



Microbiological evaluation of water during the 2011 flood crisis in Thailand



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HIGHLIGHTS

- We investigated the flood impact on the waters used for human consumption.
- Higher net levels of bacteria were found in water with a higher turbidity.
- A total bacterial load of water samples exceeded the Thai water quality standards.
- Most of the water samples were found to be contaminated with microbial pathogens.
- Cross contamination between floodwater and waters for human consumption was found.

ARTICLE INFO

Article history:

Received 16 April 2013

Received in revised form 14 June 2013

Accepted 14 June 2013

Available online 17 July 2013

Editor: Gisela de Aragão Umbuzeiro

Keywords:

Flood

Tap water

Bacterial contamination

DGGE

Sanitation

ABSTRACT

In 2011, a severe flood occurred in Thailand, covering nearly half the country in water for several months. The contamination of floodwater and subsequent contamination of water for human consumption could have potentially led to a widespread health crisis. However, to date, no study has been conducted to determine the safety of the waters used for human consumption in Thailand during the severe flood. Therefore, we conducted microbiological analysis of 4 kinds of water (floodwater, river water, tap water, and filtered tap water) collected from industrial and residential areas that were damaged due to flooding. Higher net levels of bacteria were found in water with a higher turbidity. No clear trend was observed in the pH value of all 4 water samples. The level of total bacterial contamination in the water samples was estimated by real-time quantitative polymerase chain reaction (PCR). Eleven of the 12 tap water samples and all of the filtered tap water samples had a total bacterial load that exceeded the Thai water quality standards. One of the tap water samples and one of the filtered tap water samples were found to be positive for *Shigella* sp., although none of the floodwater samples showed detectable levels of this pathogen as determined by PCR analysis. One of the samples of floodwater was also found to be positive for *Leptospira* sp., but none of the tap water or filtered tap water samples were positive. Most of the tap water samples and all filtered tap water samples were found to be contaminated with *Vibrio cholerae*. Bacterial contamination in water samples was also analyzed by denaturing gradient gel electrophoresis (DGGE) analysis. These results revealed that several microorganisms were transferred via floodwater to different areas in the central part of Thailand and cross-contaminated between floodwater and water for human consumption.

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1. Introduction

Floods are natural phenomena that commonly occur in numerous parts of the world. However, the occurrence of flood incidents is expected to increase globally in the future, as climatic changes will induce more intense rainfall in some regions (Lenderink and van Meijgaard, 2008). The increasing level of human land utilization for

agriculture and urbanization, which removes forests and other green spaces and develops flood plains that facilitate reduced storm water retention and increased runoff, further complicates this problem (Taylor et al., 2011). Amongst the problems associated with the increasing flood frequencies and growing population densities, is the increased health risk associated with exposure to contaminated floodwaters (ten Veldhuis et al., 2010).

In 2011, Thailand's worst flood in more than 50 years affected the lives of 13.6 million people (~20% of the population). The flood began in late July and devastated an area of approximately 240,000 km² (~47% of the total land area), which included agricultural, industrial,

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and urban land areas in 65 of the 77 provinces of Thailand. Flooding persisted in some areas until mid-January 2012. The severe floods led to diarrheal epidemics before the water levels receded. A total of 1,157,861 cases were recorded with 50 of them leading to diarrhea-related deaths. Diseases related to food and water contamination, as well as living hygiene, were of primary concern. However, pathogens could only be identified in 16,792 cases (Fig. 1) (Thailand's Department of Disease Control, Ministry of Public Health, 2011). Many people were affected by a lack of clean water for drinking and sanitation during the flood. In addition, turbid (containing sediment) tap water was found at or near the areas affected by the flood. This turbidity increased during the flood and made the water unfit for human consumption.

One of the great dangers of floods is not only the physical damage from floodwaters but also the health hazards of large-scale contamination of water for human consumption. Floods can result in the mixing of clean water with unsafe water. Depending on the location and sanitation conditions, tap water and drinking water can become contaminated by floodwater at the sources where the water is supplied or through the distribution systems. Following such contamination, the incidence of certain illnesses such as cholera, typhoid fever, leptospirosis, and hepatitis A can increase and affect large numbers of people (WHO, 2012). The contamination of floodwaters and subsequent contamination of drinking water sources was reported for severe flooding events that occurred in Bangladesh in 2004 (Islam et al., 2007) and in Mozambique in January–March 2000 that led to an increase in the incidence of diarrhea. Contamination also occurred in a 1998 flood in West Bengal, which led to a large cholera epidemic. In addition, epidemics of leptospirosis can spread directly from contaminated water through contact of broken skin or mucous membranes with water that is contaminated with *Leptospira*, usually from rodent urine. Outbreaks of leptospirosis have recently occurred in Brazil (1983, 1988 and 1996), Nicaragua (1995), the Krasnodar region of the Russian Federation (1997), Santa Fe, USA (1998) Orissa, India (1999), Thailand (2000) (Gayer and Connolly, 2005), and the Philippines (2008).

A good understanding of the impact of floods on population health is necessary to limit its impact on health. However, to date, no study has been conducted to determine the safety of tap water and filtered tap water that were used for human consumption in Thailand were during the severe flood. This study, therefore, forms the first investigation of the flood impact on tap water and filtered tap water used for drinking in Thailand. The study was therefore conducted to evaluate the microbial quality of tap water and filtered tap water used for drinking in the urban and suburban flood-affected areas. The possible association of

microbial contamination of the floodwater with subsequent dispersal and contamination of tap water and filtered tap water used for drinking were also investigated.

2. Materials and methods

2.1. Water sample collection

Severe flooding began at the end of July 2011 from the northern and northeastern regions of Thailand and spread through the provinces in the Central part of Thailand along the Mekong and Chao Phraya river basins. Floodwaters reached the mouth of the Chao Phraya in October, and inundated parts of the capital city of Bangkok and persisted in some areas for almost 6 months. Water samples were collected from accessible areas in industrial and residential areas that were heavily damaged (Fig. 2) due to flooding in mid-November. The sampling areas were located in 6 provinces in the central part of Thailand (Lopburi, Angthong, Ayuthaya, Nonthaburi, Pathumthani, and Bangkok), as shown in Fig. 2. Thirty samples of 4 kinds of water, namely floodwater, river water, tap water, and filtered tap water, were collected from different sources (Table 1). In this study, the filtered tap water samples were filtered using in-house water filter systems and consumed as drinking water.

