原 著

Changes in the Concentrations of Inorganic Compounds in Domestic Bath Water in Japan with Re-use of the Water

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Abstract : For bathing, water is heated to warm the body, and is used to remove cleaning agents from the skin. Usually this water does not enter the body, but there have been cases where people have caught infectious diseases through inhalation of aerosols during bathing. In this study, as a model case of circulating bath, we examined the change in hygiene and chemical composition of the bathtub water in various conditions. To investigate the potential for bacterial growth in domestic bath water in Japan, changes in the concentrations of metal and non-metal inorganic compounds were studied as the number of days the water was re-used for increased. With re-use of bath water over 1 week, the concentrations of most of the compounds increased. However, the concentration of phosphorus decreased, and the concentrations of manganese and titanium showed both increases and decreases. Increasing concentrations could be attributed to sweat secreted from the skin of the bather, compounds eluted from the water heater as the bath water was re-heated, and dissolution of added bath salts. Consumption of inorganic compounds by bacteria and precipitation of metal hydroxides could contribute to the decreasing concentrations. Common bacteria and coliform bacteria were measured simultaneously, and only the coliform bacteria increased in the water on the second day the water was used for bathing. Bacteria probably grew in the pipes between the water heater and the bathtub.

Key words : bathing, coliform group, inorganic compound

Bull. Nippon Vet. Life Sci. Univ., **63**, 40-47, 2014.

Introduction

For bathing, water is heated to warm the body, and is used to remove cleaning agents from the skin. It is assumed that the water does not enter the body during bathing. However, in a shower, water can be unintentionally inhaled as an aerosol. Water could also enter the body through wounds in the skin. In public baths, the water may not be replaced with fresh water for several days, and this could result in spread of infectious diseases.

Bacteria such as *Legionella* can grow in water heaters if they are not cleaned properly, and the addition of inorganic compounds to the bath water from the water source or the skin of bathers can promote bacterial growth. Consequently, the Ministry of Health, Labour and Welfare in Japan recently declared that the bath water in public baths must be tested for *Legionella* at least once a year, and the results should be made public for consumer safety[1].

Legionnaires' disease, which causes pneumonia, can be spread through contaminated water sources[2-5]. There have been many reports in Japan of Legionnaires' disease in users of public baths[6-8] or hot springs[9,10]. *Legionella* is thought to be transmitted in hot aerosols from the baths, which are inhaled[11-15]. *Legionella* can grow in soil[3,13], and in a bathroom, bacteria in the bath water will circulate throughout the water heater where they could adhere and grow. Consequently, filtration of water before it enters the water heater is a requirement at large public baths in Japan[10]. However, many domestic bathrooms in Japan are equipped with small cyclical type boilers that re-heat bath water without filtration, and bacteria could multiply in these systems[2,4]. It is a Japanese custom to soak in a bathtub after cleaning the body outside of the bathtub, and the water is re-used for subsequent baths. This custom could increase the risk for contracting such illnesses compared with in other countries. Sekine et al. showed that bacteria, such as ***Legionella***, require inorganic compounds for growth[11,12]. She added trace elements to the agar medium, and investigated the increase and decrease. It is thought that this demand nature is naturally maintained also in the bathroom, the inorganic compounds may be required for growth of bacteria in bath water. This is considered to lead to prevention of Legionnaires' disease.

In order to prevent Legionella infection, daily water quality testing is important. In this study, as a model case of circulating bath, we examined the change in hygiene and chemical composition of the bathtub water in various conditions. Changes in the concentrations of inorganic compounds domestic bath water in Japan were investigated as the number of days the water was re-used for bathing increased. The relevance of these results to growth of bacteria was considered.

