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原　著

Initial Trials of Monochloramine Disinfection of Circulating Bathtub Water at Public Hot Spring Facilities and Determining its Efficacy

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温泉を用いた公衆浴場における
モノクロラミン消毒の試行とその有用性

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要　旨

本研究では，手投入による簡易的なモノクロラミン消毒による浴用水中のレジオネラ属菌をはじめとする微生物学的，理化学的モニタリングを行うことにより，その消毒効果の検証を行った．従来の消毒方法である次亜塩素酸ナトリウムによる消毒効果の減弱が指摘されている高 pH と高濃度のアンモニア態窒素を含む温泉を利用する施設を対象とした．研究の結果，いずれの浴用水，配管内部からもレジオネラ属菌は検出されなかったことから，手投入の簡易的なモノクロラミン消毒であっても，レジオネラ属菌の殺菌に効果があるものと考えられた．本研究の結果から，手投入による簡易型モノクロラミン消毒であっても，レジオネラ属菌の制御が可能となることが示唆された．今後，従来型の次亜塩素酸ナトリウム消毒に阻害要因を持つ温泉水を利用している施設がモノクロラミン消毒の導入を検討する場合，初期投資を要しない本方法でその試行を行うことが可能となる．

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Abstract

In this study, the effect of monochloramine disinfection by simple manual addition was verified through the microbiological and chemical monitoring of bathtub water. The research focuses on hot spring facilities that use hot spring waters containing high pH and high NH_4-N concentrations, because these conditions diminish the effectiveness of sodium hypochlorite disinfection. Using the manual application of monochloramine as a disinfectant, *Legionella* spp. was not detected in the bathtub water or inside the pipes. Therefore, the simple manual disinfection using monochloramine was suggested to effectively control *Legionella* spp. These results imply that monochloramine disinfection by simple manual addition can control *Legionella* spp. Hot spring facilities using hot spring water unsuited to sodium hypochlorite disinfection that are considering the introduction of monochloramine disinfection, can verify this method without upfront costs.

Key words : *Legionella* spp., monochloramine, high pH, NH_4-N, manual addition, circulation filtration system

1. Introduction

In many hot spring facilities, sodium hypochlorite has been widely used as a method of disinfecting bathtub water because of its low cost and the simplicity of handling. However, it has been shown that sodium hypochlorite can be inhibited by the chemical qualities of hot spring water, thereby reducing its disinfection effects. Therefore, sodium hypochlorite may not always be appropriate as a disinfectant, depending on the chemical qualities and dissolved components of the hot spring water (Yanagimoto *et al.*, 2015).

Monochloramine is an effective disinfectant for controlling *Legionella* spp. (Jakubek *et al.*, 2013). It is reported that biofilms adhering to pipes can be controlled using monochloramine (Kool *et al.*, 2000 ; Flannery *et al.*, 2006). In 2019, the Ministry of Health, Labor and Welfare issued the “Guidelines for the Management of Environmental Health in Public Bath Facility” (Minister of Health, Labor and Welfare, Deputy Vice-Minister for Public Health and Food Safety, 2019) and the “Manual for Prevention of *Legionella* Disease in Bath of Circulating Filtration System” (Minister of Health, Labor and Welfare, Pharmaceutical Safety and Environmental Health Bureau, Environmental Health Division, 2019), recommending the use of monochloramine disinfection for hot spring water that has a high pH or contains high concentrations of ammonium ions, for which the disinfection effects of sodium hypochlorite are reduced.

Monochloramine is prepared from a formulation containing ammonium sulfate or ammonium chloride, and a formulation containing sodium hypochlorite or calcium hypochlorite. These formulations are necessary because monochloramine cannot be preserved.

In some public bath facilities, automatic addition equipment is installed before the filter in the circulation filtration system to perform automatic monochloramine addition. However, installation of the automatic addition equipment requires an upfront cost, which appears to discourage the usage of monochloramine disinfection.

