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Research Article

Recontamination of total plate count, coliforms and *Escherichia coli* in drinking water

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Abstract

The microbiological quality, total plate count (TPC), coliform and *Escherichia coli* contamination of drinking water used for general consumption at the Thaksin University, Patthalung campus was estimated. Treatments (n = 180) were tap water before the processed drinking water system (TWBP), drinking water before collection in a cooling tank (DWBT) and cold drinking water (CDW) at each drinking water site; 2 school buildings and 8 dormitories. The installed drinking water systems were found to be inefficient for reducing microbial contamination. TPC of DWBT and CDW were higher than that of TWBP ($P \leq 0.05$) in which there were 3.27, 3.44 and 1.15 log cfu ml^{-1}, respectively. Similarly, the percentage of coliform and *E. coli* contaminations of TWBP, DWBT and CDW were found to be at a level higher than the drinking water standard, being 83.33, 87.50 and 61.67% for coliform contamination and 96.33, 96.44 and 86.67%, respectively, for *E. coli* contamination. The conclusion from this research was that microbiological quality of the drinking water system depends on regular cleaning of filters and the cooling tank.

Keywords: TPC, microbiological quality, safety, Thailand.

Introduction

In many countries, microbiologically safe drinking water is considered a fundamental human right [1]. About 80% of communicable diseases in the world are waterborne [2]. To reduce the incidence of waterborne diseases and make the water suitable for human consumption, the removal of pathogenic organisms, fecal matter, suspended solids, algae, organic matter and harmful chemicals is absolutely necessary [3].

The dramatic decline in the incidence of waterborne disease in the early 1990s following the introduction of water treatment and disinfection has been well documented [4]. However, there is reason to be concerned for the future microbiological safety of drinking water, in both developing and developed countries [5]. Microbiology standards for public water supplies and industrial drinking water in Thailand are administered by the Ministry of Industry. This agency requires routine testing for the presence of coliforms and *Escherichia coli* as an indicator of human or animal waste. In addition, routine monitoring for total plate count is required for filtered or disinfected municipal water or drinking water. Standards for drinking water have been introduced for total plate count, TPC, (no more than 2.70 log cfu ml^{-1}), coliform (no more than 2.2 MPN 100 ml^{-1}) and *E. coli* (not found in 100 ml) [6]. Although public water supplies have been disinfected by chlorine solution, they are not recommended for use because of the possibility of microbial contamination. Many methods have been used to remove TPC, coliform and *E. coli* from water. Conventional methods for removal of these microbial contaminants involve coagulation followed by separation of the produced insoluble matter by direct filtration through sand beds [7]. These conventional water treatment methods are not affordable in rural communities of developing countries so other small-scale, more economical methods are needed for decentralized communities [8].

Various low-cost methods using different filters such as carbon, resin and ceramic were investigated to assess their capacity for removal of microbial contaminants, however this problem has not still been effectively resolved [9]. The purpose of this study was to investigate contamination by total plate count, coliform and *E. coli* in the drinking water system of a university in southern Thailand to assess the effectiveness of their treatment system.

Materials and Methods

To determine the microbiological quality of drinking water produced by drinking water system of a university in South of Thailand, small-scale economical methods composed of carbon, resin and ceramic filters, a randomized block design (RBD) was used with duplicate samples being taken for each replication and three replications were performed for drinking water system. Treatments composed of tap water before processed drinking water system (TWBP), drinking water before collected in cooling tank (DWBT) and cold drinking water (CDW). The different sites of drinking water system were blocked, 2 school buildings and 8 dormitories. A total of 60 TWBP, 60 DWBT and 60 CDW were sampled. All samples were taken aseptically, collected 1,000 ml in polystyrene bottle and held in an ice box (4^oC) for less than 2 h and analyzed on the day of sampling.