2.2. Sample processing and extraction of bacterial DNA from water samples

Water samples (1.5 L for river and floodwater, 4.5 L for tap water, and 6 L for filtered tap water) at each location were collected in sterile plastic containers and transported back to the laboratory. Physicochemical parameters of the water samples, such as pH and turbidity, were determined in situ at each sampling site. The water samples were left standing overnight at room temperature and then evaluated for turbidity by visualization. The water pH was measured using a pH meter HM-25R (DKK-TOA, Japan). For bacterial determination, the collected water samples were first filtered through a 0.3- μ m membrane filter (Advantec, Tokyo, Japan) to remove insoluble particulate matter (e.g., soil) and then filtered through a 0.2- μ m membrane filter (Advantec, Tokyo, Japan) to collect the bacterial cells in the samples. Bacterial cells were then harvested from the 0.2- μ m membrane filter by reverse flushing and then vortexing for 15 s in 25 mL phosphate-buffered saline at pH 7.4. Finally, the bacteria were pelleted by centrifugation at 5000 $\times$ g for 15 min, resuspended in 700 μ L of 30% (v/v) glycerol in sterile distilled water, and stored at -80°C during transportation to the laboratory at Tokyo University of Marine Science and Technology until further use.

DNA was extracted from each bacterial suspension after pelleting by centrifugation at 5000 $\times$ g for 15 min by using a NucleoSpin Tissue DNA extraction kit (Macherey-Nagel, Germany) according to manufacturer's protocol. The extracted DNA was used as the DNA template for further polymerase chain reaction (PCR)-based analysis after ascertaining the net concentration by using an ES-2 fluorescence spectrophotometer (Malcom, Japan).

2.3. Determination of total bacterial counts by quantitative PCR assay of the *tuf* gene fragment

Quantitative PCR (qPCR) of a *tuf* gene fragment (~300 bp), the sensitivity of which has been shown to be comparable to that of the aerobic plate count method (Tanaka et al., 2010), was used to determine the total bacterial count in all water samples. The method was designed to amplify a highly conserved ~300 bp region of the *tuf* gene of multiple bacterial strains by using the degenerate set of 13 forward (769f) and 6 reverse (1068r) primers, as previously reported (Tanaka et al., 2010). PCR reactions consisted of 12.5 μ L of SYBR Premix Ex Taq II (Takara Bio), 0.5 μ L of 6-carboxy-X-rhodamine (ROX) reference dye (Takara

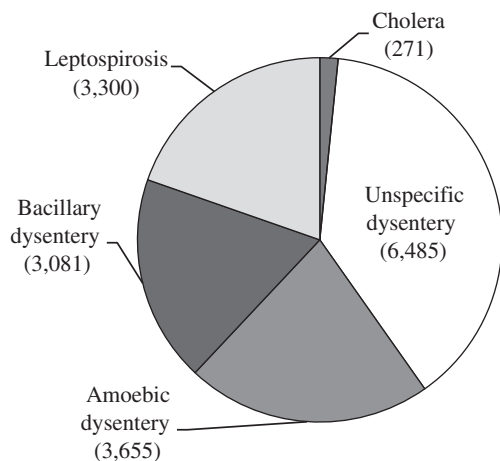


Fig. 1. The reported cases of notifiable diseases related to food and water contamination, as well as living hygiene during the 2011 flood crisis in Thailand.

