Indicator bacteria and regrowth potential of the drinking water in Alice, Eastern Cape

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Abstract

Alice drinking water has a brown colour sometimes which could imply that it is not potable. To assess the effectiveness of the purification works and ascertain whether the water distributed is safe for drinking, indicator bacteria namely, heterotrophic, total coliform, faecal coliform and injured coliform counts were performed using the membrane filtration method. In addition, the regrowth of heterotrophic bacteria, total and injured coliforms in the chlorinated water at 15°C ± 2°C and 23°C ± 2°C was recorded. All the indicator bacteria counts were above the South African acceptable standards. Moreover, heterotrophic bacteria and total coliforms showed a remarkable regrowth indicating that the treated water had possibly high biodegradable dissolved organic carbon content due to inefficient purification systems. Considering the overall microbiological indicators, it can be concluded that the Alice drinking water is of poor quality.

Introduction

The problem of distributing poor quality drinking water is not only attributed to developing countries as developed countries such as the USA, also encounter these problems (Young, 1996). Faecal contamination of drinking water supplies by untreated and/or inadequately treated sewage effluents entering rivers and dams that serve as the source of municipal water supplies create conditions for the rapid spread of pathogens. The distribution system itself can also contribute to the deterioration of the water quality, since many factors can introduce bacteria into the drinking water when it is being distributed. These include open reservoirs, enclosed reservoirs in which chlorine is not added, new construction that may disturb the existing distribution system, mains breaks, back pressure, dead ends in the mains with stagnant water, corrosion by-products and bad pipe systems (Clark et al., 1993; Hambsch and Werner, 1993).

Alice is situated in a semi-rural area in the Eastern Cape, South Africa. In many rural and growing urban areas in general and in South Africa in particular, there is widespread scarcity, gradual destruction and increased pollution of freshwater resources. The causes include untreated and/or inadequately treated sewage, loss or lack of natural water catchment areas and poor farming practices, which release pesticides and other chemicals into water, and excreta from stray animals. All these problems are applicable to the situation in Alice and almost all formerly black townships in the Eastern Cape Province. The growing population and their economic needs, as well as the poor progress in sanitation facilities in this area constitute a threat to the water resources and drinking water in particular. In addition, municipal water purification systems do not have any laboratory facilities for routine monitoring of water quality, in order to comply with standards. Alice Municipality purifies the water before supplying it to the community for domestic uses. The efficiency of the Alice purification systems is, therefore, of concern.

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Water that is contaminated constitutes a serious threat to public health world-wide because of the presence of micro-organisms, especially coliforms. Coliforms (i.e. total and faecal coliforms) are indicator bacteria used to show levels of water contamination due to faecal pollution. Faecal indicators as aquatic environment monitors are used to obtain information about the quality of water and compliance with bacteriological standards (Niemi and Niemi, 1990). These micro-organisms constitute a threat to the safety of the drinking water because they indicate a potential of the water to cause an outbreak of water-borne diseases (Young, 1996). There are also chemical and physical parameters such as temperature, pH, biochemical oxygen demand, dissolved oxygen and turbidity that are monitored to help assess the water quality (Mathuthu et al., 1995).

Allochthonous bacteria are bacteria that are transient in the ecosystem. Enteric pathogens and most coliforms are allochthonous within the aquatic ecosystems. They are not well adapted to the chemical and physical conditions in the water (McFeters et al., 1986). As a result, the use of chlorine is the major cause of sublethal bacterial injury in treated water (Bucklin et al., 1991). The coliforms can be injured by the chemical and physical conditions in water; hence these are termed injured coliforms. Chlorine injures the bacteria by inducing physiological alterations of survivors such as injuring glucose and amino acid transport systems, damaging the outer membrane and reducing adhesive ability of enterophathogenic *Escherichia coli* (Verville and Herson, 1989). The injured bacteria are susceptible to selective media (M-endo LES agar, bile salts and deoxycholate) commonly used for enumeration. They are better enumerated on a non-selective medium, such as m-T7 agar. The m-T7 agar has therefore been recommended for the enumeration of injured coliforms from treated water (McFeters et al., 1986). The injured bacteria are proof that bacteria can survive and multiply because of the resistance developed against the disinfectant chlorine (Adam and Kott, 1989).

