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Screening of faecal coliforms and heavy metals in water from the *in-situ* commercial water fitration dispensers in Skudai, Johor Bahru

Nurul Fatin Mohd Salleh and Dr Naji Arafat Mahat

Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 Johor Bahru, Malaysia
Corresponding Author: naji@kimia.fs.utm.my

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GRAPHICAL ABSTRACT

ABSTRACT

Due to easy access and hot weather conditions, consumption of filtered water from *in-situ* commercial water filtration dispensers is gaining popularity in many tropical countries including Malaysia. In Malaysia, these water filtration dispensers have been scarcely regulated by the relevant health authorities and mostly left *in-situ* without suitable surveillance and maintenance. Therefore, the compliance of the filtered water produced by these dispensers with that of the prevailing standards for drinkable water remains unclear. Hence, this present preliminary research that screened for the potential presence of faecal coliforms (a microbial indicator for drinkable water quality) and common heavy metals (lead, copper, manganese, zinc, and iron) in filtered water samples from 30 *in-situ* commercial water filtration dispensers around UTM Johor Campus acquires significance. 19 of the dispensers were appropriately maintained, while the remaining 11 were not. The possible presence of faecal coliforms was detected in 2 and 3 of the properly and poorly maintained dispensers, respectively. However, since the presence of these thermotolerant microorganisms has not been ascertained *via* Eosin-Methylene Blue agar and IMViC test, positive confirmation of such presence could not be made. While lead, manganese, and iron remained undetectable in all the dispensers, no significant differences in the concentrations of copper and zinc were observed between the properly and poorly maintained dispensers ($P>0.05$). Because minute concentrations of copper and zinc alone were found, the possibility of such contaminants being a health hazard can be ruled out. However, the fact that such water supplies may be contaminated by other types of heavy metals that may possibly be a health threat towards human health, further research in this aspect is required.

Keywords: *Escherichia coli, faecal colifoms, heavy metal, filtered water, in-situ water filtration dispenser*

1. INTRODUCTION

Water remains an integral component for various vital biochemical processes in human being (Cabral, 2010; Pirsaheb, 2013); a minimum of eight glasses of water (about 2 L) are required daily for sustaining ones good health (Valtin and Gorman, 2002). In view of the hot and humid ambient conditions prevailing in tropical countries like Malaysia, the consumption of drinkable water among its population may prove to be substantial. In addition to drinking enough volume of water, it is also found pertinent to ensure that the drinkable water resources remain unpolluted and hence safe for human consumption (Cabral, 2010; Suthar, 2010; Pirsaheb, 2013). In this context, the establishment of appropriate standards prescribing the safety parameters for drinkable water legislated by the Malaysian Food Act 1983 (Act 281) & Regulations (twenty-fifth schedule) as well as suggested by the various international entities e.g. the World Health Organization (WHO) acquire public health significance. Considering such importance and legal purview of the said act, the assessment of potential risks as well as enforcement of the act must be carried out meticulously for the benefit of the general public.

In the age of globalization, where time equals money, the rapidity of services becomes a significant factor affecting decision making; not to exclude water supplies (Riel, 2004). In meeting such demand, the supply of filtered water, presumably drinkable, *via in-situ* commercial water filtration dispensers has been gaining considerable popularity (Tomassi, 2004). Despite such popularity and convenience of supply to the general public, empirical studies for elucidating its safety for human consumption remain unreported. It is pertinent to indicate here that waterborne diseases such as cholera and typhoid (Cabral, 2010; Suthar, 2010) as well as chronic poisonings due to chemical toxicants (Suthar, 2010) are commonly associated with developing countries (Cabral, 2010; Suthar, 2010; Kostyla *et al*, 2015). Therefore, laboratory assessments for detecting pathogenic microorganisms such as faecal coliforms as well as chemical contaminants such as lead and iron in such purportedly drinkable water obtained from *in-situ* commercial water filtration dispensers deserve consideration.

2. EXPERIMENTAL

This present research included all the operational *in-situ* commercial water filtration dispensers available within all the study areas (*viz.* Taman Pulai Perdana, Taman Pulai Utama, Taman Pulai Flora, Taman Sri Pulai, Taman Universiti, and Taman Teratai) . To include the sampling process, the *in-situ* commercial water filtration dispensers must be located within the study areas and remain fully operational during the sampling period. All the operational *in-situ* commercial water filtration dispensers

were categorized into (a) the appropriately and (b) poorly maintained ones. The operational *in-situ* commercial water filtration dispensers were defined as "appropriately maintained" whenever the units have been evidently undergoing regular maintenance/repair and check up (presence of service stickers) with no evidence of physical damages such as missing parts and rusty components. In contrast, the units would be considered as "poorly maintained" in the absence of such documentation as well as judging from the presence of physical damages to the units. Considering such aspect, all the operational *in-situ* commercial water filtration dispensers included were divided into these two respective categories.