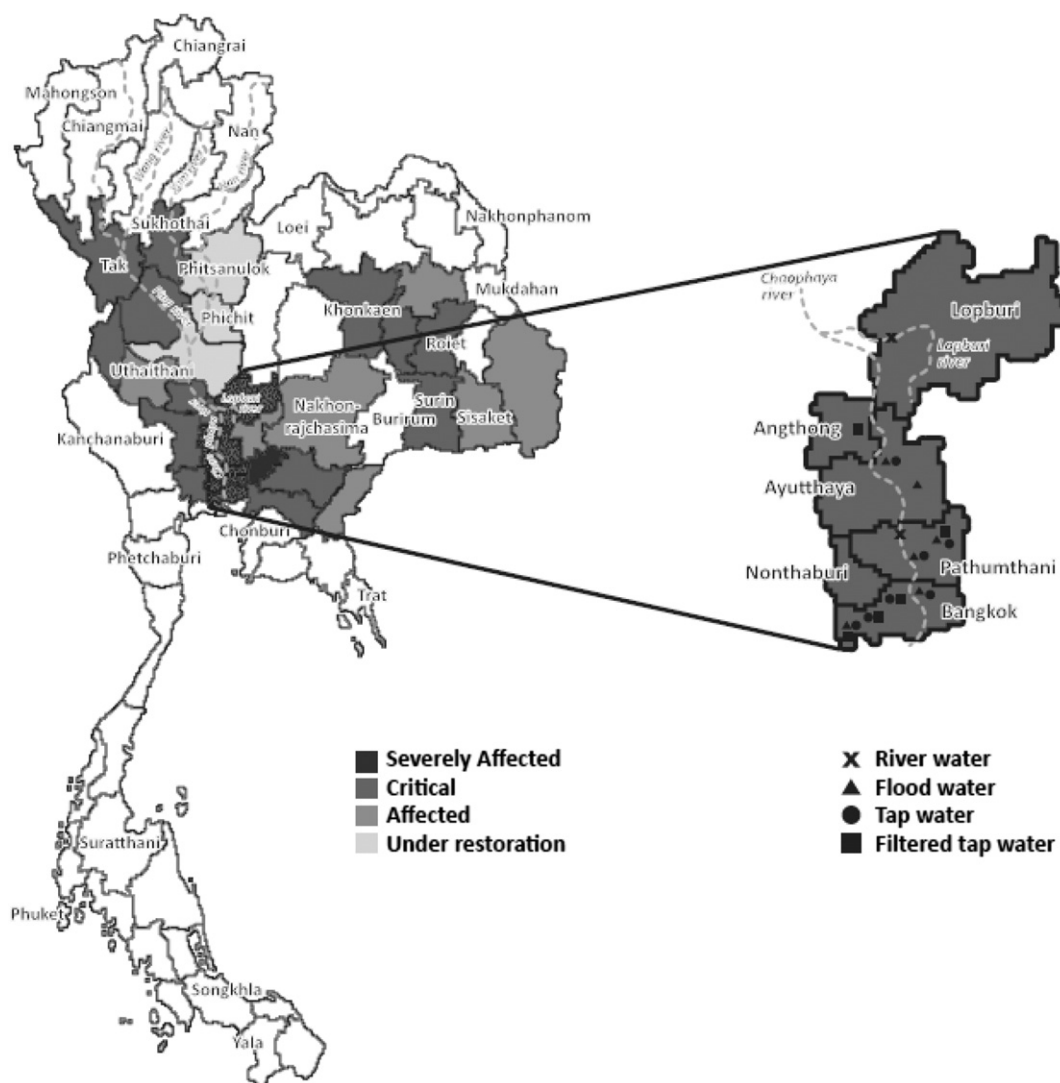


Fig. 2. Map of Thailand showing the 2011 flood-affected areas and the sampling areas from different sources in this study.

Bio), 720 nM 769f primer and 560 nM 1068r primer, and 2 μ L (~50 ng) of DNA template in a total volume of 20 μ L. Thermal cycling was performed at 95 $^{\circ}$ C for 15 s, followed by 35 cycles at 95 $^{\circ}$ C for 10 s and 60 $^{\circ}$ C for 1 min on an ABI 7900HT sequence detector (Applied Biosystems). The total bacterial count, as colony forming units (cfu), was then calculated from the derived critical threshold value (Ct) by using the following formula: $\log \text{cfu g}^{-1} = 12.491 - (0.310) \text{ Ct}$ (Tanaka et al., 2010).

2.4. Detection of pathogenic bacteria in water samples

The presence of the 3 pathogenic bacterial species, *Shigella* sp., *Leptospira* sp., and *Vibrio cholerae*, in the 0.2- μ m filter used to extract bacteria from each water sample was screened by PCR amplification of the extracted DNA (Section 2.2) with species-specific primers for the *ipaH*, *ligA*, and *hlyA* genes for *Shigella* sp., *Leptospira* sp., and *V. cholerae*, respectively (Table 2). Samples found to be positive for *V. cholerae* (*hlyA*) were then further screened for virulent strains by PCR amplification of the toxigenic *ctxA* gene from the virulence cassette by using the VCT1/2 primers (Table 2). PCR reactions were performed in a 20- μ L reaction volume containing 2 μ L of Ex Taq buffer (Takara Bio), 1.6 μ L of dNTPs (Takara Bio), 20 μ M of each of the respective F and R primer pairs (Table 2), 0.5 U Ex

Taq, and 2 μ L (~50 ng) of DNA template (from Section 2.2). PCR tests were performed in a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). Thermal cycling was performed at an initial 95 $^{\circ}$ C for 5 min followed by 35 cycles of 95 $^{\circ}$ C for 30 s, T_m (Table 2) for 45 s and 72 $^{\circ}$ C for 30 s, and then followed by a final extension at 72 $^{\circ}$ C for 7 min. PCR amplicons were resolved through 2% (w/v) agarose-TBE electrophoresis and visualized by ethidium bromide staining and UV transillumination.

2.5. PCR-denaturing gradient gel electrophoresis (PCR-DGGE) analysis of bacterial communities in water samples

2.5.1. PCR amplification

PCR of the 16S rRNA was performed using a nested protocol as previously described (Takahashi et al., 2004). The V3 region of the 16S rRNA gene (~220 bp) was amplified using the 5'-GC clamped (41 bp) 339f and unclamped V3-53r primers (Table 2). PCR amplifications were performed in a 100- μ L reaction mixture composed of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 50 pmol each of the GC-339f and V3-53r primers, 0.2 mM each of the 4 dNTPs, 2.5 U of Taq DNA polymerase (Takara Bio) and 50 ng of template DNA (from Section 2.2). Thermal cycling was performed in a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA) with 25 cycles at

Table 1

Sample details, physical and chemical properties, and microbiological contamination level.