Methods

Instrument and reagents

The target analytes were inorganic elements (B, Ba, Ca, Cu, Fe, Ge, K, Li, Mg, Mn, Mo, Na, P, Pb, Rb, Sc, Se, Si, Sr, Th, Ti, Tl, U, V, W, Zn), inorganic anions (Cl^-, NO_3^-, SO_4^{2-}), coliform bacteria, common bacteria, residual chlorine, chemical oxygen demand (COD), and pH. In these, the inorganic element was selected based on the report of Sekine[11,12]. The inorganic elements were analyzed at ppm levels by inductively coupled plasma emission spectrometry (ICP-AES, Optima 4300 DV; PerkinElmer, Waltham, MA), and at ppb levels by inductively coupled plasma mass spectrometry (ICP-MS, Elan 6100 DRC; PerkinElmer). Reference solutions (XSTC-1, XSTC-7, XSTC-13, XSTC-622; Spex Certiprep, Metuchen, NJ) were used for the inorganic element analyses by ICP-AES and ICP-MS. Anion chromatography was conducted using a AI 450 (Dionex, Sunnyvale, CA). A negative-ion mixed reference solution IV (Kanto Kagaku, Tokyo, Japan) was used as the standard for anion chromatography. Levels of coliform bacteria and common bacteria were measured using experimental paper (Sibata Scientific Technology Ltd., Soka, Japan) according to the manufacturer's instructions. Packtests including *N*,*N*-diethyl-1,4-phenylenediamine and alkali permanganic acid (Kyoritsu Chemical-Check Lab, Tokyo, Japan), were used for measurement of residual chlorine and COD, respectively. The pH was measured with a pH meter (F-22; Horiba, Kyoto, Japan). High purity nitric acid (TAMAPURE AA-100, Tama Chemicals, Kawasaki, Japan) was used in the experiments. A membrane filter (MILLEX HA, Millipore, Billerica MA; pore size 0.45 μm) was used for filtration of the samples. Purified water (>18 MΩ cm, Elix 3/Element A10, Millipore) was used for reagent preparation. All other reagents were of special grade and available commercially.

Sampling and pretreatment

Samples of domestic bath water were obtained from one residence in Chiba, Japan, from June to July 2008. The gas heater at this residence was equipped with a hot-water supply, a system for re-heating the water, and controls for the volume and temperature of water entering the bath. Bath water samples were collected before bathing every day for 1 week for each condition. For each day of the week, the same water was used for bathing and not exchanged for fresh water until the next week. The bathtub was cleaned on the first day of each week, and then water (42 ℃) was added. A sample of this water was collected from the bath before bathing, and a sample of tap water was collected from the bath faucet. Measurements for common bacteria, coliform bacteria, and residual chlorine were conducted. Another sample of the bath water was taken after the water had been re-heated for bathing on subsequent days, and the same measurements as above were conducted. Experiments were conducted over 8 weeks, and each week was used to investigate different bathing conditions, including addition of bath water to maintain the water at the same level as the day before, addition of bath salts or a chlorine disinfectant, and cleaning of the water heater. The experimental conditions are shown in Table 1. COD and pH measurements were conducted on all the bath water samples. Samples were filtered before anion chromatography, and acidified with concentrated nitric acid after filtration for ICP-AES/MS. The prepared samples were kept in a refrigerator before use.

Table 1. Experimental conditions investigated during bathing over eight weeks（I－VIII）

Sample No.	Water adding*1	Bathing	Bath additive*2	Tub cleaning*3	Boiler cleaning*4	Chloric disinfectant*5
I		○		○		
II	○	○	○	○		
III	○	○		○		
IV				○		
V		○*6		○		
VI		○		○	○	
VII		○	○	○	○	
VIII		○		○		○

*1 Hot water was added to adjust the level of the bath water to the same as that the day before, and the water in the tub was heated again before bathing.
*2 Bath salts were added at 2 day intervals over the week.
*3 Before starting the weekly experiment, the bathtub was cleaned using detergent and a brush.
*4 The water heater was cleaned with a commercial detergent that was circulated through the bath water.
*5 A chlorine disinfectant was added each day after bathing.
*6 Before bathing, the bather did not rinse soap from their body.