However, monochloramine disinfection by manual addition does not involve an upfront cost.

It is considered that the effect of monochloramine disinfection on hot spring water can be experimentally verified. Therefore, in this study, we performed monochloramine disinfection by manual addition and monitored *Legionella* spp. in the bathtub water to determine its disinfection effect.

2. Methods

2.1 Target facilities and characteristics of hot spring water quality

This study was carried out in cooperation with two hot spring facilities that have chemical compositions that affect the disinfection efficacy of sodium hypochlorite. In selecting these target facilities, we focused on hot spring water that has a high pH and contains high ammonium ion concentrations, which can significantly reduce the efficacy of sodium hypochlorite disinfection.

Table 1 shows an overview of the hot spring water quality used in these targeted hot spring facilities.

Station A is a hot spring facility located in Yamanashi Prefecture in Japan. It uses hot spring water with an extremely high pH (i.e., exceeding 10). The concentration of dissolved components in the hot spring water is low, and the concentrations of ammonium nitrogen and metal components, which inhibit sodium hypochlorite disinfection, are also low.

Conversely, Station B is a hot spring facility located in Mie Prefecture in Japan. The hot spring water here is characterized by its high ammonium nitrogen concentration (NH_4-N) of 3.3 mg/L.

In hot springs containing more than 1 mg/L of ammonium nitrogen, it is difficult to adjust the sodium hypochlorite concentration on site, and problems, such as disinfecting odor and disinfection by-products, can arise. This is further described in the "Manual for Prevention of *Legionella* Disease in Bath of Circulating Filtration System" (Minister of Health, Labor and Welfare, Pharmaceutical Safety and Environmental Health Bureau, Environmental Health Division, 2019). Therefore, it can be inferred that the ammonium nitrogen (3.3 mg/kg) in Station B had slightly higher concentration for adequate sodium hypochlorite disinfection.

It is also rich in metal ions, with a total Fe ion ($Fe^{2+}+Fe^{3+}$) concentration of 4.5 mg/L, and a Mn ion (Mn^{2+}) concentration of 2.3 mg/L. The hot spring water gradually becomes colored as the metal ions are oxidized after emerging onto the ground surface. Neither hot spring contains sulfur components such as hydrogen sulfide (H_2S), hydrogen sulfide ions (HS^-), or thiosulfate ions ($S_2O_3^{2-}$), which contribute to total sulfur content.

In general, these facilities are disinfected using sodium hypochlorite ; however, disinfecting

Table 1 Water quality parameters of targeted hot springs

Parameter	Station A	Station B
pH	10.2	7.3
NH_4-N	<0.1	3.3
Total-Fe	0.03	4.5
Mn^{2+}	0.01	2.3
Total-S	<0.1	<0.1

using this method can be difficult. As an evidence, in Station A, 100 CFU/100 mL of *Legionella* spp. was detected in the bathtub water in a preliminary investigation prior to this study. Moreover, predominantly in Station B, several cases of *Legionella* spp. were detected in the past, and there were cases of *Legionella* infection. *Legionella* spp. was tested at Station B at the time of the outbreak and as a result, *Legionella* spp. in the bathtub water was detected to be 98–9,500 CFU/100 mL, and more than 30,000 CFU/100 mL *Legionella* spp. was detected in the hot spring tank (Ohno *et al*., 2016). In this study, the effects of monochloramine disinfection in the hot spring facilities was verified with an aim to drastically improve these situations.

2.2 Monochloramine disinfection method

Table 2 shows an overview of the bathtubs and circulation filtration system used in this field test.

For the purposes of this study, Station A selected an open-air bathtub (approximately 3 m^3) and Station B selected an indoor bathtub (approximately 5.6 m^3). At Station A, the bathtub water was changed completely every day and the bathtub was cleaned each time.

Station B normally exchanged the bathtub water daily. However, at our request, during the test period of this study, the bathtub water was not changed.

