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Effect of Reservoirs on Microbiological Water Qualities in a Drinking Water Distribution System

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Abstract This study was undertaken to determine the effect of reservoirs on water quality and the distribution of pathogenic and indicator bacteria in a drinking water distribution system (total length 14 km). Raw water, disinfected water, and water samples from the distribution system were subjected to physicochemical and microbiological analyses. Most factors encountered at each season included residual chloride, nitrate, turbidity, and phosphorus for heterotrophic bacterial distribution, and hardness, heterotrophic bacteria, sampling site, and DOC (dissolved organic carbon) for bacteria on selective media. No *Salmonella* or *Shigella* spp. were detected, but many colonies of opportunistic pathogens were found. Comparing tap water samples taken at similar distances from the water treatment plant, samples that had passed through a reservoir had a higher concentration of heterotrophic bacteria, and a higher rate of colony formation with 10 times as many bacteria on selective media. Based on the results with m-Endo agar, the water in reservoirs appeared safe; however, coliforms and opportunistic pathogenic bacteria such as *Pseudomonas aeruginosa* were identified on other selective media. This study illustrates that storage reservoirs in the drinking water distribution system have low microbiological water quality by opportunistic pathogens, and therefore, water quality must be controlled.

Key words: Reservoir, selective media, drinking water distribution system, coliform, *Pseudomonas aeruginosa*

Drinking water reservoirs such as distribution reservoirs are crucial in order to meet the diverse demands of consumers [17]. Disinfection is also essential for supplying clean and safe drinking water. In 2000, human deaths caused by non-disinfected drinking tap water were announced in Walkerton, Canada, and the agent responsible for this incident was *E. coli*, which spread via the distribution system.

However, it is still possible for pathogenic bacteria to invade drinking water distribution systems, despite the fact that the drinking water has been disinfected. In addition to biofilms [19], Geldreich [9] described that birds could carry pathogens, and Fass *et al.* [8] reported with the test of *E. coli* that external contamination could develop into an internal one.

To assess the safety of tap water, viable HPCs (heterotrophic plate counts) and coliforms are widely used in many countries, including Korea. The presence of bacterial regrowth and indicators has been regarded as signaling the possible existence of pathogenic bacteria in distribution systems, and therefore, a potential threat to public health [3, 10]. Few reports have confirmed the existence of pathogenic bacteria in distribution systems, where coliforms exist [14].

This study assessed bacteriological change in a drinking water distribution system. The objectives were to determine (i) the distribution of indicator and pathogenic bacteria in a drinking water distribution system, (ii) the status of water quality in reservoirs, and (iii) factors controlling the bacterial concentration in each season, so that microbiological water quality can be determined and controlled.

Materials and Methods

Sampling Sites

Samples were collected from the distribution system of a water treatment facility supplying a part of Seoul, Korea. The raw water was surface water from the Han River, which traverses the city (Fig. 1). The raw water was piped from the intake point in the Han River to a drinking water

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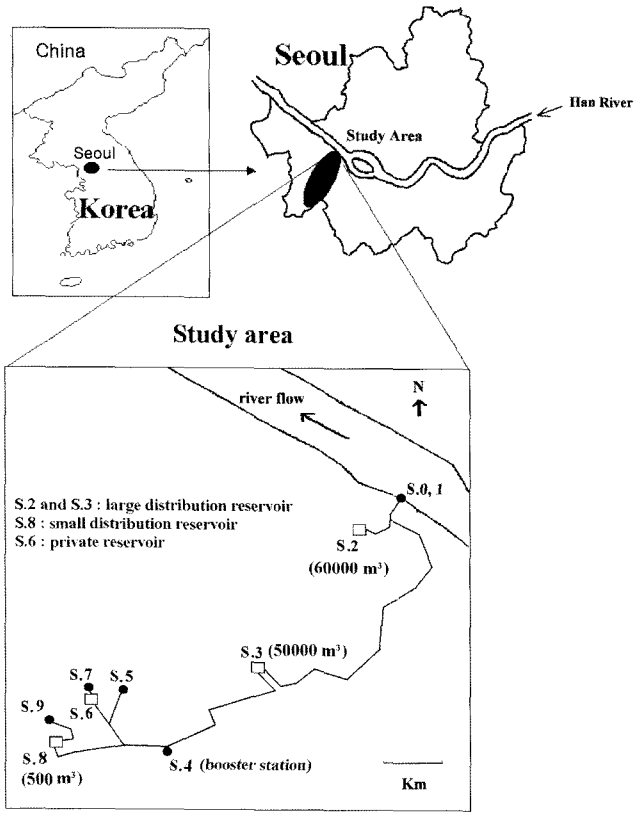

