disinfection efficacy of chlorine, chloramine, ozone and UV irradiation was found to be equally effective in the elimination of coliform bacteria during the first hours (20 min–2 h) after disinfection. Complete elimination of coliforms in hydrogen peroxide treated water occurred after 48 h. More than 4 log cfu . ml^{-1} (average killing rate) heterotrophic bacteria were killed by all the disinfectants with the exception of hydrogen peroxide (average killing rates: 3–2 log cfu . ml^{-1}). However, ozone was highly effective within the first 2 h as shown by the average killing rate of 4 log cfu . ml^{-1} heterotrophic bacteria in both source waters. The phenomenon of bacterial regrowth was linked to the absence of concentrations of disinfectant residuals. Bacterial regrowth, however, could be detected earlier with chlorine (after 20 min–average regrowth rate 0.064 h^{-1}, average generation time 10.95 h), ozone (after 2 h–average regrowth rate 0.202 h^{-1}, average–generation time 5.04 h) and UV treated water (after 2 h–average regrowth rate 0.263 h^{-1}, average generation time 2.70 h) than chloramine (between 24 h and 48 h–average regrowth rate 0.057 h^{-1}, average generation time 13.87 h) and hydrogen peroxide treated water (after 48 h–average regrowth rate 0.063 h^{-1}, average generation time 12.74 h). The greater persistence of monochloramine (7 days) and hydrogen peroxide (14 days) residuals were found to inhibit bacterial regrowth in potable water.

Key words | disinfection processes, groundwater, microbial quality

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INTRODUCTION

In semi-arid areas such as parts of South Africa, groundwater remains the main water supply source for many small communities. Although groundwater only contributes about 15% to South Africa's water supply it is of great importance to note that more than 280 towns and villages use groundwater for their water supply (Kok & Simons 1989).

Naturally groundwater is of excellent microbiological quality and generally of adequate chemical quality (Foster 1995). This is due to the soil barrier providing effective isolation from surface pollutants. In recent years there has been a growing suspicion that the protection of groundwater quality is not always assured by a soil barrier and therefore the contamination of groundwater from different sources occurs worldwide. This contamination is due to the large variety of materials used by industries and households. The disposal of toxic waste, municipal solid waste, sewage sludge, industrial waste and effluent are some examples of groundwater pollution sources (Engelbrecht 1993).

As a result of contamination, heterotrophic and coliform bacteria can be found in groundwater. Pathogens

may exist in groundwater for up to 40 days given favourable conditions and reasonable levels of nutrients (Chilton *et al.* 1995). These organisms may not only be an interference factor in coliform detection, but a population of unique organisms that may create undesirable taste, odour or degradation reactions when introduced into food products, beverages and pharmaceuticals (Geldreich *et al.* 1972). Reports of outbreaks of waterborne disease and unsatisfactory coliform results in groundwater have led to the recommendation to disinfect many well waters and springs used for potable water (Craun 1985; Lippy & Waltrip 1984).

The realization that groundwaters are the cause of waterborne disease outbreaks has forced, many rural communities in South Africa to adapt some measure of disinfection (chlorine) which often coincides with small-scale water distribution or storage systems. Effective disinfection processes for the purification of potable water are an important consideration in the prevention of waterborne diseases and their transmission. This may not only concern chlorination, but also includes chloramination, ozonation, UV irradiation and hydrogen peroxide.

It is well known that bacterial survival after disinfection is a problem for potable water utilities. The potential of bacterial growth was observed during distribution of potable surface water disinfected with chlorine (LeChevallier & McFeter 1985; Lippy & Waltrip 1984; Neden *et al.* 1992) chloramine (Mathieu *et al.* 1992), Ozone (Dietlicher 1970; Stalder & Klosterkötter 1976) and UV irradiation (Hengesbach *et al.* 1993). Research has shown that bacterial regrowth is related to a number of mechanisms, which include contamination due to mechanical failures of distribution systems (Clark *et al.* 1993), growth of planktonic bacteria in the water (LeChevallier *et al.* 1984) and the recovery of dormant bacteria or injured bacteria during treatment (LeChevallier *et al.* 1988; McFeters *et al.* 1986). In addition to these mechanisms, other factors such as the nature and concentration of biodegradable compounds in freshly prepared drinking water (Joret *et al.* 1991; Stanier *et al.* 1976; van der Kooij 1992), the kind of piping material (Momba *et al.* 1998; Roger *et al.* 1994), the water temperature (LeChevallier *et al.* 1996), the residence time of water in the distribution system and the residual disinfectant (Gibbs *et al.* 1990; Momba *et al.* 1998; Watters *et al.* 1989) have also been reported to enhance bacterial regrowth in potable surface water.