Sample no.	Origin	Location			Physical appearance	pH	Total count (log cfu/mL)	Detection of pathogen		
								<i>Shigella</i> sp.	<i>Leptospira</i> sp.	<i>V. cholerae</i>
1	River	Lopburi River, Lopburi	Rural	Industry	+++	7.66	4.25	—	—	+
2	River	Chaophaya River, Pathumthani	Rural	Resident	++++	6.39	5.24	—	—	—
3	Flooding	Rodjana, Ayuthaya	Rural	Industry	++	6.70	4.86	—	—	+
4	Flooding	Rodjana, Ayuthaya	Rural	Industry	++	6.87	5.36	—	—	+
5	Flooding	Rodjana, Ayuthaya	Rural	Industry	++	7.01	5.00	—	—	+
6	Flooding	Rodjana, Ayuthaya	Rural	Industry	++	7.06	5.49	—	—	+
7	Flooding	Bangpahan, Ayuthaya	Rural	Resident	++++	7.54	4.51	—	—	—
8	Flooding	Klongluang, Pathumthani	Rural	Resident	++	7.73	4.08	—	—	+
9	Flooding	Pathumthani	Rural	Industry	++++	7.66	6.12	—	—	—
10	Flooding	Pathumthani	Urban	Resident	++++	7.68	5.49	—	—	+
11	Flooding	Nongkaem, BKK	Urban	Resident	+++	8.05	5.74	—	+	+
12	Flooding	Nongkaem, BKK	Urban	Resident	+++	7.43	4.81	—	—	+
13	Flooding	Nongkaem, BKK	Urban	Resident	+++	7.61	6.44	—	—	—
14	Flooding	Watcharapon, BKK	Urban	Resident	++	7.30	4.69	—	—	+
15	Tap water	Bangpahan, Ayuthaya	Rural	Resident	0	8.16	3.24	—	—	+
16	Tap water	Klongluang, Pathumthani	Rural	Resident	+	8.19	3.72	—	—	+
17	Tap water	Klongluang, Pathumthani	Rural	Resident	+	8.27	2.66	—	—	+
18	Tap water	Klongluang, Pathumthani	Rural	Resident	++	7.95	4.63	—	—	—
19	Tap water	Klongluang, Pathumthani	Rural	Resident	++	7.96	4.69	—	—	+
20	Tap water	Pathumthani	Rural	Resident	0	7.52	3.79	—	—	+
21	Tap water	Nonthaburi	Rural	Resident	+	7.01	4.47	—	—	+
22	Tap water	Watcharapon, BKK	Urban	Resident	++	7.54	4.72	—	—	+
23	Tap water	Pibulsongkarm, BKK	Urban	Resident	+	6.04	3.03	+	—	+
24	Tap water	Ratanathibeht, BKK	Urban	Resident	+	7.84	3.86	—	—	+
25	Tap water	Nongkaem, BKK	Urban	Resident	0	6.79	3.36	—	—	+
26	Filtered tap water	Anghthong	Rural	Industry	0	6.77	2.92	—	—	+
27	Filtered tap water	Ratanathibeht, BKK	Urban	Resident	0	7.28	3.93	—	—	+
28	Filtered tap water	Pibulsongkarm, BKK	Urban	Resident	0	7.17	2.75	+	—	+
29	Filtered tap water	Pathumthani	Rural	Resident	0	6.39	3.58	—	—	+
30	Filtered tap water	Nongkaem, BKK	Urban	Resident	0	6.88	3.54	—	—	+

94 °C for 1 min, X °C for 1 min, and 72 °C for 1 min (25 cycles in total), where X was initially 68 °C and was decreased by 0.8 °C every second cycle to 60 °C and held at that temperature for the last 5 cycles. Aliquots (5 µL) of the PCR products were analyzed first by electrophoresis in agarose gels (Section 2.4) to confirm successful amplification and then by DGGE (see Section 2.5.2).

2.5.2. DGGE profiling

DGGE was performed as previously described (Takahashi et al., 2004) by using the DCode System apparatus (Bio-Rad Laboratories, Hercules, CA, USA). Polyacrylamide gels (8% [w/v] acrylamide–bisacrylamide [37.5:1]) in Tris–acetate–EDTA buffer with a denaturing gradient ranging from 20% to 50% denaturant (100% is 7 M urea, 40% [w/v] formamide) were prepared with the model 475 gradient delivery system (BioRad). Polymerization was achieved by adding 0.9% (v/v) ammonium persulfate (10% [w/v] solution) and 0.09% (v/v) *N,N,N,N*-tetramethylethylenediamine.

The gels were subjected to electrophoresis at a constant 200 V at 60 °C for 3 h, after which the DNA fragments were stained with GelRed™ (Biotium, USA) and washed with distilled water prior to UV transillumination.

2.5.3. DGGE profile analysis, recovery of selected bands, and sequence analysis

To identify the potential bacterial origins of the DGGE bands, a small piece of gel from the target band was excised from the DGGE gel and eluted in 50 µL of Tris EDTA buffer at 100 °C for 10 min. The extracted DNA was used as the template for PCR amplification by using the same primers and the reaction product(s) (Section 2.5.1), and then purified using the Agencourt AMPure XP kit (Beckman Coulter, USA) prior to direct sequencing with the same primers (separately) by using an ABI3130 genetic analyzer (Applied Biosystems) with the Big Dye terminator v3.0 cycle sequencing kit (Applied Biosystems). The obtained sequences of the V3 region of the 16S rRNA were used to search the DDBJ and NCBI nucleotide databases with the BLAST 2.0 algorithm. Tentative

Table 2

Primers used for the PCR reactions in this study.

Primer	Primer sequence (5' to 3')	T _m (°C)	Target gene	Pathogen	Reference
ipaH-U1	CCITTTCCGCGTTCCTTGA	58	<i>ipaH</i>	<i>Shigella</i>	Vu et al. (2004)
ipaH-L1	CGGAATCCGGAGGTATTGC				
Lig1	TCAATCAAAACAAGGGGCT	48	<i>ligA</i>	<i>Leptospira</i>	Palaniappan et al. (2005)
Lig2	ACTTGCAATTGGAAATTGAGAG				
hlyA-f	TGCGTTAAACACGAAGCGAT	58	<i>hlyA</i>	<i>V. cholerae</i>	Lyon (2001)
hlyA-r	AAGTCTTACATTGTCTGGGTCA				
VCT1	ACAGAGTGAGTACTTTGACC	55	<i>ctxA</i>	<i>V. cholerae</i>	Hoshino et al. (1998)
VCT2	ATACCATCCATATATTGGGAG				
GC-339f	[GC clamp]-TCCTACGGGAGGCAGCAG	60	V3 region	DGGE	Takahashi et al. (2004)
Vs-53r	GTATTACCGCGGCTGCTGG		16S rRNA	profiling	

[GC clamp] sequence is 5'-CGCCCGCGCGCCCGCGCCCGTCCCGCGCCCGCGCCCG-3'.

species identification was made on the basis of the percentage similarity to the species-designated sequences in the database (95.0–100.0% similarity).

3. Results

3.1. Physical appearance

The turbidity level of the water samples collected from the Chao Phraya River (central part—a residential area) was higher than those collected from Lopburi River (a rural area), but both were very turbid (Table 1). From a total of 5 urban and 7 rural floodwater samples, those collected from the urban residential areas (samples 10–14) had a higher turbidity level than those collected from the rural areas (samples 3–9). The turbidity of 8 of the 11 tap water samples was noticeable by eye, while tap water samples 3 and 5 were clear or had lower turbidity than the floodwater, respectively (Table 1). However, the other 3 tap water samples were as turbid as the floodwater being cloudy and containing fine black particles, which indicated that the water quality was inappropriate for drinking or daily use. With respect to the filtered tap water samples, the turbidity was not noticeable in any of the 5 samples assayed (Table 1).