For microbiological analyses, 200 ml of water was used. Sampling was performed weekly for one consecutive month. Enumeration of total plate count (TPC) and most probable number (MPN) of coliforms and *E. coli* was performed using methods described in the Bacteriological Analytical Manual online 2001 [10]. All culture media and chemical were purchased from Merck & Co., Inc, (Whitehouse Station, NJ, USA). All samples (1 ml) were 10-fold serially diluted in 0.85% NaCl solution. Three consecutive dilutions (from 10^0 to 10^{-2} for water) of 1 ml were transferred to two plates and immediately add tempered plate count agar and subsequently incubated at 35^oC for 48 h. While, coliform and *E. coli* isolation, 10 ml of all samples were used to inoculate ten tubes containing doubled concentration of Fluorocult LMX broth (LMX) and incubated at 35°C for 24 h. All the color changing positive tubes were then transferred using a 5-mm inoculating loop in Briliant Green Lactose Bile broth and incubated at 35°C for 24 h and then the tubes were observed for gas production for completely coliform test. Furthermore, all the fluorescent in UV light positive LMX tubes were streaked onto L-EMB agar and incubated at

35°C in a water bath for 48 h and inoculated Kovac reagent. As a confirmation test for the presence of 5 colonies of *E. coli*, dark centered and flat, with or without metallic sheen, a biochemical test was performed by IMVic reactions that were indole production, voges-proskauer (VP)-reactive compounds, methyl red-reactive compounds, citrate and gas from lactose.

A randomized block design (RBD) was used to evaluate the water types for TPC. Means were considered significantly different when $P \leq 0.05$. For coliform and *E. coli*, the Chi-square method in non-parametric analysis was used to evaluate the water types by SAS software [11].

Results and Discussion

The TPC count is shown in Table 1. TPC in both DWBT and CDW were higher than those in TWBP ($P \leq 0.05$) which were 3.27, 3.44 and 1.15 log cfu ml^{-1}, respectively. This may result in cleaning of drinking water system in each site which has been not cleaned more than 3 months, was not enough. Moreover, algae grew up in ceramic filter, the last filter, when collected samples. In the same way, the percentages of sample showing the presence of TPC at levels lower than the drinking water standard in TWBP was higher than those in both DWBT and CDW which were 83.33, 26.79 and 21.67%, respectively (Table 2.). This result is in agreement with this reported for drinking water, produced in Ratchaburi province, Thailand, the percentages of sample showing the presence of TPC at levels lower than the drinking water standard was 27.5% [12]. This result showed that microbial contamination in filter of drinking water system may result in TPC recontamination of drinking water.

Table 1. TPC load for different types of water [1]

Type of Water	TPC Loading (log cfu ml^{-1})[2,3]
TWBP	1.15 ± 0.51^{a}
DWBT	3.27 ± 0.10^{b}
CDW	3.44 ± 0.14^{b}

[1] TWBP, tap water before processed drinking water system; DWBT, drinking water before collected in cooling tank and CDW, cold drinking water

[2] Microbiological standards for drinking water (Thai Industry Standard no. TIS. 257-2549, 2006) has been introduced for TPC (≤ 2.70 log cfu ml^{-1})

[3] Different letters indicate that values are significantly different ($P \leq 0.05$)

Faecal contamination is evidenced by the presence of coliforms and *E. coli*. Their presence in food possibly indicates the presence of enteric pathogens [13]. The levels of coliforms and *E. coli* contamination in both DWBT and CDW were higher than those in TWBP (Fig. 1a and b, respectively). However, the percentages of sample showing the presence of coliforms and *E. coli* at levels lower than the drinking water standard in both TWBP and DWBT were higher than those in CDW which was 83.33, 87.50 and 61.67%, respectively, for coliform contamination and 96.33, 96.44 and 86.67%, respectively, for *E. coli* contamination (Table 2). Microbiologically unsafe drinking water can be caused by water treatment and distribution systems aging and deteriorating [5]. This result showed that microbial contamination in a cooling tank of a drinking water system may result in TPC recontamination of drinking water.