From the above information, it can be noted that most studies on the survival of bacteria in potable water distribution systems have been conducted using surface water. No investigation has yet addressed with complete information the situation pertaining to bacterial regrowth in potable groundwater distribution systems. However, it is well known that the treatment methods for potable water depend on the quality of the raw water. Disinfection of surface waters is often more difficult because of fluctuating water quality. Conventional treatment most commonly used for surface waters includes the chemical and physical processes of coagulation, flocculation, gravity separation, rapid sand filtration and disinfection. As to groundwater, conventional treatment is mostly limited to water hardness reduction, taste and odour removal and disinfection. This suggests that the situation pertaining to bacterial regrowth in potable groundwater could be different from that of potable surface water.

To investigate the pattern of bacterial regrowth and to limit the accumulation of bacteria in potable groundwater, the disinfection efficiency and the presence of the disinfectant residual must be taken into account. This study aims to compare a number of disinfections commonly used in the South African potable water industry (chlorination, chloramination, ozonation, UV irradiation and hydrogen peroxide disinfection process) for their efficacy in rendering high quality potable water after the retention of water in the water distribution systems under South African conditions. The role that disinfection processes play in the deterioration of the water quality in treated water distribution systems was therefore investigated using a laboratory-scale unit and emphasizing disinfection efficacy, the presence of disinfection residual and bacterial regrowth.

MATERIALS AND METHODS

Water source

Groundwater from wells in a rural area was collected in 4×100 l sterile polyethylene drums and transferred into

6 × 50 l sterile polyethylene drums. To obtain statistically meaningful results, two sources of water (source water 1 and source water 2) were used during the study period. The experimental data are based on two replications for each source water.

Laboratory-scale system model

This study was performed using the laboratory-scale unit described for the first time for the study of biofouling by Jacobs *et al.* (1996) and used by Momba (1997) and Momba *et al.* (1998) for the study of biofilm in potable water distribution systems. The laboratory-scale system model consisted of 6 × 50 l polyethylene drums which were used as batch reactors. The drums, peristaltic pump, flow through glass, Pedersen device and tap were connected between them using latex tubing (8 mm diameter, 4 m length) which also allowed the circulation of water in the system.

Six laboratory-scale units were used, one for each of the treatments and a separate unit for the control experiments (non-disinfected water). The disinfected and the control waters (50 ml water for each system) circulated in the systems at a flow rate of 2.8 l . h^{-1} for a period of 35 days. Figure 1 illustrates schematically the laboratory-scale unit used as batch reactor.

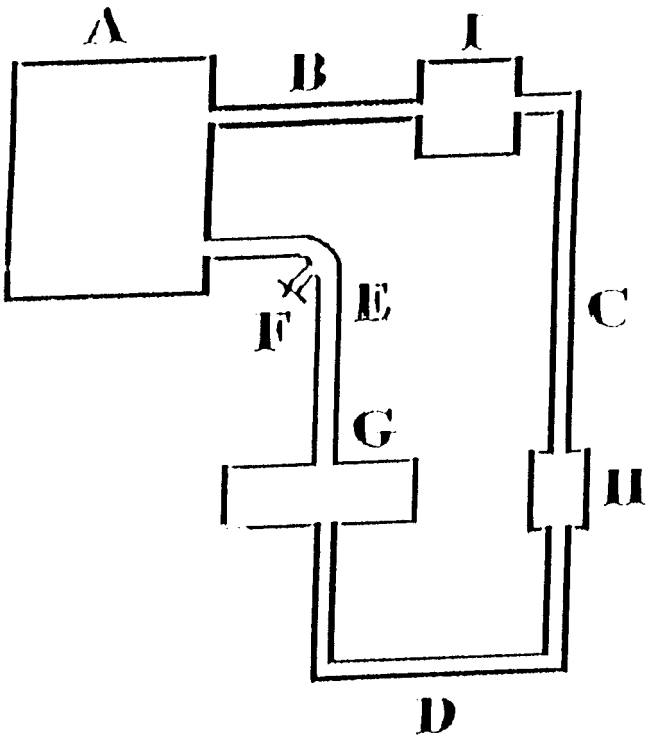

Figure 1 | Schematic diagram of the laboratory-scale unit. (A) Sterile drum, (B), (C), (D), (E) latex tubing, (F) tap facilitating for sampling, (G) Pedersen device, (H) flow-through glass tube and (I) peristaltic pump.

Disinfection processes

Disinfection was carried out using initial disinfectant concentrations which were as close as possible to those used in practice (free chlorine: 2 and 2.5 mg l^{-1}, monochloramine: 2.5 and 3.5 mg l^{-1}, ozone: 2.07 and 2.6 mg l^{-1}, hydrogen peroxide: 20.70 and 17.73 mg l^{-1}). The non-disinfected water and the 5 disinfected waters were transferred into laboratory-scale units designed for the experimental study. No neutralization of any disinfectants used for the source waters was done during the experimental period, with the exception of samples on which microbiological analyses were conducted.