Fig. 1. Map of sampling sites (S.0, chlorinated raw water; S.1, after disinfection step in water plant; S.2–S.9, drinking water distribution system).

treatment plant after prechlorination. The drinking water treatment plant, situated on the riverside, uses conventional water treatment methods, including precipitation, filtration, and disinfection. Only water produced by this drinking water treatment plant was supplied to the study area. A description of the sampling sites is in Fig. 1. Produced water flowed from S.1 to S.4 and was distributed to the other sites (Fig. 1). S.5, 6, 7, 8, and 9 were similar distances from the water treatment plant. Hence, these sites were used for comparison of the water qualities between direct distribution (S.5), reservoir, and its following tap (S.6, S.7, S.8, and S.9) in the end region of the distribution system. The storage reservoirs at S.2, 3, and 8 were at ground level, whereas that at S.6 was on the roof of a three-story house. Reservoirs of S.2, 3, and 8 were concrete enclosed tanks, and the reservoir of S.6 was made up of plastic material including a cover.

Sampling

Samples were collected once a month from January to October, except in March (spring) and August (summer), when they were collected twice a month. Sampling from S.9 began in June, and six samples were obtained. Samples were collected in autoclaved polypropylene bottles or in sterilized disposable bottles with 0.5 ml of 10% $Na_2S_2O_3$ added per liter. They were refrigerated and analyzed within six hours of arrival in the laboratory.

Physicochemical Factors

Water temperature (°C) and pH were measured using Check-Mate 90 (Corning, U.S.A.). Free residual chlorine was measured using the colorimetric method (Advantec, Japan), and TOC (total organic carbon), DOC (dissolved organic carbon), and BDOC (biodegradable DOC) were measured with a TOC5000A (Shimadzu, Kyoto, Japan) as non-purgeable organic carbon. Turbidity, alkalinity, hardness, conductivity, NH_3-N, NO_2^--N, NO_3^--N, and PO_4^{3-}-P were also determined [1].

HPC, Indicator, and Pathogenic Bacteria

Viable HPC was enumerated in triplicate on PCA (plate count agar) after culture at 37°C for 48 h, and on R2A agar [1] after culture at 20°C for 1 week. In Korea, regulations require that tap water produces no colonies on m-Endo agar after filtration of 50 ml and 100 cfu (colony forming unit) per ml or less on PCA.

A membrane filtration method was applied for m-Endo (total coliforms), m-Enterococcus (fecal streptococci), bismuth sulfite (*Salmonella* spp.), and m-Hektoen enteric (*Shigella* spp.) agar. With an exception of the chlorinated raw water, the filtration volume was 250 ml, in triplicate. After a 24-h culture at 37°C, colonies were counted. Presumptive colonies were selected according to the manufacturer's manual. All media were purchased from Difco and BBL (Sparks, MD, U.S.A.).

Identification

To exclude false positives and negatives, all colonies with typical morphology and atypical colony representing a different morphology were isolated. Gram staining, catalase and cytochrome tests, and biochemical kits (*i.e.*, API20E, API20NE, and API20STREPT kits, bioMérieux, Marcy l'Etoile, France) were used for identification [4] after enrichment on nutrient agar (Difco).

Statistical Analyses

All statistical analyses were performed with the SAS (Statistical Analysis System) software package for Windows (ver. 6.12), based on 95% confidence levels. Analyses were done after normalization.

Results and Discussion

Physicochemical Factors

Table 1 shows the water quality in the drinking water distribution system. Phosphorus was slightly elevated in the distribution system (Table 2), as compared with the

Table 1. Quality of chlorinated raw water, treated water, and water samples of the distribution system in 1996. Ranges are from the data of all tested seasons, and the data of the column "Distribution System" are from all tested sites (S.2–S.9) and season. Values in parentheses represent mean values.