While both hot spring facilities normally use sodium hypochlorite as a disinfectant, they did not during the test period. Notices that monochloramine disinfection was implemented were shown near the bathtub at targeted facilities, out of consideration for the facility users.

The disinfectant to be added to the bathtub water for monochloramine disinfection was prepared on-site at the time of use. Granules of ammonium chloride covered with a polyethylene glycol and calcium hydroxide (hereafter referred to as "Agent A") and granules of calcium hypochlorite (hereafter referred to as "Agent B") were prepared. The weight ratios of ammonium chloride, polyethylene glycol, and calcium hydroxide of Agent A are 52.4%, 17.7%, and 29.9%, respectively.

Tap water (5 L) was placed in a plastic container, and 24 g of Agent A and 14 g of Agent B were added. The container was shaken to allow both agents to dissolve completely. This mixture was added manually to the bathtub water through the circulation filtration system. The control standard monochloramine concentration was set at 3 ppm so as not to fall below the

Table 2　Details of targeted hot spring facilities and equipment during the test period

	Station A	Station B
Targeted bathtub in this study	Open-air bathtub	Indoor bathtub
Disinfectant used prior to the field test	Sodium hypochlorite	Sodium hypochlorite
Circulation filtration system	Yes	Yes
Air bubble bath	No	No
Bathtub volume	3 m^3	5.6 m^3
Filtration method	Sand filter	Sand filter
Change bathtub water	Once daily	Once weekly[※1]
Number of users	Approximately 100 - 200 / day	Approximately 20 /day

[※1] Only during the field test period. Station B normally exchanges the bathtub water daily. However, at our request, during the test period of this study, the bathtub water was not changed.

control standard.

Sugiyama (2019) reported that when the bathtub water was not disinfected, various bacteria were detected on the first day, amoeba were detected on the second day, and *Legionella* spp. on the third day. Based on this research, the examination period of monochloramine disinfection was set to be 4 to 5 days longer than the period required for the appearance of *Legionella* spp. as this study's purpose was to verify whether the appearance of *Legionella* spp. could be suppressed by monochloramine disinfection during the test period.

The frequency of addition was approximately once in the morning and once in the afternoon. Meanwhile, monochloramine measurements were recorded once every 1–2 hours. If the measurement result was < 3 ppm, a supplemental addition was performed.

2.3 Sampling and analysis methods

Samples were collected at each facility by the methods described in 1). These samples were immediately transported to the laboratory and analyzed using the methods described in 2).

Monochloramine concentrations in the bathtub water were measured with a pocket colorimeter (HACH DR-300), using the indophenol method.

1) Sampling method

Sample water :

Water samples were collected from the bathtub outside operating hours.

Wiping sample (only Station A) :

Wiping samples were collected by wiping the pipe inside the circulation filtration system (i.e., inside the hair catcher).

Transportation and storage :

Samples were stored under refrigeration, except for the samples for amoeba culture, which were stored at room temperature, and were immediately brought into the laboratory for analysis.

2) Analysis method

pH :

Glass electrode method.

NH_4-N :

Indophenol method.

Total Organic Carbon (TOC) (only Station B) :

Wet oxidation method.

Legionella spp. :

Sample water (500 mL) was concentrated 100 times with a polycarbonate filter with a 0.2 μm pore size. The concentrations and wiping samples were heat-treated or acid-treated and inoculated on a GVPC agar medium and a BCYEα agar medium and cultured at 36℃ for 7 days. If necessary, an identification test using PCR (polymerase chain reaction) was performed.

Viable bacteria :

Sample water (1 mL) was diluted at an appropriate magnification. The diluted sample was inoculated on a standard agar medium by a pour medium method and cultured at 36℃ for 24 h.

Heterotrophic bacteria :

Sample water (1 mL) was diluted at an appropriate magnification. The diluted sample was inoculated on a R2A agar medium by a pour medium method and was cultured at 42℃ for 2 weeks.

Amoeba :

Sample water (1 mL) was inoculated on an E. coli-coated nutrient agar medium and was cultured at 42℃ for 7 days.