Concentrations of disinfectants were measured using the N,N′-diethyl-p-phenylenediamine (DPD-Sigma) ferrous titrimetric method for free chlorine and monochloramine, and the indigo colorimetric method for ozone, according to a standard method (APHA 1989). The hydrogen peroxide residual was determined using the Rqflex reflectometer (Reflectoquant 16974, Merck). As to the UV irradiation, an ultraviolet irradiation unit (Willand UV systems) able to disinfect water at a flow rate of 6 l/min, at a 107 m Ws/cm^{-2} dosage was used.

Sampling and microbial analyses

Sampling frequency is indicated in the results and was the same for all experiments. Chlorinated, chloraminated and ozonated samples were collected in sterile bottles previously rinsed with MilliQ water, which contained sodium thiosulphate (ca. 17.5 mg l^{-1}) in order to neutralize any residual disinfectant.

Coliform, faecal coliform and injured coliform bacteria were enumerated by the membrane filter procedure

Table 1 | The physico-chemical values (average) of groundwater in different laboratory-scale units.

	Source water 1					
Parameters	**Control**	**Chlorine**	**Chloramine**	**H_2O_2**	**Ozone**	**UV**
pH	7.9	7.9	7.7	7.6	8.2	7.8
T°C	24.0	24.0	24.0	23.0	24.0	24.0
Turbidity (NTU)	1.2	1.8	1.4	1.2	1.2	1.4
SS mg/l	5.1	5.0	4.8	4.9	5.8	4.5
	Source water 2					
pH	7.9	7.8	7.8	7.9	8.3	7.9
T°C	25.0	24.0	25.0	24.0	24.5	25.0
Turbidity (NTU)	1.8	2.4	1.9	2.4	2.3	2.0
SS mg/l	8.3	7.5	8.0	11.8	10.5	7.0

SS=suspend solids.

using m-Endo Les agar (Difco), m-FC agar (Merck) (APHA 1989) and m-T7 agar (LeChevallier *et al.* 1983) respectively. Heterotrophic plate count (HPC) bacteria were enumerated by the standard spread plate procedure using R2A agar (Difco), incubated at 28°C for 7 d (Reasonner and Geldreich 1985). Analyses were carried out in triplicate. The total bacterial (TB) numbers were determined using epifluorescence direct count procedure, involving DAPI (Boehringer Mannheim GmbH) according to Kepner and Pratt (1994). The regrowth rate of bacteria was calculated according to Stanier *et al.* (1976).

Statistical analyses

To compare variations in treatments, ANOVA ($\alpha = 0.05$) was applied to the bacterial counts with the latter as the dependant variable. The counts were transformed by taking logarithms base 10 (Lcounts) in order to stabilize the variance. The number of days from the outset of the experiments was included as a co-variant. If significant factors (e.g. treatments) were found, then the multiple comparison technique based on Least Squares Means (LSMEANS) was applied to establish which particular levels of a factor were different, in terms of the bacterial counts.

Physico-chemical analyses

Temperature, pH, total suspended solids and turbidity were determined according to a standard method (APHA 1989). Table 1 summarizes the physico-chemical values of disinfected and untreated (control) surface water during the experimental period.

RESULTS

Disinfectant effectiveness

With the exception of hydrogen peroxide, all disinfectants were equally effective in the inactivation of coliform

Table 2 | Recovery of coliform bacteria in potable groundwater.

		Source water 1					
Parameters	**Residual (*mg*/l)**	**BD (cfu/100 ml)**		**AD (cfu/100 ml)**		**Recovery (cfu/100 ml)**	**Time**
		TC	FC	TC	FC	IJ	
Chlorine	1.25	7.7×10	2×10	0	0	0	20 min AD
Chloramine	1.70	7.7×10	2×10	0	0	0	20 min AD
Ozone	0.22	7.7×10	2×10	0	0	0	2 h AD
UV	—	7.7×10	2×10	0	0	0	2 h AD
H_2O_2	20.00	7.7×10	2×10	37	3	7	2 h AD
	19.00	—	—	4	0	4	24 h AD
	18.10	—	—	0	0	0	48 h AD
		Source water 2					
		TC	FC	TC	FC	IC	
Chlorine	1.40	6.0×10	1.2×10	0	0	0	20 min AD
Chloramine	2.00	6.0×10	1.2×10	0	0	0	20 min AD
Ozone	0.30	6.0×10	1.2×10	0	0	0	2 h AD
UV	—	6.0×10	1.2×10	0	0	0	2 h AD
H_2O_2	17.	6.0×10	1.2×10	55	11	4	2 h AD
	12.50	—	—	14	3	13	24 h AD
	10.70	—	—	0	0	0	48 h AD

BD: Before disinfection; AD: after disinfection; TC: total coliform; FC: faecal coliform; IJ: injured coliform.

bacteria within the first hours after disinfection. The killing rates of coliform bacteria are summarized in Table 2.