A sample of filtered water (> 0.5 L) from each *in-situ* commercial water filtration dispenser (February 2016) was collected using a sterile Schott bottle and examined for the presence of faecal coliforms as well as heavy metal elements namely lead, copper, manganese, zinc, and iron. For comparison purposes, samples of treated tap water obtained within UTM (Control 1) as well as the commercially bottled drinking water (Control 2) were included as controls. The collected samples were placed in a sterile cold box, transported immediately to the laboratory and analyzed within 2-3 hours upon collection. Following the procedure prescribed by Mahat *et al.* (2015), each sample was divided into 2 portions of 0.3 L and 0.2 L for faecal coliforms (membrane filtration technique) and heavy metals analyses (AAS), respectively

2.1. Faecal Coliforms Count

This present research utilized the selective MacConkey agar for detecting the presence of faecal coliforms *via* membrane filtration technique. Following the manufacturer's instruction manual, the MacConkey agar was prepared in a laminar flow. The agar (50.0 g) was suspended in distilled water (1 L), mixed and stirred using a glass rod. For dissolving the mixture, the suspension was gradually heated and boiled followed by autoclaving at 121°C for 15 minutes.

Next, the prepared solution was poured into a sterile plate and left to cool to room temperature for about 15 minutes. A volume (100 mL) of filtered water sample was mixed thoroughly by inverting the container for several times followed by filtrating it through a sterile cellulose acetate membrane filter (pore size: 0.45 µm; diameter 47 mm) attached to a vacuum pump. Replicating the above procedure, 3 replicates were analyzed for each sample.

After completion of the filtration process, the cellulose acetate membrane filter was removed from the chamber using a sterile forcep, placed in a MacConkey agar plate and incubated at 44.5°C for 24 hours. Considering that the faecal coliforms (e.g. *Escherichia coli*) are lactose fermenters, they would form small and smooth convex reddish or pinkish colonies (1-3 mm) surrounded by precipitated bile on the MacConkey agar upon completion of the incubation period (Cheesbrough, 2006). Following the suggestion made by the same author, the number of such colonies was recorded and whenever the number of colonies exceeded that of 50, such confluent growth was reported as 'numerous to count'.

2.2. Heavy Metal Analysis

Using the commercial standard solutions of $Pb(NO_3)_2$, $CuSO_4.5H_2O$, $MnSO_4.H_2O$, $ZnSO_4.7H_2O$, and $Fe(NH_4)_2(SO_4)_2$, stock solutions (1000 ppm) of lead, copper, manganese, zinc, and iron were prepared using distilled water. Using the FAAS, analysis of such heavy metal elements in the filtered water samples was attempted. For each analyte, a calibration curve was plotted at 6 different concentrations *via* serial dilutions with distilled water. The calibration curve was only accepted whenever the coefficient of determination exceeded that of 0.994 with the coefficient variation (CV %) of no more than 20% (Man *et al*., 2006). Distilled water alone was used as blanks. Using the formula described by Sanagi *et al*. (2009), the limit of detection (LOD) and limit of quantitation (LOQ) were mathematically determined from the accepted calibration curve detailed below.

2.3. Statistical Analysis

The statistical analysis was performed using the IBM SPSS version 20.0 software and the normality of the data was ascertained using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Since the data for heavy metals (lead, copper, manganese, zinc, and iron) in water samples from the appropriately and poorly maintained *in*-situ water filtration dispensers included here violated the assumption of normality, the non parametric Mann-Whitney U test was used for hypothesis testing. Therefore, unless specified otherwise, the concentrations of heavy metals were presented as median ± interquartile range. For inferring the significance of the finding, the level of significance of 0.05 was chosen.

3. RESULTS AND DISCUSSION

In this present research, samples of filtered water from 30 operational permanent *in-situ* water filtration dispensers identified around the UTM, Johor Bahru Campus were analyzed for the possible presence of faecal coliforms (Table 1). Following the qualitative assessment of individual *in-situ* water filtration dispensers revealed that 19 and 11 of them were of appropriately and poorly maintained, respectively. The possible presence of faecal coliforms was detected in 5 of the *in-situ* water filtration dispensers, ranging from 1 to >50 CFU/100 mL of filtered water (Table 1). While the possible presence of

faecal coliforms was not detectable in all the 19 appropriately maintained *in-situ* water filtration dispensers, confluent growth of possibly faecal coliforms (>50 CFU/100 mL) was detected in 2 of the poorly maintained ones (Table 1). Such possible presence of faecal coliforms was not detectable in both the treated tap water and commercially bottle mineral water samples (Figure 4.1a). A photograph representing the possible presence of faecal coliform colonies, in a representative presumably contaminated sample, on the McConkey agar incubated at 44.5°C for 24 hours.

Table 1 Condition of *in-situ* commercial water filtration dispensers and counts of possible presence of faecal coliform in the filtrated water samples (February 2016).