Coliform group :

Sample water (100 mL) was inoculated into a specific enzyme substrate medium (X-Gal MUG medium supplemented with pyruvate) and was cultured at 36℃ for 24 h.

3. Results and Discussions

3.1 Monochloramine concentration

Figure 1 shows the variations of monochloramine concentration at Station A (A) and Station B (B).

Monochloramine concentrations were measured on-site every 1–2 hours during operating hours and remained stable at approximately 3 ppm or more.

3.2 Microbiological and chemical feature

Table 3 lists the microbiological and chemical analysis results, including *Legionella* spp. detection.

In Station A, which has a high pH hot spring water, *Legionella* spp. was not detected in the bathtub water during the test period. Similarly, *Legionella* spp. was not detected in any of the wiping samples collected inside the circulation filtration system of Station A. In addition, amoeba

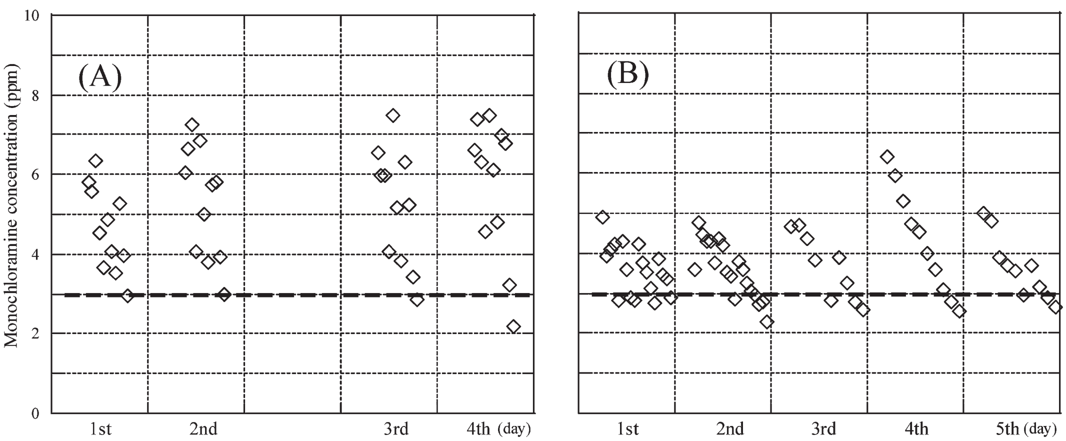

Fig. 1 Changes in the monochloramine concentrations in bathtub water for Station A (A), and Station B (B). Broken lines indicate the control standard of 3 ppm monochloramine. The blank space between the second and third days of (A) indicates the closing of the facility. Monochloramine was added once in the morning and once in the afternoon. As a result, the control standard of the monochloramine concentration was almost satisfied.

Table 3 Water quality investigation results

Station A	*Legionella* spp. (CFU/100mL)	Viable bacteria※1 (CFU/mL)	Coliform group (/100mL)	Amoeba (/50mL)	Heterotrophic bacteria※1 (CFU/mL)	pH
1 day later	N.D.	1	N.D.	N.D.	N.D.	10.94
2 days later	N.D.	9	N.D.	N.D.	5	9.85
3 days later	N.D.	6	N.D.	N.D.	1	10.02
4 days later	N.D.	5	N.D.	N.D.	9	9.87

Station B	*Legionella* spp. (CFU/100mL)	Viable bacteria※1 (CFU/mL)	Coliform group (/100mL)	Amoeba (/50mL)	Heterotrophic bacteria※1 (CFU/mL)	pH	NH_4-N (mg/L)	TOC (mg/L)
1 day later	N.D.	N.D.	N.D.	1	4	8.15	6.6	8.2
2 days later	N.D.	27	N.D.	3	31	8.34	7.2	10.4
3 days later	N.D.	16	N.D.	N.D.	14	8.44	7.7	14.1
4 days later	N.D.	4	N.D.	N.D.	26	8.50	8.6	16.4
5 days later	N.D.	1,080	N.D.	N.D.	210	8.54	8.9	19.0