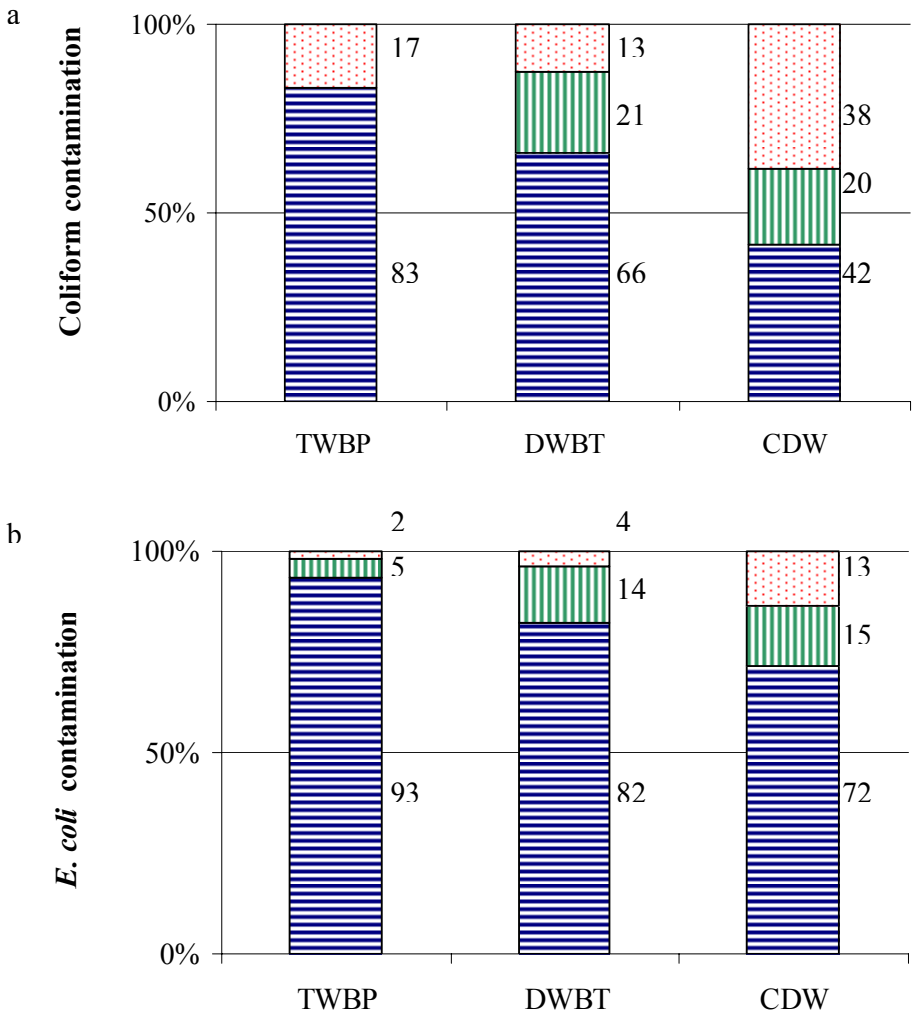

Figure 1. Percentage of coliforms (a) and *E. coli* (b) contamination in TWBP.
tap water before processed drinking water system; DWBT, drinking water before collected in cooling tank and CDW, cold drinking water by 3 range levels; <1.1, 1.1-2.2, >2.2 MPN 100 ml^{-1}

Table 2. Percentage of microbial contamination at levels lower than the drinking water standard for different types of water.

Type of Water[1]	Percentage of Microbial Contamination at Levels Lower than the Drinking Water Standard[2]		
	TPC	Coliform	*E. coli*
TWBP	83.33	83.33	96.33
DWBT	26.79	87.50	96.44
CDW	21.67	61.67	86.67

[1] TWBP, tap water before processed drinking water system; DWBT, drinking water before collected in cooling tank and CDW, cold drinking water.
[2] Microbiological standards for drinking water (Thai Industry Standard no. TIS. 257-2549, 2006) has been introduced for TPC (≤ 2.70 log cfu ml^{-1}), coliform (≤ 2.2 MPN 100 ml^{-1}) and *E. coli* (not found in 100 ml)

Conclusion

These results indicate that the various low-cost methods like deferent filters such as carbon, resin and ceramic cannot control microbial load. As microorganisms readily propagate in the drinking water, proper sanitary care should be taken during processing and storage of the water.

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