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African Journal of Agronomy ISSN: 2375-1185 Vol. 12 (6), pp. 001-006, June, 2024. Available online at www.internationalscholarsjournals.org © International Scholars Journals



*Full Length Research Paper*

# The Role of Environmental Disturbances in Pathogen Dispersion: A Study of the 2012 Thailand Floods

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Received 9 January, 2024; Accepted 19 May, 2024

**Fungi and bacteria in water samples taken from various flooded areas in Narathiwat Province, Thailand, were investigated. They were isolated by filtration from water samples, and identified by examining macroscopic and microscopic features for fungi and using biochemical methods for bacteria. Nine species of filamentous fungi and two yeast species were isolated. Water contaminated with dermatophytes contained *Trichophyton mentagrophytes* (44%), *Trichophyton rubrum* (19%) and *Microsporum canis* (15%). The yeast *Candida albicans* was also found (75.5%). Water samples were contaminated with fungi, identified as non-cutaneous mycoses. The dominant fungi were *Aspergillus niger* (73%), *Cladosporium* spp. (58%) and *Aspergillus flavus* (41%). Thirteen bacterial strains were isolated from the samples; Gram-negative bacteria were most prevalent. The three dominant Gram-negative bacteria were *Escherichia coli* (62.5%), *Klebsiella pneumoniae* (61%) and *Enterobacter* spp. (59.5%). The two most abundant Gram-positive bacteria were *Corynebacterium* spp. (59.5%) and *Bacillus* spp. (not *Bacillus cereus*) (52.5%). These results suggest contaminated flood areas may be a transmission route for pathogens, and increase the risk of abnormal skin conditions among people exposed to the area.**

**Key words:** Flood, Thailand, fungus, bacteria, yeast.

## INTRODUCTION

Climate change represents a serious problem. There is an increase in the Earth's surface temperature, heat waves, droughts, storms and floods, as well as other frequent and costly natural disasters (Friel et al., 2011). Flooding causes many different health problems, including shortage of food and clean water, and a decreased sense of general well-being (McMichael et al., 2006; Bich et al., 2011; Friel et al., 2011). A study on the health impacts of the devastating flood that occurred in 2008 in Hanoi, Vietnam, revealed higher incidences of dermatitis, pink eye, dengue fever and psychological problems, in the communities that were severely affected, when compared with less-affected communities (Bich et al., 2011). A previous report on the health effects of flooding in Pakistan, in 2010, found that the most frequently reported conditions were skin diseases (18.3%), acute respiratory

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infections (15.1%) and acute diarrhea (13.3%) (CDC, 2012).

In Thailand, floods are frequent natural disasters. Regions to the south are faced with flash floods from heavy rains all year round. Southern Thailand is located on the peninsula bordered by the Andaman Sea to the West and the Gulf of Thailand to the East. The South has a tropical monsoon climate. Due to its geographical and climatic features, the South has become extremely vulnerable to flood disasters. Narathiwat Province is among those regularly affected. According to the Office of Natural Resources and Environmental Policy and Planning, Ministry of Natural Resources and Environment, Thailand (http://www.onep.go.th/index.php?option=com_content&task=view&id=4457&Itemid=266), in December 2012, devastating floods hit Narathiwat, affecting 25,158 people. Floods covered large areas of agricultural plantations (rubber, coconut, tropical fruit, rice), animal farms, water resources, roads and residential land. Heavy rain continued across its 13 districts, and in many parts of the province, flood water remained stagnant for weeks. People living in areas prone to flooding are at higher risk of contracting skin infections because of exposure to stagnant pools of flood water, which represent breeding sites for pathogens that cause waterborne skin infections. Poor hygiene standards among the displaced also contribute to this problem. Skin problems are among the most common diseases caused by flooding, especially superficial fungal infections. A previous study conducted in Thailand showed that the most commonly occurring dermatosis during the 2006 flood crisis was eczema. It accounted for 34.3% of the total skin problems and the great majority of these cases comprised chronic irritant foot dermatitis. Skin maceration of toe web spaces (Hong Kong foot), due to chronic irritant dermatitis with associated bacterial infections were prevalent. Only a few cases of fungal infection were reported. Topical anti-inflammatories, with antibacterial and antifungal agents, were used to treat skin conditions (Vachiramon et al., 2008). The highest level of total bacterial contamination was found in samples of flood water collected during the 2011 Thai flood. Moreover, one sample of flood water was found to be positive for *Leptospira* spp. (Chaturongkasumrit et al., 2013). A study carried out in Chiang Mai, Thailand, in 2011, found that water samples collected from seven flood crisis areas in Chiang Mai were contaminated with free-living amoebae (Wannasan et al., 2013). Flood water samples collected from flood zones in central Thailand, in 2011, were contaminated with human enteric viruses-norovirus (14%), rotavirus (9%), and hepatitis A (7%) (Ngaosuwankul et al., 2013).