※ CFU means "colony forming unit".
※ N.D. means "not detected."
※ Sampling dates are 30/Jul/2018–3/Aug/2018 (Station A), and 11/Jun/2018–15/Jun/2018 (Station B).
※1 "Viable bacteria" in this table shows the colony counts by standard agar medium, and "Heterotrophic bacteria" in this table shows the colony counts by R2A agar medium.

and the coliform group were not detected.

In Station B, which has hot spring water containing a high NH_4-N concentration, no *Legionella* spp. was detected in the bathtub water during the test period.

These results imply that monochloramine disinfection was effective in the selected facilities.

3.3 Levels of viable and heterotrophic bacteria

Regarding *Legionella* spp., all samples were negative. However, all the viable bacteria samples were positive in Station A and Station B except for one sample after a single day. This result was the same for heterotrophic bacteria. In particular, in Station B, the number of viable and heterotrophic bacteria gradually increased over the test period (Fig. 2).

Previous studies have reported that bacteria in bathtub water cannot be effectively disinfected with monochloramine (Mori *et al.*, 2019) because some bacteria can be resistant to monochloramine disinfection. The results in this study are consistent with these findings.

Watanabe *et al.* (2018) analyzed circulating bathtub water that underwent monochloramine disinfection and found that the dominant species of heterotrophic bacteria detected was *Mycolibacterium phlei.* (hereinafter referred to as "*M. phlei.*"). Furthermore, comparing the resistance of *M. phlei.* to monochloramine and sodium hypochlorite disinfectants, it was reported that the number of bacteria increased under the monochloramine disinfection environment. As *M. phlei.* uses ammonia as a substrate (Kuenen and Robertson, 1994), they found that excess ammonia under monochloramine disinfection may have attributed to the growth of *M. phlei.* Although heterotrophic bacteria were not identified in this study, it is highly possible that heterotrophic bacteria, such as *M. phlei.*, increased.

Analyzing the results further by each facility, both viable and heterotrophic bacteria were detected at Station A, although the number of bacteria did not increase. Conversely, Station B

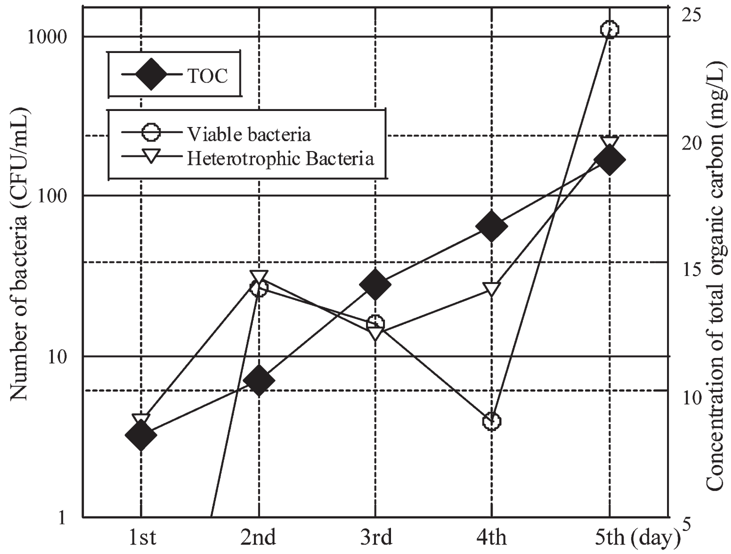

Fig. 2　Changes of the number of viable and heterotrophic bacteria and total organic carbon (TOC) concentrations in bathtub water at Station B. "Viable bacteria" in this figure shows the colony counts of colony by standard agar medium, and "Heterotrophic bacteria" in this figure shows the colony counts by R2A agar medium (see text).

contained 1,080 CFU/mL for viable bacteria after 4 days, and 210 CFU/mL for heterotrophic bacteria after 4 days. Based on these results, we determined that it was more difficult to control the bacteria in Station B than Station A.