Sample Code Number	Maintenance Condition	Faecal Coliforms Counts (CFU/100 mL)	Sample Code Number	Maintenance Condition	Faecal Coliforms Counts (CFU/100 mL)
1	Appropriate	ND	16	Appropriate	ND
2	Poor	ND	17	Appropriate	ND
3	Appropriate	1	18	Appropriate	ND
4	Appropriate	ND	19	Poor	>50
5	Appropriate	ND	20	Appropriate	ND
6	Poor	ND	21	Appropriate	ND
7	Appropriate	2	22	Poor	ND
8	Poor	2	23	Appropriate	ND
9	Appropriate	ND	24	Poor	ND
10	Poor	ND	25	Appropriate	ND
11	Poor	ND	26	Poor	>50
12	Appropriate	ND	27	Appropriate	ND
13	Appropriate	ND	28	Appropriate	ND
14	Appropriate	ND	29	Appropriate	ND
15	Poor	ND	30	Poor	ND

Note: In this present research, the detection of the possible presence of faecal coliform was made using the McConkey agar incubated at 44.5°C for 24 hours. Since the confirmatory tests of Eosin-Methylene Blue and IMViC were not performed, the absolute presence of faecal coliform could not be positively concluded.

Apart from faecal coliforms, five heavy metal elements commonly found in water *viz.* lead, copper, manganese, zinc and iron were analyzed in all the filtered water samples included in this present research too, and the partial validation data *i.e.* calibration curves, LODs and LOQs are presented in Table 2. The coefficient of determination (R^2) for all the calibration curves range between

Table 2 Range of calibration curves, limit of detection and limit of quantitation for lead, copper, manganese, zinc, and iron analyzed in this study

Heavy Metals	Range of Standard Concentrations (ppm)	Coefficient of determination (R^2)	Calibration Equation	LOD (ppm)	LOQ (ppm)
Lead	0.00-5.00	0.9992	y = 0.9889x – 0.9495	0.17	0.51
Copper	0.00-2.00	0.9969	y = 0.3895x – 0.3525	0.05	0.14
Manganese	0.00-1.00	0.9979	y = 0.1954x – 0.1795	0.04	0.12
Zinc	0.00-1.00	0.9963	y = 0.1954x – 0.1795	0.04	0.11
Iron	0.00-5.00	0.9957	y = 0.9889x – 0.9495	0.06	0.18

Result revealed that lead, manganese and iron were not detectable in all the filtered water samples. Minute concentrations of copper and zinc were found in filtered water samples obtained from both the appropriately (Cu: 0.003 ± 0.011 µg/mL; Zn: 0.001 ± 0.018 µg/mL) and poorly (Cu: 0.013 ± 0.011 µg/mL; Zn: 0.001 ± 0.018 µg/mL) maintained *in-situ* water filtration dispensers (Table 3). However, statistical analysis (Mann-Whitney U test) revealed insignificant differences ($P>0.05$) in the concentrations if both analytes between the appropriately and poorly maintained dispensers (Table 3).

Table 3 The range values of concentration of copper, lead, manganese, zinc, and iron observed in all filtered water samples (Appropriately Maintained and Poorly Maintained)

Heavy Metal	Lead	Copper	Manganese	Zinc	Iron
Appropriately Maintained (n=19)	ND	0.003±0.011 (0.003 – 0.018)	ND	0.001±0.018 (0.001 – 0.111)	ND
Poorly Maintained (n=11)	ND	0.013±0.011 (0.001 – 0.021)	ND	0.001±0.018 (0.003 – 0.046)	ND

Note: The concentrations of heavy metals (lead, copper, manganese, zinc, and iron) were expressed as median ± interquartile range and values in parentheses represent the range of concentrations. Statistical comparison made using Mann-Whitney U test revealed no significant differences in concentrations of heavy metals between the appropriately maintained *in-situ* water filtration dispensers with that of poorly maintained ones.

4. CONCLUSION

While consumption of filtered water from the *in-situ* water filtration dispensers are gaining considerable popularity in community in Malaysia, review of literature reveals no specific studies ever reported pertaining to its status of contamination by pathogenic microorganisms as well as chemical contaminants such as lead and iron. Therefore, this preliminary research for investigating in such aspect proves to be important. Although the possible presence of faecal coliforms in such water samples was detected *via* the screening procedure employed in this present research, confirming the contamination by such microorganisms could not be ascertained since the confirmatory tests were not carried out. Hence, further studies utilizing the confirmatory tests such as IMViC and Eosin-Methylene Blue agar are required. The fact that the concentrations of copper and zinc alone were found in several samples and the amounts prove to be minute, the possibility of such contaminants for being a health hazard can be ruled out. However, the fact that such water supplies may be contaminated by other types of heavy metals that may possibly be a health threat towards human health, further research in this aspect is required.

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