Some data exist on the identification of microorganisms in skin specimens from patients during the floods in Thailand. Other studies have shown the presence of different types of microorganisms in water samples during flood crises. However, the microorganisms responsible for skin problems in flooded areas have not been examined. Therefore, the purpose of this study was to identify microorganisms from flooded areas of Narathiwat Province in December 2012.

## MATERIALS AND METHODS

### Sample collection

This study was conducted in Narathiwat Province, southern Thailand. Four main flood-affected districts of Narathiwat (Chanae, Waeng, Su-ngai Kolok, and Sukhirin) were selected for water sampling and testing. Sampling locations in each district included rubber plantations, water resources, roads and houses. A total of 200 water samples (200 mL each) were taken from flooded areas in December 2012, and stored in clean, sterile, 250 mL bottles with screw caps that were sealed and sent to the laboratory for the identification of any microorganisms. With the naked eye, moderately turbid water from rubber plantations, water resources, roads, and houses, were observed. Turbid water samples were allowed to settle for 30 min at room temperature. The water samples were fairly clear after the sedimentation process. Only clear water samples with virtually no sediment were collected and examined.

### Isolation and identification of fungi

Water samples (100 mL each) were filtered through sterile 0.45 µm membrane cellulose nitrate filters (47 mm diameter). The filters were transferred to Petri dishes containing sterile Sabouraud Dextrose Agar (SDA) medium supplemented with chloramphenicol (50 mg/L) and gentamycin (25 mg/L) after autoclaving. Three replications were used per water sample. Petri dishes were incubated at 25°C for 7 days and examined daily for the presence of fungal colonies. The number of colonies was recorded and the fungi were subcultured for purity and identification (Gonçalves et al., 2006; Mbata et al., 2008). Fungi were identified by examining their macroscopic and microscopic structures.

***Yeasts were identified by the following tests:***

Germ tube test: For the identification of *Candida albicans*. The suspected yeast colonies were inoculated into 0.5 mL human serum in a small tube and incubated at 37°C for 2 h. After incubi-tion, a drop of the yeast-serum mixture was placed on a glass slide, covered with a cover slip, and examined (Bhavan et al., 2010).

Carbohydrate assimilation test measures the ability of yeast to utilize different carbohydrates as the sole source of carbon aerobically. The sugars used were dextrose, maltose, sucrose, lactose, galactose, melibiose, cellobiose, inositol, xylose, raffinose, trehalose and dulcitol. Yeast cultures were suspended in saline, and basal medium (1.5 mL) containing 67.8% yeast nitrogen base was added. This was then added to 13.5 mL of molten, cooled agar containing 2% agar powder, mixed well, poured into a Petri dish and allowed to solidify. Then, paper disks soaked in 20% solutions of the various sugars were placed on the plates. Yeast growth was examined after incubating the plates at 25°C for 10-24 h (Bhavan et al., 2010).