Factors	Site and the determined values		
	Raw water (S.0)	Water plant (S.1)	Distribution system (S.2–S.9)
Residual Chloride (Cl_2 mg/l)	0.05–0.59 (0.20)	0.48–1.23 (0.86)	0.01–1.09 (0.48)
Water Temperature (°C)	N.D.[c]	2.0–25.4 (15.7)	2.0–29.7 (16.8)
TOC (mg/l)	0.74–11.72 (2.33)	0.89–2.88 (1.79)	0.44–3.59 (1.98)
DOC (mg/l)	0.11–3.82 (1.42)	0.09–2.82 (1.09)	0.07–3.22 (1.08)
BDOC (mg/l)	0.06–3.06 (1.32)	0.12–1.98(0.65)	0.05–2.69 (0.62)
Turbidity (NTU)*	N.D.	0.06–0.35 (0.16)	0.05–0.60 (0.18)
Alkalinity (mg/l)	N.D.	15.0–37.0 (23.8)	10.0–37.0 (23.3)
Hardness (mg/l)	N.D.	43.0–75.0 (59.3)	38.0–81.0 (58.98)
Conductivity (mS/cm)	N.D.	0.10–0.22 (0.16)	0.10–0.24 (0.17)
pH	6.72–7.47 (7.07)	6.60–7.47 (6.94)	6.65–7.49 (7.02)
NH_3-N (μg/l)	0.00–30.08 (4.96)	0.00–6.92 (1.42)	0.00–15.34 (2.47)
NO_2^--N (mg/l)	0.00–9.16 (2.11)	0.00–6.52 (0.87)	0.00–7.31 (0.86)
NO_3^--N (mg/l)	0.00–1.91 (1.14)	0.51–2.23 (0.94)	0.13–3.13 (1.04)
PO_4^{3-}-P (μg/l)	0.00–31.92 (8.69)	0.00–8.81 (2.50)	0.00–21.19 (6.91)
HPC[a] in R2A (cfu/ml)	1.9×10–3.4×10^4 (5.5×10^2)	0.0–2.8×10^1 (7.3×10^0)	1.0–4.0×10^2 (2.6×10^1)
HPC in PCA (cfu/ml)	1.4×10–3.8×10^3 (3.7–10^2)	0.0–8.5×10^0 (2.7–10^0)	0.0–2.4×10^2 (1.5–10^1)
Total Coliform (cfu/250 ml)[b]	0.3–3.0 (0.4)	0.0–0.0 (0.0)	0.0–36.0 (0.5)
Fecal Streptococci (cfu/250 ml)[b]	0.3–17.0 (1.8)	0.0–1.0 (0.1)	0.0–2.0 (0.1)
Salmonella spp. (cfu/250 ml)[b]	0.3–12.0 (2.1)	0.0–1.0 (0.3)	0.0–4.0 (0.5)
Shigella spp. (cfu/250 ml)[b]	0.3–18.0 (2.2)	0.0–1.0 (0.1)	0.0–2.0 (0.1)

*Nephelometric turbidity units.
[a]Heterotrophic plate count.
[b]Presumptive positive colonies on each selective agar before identification.
[c]Not determined.
Zero means not detected.

water treatment plant; an anticorrosion agent was suspected as the cause [22]. Since microbial growth was dependent on phosphorus in drinking water, the growth could be regulated by limiting the availability of phosphorus [2, 11, 13].

TOC, DOC, and BDOC levels did not significantly change with water flow; however, they differed with sampling times. The BDOC concentrations exceeded 0.05 mg/l (Table 2), which is enough to sustain bacterial growth [12]. As for the percentage of DOC, the BDOC was 46% at S.0, 59% at S.1, and 47% in the remainder of the distribution system. The seasonal change in water temperature was pronounced. There were no marked differences in the physicochemical parameters (Table 1) between S.2 and S.9, except for residual chlorine, NH_3-N, and PO_4^{3-}-P. Levels of residual chlorine were high in the summer and low in the winter. In a distribution system, disinfectants react with the wall of the system and materials in water [12]. The reduction in disinfectant concentrations might be proportional to the distance travelled: Obvious decrease was observed downstream from S.4 (9.2 km from the water plant) and at S.9 (Fig. 2). At S.9, the free residual chlorine ranged from 0.01 to 0.51 Cl_2 mg/l, and the difference from S.8 was 0.0–0.5 Cl_2 mg/l (mean 0.29 Cl_2 mg/l). Low chlorine concentration and rather high BDOC levels imply possible internal bacterial contamination of drinking water.