Heterotrophic bacteria are indicators of the general microbiological quality of the water. The multiplication of bacteria in water rich in biodegradable organic substances is called regrowth (Hambsch and Werner, 1993). Factors that contribute to the regrowth of bacteria are chlorine residuals and biodegradable

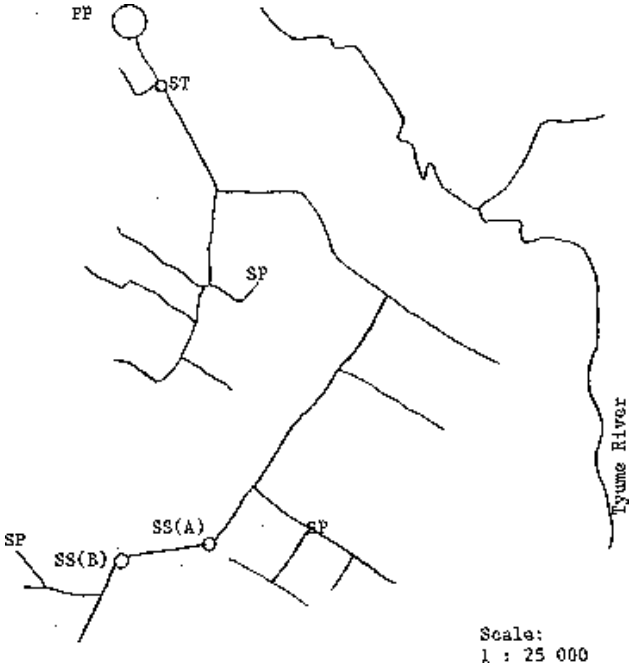

Figure 1

Representation of the Alice water distribution systems. PP designates purification plan, ST storage tank, SP sampling point, SS(A) substorage (A), SS(B) substorage (B)

dissolved organic carbon (BDOC)(Gatel et al., 1995). BDOC is the actual signal to which growth and activity of heterotrophic micro-organisms respond in natural waters. The knowledge of BDOC is required for modelling bacterial activity in aquatic systems (Servais et al., 1987).

The aim of this study was to assess the potability and the effectiveness of the drinking water purification systems in Alice, Eastern Cape. In addition, the potential of treated water to support the regrowth of heterotrophic bacteria, total and injured coliforms was investigated.

Materials and methods

Study site

The study site covered the Alice purification system, the main storage tank and its distribution network. The purification system gets its water from a catchment dam that draws the water from the Tyume River. The water is drawn from the middle layer of the catchment dam area to the purification system, as the top layer is exposed to air and contains leaves and dust, and the bottom layer may be anaerobic. The water from the dam undergoes purification, which involves the following steps: sedimentation, sand filtration and chlorination, using chlorine gas. Chlorine gas has been reported to be an efficient disinfectant in the treatment of drinking water (Mitchell, 1972; Venkobachar et al., 1977; Verville and Herson, 1989; Bucklin et al., 1991; Du Preez et al., 1995). Flocculation and sedimentation are enhanced by the addition of alum [$Al_2(SO_4)_3$] and lime for coagulation of the solids. The flocculation and sedimentation, and filtration steps are performed outdoors in open reservoirs. The chlorinated water is stored in the main storage tank until required. The main storage tank supplies water to a substorage (A) tank and Network 1. Network 2 is supplied by substorage (A) which also supplies water to substorage (B). Network 3 is supplied by substorage (B). The distribution systems that supply water from the main storage tank including distribution Networks 1 and 2 are metal piping systems. The Network 3 system was recently built and is a polyvinyl chloride (PVC) plastic distribution system. Figure 1 illustrates the summary diagram of the Alice water distribution systems.