Carbohydrate fermentation test detects the ability of microorganism to ferment a specific carbohydrate. Fermentation is noted by the production of acid and gas. The sugars used were dextrose, maltose, sucrose, lactose, galactose, and trehalose. 5 mL of carbohydrate (pH 7.4) containing 1% peptone, 1% sugar, 0.3% beef extract and 0.5% NaCl, 0.2% bromothymol blue in distilled water medium were prepared in a sterilized Durham tube. 0.2 mL of saline suspension was added to the tube of the suspected yeast, and incubated at 37°C for 10 days. After incubation, the tubes were exa-

examined for acid and gas production (Bhavan et al., 2010).

Chlamydospore formation: The suspected yeast cultures were inoculated on corn meal agar plates containing 1% Tween 80 and covered with cover slips. After incubation at 25°C for 3 days, the plates were examined for chlamydospores (Kim et al., 2002).

**Isolation and identification of bacteria**

Water samples (100 mL each) were filtered to isolate any bacteria. The filters were then placed on Trypticase Soy Agar (TSA) plates, and incubated to obtain a bacterial colony count. Distinct colonies were selected from the plates and subcultured on TSA to isolate a pure, single colony for identification. Unknown bacteria were identified to genus and species level busing biochemical methods as follows:

Gram staining is a common method used to differentiate two large groups of bacteria. It involves three steps: staining with crystal violet, decolorization and counterstaining with safranin. Gram-positive bacteria appear blue-black or purple, while gram-negative bacteria appear red or pink (Nester et al., 2007).

Catalase test is used to detect the existence of catalase enzyme in bacteria. Catalase activity is revealed by bubble formation after the addition of a drop of 3% hydrogen peroxide (Alexander and Strete, 2001).

Oxidase test is used to differentiate bacteria that produce cytochrome oxidase enzyme. Oxidase activity was determined by the oxidation of N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride (TMPD). The filter paper was moistened with 1% TMPD. Then, the bacterial colony was picked up and smeared onto the filter paper. If oxidase was present in the colony, it would oxidize the reagent and the colony on the filter paper would turn dark blue (Alexander and Strete, 2001).

Methyl red test is used to identify bacteria that ferment glucose to stable acid. Methyl Red Voges Proskauer Broth (MRVP Broth) was used. Broth was inoculated and incubated at 30°C for 5 days to allow stable acid to be produced. After incubation, methyl red indicator was added. Red indicates a positive result, while yellow is negative (Leboffe and Pierce, 1995).

Gelatin hydrolysis test detects the capacity of bacteria to produce enzyme gelatinases. Gelatin is solid at room temperature. If bacteria make gelatinase, gelatin is hydrolyzed and turns to liquid. To carry out the test, gelatin was set in a test tube. The gelatin was stabbed with an inoculated needle to the base of the tube, and incubated at 25°C for 2 days. The tube was then placed in a refrigerator for 30 min or on ice for 15 min. The result is positive if the gelatin remains liquid (Haas and Defago, 2005).

Citrate test detects the ability of bacteria to use citrate as carbon and energy source. A single isolated colony was selected and lightly streaked onto the surface of an agar slant. The agar was then incubated at 35°C for 24 h. After incubation, bromothymol blue indicator was added. The result is positive if the agar turns blue and negative if no color change is observed (Forbes et al., 1998)

Nitrate test is used to determine the ability of bacteria to reduce nitrate to nitrite. Nitrate test tubes were arranged using premade nitrate broth, inoculated, and incubated at 37°C for 24 h. After incubation, several drops of N-N-dimethyl-1-napthylamine and an equal amount of sulfanilic acid were added and mixed. A red color, which should develop within a few minutes, indicates a positive result for nitrate reduction. If no color develops, zinc dust is added. Zinc catalyzes the reduction of nitrate to nitrite. A color change to red indicates a negative result and no color change indicates a positive result (Gusberti and Syed, 1984).