Initial average numbers of 5 log and 4 log cfu . ml^{-1} HPC were recorded in raw waters from source water 1 and source water 2, respectively, (Figure 2a). Within the first hours (20 min–24 h) after disinfection, chlorine (Figure 3), monochloramine (Figure 4), ozone (Figure 5) and UV irradiation (Figure 6), exhibited an average killing rate of 4 log cfu . ml^{-1} HPC when using source water 1. For source water 2, all the above-mentioned disinfectants exhibited an average killing rate of 3 log cfu . ml^{-1} HPC with the exception of ozone (4 log cfu . ml^{-1} HPC). In both source waters, a total of 48 h was required for hydrogen peroxide to achieve killing rates of 3 log cfu . ml^{-1} HPC (for source

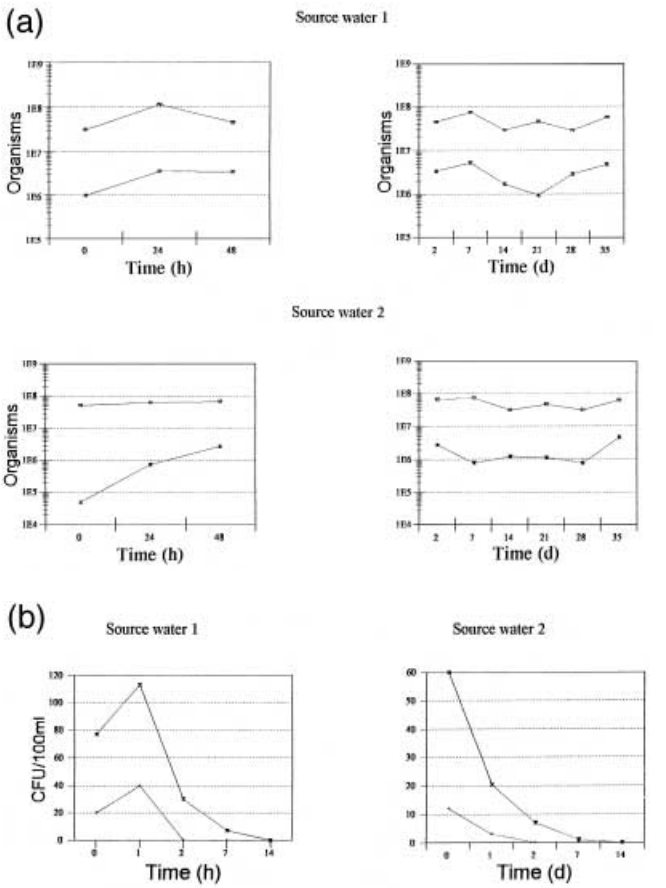

Figure 2 | (a) Number of total bacteria (TB—epifluorescence) and heterotrophic plate count (HPC) bacteria in the raw water —×— HPC (CFU/ml), —□— TB (cell/ml); (b) recovery of coliform bacteria in the raw water during the experimental period. —×— total coliform, —— fecal coliform.

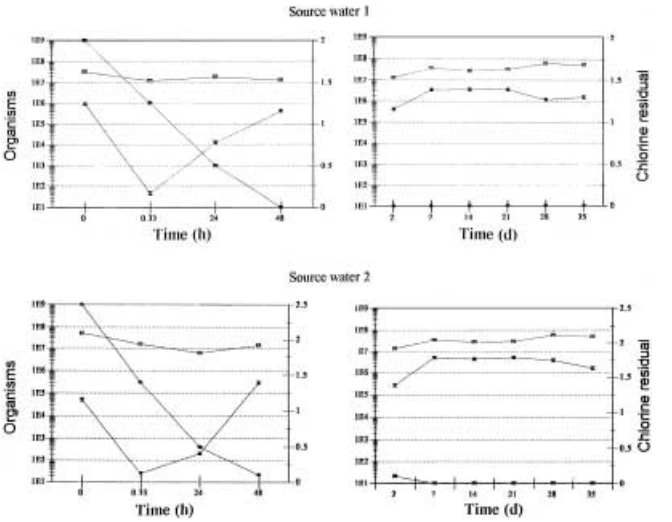

Figure 3 | Number of total bacteria (TB—epifluorescence) and heterotrophic plate count (HPC) bacteria in the chlorinated water —×— HPC (CFU/ml), —□— TB (cell/ml) —◇— residual chlorine.