Sample collection

Water samples were collected in autoclaved 1 l-glass bottles from the purification plant before treatment and after each purification step except sedimentation. Samples were also collected from the main storage tank and the three distribution networks. Samples from the purification and storage tank were collected as grab samples and those from the three distribution networks as running water. Samples were transported in cooler boxes to the laboratory and analysis proceeded immediately.

Chemical and physical analysis

The turbidity of the different samples was determined using a Hach 2100P turbidimeter within one hour following the collection. The temperature and the pH of the sample were determined on the site of collection with a thermometer and a pH meter. Free chlorine was determined within 24 h of collection with a Lovibond 1000 Comparator system using a DPD n°3 chlorine tablet.

Microbiological analysis of water samples

Chlorinated water samples for microbiological analysis were treated with sodium thiosulphate to stop the chlorination process at the moment of taking the sample (i.e. 100 mg sodium thiosulphate/l of water). Heterotrophic bacteria, total coliforms, faecal coliforms and injured coliforms were enumerated by the membrane filtration method performed under a laminar flow hood (i.e. 10 ml for untreated water and 100 ml for treated water) (*Standard Methods*, 1989). The following culture media were used: m-Endo LES agar was used to enumerate total coliforms, m-T7 to enumerate injured coliforms (LeChevallier et al., 1983) and mFC to enumerate faecal coliforms. Heterotrophic bacteria were recorded using two different media i.e. R2A (low nutrient medium) and 1/4 strength nutrient agar (prepared by diluting nutrient agar 4 times and adjusting the agar concentration to 1.6% with Bacto agar). All the media used were from Difco unless indicated otherwise. Millipore membrane filters (dia. 47 mm, pore size 0.45 μm) were used for total, faecal and injured coliform enumeration. For heterotrophic bacteria, Millipore membrane filters (dia. 47 mm, pore size 0.22 μm) were used. The total and injured coliform colonies per 10 ml or 100 ml were counted after incubation at 35°C for 24 h. Faecal coliform colonies were counted after incubation at 44.5°C for 24 h. The heterotrophic plate counts were recorded after incubation at 35°C for 48 h. The experiments were conducted in duplicate and repeated four times.

Assessment of regrowth potential of chlorinated water samples

Experiments simulating the conditions in the storage tank were conducted to assess the maximum growth (Nmax) of "indigenous" heterotrophic, total and injured coliforms in chlorinated drinking water samples from the water purification plant. Five

TABLE 1
TEMPERATURE (°C), pH, TURBIDITY (NTU) AND CHLORINE (mg/l) OF THE ALICE DRINKING WATER SAMPLES, AUGUST TO SEPTEMBER 1996

Dates	Samples	Temperature (°C)	pH	Turbidity (NTU)	Cl_2 (mg/l)
27/8	S3	15.5	7.3	-	-
	S4	18.0	7.3	-	-
	Net 1	19.0	7.3	-	-
	Net 2	19.0	7.0	-	-
10/9	S3	14.8	7.4	7.18	-
	S4	16.0	7.4	3.70	-
	Net 1	16.5	7.4	5.72	-
	Net 2	18.0	7.5	6.71	-
	Net 3	18.0	7.0	0.81	-
17/9	S3	14.5	7.3	10.70	0.2
	S4	14.5	7.3	6.16	0.2
	Net 1	19.9	7.3	7.80	0.1
	Net 2	18.0	7.3	9.51	0.1
	Net 3	18.0	7.4	3.00	0.1
24/9	S3	15.0	7.2	4.97	0.2
	S4	15.5	7.0	1.59	0.2
	Net 1	18.0	7.2	2.27	0.1
	Net 2	17.5	7.2	4.36	0.1
	Net 3	18.0	7.4	3.10	0.1