## RESULTS

The identification of fungi and bacteria isolated from water taken from different flood zones in Narathiwat Province, in December 2012, is shown in Tables 1 and 2, respectively. In this study, we tested 200 samples from a range of flooded areas. Nine species of filamentous fungi and 2 yeast species (*C. albicans* and *Trichosporon* spp.) were isolated from the water samples by filter methods. The results showed the dominant isolated dermatophyte was *Trichophyton mentagrophytes* (44%). *Trichophyton rubrum* and *Microsporum canis* accounted for 19 and 15%, respectively. Other cutaneous mycoses found in the water samples were *C. albicans* (75.5%). The dominant isolated non-cutaneous mycoses were *Aspergillus niger* (73%), *Cladosporium* spp. (58%), and *Aspergillus flavus* (41%). Other fungi, including *Trichosporon* spp., *Penicillium* spp. (not *Penicillium marneffei*), *Aspergillus nidulans* and *Rhizopus* spp. were also isolated. The distribution of each of the 11 fungi across 4 different flood zones is shown in Table 1 and Figure 1. These fungi were mainly found in water samples taken from rubber plantations and houses.

Bacteria were isolated by filtration from water samples. Isolated strains were identified by conventional biochemical testing. A list of the 13 isolated bacterial strains is shown in Table 2 and Figure 2. Five Gram-positive bacterial strains were isolated (*Bacillus* spp. (not *Bacillus cereus*), coagulase-negative staphylococci, coagulase-positive staphylococci, *Corynebacterium* spp., and *Micrococcus* spp.). The dominant isolated Gram-positive bacteria in the water were *Corynebacterium* spp., which accounted for 59.5%, and *Bacillus* spp. (not *B. cereus*), which accounted for 52.5%. Eight gram-negative bacterial strains were isolated: *Acinetobacter baumannii*, *Enterobacter* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *Morganella morganii*, *Pseudomonas aeruginosa*, *Pseudomonas* spp. and *Vibrio cholerae*. The most abundant Gram-negative bacteria in the water were *E. coli*, accounting for 62.5%, *K. pneumoniae*, accounting for 61%, and *Enterobacter* spp., accounting for 59.5%. Both Gram-positive and negative bacteria were found mostly in water samples from rubber plantations and houses.

## DISCUSSION

During flood events, several types of microorganisms are present in the flood water, such as human enteric viruses (Ngaosuwankul et al., 2013), free-living amoebae (Wannasan et al., 2013), and bacteria (Chaturongkasumrit et al., 2013). Contaminated flood water indicated a high risk of health problems. This is the first study to investi-gate the prevalence of both fungi and bacteria in affected areas during the flood crisis in Thailand. This research found that water samples collected from various flooded areas in Narathiwat Province, in December 2012, con-tained high levels of microorganisms (fungi and bacteria). Flooded areas are a source of potential pathogen trans-mission to humans.

Flood conditions contribute to the growth and spread of pathogens, and direct contact with contaminated water

**Table 1.** Fungi identified in water from different flood zones in Narathiwat Province, December 2012.

| Fungi | Rubber plantation (n=50) | Water resource (n=50) | Road (n=50) | House (n=50) | Total | Percentage (%) |
|---|---|---|---|---|---|---|
| *Aspergillus flavus* | 28 | 16 | 15 | 23 | 82 | 41 |
| *Aspergillus nidulans* | 9 | 6 | 4 | 1 | 20 | 10 |
| *Aspergillus niger* | 45 | 38 | 22 | 41 | 146 | 73 |
| *Candida albicans* | 47 | 35 | 30 | 39 | 151 | 75.5 |
| *Cladosporium* spp. | 40 | 37 | 15 | 24 | 116 | 58 |
| *Microsporum canis* | 11 | 3 | 6 | 10 | 30 | 15 |
| *Penicillium* spp. (not *P. marneffei*) | 17 | 8 | 2 | 14 | 41 | 20.5 |
| *Rhizopus* spp. | 8 | 6 | 0 | 2 | 16 | 8 |
| *Trichophyton mentagrophytes* | 26 | 13 | 16 | 33 | 88 | 44 |
| *Trichophyton rubrum* | 13 | 6 | 8 | 11 | 38 | 19 |
| *Trichosporon* spp. | 15 | 10 | 11 | 14 | 50 | 25 |