3.2. Chemical parameters

No clear trend was observed in the pH value of the river, flood, tap, or filtered tap water samples (Table 1). For the river and floodwater samples, the pH values ranged from 6.39 to 8.05, whereas the pH values for the tap and filtered tap waters ranged between 6.04–8.27 and 6.39–7.28, respectively. However, these pH values for all the tap and filtered tap water samples were above the acceptable pH range according to the water quality standards of the Ministry of Industry of Thailand (2012), which requires the pH to be between 6.0 and 8.5.

3.3. Bacteriological contamination level

The level of total bacterial contamination in the water samples was estimated by real-time quantitative PCR (rt-qPCR) amplification of a fragment of the *tuf* gene, which has previously been shown to be comparable in sensitivity to the total plate count method (Tanaka et al., 2010), subject to the taxa coverage level of these primers across all species. The total bacterial load (level) in the different water samples, measured as log cfu/mL, was high (Table 1). The highest contamination level was in the river and floodwater samples (4.08–6.44 log cfu/mL), as expected. Although lower, the levels in the tap (2.66–4.72 log cfu/mL) and filtered tap (2.75–3.93 log cfu/mL) water were still high. Indeed, these were above the water quality standards of the Ministry of Industry (2012), which requires the amount of bacteria in tap and drinking water to be less than 500 cfu/cm³ (~2.7 log cfu/mL). Thus, only one of the 12 tap water samples, and none of the filtered tap water samples, had a total bacterial load that was within the Thai standard and could be used for drinking.

3.4. Detection of pathogens

One tap water and one filtered tap water sample were found to be positive for the presence of *Shigella* sp., although none of the floodwater samples showed detectable levels of this pathogen as determined by PCR analysis (Table 1). For *Leptospira* sp., one sample of floodwater collected from a residential area was found to be positive, but none of the tap or filtered tap water samples was positive. However, for *V. cholerae*, all 5 filtered tap water samples and 10/11 of the tap water samples were found to be contaminated, along with 9/12 of the floodwater samples (Table 1). Subsequent PCR amplification of the *ctxA* gene fragment was negative in all cases and, therefore, most to all of the *V. cholerae* present in these samples did not contain the virulence cassette.

Nevertheless, *V. cholerae* without the virulence cassette can still cause morbidity and therefore remains a health concern. According to the Thai drinking water standard (Ministry of Industry, 2012), pathogens must not be detected in water used for human food preparation or consumption (tap and drinking water), and so all the tap and filtered tap water samples analyzed in this study were not appropriate for human consumption or food preparation. Note that with respect to the single sample of tap water in which all 3 pathogens (*Shigella* sp., *Leptospira* sp., and *V. cholerae*) were not detected (sample no. 18), the total bacterial load and turbidity still exceeded the acceptable levels for the Thai water quality standard.

3.5. Relationship of bacterial contamination in the floodwater, tap water, and drinking water

According to the obtained DGGE profiles (Fig. 3), the selected bands were excised and sequenced to determine likely bacterial species (Table 3), which were then compared to the water samples. The results revealed 4 potential patterns of contamination (schematically shown in Fig. 4). The first pattern showed no apparent cross-contamination between the floodwater and the water for human use (tap and filtered tap waters) and no apparent transfer of microorganisms to different areas by the floodwater. Accordingly, *Arthobacter phenanthrenivorans* and *Acinetobacter towneri* were found only in the floodwater collected in the Ayutthaya and Pathumthani provinces, respectively, and not in other provinces downstream of the floodwater, or in the tap and filtered tap waters. The second pattern has also showed no evidence of cross-contamination between the floodwater and water for human use, but with potential transfer of the microorganisms to different areas. Within this pattern, *Azovibrio restrictus* was detected in the floodwater in the Ayutthaya and Pathumthani provinces, and in Bangkok, whereas *Kineococcus xinjiangensis* was detected in the river water in Lopburi province and in the floodwater in Pathumthani province. The third pattern indicated the absence of contamination in the floodwater but the existence of potential cross-contamination between tap water and filtered tap waters. This likely occurred with the contamination of *Bosea minatitlanensis*, *Proteus mirabilis*, and *Novosphingobium indicum*. *B. minatitlanensis* was found in tap and filtered tap waters in the Bangkok area. *P. mirabilis* was detected in the tap water in Bangkok and the filtered tap water in Bangkok and Anghong province, whereas *N. indicum* was found in the tap water of Anghong province and Bangkok and in the filtered tap water in Bangkok. However, the fourth pattern, that of both potential cross-contamination between floodwater and water for human consumption (tap water and filtered tap waters) and the transfer of the microorganisms to different areas, is the pattern of most concern. This pattern was potentially found for *Acinetobacter junii*, *Thiobacillus thioparus*, and *Prochlorococcus marinus*. Thus, *A. junii* was found in the flood, tap and filtered tap waters in Bangkok and in the tap and filtered tap waters in the Pathumthani province. *T. thioparus* was found in the flood and tap waters in Pathumthani province, whereas *P. marinus* was found in Lopburi river water, in floodwater in Ayutthaya province and in tap water in Bangkok.