Legends: S3 - after chlorination; S4 - main storage tamk; Net 1 - distribution Network 1; Net 2 - distribution Network 2; Net 3 - distribution Network 3; - not determined.

litre glass bottles were cleaned and rinsed 10 times with boiled distilled water to remove the carbonaceous materials, and autoclaved at 121°C for 15 min. The glass bottles were each filled with 5 l of freshly chlorinated water sample and incubated at 15°C ± 2°C and 23°C ± 2°C, respectively. These temperatures were selected because they are close to the temperatures observed under practical conditions in drinking water during distribution (i.e. about 15°C during the night and early morning, and 23°C in the afternoons in summer time). The growth of heterotrophic bacteria (using R2A agar), total and injured coliforms was recorded at a 12 h interval for a period of 84 h, starting from time zero. The enumeration was done in duplicate by the membrane filtration method.

Results and discussion

The chemical and physical values, and indicator bacteria counts of the Alice drinking water are given in Tables 1 and 2 respectively and the regrowth potential of the chlorinated water is illustrated by Fig. 2.

The turbidity for the chlorinated water samples used in the regrowth potential experiment fluctuated between 7.62 to 8.37 with an average of 7.95; and 7.45 to 9.69 with an average of 7.60 for the samples stored at 15°C ± 2°C and 23° ± 2°C, respectively. These samples were collected on rainy days.

The pH values of Alice drinking water were within the South African recommended limits (pH 6.0 to 9.0). However, only about 50% of the samples complied with the allowable standard for turbidity (1 to 5 NTU) (Department of Water Affairs and Forestry, 1993). Samples collected after chlorination, at the main storage tank and from Networks 1 & 2 showed generally high turbidity values. Distribution Network 3 turbidity values were 100% within the allowable standards compared to Networks 1 & 2 which hardly complied with the standards (about 30% only complied). These high values can be accounted for by the distribution system pipes used for transporting the water in addition to bacterial contamination. The high turbidity after purification may be attributed to the rusted metal pipes that are used for the distribution of treated drinking water from the main storage tank to the distribution Networks 1 and 2. Distribution Network 3 was recently built and it uses PVC piping. The high turbidity values observed in the chlorinated water samples used in the regrowth studies (7.45 to 9.49 NTU) were probably enhanced by rain since these samples were collected after rainy days. This further illustrated the inefficiency of the filtration steps.

A decrease of the levels of bacteria, i.e. heterotrophic bacteria, total, faecal and injured coliforms from samples S1 and S2 is shown in Table 2. The decrease in the number of bacteria between the incoming water and water after filtration is partly attributable to sedimentation rather than filtration alone. There was not much difference in colony/100 ml (HPC were reported in colony per 100 ml for easy comparison with other indicators) for heterotrophic bacteria, total and injured coliforms respectively for the samples collected after chlorination (S3) and up to those from Network 3 (Table 2). This may imply that the steps that

TABLE 2
HETEROTROPHIC BACTERIA (HPC), TOTAL COLIFORMS (TC), FAECAL COLIFORMS (FC) AND INJURED COLIFORMS (IC) IN ALICE DRINKING WATER SAMPLES, AUGUST TO OCTOBER 1996 (COLONY/100 ml FOR THE TREATED WATER AND COLONY/10 ml FOR INCOMING WATER)