Results and Discussion

Common bacteria and coliform bacteria

Bacteria were detected in the water sample from the faucet. In each of the eight weeklong experiments, the number of bacteria decreased initially and then increased again（Fig. 1）. All bacterial levels were less than 100 cells per milliliter, which is the standard for domestic water supply in Japan[1]. Coliform bacteria were not detected in the water samples on the first day, but they were detected on the second day, which was the first day the water was heated for re-use after bathing. The levels of coliform bacteria then decreased and increased at different points after the second day. Increases in the viable cell counts show that bacteria were present on the body of the bather or in the bathroom. Changes in the coliform levels were probably affected by circulation of the bath water through the water heater, piping, and bathtub. Because cleaning the bathtub before each experiment was sufficient to remove any bacterial growth, there were very few places that bacteria could adhere and grow within the bathtub. Therefore, bacteria must have grown inside the water heater or pipes between the bathtub and heater[9]. Some bacteria, such as *Legionella*, are protected from detergent by formation of a biomembrane, and cannot be easily removed[3,15]. Mold growth can occur at the faucet and enter the bath water when the bath is filled[16]. Bacteria were detected in the bath water immediately after the water heater was cleaned, which indicates that the bactericidal effect of chlorine in the bath water was not maintained for a long time.

COD, residual chlorine and pH

In the water sample taken before bathing, the COD was not 0 mg/dL. This was attributed to the tap water source, which was swamp water. The water sample also had a swamp-like smell when it was heated. Increases in the COD during the experiments（Fig. 2）could be cause by sweat and dirt from the bather. Consumption of organic matter by bacteria was thought to causes the decreases observed in the COD. Additionally, the pipes and water storage area could have affected the COD. Compared with the other experimental conditions, V（no rinsing）and VIII（addition of chlorine disinfectant）had higher COD values. These results could be attributable to soap and the chlorine disinfectant in the water. The residual chlorine concentration was highest on the first day of each week, and then decreased gradually as there was no further addition of chlorine disinfectant during the week. This decrease was probably caused by volatilization of the chlorine with heating of the water. However, even with these decreases, a free chlorine concentration of 0.1 mg/L was obtained on the seventh day. Because other results showed that bacterial growth occurred, these chlorine measurements were not useful for determining if disinfection of the water occurred[17,18]. Measurement of residual chlorine is recommended at

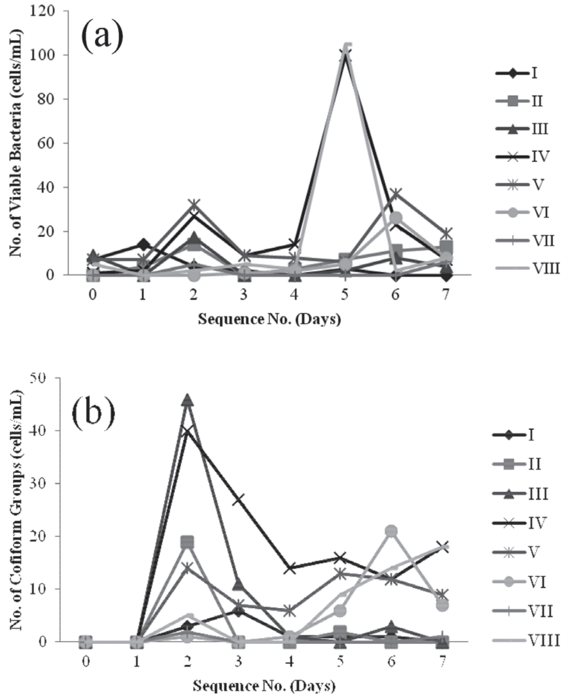

Fig. 1. Changes in the number of viable bacteria (a) and coliform bacteria (b) over the 7 days

The same bath water was used in each of the eight experiments (I−VIII).
The experimental conditions are shown in Table 1.