Table 2. Range of BDOC (mg/l) and phosphorus (μg/l) in chlorinated raw water (S.0), treated water (S.1), and water samples of the distribution system (S.2–S.9). Values in parentheses represent mean values.

Site	BDOC (mg/l)	Phosphorus (μg/l)
S.0	0.06–3.06 (1.32)	0.00–31.92 (8.69)
S.1	0.12–1.98 (0.65)	0.00–8.81 (2.50)
S.2	0.13–2.02 (0.64)	0.00–12.11 (5.27)
S.3	0.18–2.32 (0.61)	0.00–12.94 (5.66)
S.4	0.11–1.63 (0.54)	1.38–21.19 (7.53)
S.5	0.13–1.29 (0.40)	3.03–14.58 (8.73)
S.6	0.11–1.63 (0.59)	0.55–14.58 (7.75)
S.7	0.05–2.04 (0.60)	0.55–10.46 (6.40)
S.8	0.07–2.69 (0.74)	0.00–13.76 (5.90)
S.9	0.55–2.16 (0.82)	0.55–16.23 (8.04)

Distribution of Viable HPC

Although S.0 contained residual chlorine (0.05–0.59 Cl_2 mg/l), viable HPCs ranged from 1.9×10^1 to 3.4×10^4 cfu/ml on R2A agar (Table 1). The high viable HPCs were

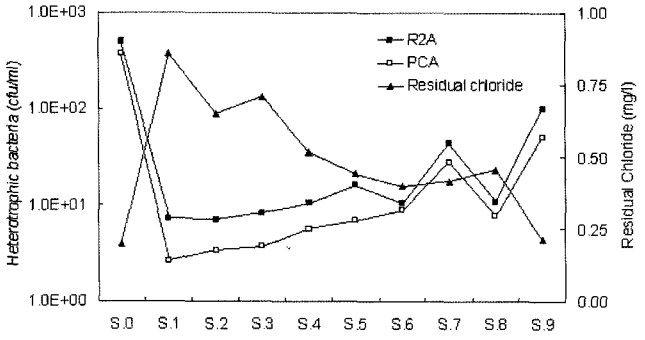

Fig. 2. Mean values of residual chlorine level (Cl_2 mg/l) and heterotrophic bacterial levels (cfu/ml) in R2A and PCA media.

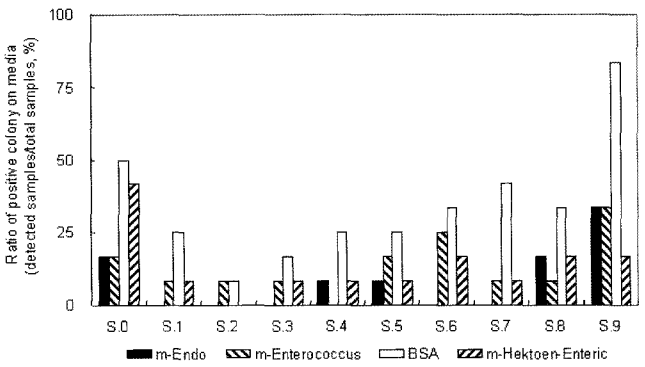

Fig. 3. Rates of presumptive positive colony formation on selective media (%).
The media included m-Endo (total coliform), m-Enterococcus (fecal *Streptococcus*), bismuth sulfite (*Salmonella* spp.), and m-Hektoen enteric (*Shigella* spp.) agar.

significantly reduced after water purification (Fig. 2). At S.5, an increase of HPC and a decrease of residual chlorine and nitrite were observed: Residual chlorine was below 0.5 Cl_2 mg/l at S.5, and similar values were observed at S.5, 6, 7, and 8 (Fig. 2). Ollos *et al.* [16] reported that higher than 0.5 Cl_2 mg/l free chlorine is needed to reduce a biofilm. The mean colony counts in R2A agar exceeded 10 cfu/ml in water from S.5. The HPC levels on both PCA and R2A agars increased as the drinking water distribution system was farther downstream. The HPC levels were elevated in tap water (S.9), downstream from a small distribution reservoir (S.8), and in tap water (S.7), downstream from a private reservoir (S.6). Their maximum levels on PCA agar exceeded 2.0×10^2 cfu/ml, which violates the regulatory guidelines of Korea (Table 1). The increase of HPC occurred severely at the household distribution system or the household tap rather than from source waters or the distribution system [20]. S.5, 7, and 9 were taps in the household; however, S.5 was the only station not supplied by a reservoir. S.5 revealed lower HPC than those of S.7 and S.9 in all sampling times, thus indicating that microbiological water quality could deteriorate by passing reservoirs.