Dates	Indicator bacteriar	S1	S2	S3	Main storage tank	Net 1	Net 2	Net 3
27/8	HPC (R2A)	24	185	205	52	58	37	-
	HPC ($_{1/4}$NA)	13	119	110	90	41	37	-
	TC12	6	1	4	4	4	-	
	FC 4	2	1	1	1	1	-	
	IC	-	-	4	4	5	7	-
10/9	HPC (R2A)	58	350	275	270	206	65	440
	HPC ($_{1/4}$NA)	54	325	285	280	220	67	447
	TC	21	6	2	3	5	0	8
	FC	1	1	1	3	2	0	2
	IC	-	-	2	3	4	4	6
17/9	HPC (R2A)	53	475	265	255	131	221	440
	HPC ($_{1/4}$NA)	49	425	260	265	137	233	447
	TC	24	25	8	5	5	7	7
	FC	1	2	2	4	1	0	2
	IC	-	-	3	2	5	4	6
24/9	HPC (R2A)	54	330	245	220	275	53	360
	HPC ($_{1/4}$NA)	64	410	370	240	308	63	360
	TC	47	11	2	2	4	0	10
	FC	1	5	1	0	2	0	1
	IC	-	-	1	2	2	4	4

Legends: S1 - incoming water; S2 - water sample after filtration; S3 - chlorinated water; - not determined;
Net 1 - distribution Network 1; Net 2 - distribution Network 2; Net 3 - distribution Network 3;
$_{1/4}$ NA - quarter-strength nutrient agar.

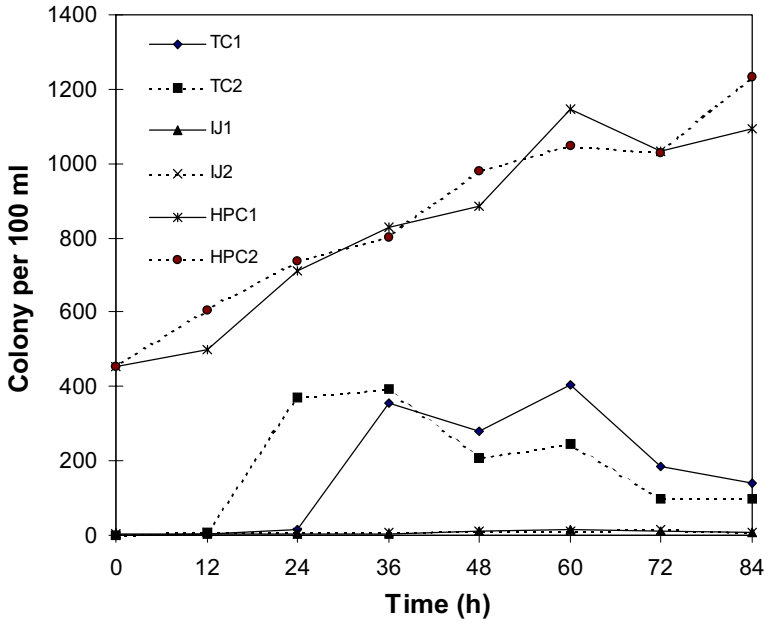

Figure 2
Growth of heterotrophic bacteria (HPC), total coliforms (TC) and injured coliforms (IC) in Alice chlorinated water samples incubated at 15°C ± 2°C and 23°C ± 2°C for 84 h. TC1, IC1 and HPC1 refer to samples incubated at 15°C ± 2°C, and TC2, IC2 and HPC2 to those incubated at 23°C ± 2°C.

follow sedimentation were not effective. A clear decrease in colony/100 ml should be expected if filtration and chlorination were effective. The inefficiency of the filtration and chlorination steps in the water purification procedures is demonstrated by high values of indicator bacteria (considering the total coliform count for instance: Network 1 = 4 to 5 colonies/100 ml, Network 2 = 0 to 7 colonies/100 ml and Network 3 = 7 to 10 colonies/100 ml). The South African standards limit is zero for both total and faecal coliforms (Department of Water Affairs and Forestry, 1993). Both values of total and faecal coliforms exceeded the recommended limits by far. The S3 samples were from water that had just been chlorinated, yet they were contaminated with faecal coliforms that exceeded the allowable limits (Table 2). The high faecal coliform levels in S3 demonstrate the inefficiency of chlorination. This constitutes a health risk to the consumers. The water samples from Network 3 show exceedingly high values of total coliforms probably because of perturbation due to recent construction work since this is a newly built distribution system (Clark et al., 1993).