**Table 2.** Bacterial organisms identified in water from different flood zones in Narathiwat Province, December 2012.

| Bacteria | Rubber plantation (n=50) | Water resource (n=50) | Road (n=50) | House (n=50) | Total | % |
|---|---|---|---|---|---|---|
| *Acinetobacter baumannii* | 1 | 0 | 0 | 3 | 4 | 2 |
| *Bacillus* spp. (not *B. cereus*) | 36 | 22 | 19 | 28 | 105 | 52.5 |
| Coagulase-negative staphylococci | 25 | 19 | 16 | 21 | 81 | 40.5 |
| Coagulase-positive staphylococci | 28 | 17 | 11 | 22 | 78 | 39 |
| *Corynebacterium* spp. | 38 | 25 | 23 | 33 | 119 | 59.5 |
| *Enterobacter* spp. | 30 | 36 | 21 | 32 | 119 | 59.5 |
| *Escherichia coli* | 34 | 38 | 20 | 33 | 125 | 62.5 |
| *Klebsiella pneumoniae* | 33 | 31 | 22 | 36 | 122 | 61 |
| *Micrococcus* spp. | 19 | 10 | 11 | 17 | 57 | 28.5 |
| *Morganella morganii* | 10 | 6 | 8 | 12 | 36 | 18 |
| *Pseudomonas aeruginosa* | 24 | 9 | 11 | 16 | 60 | 30 |
| *Pseudomonas* spp. | 27 | 15 | 14 | 15 | 71 | 35.5 |
| *Vibrio cholerae* | 5 | 7 | 11 | 10 | 33 | 16.5 |

can cause skin conditions. People exposed to flood water contaminated with pathogens are at increased risk of developing skin problems.

Fungi are eukaryotic organisms that are ubiquitous in nature. Most fungi grow terrestrially, but they can be found in every habitat, including aquatic environments. The results of this study showed that water samples collected from different flooded areas were contaminated with 9 species of filamentous fungi and 2 yeast species. Cutaneous mycoses are superficial fungal infections of the skin, hair or nails. Dermatophytes are a group of filamentous fungi that are the most common cause of cutaneous mycoses. The present study showed that the examined water was contaminated with the dermatophytes *T. mentagrophytes* (44%), *T. rubrum* (19%), and *M. canis* (15%). *T. mentagrophytes* and *T. rubrum* are the two main causative agents of tinea pedis (Hong Kong foot) (Rippon, 1988). However, causative agents of tinea pedis in outpatients attending the Institute of Dermatology, Bangkok, Thailand, differed from previous reports. The dermatophytes were the secondary cause of tinea pedis (36.8%), comprising *T. mentagrophytes* (18.4%), *T. rubrum* (13.2%), and *E. floccosum* (5.2%) (Ungpakorn et al., 2004).

A report from Nigeria showed that the most common dermatophytes isolated from athletics kits stored in Nigeria University's Sports Center were *T. mentagrophytes*, *T. rubrum* and *E. floccosum*. These fungi are often asso-ciated with tinea pedis among athletes in Nigeria (Essien et al., 2009). Rafiei and Amirrajab (2010) found that dermatophytes isolated from indoor public swimming pools in Ahwaz, Iran, were *T. mentagrophytes*, *T. rubrum*,