4. Discussion

This assessment of various water sources demonstrated that the tap water and filtered tap water, which people consumed or used for food preparation during the period of the 2011 flood in Thailand, contained a high level of soil microorganisms and potential pathogens. This could have posed a public health threat. Water turbidity, is commonly used as a proxy measure for the risk of microbial contamination and the effectiveness of the treatment of public drinking water (Schwartz et al., 2000). Several studies have shown a correlation between turbidity levels and microbial contamination of water, where higher turbidity levels are often associated with higher levels of pathogenic microorganisms (Clark et al., 1992; LeChevallier et al., 1991). Accordingly, in this study, a higher net

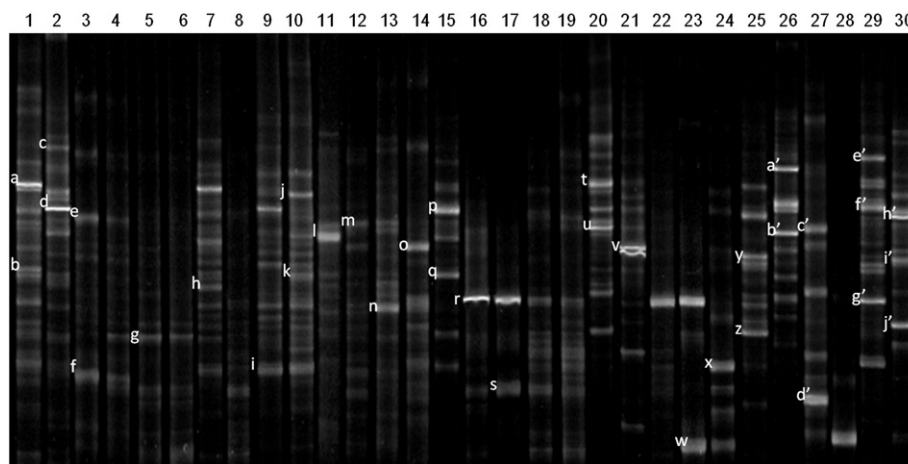


Fig. 3. Representative DGGE profile of bacteria in the sampled river, flood, tap and filtered tap waters. Lanes 1–30 represent the 30 samples listed in Table 1. Lowercase letters (a–z and a'–j') next to bands indicated the bands that were excised for sequencing.

level of bacteria were found in water with higher turbidity, with the level of both bacteria and water turbidity being ranked (highest to lowest) as river water > floodwater > tap water > filtered tap water. However, the turbidity and total bacterial contamination levels of the filtered tap water did not appear to be related to the presence or absence of the 3 tested potential pathogens.

Pathogenic contamination in floodwater indicated a high health risk during flooding events, especially for the people who walked through the floodwater (e.g., exposure to cuts or food handling at a later instance) and for children playing in it (e.g., oral or nasal ingestion). In addition, due to the lack of clean water, many people used the floodwater for washing and other domestic purposes, which increased their risk of infection. Thus, unsurprisingly, the 2011 severe flood in Thailand resulted in an extensive diarrhea epidemic that was attributed to the widespread heavy contamination of tap and drinking water. The Department of Disease Control Authorities had advised residents in flooded areas to boil tap water before drinking after 72 cases of diarrhea were reported at a condominium. The investigation confirmed that all those who fell ill had consumed tap water without boiling it (International Society for Infectious Diseases, 2011). These results indicate that the tap water supply sources need to be cleaned during and after floods in order to prevent the accumulation of harmful microorganisms in the water system. Furthermore, public health authorities should make the public aware of the potential danger of tap water supply, and encourage in-house treatment of the water before consumption. Specifically, the public should be informed that even if the water smells and looks clean, it might contain infectious bacteria such as *V. cholerae*, *Shigella* sp., and *Leptospira* sp. that can cause cholera or other forms of diarrhea, shigellosis and leptospirosis, respectively.

V. cholerae serogroups O1 and O139 are recognized as causing outbreaks of epidemic cholera (Hoshino et al., 1998) because they possess a virulence gene cassette (Kurazono et al., 1995) encoding the cholera toxin (*ctx*), zonula occludens toxin (*zot*), accessory cholera toxin (*ace*), toxin-coregulated pilus (*tcp*), and central regulatory protein (*toxR*). In contrast, other serogroups of *V. cholerae*, including non-O1 and non-O139 strains, lack these virulence genes (i.e., *ctx*, *zot*, and *ace*), but they contain genes that encode other products such as NAG-specific heat-stable toxin (*st*), thermostable direct hemolysin, shigella-like toxin, and the classical hemolysin that can cause a cholera-like illness (Calia et al., 1994). Therefore, detection of *V. cholerae* in the water samples collected in this study was performed by PCR by using the non-classical specific *hlyA* gene of *V. cholerae* to detect (essentially) all *V. cholerae* isolates independent of their O1, O139, non-O1 and

non-O139 serotype grouping, and further, to check for the presence of the cholera toxin gene cassette by PCR amplification of the *ctxA* gene. No *ctxA* amplicons were detected in all the *V. cholerae*-contaminated water samples; hence, the majority of the *V. cholerae* present were unlikely to carry this gene (or the virulence gene cassette). This finding is consistent with a report by Singh et al. (2001) that most O1, O139, non-O1, and non-O139 strains of *V. cholerae* from environmental sources do not possess the virulence gene cassette. However, the presence of *V. cholerae* is still of public health concern.

In this study, one sample of tap water and one sample of filtered tap water were found to be contaminated with detectable levels of *Shigella* sp. No tap water or filtered tap water sample was contaminated with *Leptospira* sp., although the pathogen was found in floodwater. Although *Shigella* sp. and *Leptospira* sp. were found in only a few samples in this study, the presence of these pathogens in tap and filtered tap waters is of concern. Later study was performed and shown that the presence of these pathogens in tap and filtered tap waters is unusual compared with the waters in non-flooding situation. Four samples of tap water and 3 samples of filtered tap water were collected from the same sampling locations in the Bangkok areas in June 2013, about 1 year after the flood crisis. The levels of total bacterial contamination in all samples of both tap water (average 1.8 log cfu/mL) and filtered tap water (average 1.1 log cfu/mL) were within the water quality standard of the Ministry of Industry of Thailand (2012). Also none of any pathogens was detected in both tap and filtered tap waters collected in non-flooding situation.