Van der Bruggen and Vandecasteele [24] discussed the importance of control against bacteria in the drinking water production. Our results indicated that reservoirs increase the concentration of bacteria, which may be important in drinking water consumption and management.

Distribution of Bacteria on Selective Media

Figure 3 shows the distribution of presumptive positive colonies on each selective medium before identification. Presumptive positive colonies are defined as colonies of typical morphology on selective medium, but they are not necessarily target colonies. The numbers of presumptive positive colonies in 250 ml of water were 0–8 cfu (S.0), 0–3 cfu (S.1), and 0–36 cfu (S.2–9). Coliform and fecal streptococcus have been found in treated drinking water, having 0.05 to 1.5 Cl_2 mg/l free chlorine, similar to our results [8].

Figure 4 shows the distribution of coliforms and Enterobacteriaceae on the four selective media after identification. For the selective media studied, the rate of presumptive positive colony formation was the highest on bismuth sulfite agar and lowest on m-Endo agar (Fig. 3). However, no *Salmonella* or *Shigella* species were detected on bismuth sulfite or m-Hektoen enteric agar. After identification, it was found that bismuth sulfite agar grew many coliforms; sometimes it cultivated coliforms, whereas m-Endo agar did not (data not shown). Identification of colonies revealed Enterobacteriaceae from all sites (Table 3 and Fig. 4), and this would not happen if only m-Endo agar had been used (Fig. 3). *Pseudomonas aeruginosa* (S.0, 3, 7, and 8) and many opportunistic pathogens, such as *Enterobacter sakazakii* and *Aeromonas hydrophila*, were identified. The genera identified included *Enterobacter*, *Citrobacter*, *Serratia*, *Klebsiella*, *Kluyvera*, *Erwinia*, *Proteus*, and *Rahnella* (all Enterobacteriaceae), and *Aeromonas*, *Chryseomonas*, *Pseudomonas*, and *Acinetobacter* (Table 3).

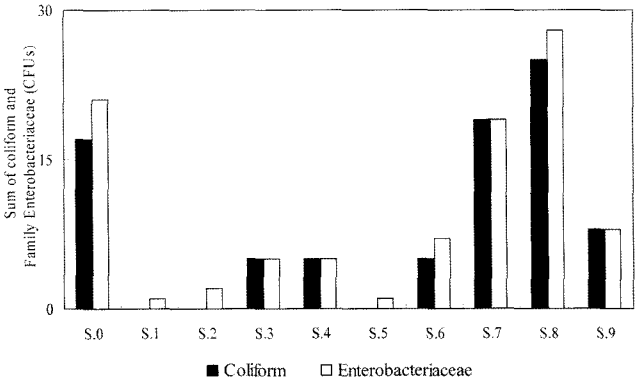

Fig. 4. Sum (cfu) of coliform and family Enterobacteriaceae on four selective media and mean values of HPC in R2A agar (cfu/ml). Each was done in triplicate. S.9 was sampled six times and the others twelve times.

Table 3. Bacteria isolated from prechlorinated raw water (S.0), treated water (S.1), and distribution system water (S.2–S.9). S.5, 6, 7, 8, and 9 had similar distance from the water plant, and only S.5 was supplied without a reservoir. The sampling number was six in S.9 and 12 in the others.