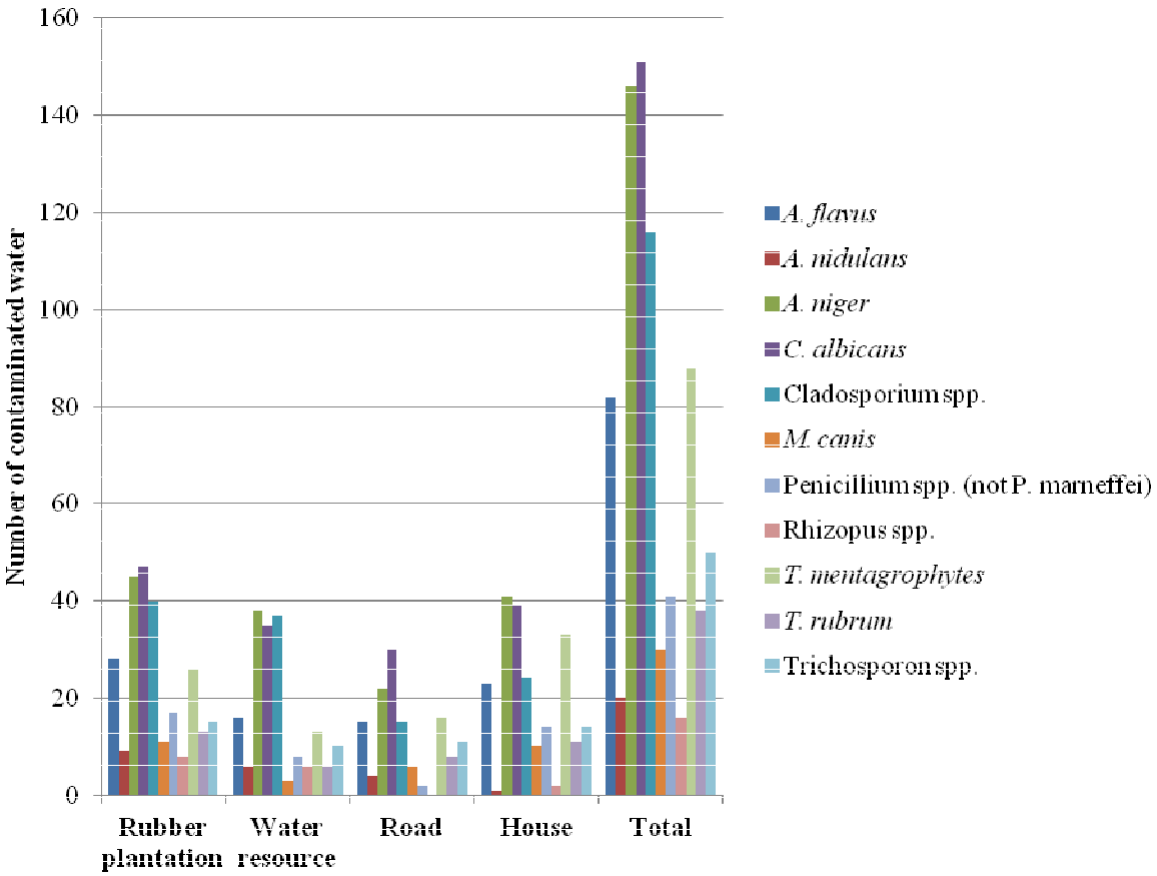


**Figure 1.** Prevalence of fungal species in water from different flood regions in Narathiwat Province, December 2012.

*T. verrucosum* and *E. floccosum*. The results from skin specimens from patients presenting with itching and skin maceration at the web spaces of the toes (16 cases) revealed 12.5% positive fungal growth (2 cases), comprising *Trichosporon mucoides* and non-spore forming hyaline fungi (Vachiramon et al., 2008).

Cutaneous mycoses can also be caused by the yeast *Candida* spp. From water tests, the highest frequency of fungi in our samples was *C. albicans*, accounting for 75.5%. The presence of *C. albicans* in this study was significant; the level is similar to that recorded in other studies. *Candida* species have been reported as common pathogens in community and hospital tap-water samples. Nine of the 14 isolated yeasts discovered in water were *Candida* species (Arvanitidou et al., 1999).