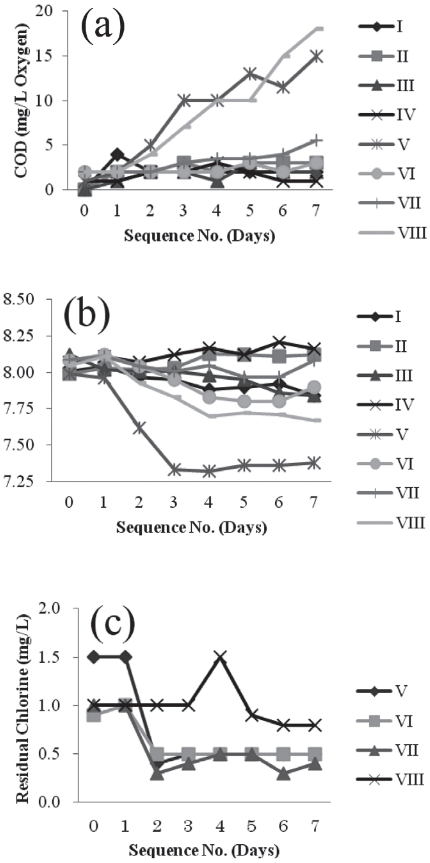

Fig. 2. Changes in the COD (a), pH (b) and residual chlorine (c) over the 7 days

The same bath water was used in each of the eight experiments (I−VIII).
The experimental conditions are shown in Table 1.

public baths and hot springs in Japan. In the present study, the Packtest was used for the measurement. However, this kit can react to substances other than chlorine[19]. Therefore, the chlorine concentration should be measured by other methods[20]. Over the week, the pH decreased gradually under all experimental conditions. The decrease under the conditions in experiment V was particularly remarkable, and this could be attributed to sweat remaining on the skin of the bather[21].

Inorganic components

The concentrations of Ba, Ca, Cu, K, Na, Rb, Zn, Cl^-, and SO_4^{2-} increased over each week (Fig. 3). By contrast, the concentration of P decreased (Fig. 4), and the concentrations of Mn and Ti showed both increases and decreases (Fig. 5). The concentrations of B, Fe, Ge, Li, Mg, Mo, Sc, Si, Sr, Th, V, W, and NO_3^- did not change. ICP-MS measurements were performed for Pb, Se, Tl, and U. However, the measured values were close to the limit of detection, and the results cannot be considered as reliable. The bathtub, water heater, bather, and bath salts, were all considered as potential sources of the inorganic elements. The concentrations of Ba and Cu increased in all experimental conditions (I−VIII), and the bathtub and the water heater were likely sources for these elements. To support this, the water heater was known to contain Cu. The concentrations of Ca, K, Rb, Zn, and Cl^- increased under all conditions, except for IV. Therefore, these elements are likely secreted by the bather. Sweat is known to contain Ca, K, Na, and Cl^-[22,23], and a small amount of Zn[24]. Rb is thought to behave like Na and K because it is an alkaline metal. When bath salts were added to the bath, the concentrations of Na, Ti, Cl^- and SO_4^{2-} increased dramatically. The ingredients label of the bath salts confirmed it contained Na and SO_4^{2-}, and Ti is also a known ingredient of bath salts[25]. The Ti