Cholera, shigellosis and leptospirosis often occur after natural catastrophes such as floods, earthquakes, and tsunamis, and the spread of these disease-causing pathogens has been widely reported following floods. *Shigella* spp. infections are transmitted from the feces of an infected person or by drinking contaminated water (Ashbolt, 2004). When a flood occurs in urban areas, the flood water can combine with the sewer system, which leads to fecal contamination of the floodwater that may pose health risks to people exposed to these waters. Pathogenic *Leptospira* sp. (causative agent of leptospirosis) can be transmitted to humans and animals by direct contact with the urine of infected rodents as well as through contaminated flood water (Bharti et al., 2003). Major outbreaks of leptospirosis in Thailand were last reported between 1997 and 1999 and involved ~15,000 cases and ~400 deaths. In Thailand, an estimated 2000–3000 people are annually infected with leptospirosis, and the number of cases usually peaks during the monsoon season (Bureau of Epidemiology, 2012). Flooding occurs in Thailand every year during the rainy season, although not always in urban areas. Poor sanitation and garbage disposal (that

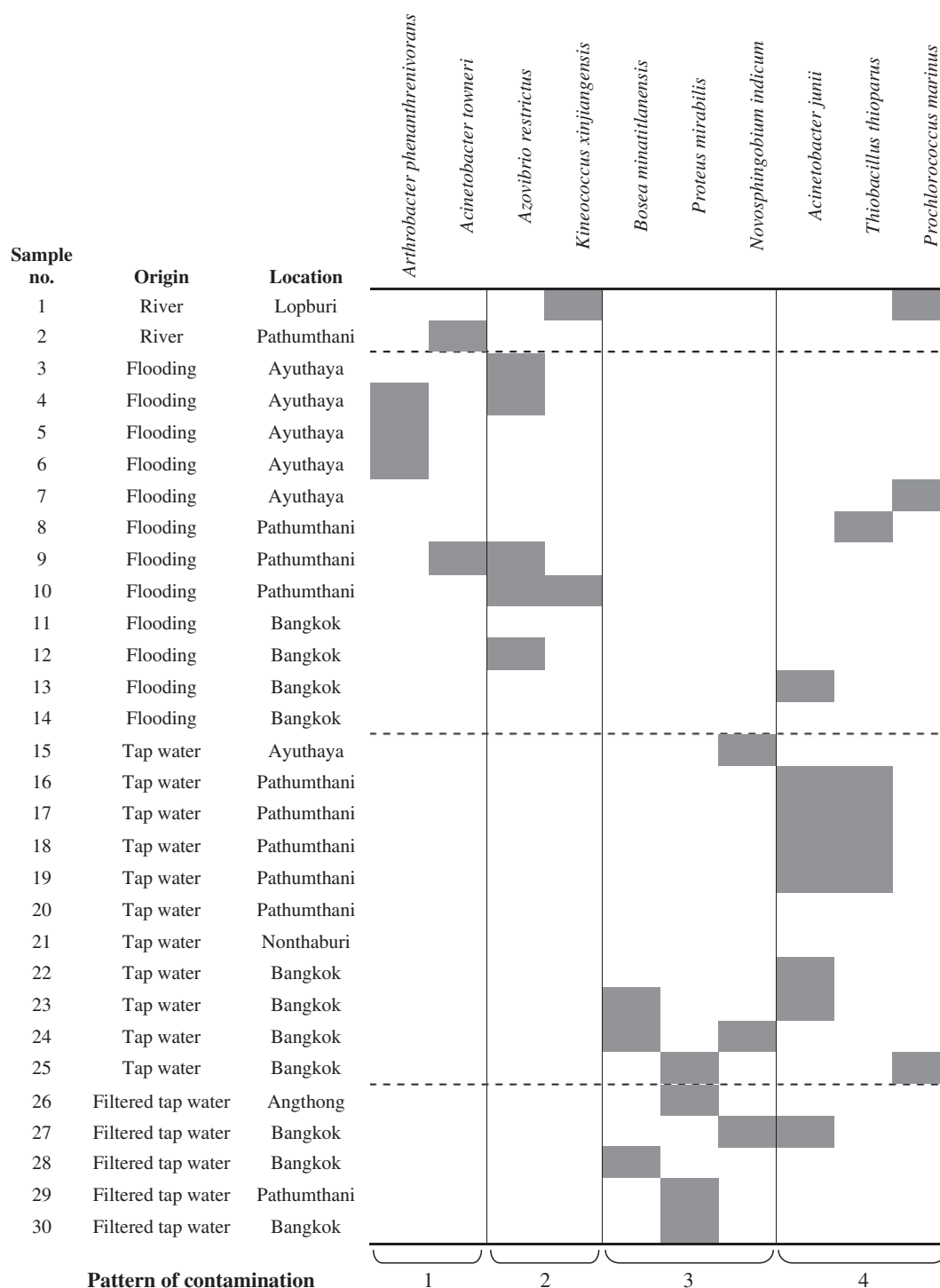


Fig. 4. Relationship of the bacterial species found in the river, flood, tap, and filtered tap waters in the different regions. Sample numbers refer to those in Table 1. Gray band refers to each bacteria (column) positive result in each sample (row).

support high-density rodent populations and therefore act as reservoirs of *Leptospira* sp.) in flood-affected communities of urban areas such as Bangkok could result in an outbreak of leptospirosis following flooding.

The 2011 flood in Thailand occurred in the North and spread down the Chao Phraya River through the central plains. As the floodwaters drained southward from the provinces of Lopburi and then Ayutthaya, the capital city Bangkok was seriously threatened by flooding in mid-October 2011. Several districts in eastern Bangkok, as well as parts of the surrounding Nonthaburi and Pathumthani provinces, became flooded as water was diverted from the Chao Phraya River. In

the Pathumthani province, which borders Bangkok to the north, the flood barriers failed and industrial estates as well as suburban residential areas became flooded, whereas disruption of a barrier protecting the Khlong Prapa raw water-supplied canal in this province allowed floodwaters to enter the canal. The floodwaters rapidly flowed down from Pathumthani province to central Bangkok. Through this survey of bacterial loads in the different water sources across the flood-affected regions, the potential transfer of microorganisms with the floodwater was determined. For example, *A. restrictus* was found in floodwaters in Ayutthaya province and in the downflow in Pathumthani province

Table 3

Likely identity of the DNA fragments (V3 16S rRNA) obtained from the DGGE analysis of water samples.