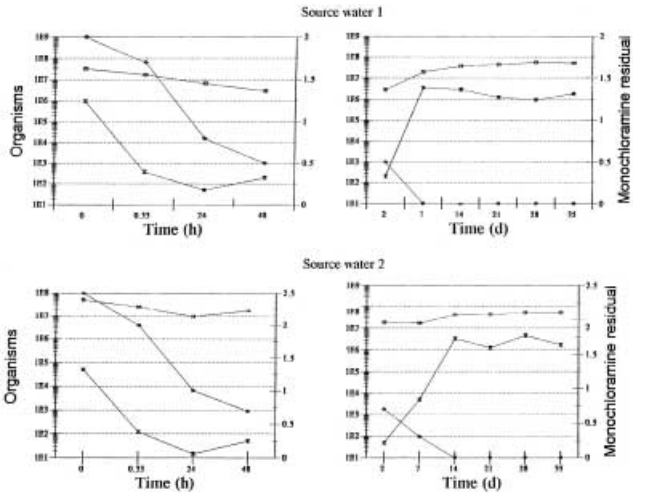

Figure 4 | Number of total bacteria (TB—epifluorescence) and heterotrophic plate count (HPC) bacteria in the chloraminated water —×— HPC (CFU/ml), —□— TB (cell/ml), —◇— monochloramine residual.

water 1) and 2 log cfu . ml^{-1} HPC (for source water 2) (Figure 7a). As to the total bacteria, no specific decrease in bacterial number was observed in chlorinated, UV irradiated water and hydrogen peroxide treated water. An average cell number of 7 log cells . ml^{-1} was noted within the first hours in these treated waters (Figures 3, 5–7a) while bacterial numbers decreased from 7 log to 6 log cells . ml^{-1} in chloraminated water 24 h after disinfection (Figure 4).

Effect of disinfectant residuals on bacterial regrowth

Results of this study confirm the instability of ozone in terms of providing a residual effect (Figure 5). Chlorine was relatively unstable and also did not provide a sustainable residual (Figure 3). Monochloramine (Figure 4) and hydrogen peroxide (Figure 7) gave a relatively stable residual. Table 3 illustrates the effect of disinfection processes on bacterial regrowth.

The phenomenon of bacterial regrowth was therefore related to the absence of concentrations

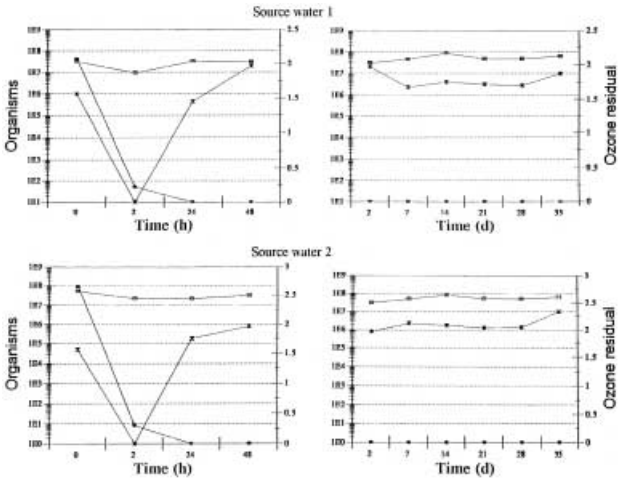

Figure 5 | Number of total bacteria (TB—epifluorescence) and heterotrophic plate count (HPC) bacteria in the ozonated water —×— HPC (CFU/ml), —□— TB (cell/ml), —◇— ozone residual.

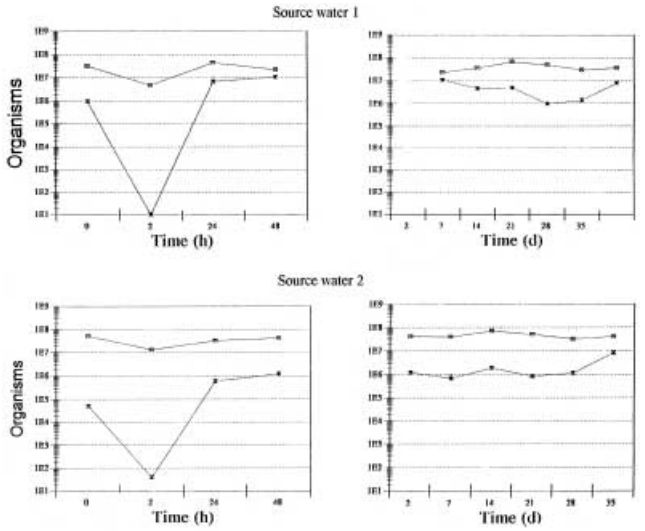

Figure 6 | Number of total bacteria (TB—epifluorescence) and heterotrophic plate count (HPC) bacteria in the UV irradiated water —×— HPC (CFU/ml), —□— TB (cell/ml).