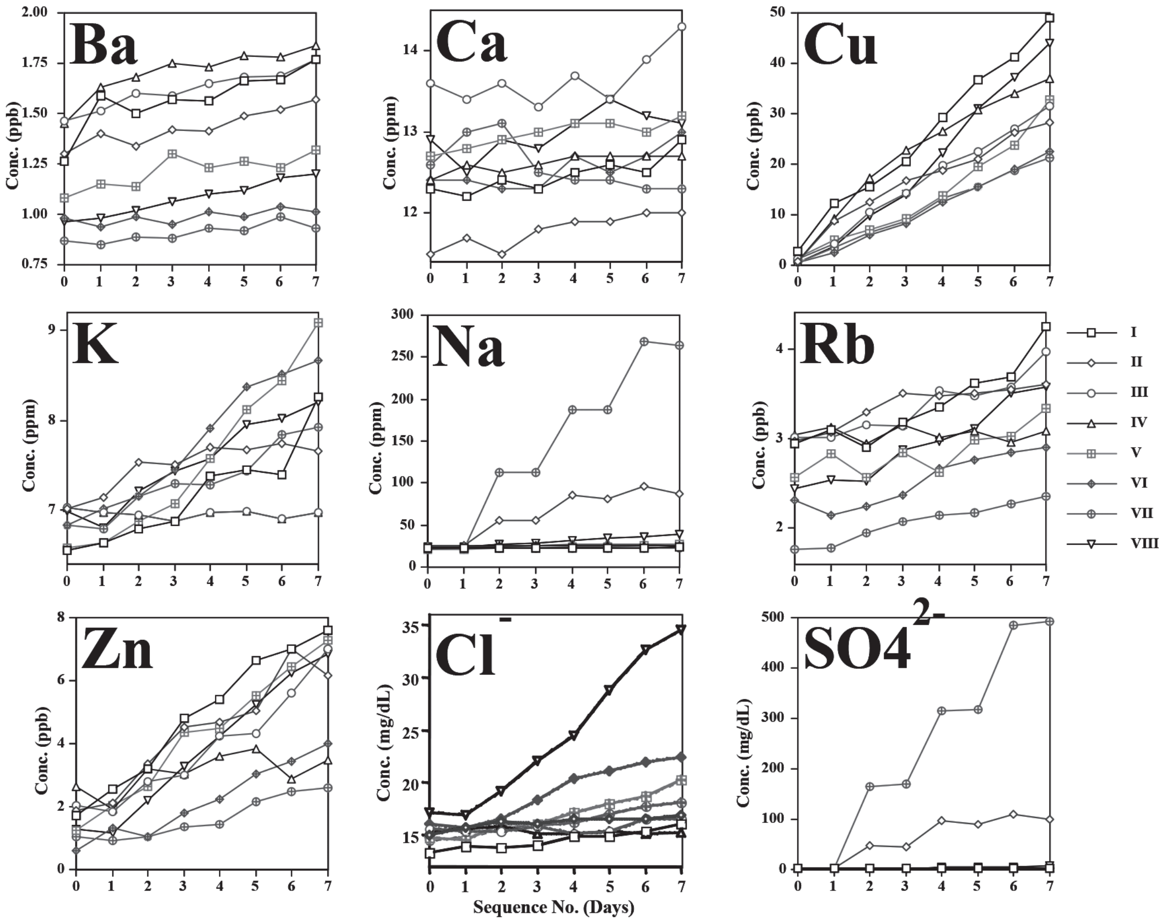

Fig. 3. Changes in the concentrations（increasing concentrations）of inorganic compounds over the 7 days

The same bath water was used in each of the eight experiments（I－VIII）.
The experimental conditions are shown in Table 1.

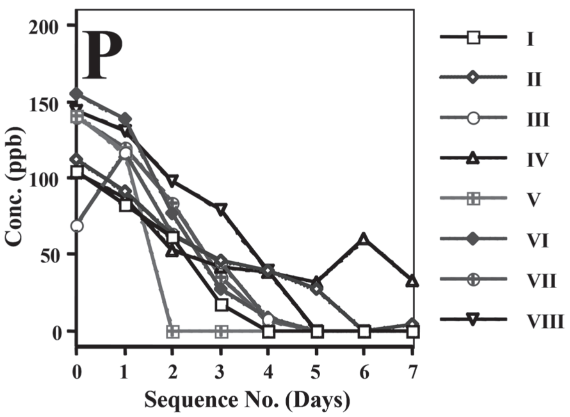

Fig. 4. Changes in the concentrations（decreasing concentrations）of inorganic compounds over the 7 days

The same bath water was used in each of the eight experiments（I－VIII）.
The experimental conditions are shown in Table 1.

concentration decreased in some conditions and the P concentration decreased in all the conditions. Bacteria in the water could have consumed these elements. The changes in the concentration of P could be attributed to the source water as with the COD. In experiment IV, where bathing did not occur, the decrease in the P concentration was slow. This is because bacteria that would have entered the bath water from the bather were not present to consume P. By contrast, Ti can be consumed rapidly by only a few bacteria, and may have a catalytic function. Furthermore, the possibility that Mn was oxidized to oxyanions was investigated. Sekine et al. reported that Mn and Se are co-factors of *Legionella* growth[11,12]. In the present study, *Legionella* was not added to the water, and the investigations used an actual bathing process. The Mn concentration did not decrease, and the bather did not develop Legionnaires' disease. Therefore, there were few *Legionella* in the bath water. To support this, the concentration of Fe,