Bacteria	Sum	Site and the number of bacteria									
		S.0	S.1	S.2	S.3	S.4	S.5	S.6	S.7	S.8	S.9
Pseudomonas aeruginosa	4	1			1				1	1	
Enterobacter cloacae	17	7			1			1	5	1	2
Enterobacter sakazakii	32	4			2	4		3	10	8	1
Enterobacter aerogenes	5										5
Enterobacter amnigenes	3								1	2	
Enterobacter agglomerans	14	6			2			1	1	4	
Enterobacter intermedium	3	1							1	1	
Klebsiella ascorbata	3									3	
Klebsiella pneumoniae	5					1				4	
Klebsiella gr.47	1									1	
Citrobacter freundii	2								1	1	
Erwinia spp.	1			1							
Kluyvera spp.	1							1			
Proteus penneri	1			1							
Rahnella aquatilis	1									1	
Serratia plymuthica	6	3					1	1		1	
Serratia liquefaciens	1	1									
Serratia ficaria	2		1							1	
Acinetobacter spp.	2		1				1				
Aeromonas hydrophila	16	8		1		1		2	2	2	
Aeromonas sobria	7	3				3			1		
Aeromonas salmonicida	2										2
Chryseomonas luteola	18	2	1		2	1	2	4	1	3	2
Flavimonas oryzihabitans	12	1	4		2	1	4				
Flavobacterium odoratum	1									1	
Leclercia adecarboxylata	2				1					1	
Pasteurella spp.	1	1									
Pseudomonas putida	2									2	
Pseudomonas fluorescens	28	5		2	3	2	4	4	3	4	1
Pseudomonas paucimobilis	1									1	
Pseudomonas putrefaciens	1										1
Pseudomonas spp.	3		1			1				1	
Sphingobacterium multtivorum	1						1				
Vibrio metschnikovii	1					1					
Xanthomonas maltophila	3							2	1		
Not identified Gram (-)	86	32	3	3	5	11	7	11	1	2	11
Not identified Gram (+)	124	41	9	6	14	6	10	20	3	9	6
Total	413	116	20	14	35	32	30	46	32	57	31

S.9 seemed to have a small number of coliforms (Fig. 4); however, the number of samplings was half that of the other sites. Identification revealed the highest coliform detection ratio at S.9 and no colony formation at the other sites, and colonies were still observed at S.9 in September and October.

Factor Analysis

Factor analyses (Table 4) revealed that diverse factors were involved in the distribution of bacteria, since no factor played a major role throughout the study period. Residual chloride, nitrate, turbidity, and phosphorus were related to the distribution of HPC at all seasons, and bacteria on selective media were found to have different factors in this study (Table 4). This implied the necessity of integrated or case-by-case management for the control of microbiological water qualities.

Phosphorus (PO_4^{3-}-P) and free residual chlorine were related to bacterial concentration throughout the study period, whereas the other factors differed with season.

Table 4. Factors affecting the concentration of bacteria, in the water plant and distribution system water, on R2A media and selective media. Factors were analyzed for total period as well as each season studied.

	R2A media				Selective media			
	Factor	Partial R^2	F Value	$P>F$	Factor	Partial R^2	F Value	$P>F$
Jan–Oct (n=126)	PO_4^{3-}-P	0.108	11.58	0.001	WT[a]	0.103	10.97	0.001
	RC*	0.068	7.82	0.006	R2A	0.070	8.04	0.006
					PO_4^{3-}-P	0.108	14.14	0.000
Jan–Feb (Winter, n=20)	RC	0.541	20.02	0.000	Hardness	0.209	4.49	0.049
	NO_3^- -N	0.105	4.73	0.045	WT	0.190	5.05	0.039
	NH_3-N	0.072	3.85	0.067	PO_4^{3-}-P	0.126	3.96	0.065
	Site	0.124	10.93	0.005	Site	0.207	10.31	0.005
Mar–May (Spring, n=40)	NO_3^--N	0.323	15.29	0.001	PCA	0.170	6.54	0.016
	WT[a]	0.129	7.32	0.011	NH_3-N	0.164	7.62	0.010
	Conductivity	0.109	7.44	0.010	WT	0.085	4.38	0.045
	RC	0.038	2.76	0.107	R2A	0.059	3.26	0.081
	Turbidity	0.034	2.63	0.116	Conductivity	0.116	8.01	0.009
June–Aug. (Summer, n=44)	Turbidity	0.127	5.37	0.026	Site	0.123	5.20	0.028
	Site	0.052	2.26	0.141	Hardness	0.082	3.70	0.062
					R2A	0.112	5.74	0.022
Sep.–Oct. (Autumn, n=22)	PO_4^{3-}-P	0.251	5.70	0.029	DOC	0.119	2.29	0.149
					WT	0.128	2.72	0.118
					Hardness	0.228	6.49	0.022

*Free residual chlorine.
[a]Water temperature.