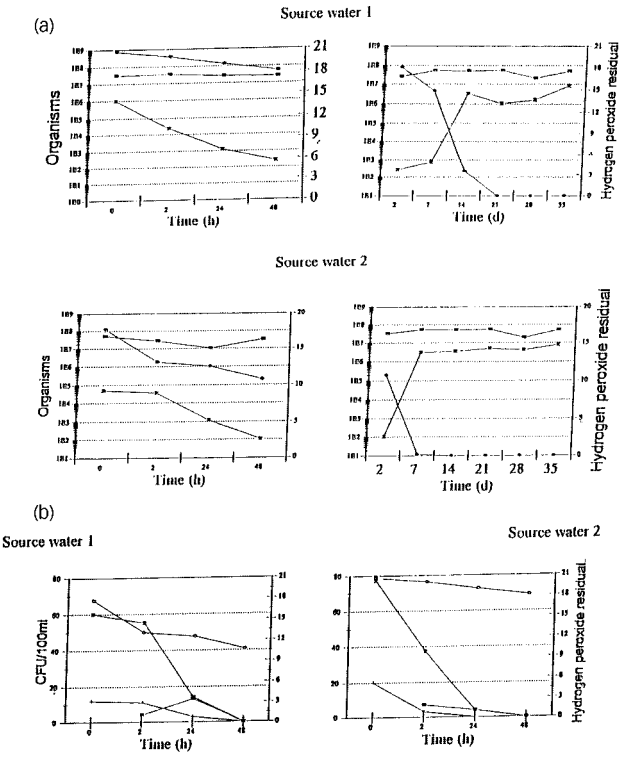

Figure 7 | (a) Number of total bacteria (TB—epifluorescence) and heterotrophic plate count (HPC) bacteria in the hydrogen peroxide treated water —×— HPC (CFU/ml), —□— TB (cell/ml) —◇— hydrogen peroxide residual; (b) Recovery of coliform bacteria in hydrogen peroxide treated water during the experimental study —×— Total coliform, —□— fecal coliform, —◇— injured coliform, —○— hydrogen peroxide residual.

of disinfectant residual. Within the first hours after disinfection (48 h) higher heterotrophic bacteria numbers were noted in ozone (7 log cfu . ml^{-1} for source water 1, 5 log cfu . ml^{-1} for source water 2), chlorine (5 log cfu . ml^{-1} for both source waters) and UV treated water (7 log cfu . ml^{-1} for source water 1, 6 log cfu . ml^{-1} for source water 2) than in chloramine (2 log cfu . ml^{-1} for source water 1, 1 log for source water 2) and hydrogen peroxide (2 log cfu . ml^{-1} HPC for both source waters) treated water. The greater persistence of monochloramine and hydrogen peroxide was found to inhibit bacterial regrowth in treated water systems (Figures 4 and 7a).

Treated water quality also varied in laboratory-scale units in accordance with the age of water in the systems. Examples of this include the decrease of chlorine, ozone, chloramine and hydrogen peroxide residuals as well as the increase of bacterial numbers in all treated waters. Average bacterial counts of 6 log cfu . ml^{-1} for HPC and 7 log cells . ml^{-1} for total bacteria could be noted over the remainder of the study period (Figures 2a, 3–7a).

Table 3 | Effect of disinfection processes on bacterial regrowth (HPC) in potable groundwater laboratory-scale units.

Parameter		Time	Residual ($mg \cdot l^{-1}$)	Killing rate ($cfu \cdot m\ l^{-1}$)	Regrowth rate (h^{-1})	Generation time (h)	Time for regrowth
Chlorine	S1	20 min	1.25	4 log	0.067	10.35	20′–7 d
	S2	20 min	1.40	3 log	0.060	11.55	20 min–7 d
Chloramine S1	20 min	1.70	3 log				
		24 h	0.80	4 log	0.077	9.00	24 h–7 d
	S2	20 min	2.00	2 log			
		24 h	1.00	3 log	0.037	18.73	48 h–14 d
Ozone	S1	2 h	0.22	4 log	0.315	2.20	2 h–48 d
	S2	2 h	0.30	4 log	0.088	7.88	2 h–7 d
UV	S1	2 h	NR	4 log	0.302	2.30	2 h–48 h
	S2	2 h	NR	3 log	0.224	3.09	2 h–48 h
Hydr Per.	S1	2 h	20.70	1 log			
		48 h	18.10	3 log	0.041	17.33	48 h–14 d
	S2	2 h	13.10	NSD			
		48 h	10.70	2 log	0.085	8.15	48 h–7 d

NR=no residual; NSD: no specific death of bacteria.

DISCUSSION

The study performed on the influence of disinfection processes on the microbial quality of potable groundwater in the laboratory-scale units indicated that the quality of treated water was related to the disinfectant effectiveness, the concentrations of disinfectant residual, the age of water in the systems and the origin of the intake water. To perceive the specific difference to other waters to be disinfected, a comparison could be done between the present study with groundwater and our previous investigation in which surface water was used to evaluate the bacterial effectiveness of chlorine, chloramine, ozone, UV irradiation and hydrogen peroxide (Momba 1997). It is also important to note that concentrations of these disinfectants were similar for both test waters.