The average number of heterotrophic bacteria enumerated with 1/4 strength nutrient agar i.e. 272 colonies/100 ml [37 to 447] was higher than that recorded with R2A agar i.e. 199 colonies/100 ml [37 to 440]. This implies that 1/4 strength nutrient agar can be considered as an alternative medium for the enumeration of heterotrophs instead of using an expensive medium such as R2A. The number of heterotrophic bacteria and total coliforms was high in Network 3, yet this is a PVC distribution system. This observation could be accounted for by the nutrients introduced possibly through holes, back pressure and new construction or adding new connections to the system (Clark et al., 1993). These nutrients are responsible for the high growth in the Network 3 because they serve as a food source for the micro-organisms in the water (Van der Kooij et al., 1994).

The chlorine levels in the water samples were very low i.e. 0.1 to 0.2 mg/l, compared to the recommended amount i.e. 1 mg/l (Table 2). The drinking water sample contained 10 times less chlorine than the recommended concentration. The free chlorine constitutes a built-in safety measure. The low chlorine concentration are due to the fact that the chlorine demand has not been determined. There are no facilities at the plant to help measure the chlorine level even after chlorination. In addition there is no gauge chart that shows the amount of chlorine gas being pumped into the water. During rainy days the turbidity of the water becomes very high, therefore more coagulants should be added and chlorine gas dosage should be increased to produce clean and potable water consistently.

Heterotrophic bacteria started growing immediately after incubation of water samples at 15°C ± 2°C. Growth was sustained over the full experimental period (84 h). Total coliforms showed a 24 h lag phase before growing remarkably up to 60 h when they started to decline (Fig. 2). At 23°C ± 2°C, the overall growth pattern of heterotrophic bacteria remained the same as that observed at 15°C ± 2°C but there was slightly higher maximum growth (862 colonies/100 ml compared to 831 colonies/100 ml for 15°C ± 2°C and 23° ± 2°C respectively). Total coliforms at this temperature showed a rather short lag phase (12 h) compared to 15°C ± 2°C and reached maximum growth after 36 h. This rapid growth and decline might be attributed to the rising temperature. Incubation at higher temperatures has been shown to result in a rapid decline of the colony count after having reached Nmax (Van der Kooij, 1990). This indicated that the higher the temperature (with a max at 37°C) the faster the regrowth. Decline in heterotrophic and total coliform colonies/100 ml might be due to the depletion of nutrients. The number of injured coliforms remained almost within the same range in both cases, with a slight increase around 60 and 72 h. Higher values of heterotrophs are observed, than total coliforms and the injured coliforms (Fig. 2). The high heterotrophs indicate that the general quality of the chlorinated water is poor. The increase in the number of heterotrophic bacteria and total coliform incubated at both 15°C ± 2°C and 23°C ± 2°C suggests that the chlorinated water contains sufficient amounts of nutrients that can be degraded and metabolised by the bacteria for growth. This demonstrates the inefficiency of the purification systems.

Conclusion

The results indicated that 1/4 nutrient agar could be used as an alternative medium in assessing the aerobic heterotrophic plate count in treated drinking water since it displayed a high average number of heterotrophs enumerated compared to R2A agar 48 h after incubation at 35°C. The water from the Alice purification system is not safe for human consumption as its levels of contamination are high and exceed by far the South African recommended and allowable limits. This poses a health risk for the consumers. The rusted metal-piping system is probably contributing to the deterioration of the water quality by increasing turbidity. The high regrowth of heterotrophs and total coliforms occurring after chlorination indicates the inefficiency of the filtration and chlorination steps. The levels of chlorine in treated water were low and regrowth of heterotrophic and total coliforms might have been also encouraged by the presence of nutrients (sufficient BDOC content) in the water. The Alice water purification systems are inefficient and need to be upgraded before an outbreak of disease occurs.

Acknowledgements

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