Mbata et al. (2008) demonstrated the presence of *Candida* species from the Yardenit Baptismal site on the Jordan River, Israel, as highly significant. There are also clinical-study data from workers at Ahvaz University of the Medical Sciences, Iran. The prevalence of cutaneous mycoses among the workers was 10.2%. The most common fungal disease was pityriasis versicolor (Tinea versicolor). Candidiasis due to *C. albicans* was the second most-common type of cutaneous mycosis. All patients with candidiasis were exposed to humidity during the day (Mahmoudabadi and Izadi, 2011).

The present study showed the water samples contaminated with fungi are non-cutaneous mycoses. The dominant fungi were *A. niger*, *Cladosporium* spp. and *A. flavus*, which accounted for 73, 58 and 41%, respectively. The most abundant filamentous fungi in water from the Jordan River in Israel were *Aspergillus* spp., which accounted for 32.5%, followed by *Penicillium* spp. (26.2%) (Mbata et al., 2008). Similarly, *Cladosporium*, *Penicillium* and *Aspergillus* dominated the fungi isolated from raw and treated water from the municipal water supply system in sub-tropical Australia (Sammon et al., 2010).

Flood water commonly contains high levels of bacteria that can cause skin infections. In this study, a total of 13 bacterial strains (8 strains of Gram-negative and 5 of Gram-positive bacteria) were isolated from water sam-ples. The most prevalent bacteria found were gram-negative (56.44%). The three dominant isolated Gram-negative bacteria were *E. coli*, *K. pneumonia* and *Enterobacter* spp., accounting for 62.5, 61 and 59.5%, respectively. The two most frequent Gram-positive bac-teria were *Corynebacterium* spp. (59.5%) and *Bacillus* spp.