In groundwater, all bactericides were found equally effective in the inactivation of coliform bacteria within the first hours after disinfection with the exception of hydrogen peroxide (48 h after disinfection) (Figure 7b, Table 2). As to the surface water, with the exception of chloramine treated water, the study reported the presence of injured coliforms in treated waters 24 h after disinfection whereas no coliforms were noted within the first hours after disinfection (Momba 1997). The complete inactivation of

coliform bacteria occurred in all treated surface waters 48 h after disinfection. According to the author, this coincided with the regrowth of heterotrophic bacteria (HPC) in all treated waters with the exception of hydrogen peroxide treated water. Although the concentrations of organic carbon were not determined during the experimental studies, the competition for limiting organic carbon could be responsible for the elimination of coliform bacteria, as indicated by LeChevallier and McFeters (1985), who found a significant correlation between the initial level of HPC and the rate of coliform decline. Comparing both studies, it could be noted that the removal of coliform bacteria was quicker when using groundwater than when using surface water.

All disinfection processes were effective in the initial reduction of HPC as shown in Table 3 and Figures 3–7a. A killing rate of 4 log cfu . ml^{-1} of HPC was observed using chlorine, monochloramine, ozone and UV irradiation within the first hours (20 min–24 h) after disinfection of groundwater-source water 1, whereas the study performed by Momba (1997) reported similar observations only with monochloramine, 24 h after disinfection of surface water-source water 1. However, a 5 log cfu . ml^{-1} initial count was found in both test waters. This indicated that the effectiveness of chlorine, ozone and UV against HPC bacteria was greater in groundwater than in surface water. Organic materials in the intake water could be one explanation for this fact. Groundwater could be less polluted with organic compounds leading to better performance of some of the disinfectants than could be expected with surface water.

A comparison between source water 1 and source water 2 showed that in groundwater, ozone efficacy was similar (average killing rate, 4 log cfu . ml^{-1}) and greater after 2 h in both source waters than any of the other disinfectants (Table 3), whereas Momba (1997) reported a killing rate of 3 log cfu . ml^{-1} HPC when using surface water (source water 1). Although the average killing rates of 2–3 log cfu . ml^{-1} and 3–4 log cfu . ml^{-1} were, respectively, recorded in treated groundwater and surface water when using hydrogen peroxide, its bactericidal efficacy remained slower than that any of the other disinfection processes. Therefore, the use of this disinfectant as a primary disinfectant for potable water might not be recommended.

Undoubtedly, all the disinfectants did progressively undergo a process of depletion throughout the systems. Whereas the decay occurred earlier in the ozonated water (1 day for groundwater and surface water), residual disinfectant concentrations could be detected up to 2 days in the chlorinated water (groundwater and surface water), up to 7 days in chloramine and 14 days in the hydrogen treated water from groundwater (between 14–15 days in the chloraminated water, and between 7–22 days in the hydrogen peroxide treated water from surface water). Consequently, this decay of residual disinfectants resulted in bacterial regrowth.

Bacterial regrowth, therefore, could be detected earlier in the ozonated (Figure 5) , UV irradiated (Figure 6) and chlorinated water (Figure 3) than in the chloramine (Figure 4) and hydrogen peroxide treated waters (Fig. 7a). However monochloramine and hydrogen peroxide exhibited lower growth rates (Table 3) than any of the other disinfectants. These observations were similar in source water 1 as well as in source water 2 and confirmed Momba's findings with surface water (Momba 1997). While monochloramine and hydrogen peroxide in groundwater exhibited a greater residual persistence (up to 14 d), the regrowth rates were 0.037 h^{-1} (generation time 18.73 h) and 0.041 h^{-1} (generation time 17.33 h), respectively, and much lower than when there was a shorter residual persistence of monochloramine and hydrogen peroxide (24 h–7 d resulting in a growth rate of 0.077 h^{-1}, generation time 9 h for monochloramine; 48 h–7 d resulting in a regrowth rate of 0.085 h^{-1}, generation time 8.15 h for hydrogen peroxide). Chloramine, however, resulted in a lower regrowth rate than any of other disinfectants (Table 3). A comparison between groundwater and surface water showed that chloramination resulted in lower growth rates (0.077 h^{-1}, generation time 9 h–source water 1; 0.037 h^{-1}, generation time 18.73 h–source water 2) than surface water chloramination (0.13 h^{-1}, generation time 5.33 for source water 1; 0.039 h^{-1}, generation time 17 h for source water 2) whereas disinfection with hydrogen peroxide resulted in an equal regrowth rate (0.041 h^{-1}, generation time 17 h) for surface water and groundwater from source water 1, and in a lower growth rate (0.29 h^{-1}, generation time 24 h) for surface water than for groundwater (0.085 h^{-1} ,

generation time 8.15 h) from source water 2. Statistical data (ANOVA-$\alpha = 0.05$) also confirmed this fact. Heterotrophic bacteria counts were significantly higher in the ozone (LSMEANS = 6.07), UV (LSMEANS = 6.29) and chlorine (LSMEANS = 6.24) treated water than in the chloramine (LSMEANS = 5.02) and hydrogen peroxide (LSMEANS = 5.15) treated groundwaters within the first 48 h, whereas the count in the control (LSMEANS = 6.37) did not differ significantly from that in the chlorine, ozone and UV treated waters, as a result of regrowth in the treated waters. Similar observations were also noted in surface water 72 h after disinfection (Momba 1997). Findings from groundwater and surface water demonstrated that the presence of an adequate disinfectant residual, whether, it be monochloramine or hydrogen peroxide will enable water suppliers to make significant progress in compliance with bacteriological water quality standards.