3.4 *Legionella* spp. growth risk process inferred from chemical indicators

We explored the differences in the difficulty of controlling bacteria in two hot spring facilities. In this study, NH_4-N, TOC concentrations, and the pH of bathtub water were analyzed at Station B as chemical pollution indicators. Increases in pH, NH_4-N, and TOC concentrations were observed (Table 3 and Fig. 2).

It is presumed that the increase in the NH_4-N concentration was due to the addition of monochloramine, and that the increase in the TOC concentration was due to perspiration and dirt washed off the bath users. In addition, variations of these concentrations significantly depended on the number of bath users as well as the amount of additional hot spring water supplied.

However, the most prominent variation factor may be the frequency of bathtub water replacement. In many case, the concentration of dissolved components (including organic matter) increase proportionally in the bathtub water unless otherwise vaporized or precipitated (Mori *et al.*, 2010).

The higher the organic matter concentration in the bathtub water, the higher the risk of bacteria growth, as bacteria may consume that organic matter. Also, as the number of viable and heterotrophic bacteria increases, the risk of growth of amoeba preying on them may increase. Additionally, dirt accumulation may lead to an increased risk of *Legionella* spp. growth on amoeba.

The "Guidelines for the Management of Environmental Health in Public Bath Facility" (Minister of Health, Labor and Welfare, Deputy Vice-Minister for Public Health and Food Safety, 2019), specifies the following : "Change the bathtub water every day and clean the bathtub. However, even if this is difficult, clean the bathtub by cleaning water completely at least once a week." If there is an interval of several days between complete water changes, an increase in the concentration of chemical components due to bath users is unavoidable. However, it is important to understand that this leads to the risk of *Legionella* spp. growth.

In Station A, it was assumed that the chemical and microbiological contamination returned to the initial state every time the water was replaced. Therefore, it is necessary to completely exchange the water, clean the pipes of the circulation filtration system, and clean the bathtub itself in order to prevent the accumulation of microbial contamination, including biofilms.

4. Conclusion

In this study, the effects of simple manual addition of monochloramine disinfection were determined through chemical and microbiological monitoring, including an analysis of *Legionella* spp. in bathtub water. Hot spring facilities using hot spring water with a high pH, and those with hot spring water containing a significant amount of NH_4-N, were selected as target facilities since these types of spring water are both likely to reduce the disinfection effect of sodium hypochlorite.

No *Legionella* spp. was detected in any of the bathtub water samples or samples collected by wiping the inside of the pipes. However, the majority of the samples were positive for viable and heterotrophic bacteria. In particular, the numbers of viable and heterotrophic bacteria increased day by day at the hot spring facility that did not completely replace the bathtub water daily.

This growth may indicate that bacteria, such as *M. phlei.*, are resistant to monochloramine disinfection. In addition, we found a growth in bacteria that preyed on the organic matter in bathtub water. This can be inferred from the increase of TOC concentration in the bathtub water. Notably we were able to verify that TOC concentration is a good chemical indicator for the contamination of bathtub water and the risk of bacterial growth.

The growth of these bacteria increases the risk of *Legionella* spp. growth. Therefore, this study determined that proper management by frequent and complete bathtub water replacement, bathtub cleaning, and pipe cleaning is necessary.

The results of this study suggest that monochloramine disinfection by simple manual addition can control *Legionella* spp. Therefore, if a facility using hot spring water with an inhibitory factor for sodium hypochlorite disinfection would like to consider the introduction of monochloramine disinfection, it is possible to perform on-site verification using this disinfection method without incurring upfront costs.

The disinfection method presented in this study aids in the selection of the most appropriate disinfection for bathtub water with various chemical characteristics. It is likely that this research will contribute to the prevention of legionellosis caused by public baths.

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