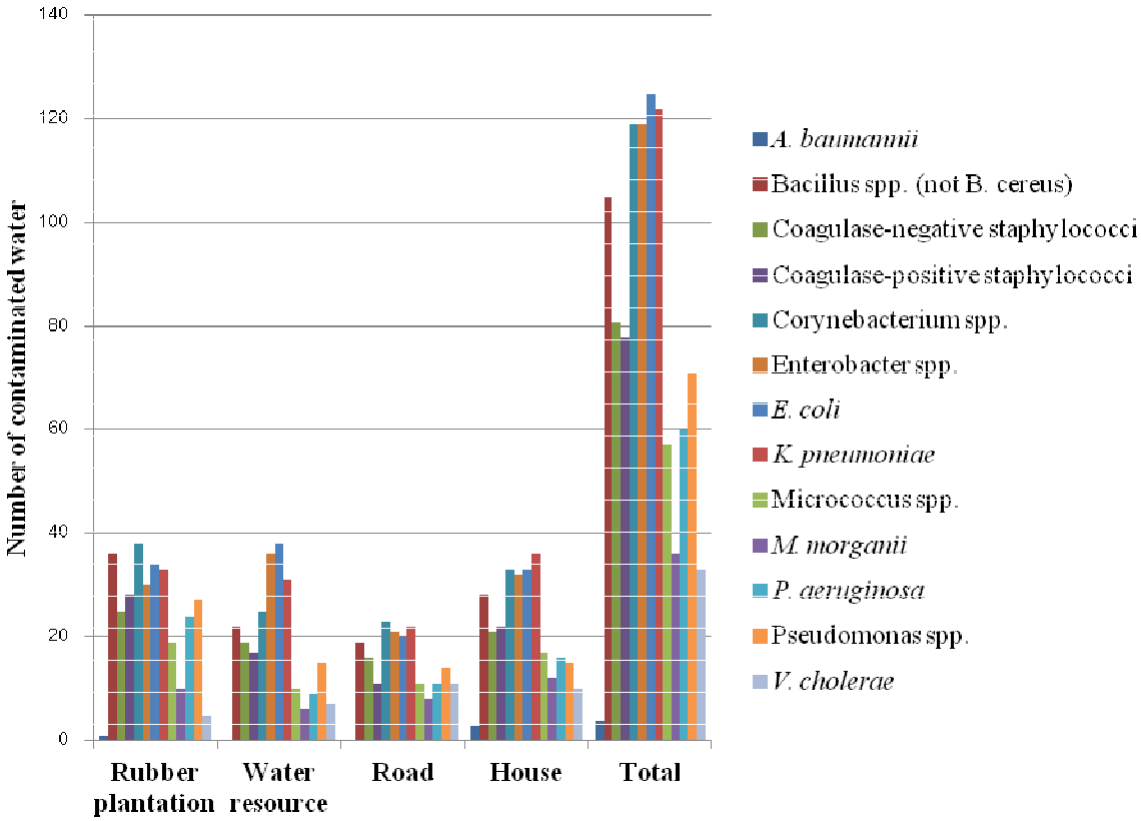


**Figure 2.** Prevalence of bacterial species in water from different flood regions in Narathiwat Province, December 2012.

(not *B. cereus*) (52.5%). Assessment of total bacterial contamination in various water sources during the 2011 Thai flooding disaster showed that the flood water and river water samples from the central part of Thailand contained the highest level of contamination (4.08-6.44 log cfu/mL) when compared with tap water (2.66 -4.72 log cfu/mL) and filtered tap water (2.75-3.93 log cfu/mL) (Chaturongkasumrit et al., 2013).

Previous studies have shown that several species of water-borne Gram-negative bacteria are linked to skin and soft-tissue infections after tsunamis and floods. One clinical study found Gram-negative bacteria were the most common bacteria isolated from the traumatic wounds of tsunami victims in southern Thailand in 2004 (95.5%). The five dominant isolated Gram-negative bacteria were *Aeromonas* spp., *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *Proteus* spp. The most common Gram-positive bacteria were *Staphylococcus* spp. (*Staphylococcus aureus* and coagulase-negative staphylococci). Most skin and soft-tissue infections among the tsunami victims were polymicrobial infections (71.8%) (Hiransuthikul et al., 2005). During the 2006 flood crisis in Thailand, Gram-negative bacilli were the most prevalent microorganism found in the skin specimens of patients presenting with itching and skin maceration at the web spaces of the toes (14 of 16 skin specimens). Gram-positive bacilli, *Corynebacterium* spp. and *Staphylococcus* spp. were also recorded. The increased prevalence of irritant dermatitis might be a result of over-exposure to contaminated water, friction, high humidity and unhygienic surroundings (Vachiramon et al., 2008).

Water turbidity, a measure of the cloudiness of water, is used to indicate water quality and measure the risk of contamination (Schwartz et al., 2000). The relationship between water turbidity and microbial contamination has been studied. Higher turbidity levels are often associated with higher contamination levels (LeChevallier et al., 1991; Clark et al., 1992; Chaturongkasumrit et al., 2013). In this study, water samples from various flooded areas appeared moderately turbid to the naked eye. When these samples were examined, several different types of fungi and bacteria were isolated. Fungi and bacteria were mainly found in water samples taken from rubber plantations and houses. There may be a correlation between the number of microorganisms and level of water turbidity in these areas. However, the turbidity level and the number of fungi and bacteria were not measured in this study.

The recommended regimen for treating skin infections is the use of topical anti-inflammatories with antibacterial

and antifungal agents. The fungi and bacteria that cause skin infections should be investigated in patients pre-treatment. Regrettably, it remains impractical because many people have skin infections during a flood disaster and need prompt treatment. Further investigation of the prevalence of fungi and bacteria in flooded areas during disasters would be beneficial for treating skin infections, and may also help physicians prepare effective medications in the future.

## Conflict of interest

The authors declare that they have no conflict of interest.

## ACKNOWLEDGMENTS

This study was supported by Trop. Med Grants and Dean Fund Grant, 2012, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

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