When comparing ground water–source water 1 and source water 2 (exceedance probability (P) = 0.3347) or different treatments in source water 1 and 2 (3 d after disinfection–P = 0.9989; 35 d after disinfection, P = 0.9218), ANOVA showed no significant difference in viable counts. This demonstrated that the phenomenon of regrowth was not influenced by the physico-chemical quality of potable groundwater (Table 1). Therefore, the phenomenon of bacterial regrowth in potable groundwater was related to the absence of concentrations of disinfectant residuals and the microbial efficacy of chloramine and hydrogen peroxide provided a persisting residual which inhibited bacterial regrowth in treated water. The greater effectiveness of monochloramine in controlling heterotrophic bacteria when compared to chlorine was also reported by Neden *et al.* (1992). Experiments with ozone have shown that ozone may also react with organic material in source water to form nutrients that allow regrowth in the distribution system and increase the growth in the bulk phase of water (Hengesbach *et al.* 1993). The potential for bacterial regrowth has also been determined in UV irradiated drinking water (Hengesbach *et al.* 1993). Results showed that as the low-molecular weight component of the total dissolved organic carbon (DOC) determines the degree of its metabolic availability to bacteria, any UV degradation of DOC is linked to the question of bacterial regrowth potential (Hengesbach *et al.* 1993).

Treated groundwater quality also varied in laboratory-scale units in accordance with the age of water in the systems. Significantly higher heterotrophic bacteria numbers (P = 0.001) occurred 35 days after disinfection than 3 days after disinfection (P = 0.0036). Gibbs and his co-workers (1990) observed a quick reduction in bacterial number, but regrowth occurred when the residual chlorine was decreased by retention in the distribution system. LeChevallier *et al.* (1980) noted that dead-end distribution lines in which no free chlorine could be detected contained 23 times the number of standard plate count compared with distribution lines with a free chlorine residual. This phenomenon was a function of time. Based on the results of this study, it is recommended that water suppliers must also consider the retention period of water in distribution systems in devising a solution to the problem of bacterial regrowth in potable water distribution systems.

Counting of bacteria by the epifluorescence technique involving DAPI staining gave a lower kill percentage than by the standard plate procedure with R2A agar (Table 3). Although bacteria in all samples were counted within a minimum of 20 microscope fields at 100 magnification, this technique also revealed higher total bacteria counts than heterotrophic bacteria (Figures 3–7a). It is well known that the numbers of bacteria observed when using fluorochromes for the direct enumeration of total bacteria depend on the staining technique, and physico-chemical characteristics of the samples (Fry 1990; Kepner & Pratt 1993) as well as on individual bias (Kepner & Pratt 1994). These factors could be one of the possible explanations for the high number of total bacteria as long as the epifluorescence direct count techniques includes non-viable and viable cells (but non-culturable cells). Moreover, during the study, the fluorescence of bacterial suspension in all treated waters varied greatly, from blue fluorescing cells to poorly fluorescing bacteria. Paquin *et al.* (1994) reported a similar observation when counting bacterial suspension from potable water disinfected with chlorine. The presence of the poorly fluorescent bacteria after DAPI staining was also observed by Saby *et al.* (1997) who interpreted it as a sign of cell death.

According to these authors, cell alterations caused by oxidative stresses led to a reduction in fluorescence of DAPI-staining bacteria and generally occurred after loss of viability of bacteria.

CONCLUSIONS

The results of this study indicated that chlorine, monochloramine, ozone and UV were generally effective in causing the death of coliform bacteria and the initial reduction of heterotrophic bacteria. This effectiveness appeared to be greater when using ozone. This study confirms hydrogen peroxide as a weak disinfectant for the initial elimination of coliform bacteria and initial reduction of heterotrophic bacteria. However, monochloramine and hydrogen peroxide were more effective in controlling heterotrophic bacteria levels if sufficient residual could be maintained. To make significant progress toward compliance with bacteriological groundwater quality standards, it is recommended that ozone be used as a primary disinfectant for the disinfection of groundwater with short duration in the distribution system or as a primary disinfectant followed by monochloramine or hydrogen peroxide as a secondary disinfectant. This would make significant progress in the reduction of contamination of potable groundwater, given that these secondary disinfectants would be able to assure the control of bacterial regrowth in the distribution systems. It is also recommended that chlorination, ozonation and UV irradiation could provide effective treatment which will inhibit bacterial regrowth and prevent the microbial deterioration of potable water in distribution systems as long as residual monochloramine or residual hydrogen peroxide persists.

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