Microbial growth in drinking water is also regulated by the availability of phosphorus [2, 11, 13]. In this study, a weak relationship between viable HPC and BDOC was apparent, unlike Niquette *et al.* [15]. Together with the data in Table 1, this suggests that the BDOC concentration was sufficient to sustain bacterial growth, and that even the minimal concentration of this study could be sufficient to maintain bacterial growth [23].

Water temperature had the most significant effect on colony formation on selective media, whereas residual chlorine was not closely related (Table 4). Coliforms were detected (Fig. 4) even when residual chlorine levels were high (Fig. 2), thus necessitating other means of control.

Effect of Reservoir on Water Quality

The present study demonstrated deterioration of drinking water quality at storage reservoirs, including service reservoirs (S.3 and 8). For S.5 (Fig. 3), despite the presumptive positive result for m-Endo agar, no coliforms were identified among the colonies that grew on the four selective media (Fig. 4). Conversely, no colonies grew on m-Endo agar from the samples of S.3, 6, and 7 (Fig. 3), but coliforms grew on other selective media (Fig. 4).

P. aeruginosa, an opportunistic pathogen, and many genera of Enterobacteriaceae were detected at these reservoirs (Table 3). S.5, 6, 7, and 8 showed similar chlorine levels (Fig. 2) and comparable distance from the water plant (Fig. 1), but the portion of detection was higher in reservoirs S.6 and 8 than in the others (Fig. 3). At the end of the distribution system, S.5 was the only station not supplied by a reservoir. At storage reservoirs where the water surface is exposed to air, a decrease in disinfectants and contamination from the outside may occur. The increased retention time might enhance these phenomena. Moreover, dead and living worms as well as spiders and grasshoppers were observed in the water at S.8. These situations would support the external contamination at reservoirs.

The possible introduction of bacteria from outside such as via worms, low disinfectant concentration, and high retention time could explain the high concentration of bacteria at S.8 and 9 (Fig. 4). Injected *E. coli* were adsorbed to the indigenous bacterial biofilm and showed growth; *i.e.*, external contamination could develop into an internal one [8]. Geldreich [9] reported human death resulted from the consumption of tap water contaminated with *Salmonella* spp. that was introduced by birds into a storage tank in a public network. In fact, the WTRI (Water Technology Research Institute of Seoul City) detected biofilms on the wall of a distribution reservoir, including one in this study, with high free residual chlorine levels (personal communication).

The large distribution reservoir (S.3) showed high levels of residual chlorine (Fig. 2), and yet showed coliforms (Fig. 4). In fact, coliforms have been detected in high residual

chlorine concentrations [21], suggesting that there is a strong probability of contaminated drinking water in regions near these contaminated reservoirs.

Internal contamination or regrowth might be another cause of the increase in the viable HPC concentration and coliforms [10]. There was no significant difference in the physicochemical parameters (Table 1) between sampling sites, meaning little or no potential for severe external contamination such as cross-connections. S.6 and S.7 were located at the same house. The water reservoir at S.6 was made of plastic and had a water surface exposed to atmosphere, and pipes from S.6 to S.7 were made of ductile cast iron and polyvinyl chloride, which have been reported to stimulate the growth of bacteria [18].

The coliform detection rate was the highest, and HPCs elevated, at S.9. Although some of the coliforms detected at S.9 were in samples collected in June (summer), September, and October, no colony formation was observed at the other sites, while colonies were still observed at S.9. The free residual chlorine at S.9 ranged from 0.01 to 0.51 Cl_2 mg/l (mean 0.21 Cl_2 mg/l). A sufficient BDOC concentration (Table 2), a decrease of free residual chlorine (Fig. 2), and diverse materials [6, 5] used in the distribution system of S.9 and external contamination at S.8 all would sustain the growth of a biofilm near the end of the distribution system [8, 16, 15, 25].

In conclusion, our results indicate that (i) water deterioration may occur at exposed storage reservoirs, (ii) re-disinfection or other means are necessary to control water quality in service reservoir, especially small one (*i.e.*, S.8), (iii) control of phosphorus and BDOC would be necessary to maintain the safety of the distribution system, and (iv) further identification of colonies on selective media is necessary.

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