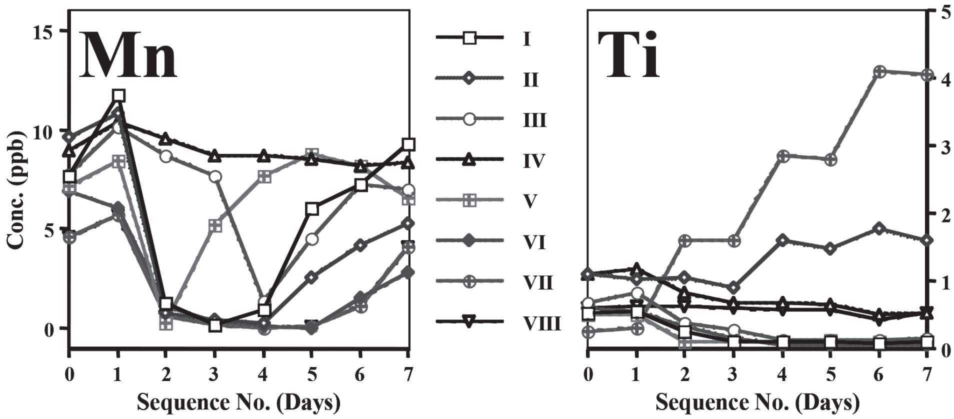

Fig. 5. Changes in the concentrations (no consistent trend) of inorganic compounds over the 7 days

The same bath water was used in each of the eight experiments (I−VIII).
The experimental conditions are shown in Table 1.

which is required for *Legionella* growth[7,15], did not change. Therefore, the results of this study did not show the *Legionella* levels increased as bath water was re-used.

Conclusions

As a result of taking a bath for relaxation and health promottion, it is putting the cart before the horse if it suffered from infection. The direct detection procedure of the legionella bacteria which is the typical infection over bath water takes time very much, and needs a special culture medium. Therefore, it is not suitable for a routine test. There are some commercial kits for measurement of standard bacteria or residual chlorine, and anyone can perform it simple. If these are strongly related from infection, such as *Legionella*, it can prevent and monitor indirectly. To clarify the potential for bacterial growth in domestic bath water, we investigated changes in the concentrations of metal and non-metal inorganic compounds as the water was re-used. The concentrations of most of the compounds increased as the water was re-used. However, the concentration of phosphorus decreased, and the concentrations of manganese and titanium showed both increases and decreases. These are considered to have been consumed by growth of a microbe.

In future, experiments with addition of *Legionella* to the water should be conducted. In addition, an investigation of the inorganic compounds present in bath water in a public bath would be useful. Measurement of residual chlorine by other methods should also be investigated. These results could be used to clarify if bath water is a suitable environment for the growth of pathogenic bacteria, and if bathing enhances or decreases bacterial growth. In cases where bacteria could enter the water from soil, such as in a hot spring, chlorination can be used to prevent bacterial growth. However, addition of chlorine could lower the quality of the water for bathing by affecting its color and/or smell. In situations where it is difficult to perform chlorination, other methods for stopping bacterial growth should be investigated.

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再利用を伴う日本での家庭浴槽水中の無機成分変化

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要　　約

入浴は，身体を温めたり，石鹸などの成分を皮膚から取り除くために行われる。通常，浴槽水は体内に入ることはないが，入浴中のエアロゾルの経気道的吸入により，感染症を引き起こすことが知られている。本研究では，循環型風呂のモデルとして，衛生学的に浴槽水の成分が様々な使用条件でどう変化するかを調べた。日本における家庭用浴槽水中での微生物繁殖の可能性を調べるため，金属，非金属無機化合物の日ごとの濃度変化を追跡した。浴槽水の再利用を１週間行ったところ，多くの化合物濃度は増加した。リンのみが減少し，マンガンやチタンは増加と減少の両方が観察された。これらの変化において，増加は入浴者の皮膚から排泄された汗，風呂釜からの溶出，入浴剤からの混入が考えられた。減少は浴槽水中細菌による消費と金属水酸化物の沈殿析出が考えられた。同時に一般細菌や大腸菌群についても測定したが，大腸菌は入浴２日目に増加し，風呂釜中に生育場所がある可能性が示唆された。

キーワード：入浴，大腸菌群，無機化合物

日獣生大研報 **63**，40-47，2014.