DGGE band ^a	Accession code ^b	Identification ^c	Identity (%) ^d
a	NR 028762.1	<i>Prochlorococcus marinus</i>	97
b	NR 044522.1	<i>Kineococcus xinjiangensis</i>	97
c	NR 028849.1	<i>Acinetobacter townneri</i>	95
d	NR 026210.1	<i>Acinetobacter radiorensistens</i>	96
e	NR 028678.1	<i>Azovibrio restructus</i>	98
f	—	Unidentified ^e	—
g	NR 042469.2	<i>Arthrobacter phenanthrenivorans</i>	95
h	NR 042564.1	<i>Rhodobacter changensis</i>	98
i	NR 028678.1	<i>Azovibrio restructus</i>	98
j	NR 028762.1	<i>Prochlorococcus marinus</i>	97
k	NR 044522.1	<i>Kineococcus xinjiangensis</i>	97
l	NR 043254.1	<i>Chryseobacterium taeanense</i>	99
m	NR 028678.1	<i>Azovibrio restructus</i>	98
n	NR 026208.1	<i>Acinetobacter junii</i>	100
o	—	Unidentified ^e	—
p	NR 044277.1	<i>Novosphingobium indicum</i>	99
q	NR 025114.1	<i>Xenophilus azovorans</i>	98
r	NR 026208.1	<i>Acinetobacter junii</i>	100
s	HM535226	<i>Thiobacillus thioiparus</i>	97
t	NR 044975.1	<i>Acinetobacter johnsonii</i>	98
u	NR 044472.1	<i>Diaphorobacter oryzae</i>	99
v	NR 040827.1	<i>Novosphingobium subterraneum</i>	100
w	NR 028787.1	<i>Bosea minititlanensis</i>	99
x	NR 044277.1	<i>Novosphingobium indicum</i>	99
y	NR 026353.1	<i>Xanthobacter taetidis</i>	99
z	NR 036953.1	<i>Bradyrhizobium elkanii</i>	100
a'	—	Unidentified ^e	—
b'	—	Unidentified ^e	—
c'	NR 026206.1	<i>Acinetobacter baumannii</i>	100
d'	NR 027188.1	<i>Zoogloea resiniphila</i>	98
e'	FJ460164	<i>Proteobacterium</i>	97
f'	NR 043997.1	<i>Proteus mirabilis</i>	100
g'	NR 044105.1	<i>Methylobacterium phyllosphaerae</i>	100
h'	NR 043997.1	<i>Proteus mirabilis</i>	100
i'	NR 025840.1	<i>Rhodoferrax fermentans</i>	98
j'	NR 028622.1	<i>Sphingobium amniense</i>	97

^a Letters indicate the excised DGGE band (V3 region of the 16S rRNA) shown in Fig. 2.^b NCBI or DDBJ/GenBank accession code for the V3 region of the 16S rRNA sequence of the DGGE resolved amplicon (band).^c Based upon the DNA sequence identity between the obtained sequence and those in the GenBank/NCBI or DDBJ data base.^d % DNA sequence identity between the sequence of this isolate at the 220 bp V3 region of the 16S rRNA and that of the indicated nearest match species in the NCBI or DDBJ data-base.^e Species that have percent similarity to database sequences $\leq 95\%$ are considered to be unidentified.

and Bangkok, although it should be noted that there is no temporal aspect, which shows the sequential detection in different areas over time (flood progression), to further support this notion. Likewise, and subject to the same caveat, *P. marinus* and *K. xinjiangensis* were detected in the water samples from Lopburi river down to Ayuthaya and Pathumthani provinces. Furthermore, the detection of *P. marinus* in the filtered tap water is consistent with the floodwater contamination of the Khlong Prapa raw water-supplied canal, which is a source for domestic water after treatment. A potentially similar contamination was seen with the presence of *T. thioiparus* in the flood and tap waters of Pathumthani province. Overflow of floodwater into the Khlong Prapa water-supplied canal may cause unusually high levels of microbial contamination in raw water that is supplied to water treatment plants. High levels of contamination may not be controlled by regular measures of water treatment, which exceeded standard level of microbial contamination in water after treatment. Moreover, tap water was transferred from centralized water treatment plants in each area via underground pipes. The authors speculated that those underground pipes might not be properly maintained, leading to pipe leakages. During floods, these pipes were in the watery damp ground resulting in post contamination of water in the pipes.

The geographical relationship of soil microorganisms in the river, flood, tap, and filtered tap waters are consistent with the transfer of these microorganisms between each type of water and from one area to another. In this study, microbial pathogens were detected in only a few water samples; therefore, in contrast with soil microorganisms, pathogen transfer between each type of water, from one area to another was not observed. In addition, a few microbial pathogens detected in water samples did not correlate with the high numbers of diarrheal disease cases that occurred during the flood crisis. This result was consistent with many previous studies, which demonstrated linkages between diarrheal disease and the fecal indicator bacteria contaminated on surface water (Emch et al., 2008; Wade et al., 2003; Walters et al., 2009). Poor correlations between fecal indicator bacteria and microbial pathogens have been previously demonstrated (Ferguson et al., 2012; Wilkes et al., 2009; Knappett et al., 2011). Unfortunately, the measurement of fecal indicator bacteria was not performed in the present study.

5. Conclusion

The 2011 flood in Thailand spread through fields and urban areas, potentially transferring various microorganisms, including pathogens of public health concern, such as *Leptospira* sp., *Shigella* sp., and *V. cholera*, to the urban areas and potentially contaminating the water supply system. During this period, both the tap and filtered tap waters were unfit for daily sanitation and consumption and potentially posed a public health risk. Therefore, the government should prepare an effective emergency response procedure to promptly supply potable quality water to people during floods in order to minimize public health risk.

Acknowledgments

We sincerely thank Ms. Krittaporn Thanatsang, Ms. Orapin Pornruangsarp, and Ms. Pinyapatch Srirongruangjit for excellent technical support, as well as Dr. Robert Butcher at the Publication Counseling Unit (PCU), Faculty of Science, Chulalongkorn University for checking the language of the manuscript.

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