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MICROBIAL QUALITY ASSESSMENT OF GREYWATER SOURCES TO REUSE FOR DIFFERENT PURPOSES

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Abstract

Water shortage is one of the most worrisome issues around the globe, especially with the increase in the human population. Nowadays, attention has been drawn towards the reuse of greywater because of its large volume and low contaminants. In the present research, greywater samples were collected from different sources including; households, restaurants, mosques, laundries, guest houses and sport clubs in Mansoura city, Egypt. The counts of total coliforms, *Escherichia coli, Enterococcus* sp., *Pseudomonas aeruginosa, Staphylococcus* sp., *Salmonella* and *Shigella* sp. were detected. The results indicated that there were variations in counts of bacterial content in greywater samples collected from different sources and sampling time. Tap water samples generally did not contain any microbial content under this study. However, greywater samples contain high counts of tested bacterial content under different sources, except laundry samples. In addition, restaurant and household samples always contained higher counts of bacterial content compared to the other samples under various locations. On the other hand, the counts of bacterial content were always higher in July samples, while was lower in January samples as compared to the other months. It could be stated that, such greywater contains significant levels of total coliforms and *E. coli* in most tested sources which determine its uses. Counts of *E. coli* in greywater collected from restaurant was the highest risk (531 CFU/100 mL), while in laundry recorded the lowest risk (24 CFU/100 mL). Overall, the study can help strategy makers in formulating biological' guidelines for reuse of greywater and the needed designs for greywater treatment technologies.

Keywords: colilert technique, *Enterococcus*, *Escherichia coli*, greywater, total coliforms

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1. Introduction

With the increasing consumption of high-quality fresh water in agricultural and urban areas increases, the pressure on available water resources where the problem is growing in arid, semi-arid and densely populated areas. Hinrichsen et al. (1998) had reported that within the next fifty years, more than 40% of the world's population will face water scarcity. So it is imperative to find out the way for development of new water resources.

As fresh water becomes increasingly scarce, it is necessary to drive the attention to alternative sources of water. That is led to a large-scale interest in the application of water treatments and reuse of domestic, industrial wastewater as alternative water supply sources (Edwin et al., 2014). Some countries have employed a proactive policy of reclaiming wastewater for productive use including the USA and Australia (Redwood, 2008). Recycling wastewater for food production is less common, more than 10% of the world's population consumes foods produced by irrigation with wastewater; this percentage is considerably higher among populations in low-income countries with arid and semi-arid climates (WHO, 2006).

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Generally, wastewater is divided into three categories, yellow, brown and greywater. Both yellow and brown water are referred to urine and faecal sewage. Greywater is usually defined as urban wastewater without any input from the toilets; it includes kitchen sinks, dishwashers, and streams from showers, baths, wash basins, and laundry (De Gisi et al., 2016). Greywater is the most suitable for water reuse, where it constitutes about 60–70% of the total domestic wastewater generated in households (DeOreo et al., 2016; Edwin et al., 2014).

The greywater contains significant concentrations of materials which has negative environmental and health impact, such as salts, surfactants, oils, synthetic chemicals as well as microbial risks (Eriksson et al., 2002; Friedler, 2004; Shi et al., 2018; Travis et al., 2008). Also, the presence of potential microbial hazards in domestic greywater has been mentioned in various literatures. Few literature data refers to the importance of microbial pathogens including *Salmonella* spp., *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, Clostridia, *Giardia*, Norovirus, Enterovirus and Rotavirus were detected in greywater from bathrooms, laundries and kitchens (Jefferson et al., 2004; Katukiza et al., 2015; Keely et al., 2015; O'Toole et al., 2012; Shi et al., 2018). Among of them, *E. coli* was the most frequently detected and widely distributed in most samples of domestic greywater (Bodnar et al., 2014; Chaillou et al., 2011; Katukiza et al., 2015; O'Toole et al., 2012). Therefore, pathogenic *E. coli* which can cause serious human diseases was chosen as the target pathogenic microorganism in the risk analysis.

Recycling of domestic greywater and its use for non-potable purposes may diminish the demand for potable water and the load on wastewater treatment plants. Several technologies for greywater treatment and recycling options are reported from the past five decades. The biological treatment process includes rotating biological contractor (RBC), sequential batch reactor (SBR), constructed wetland, upstream anaerobic sludge blanket (UASB), and some innovative technologies such as membrane bioreactor and reed bed. These techniques can generally satisfactorily eliminate the efficiency of wastewater. High-strength gray water because of its process stability and pathogens (Ansari and Shrikhande 2019; Hernandez et al., 2010). As well as Chemical processes, such as coagulation, electrical coagulation, photo oxidation, ion exchange, and granular activated carbon that can reduce organic matter to a certain level (Pidou et al., 2008). Moreover, physicochemical treatment processes such as screening, filtration, and disinfection can sufficiently reduce COD and BOD. Coagulation/flocculation is more efficient in pathogen removal rather than BOD removal. Filtration is perhaps the oldest and most feasible method for grey water recycling (Banerjee et al., 2016; Debnath et al., 2019). The selected greywater treatment process should be sustainable in view of environmental, operational, economic, and social perspectives. The quality necessary of the greywater recycling is specific to application need and require different levels of treatments, simple, advanced as well as combined processes. The treated greywater can be reused and recycled in different purposes such as for gardening, flushing toilets, car washing, fire-fighting, industrial purposes etc. (Singh et al., 2019).

There is a lack of researches on the evaluation of health risk associated with greywater reuse. Therefore the present study has the following objective to determine the bacteriological quality of greywater produced over the year from different sources. The results that will be obtained in this study may be essentially needed for better planning and design of the greywater water treatment system.

2. Methodology

Greywater was collected from different sources in Mansoura city, Egypt. Tap water sample was also collected from each source for comparison. The sources included households, restaurants, mosques, laundries, guest houses and sport clubs. The sites were sampled on four months basis (January, April, July and October) covering the four seasons and six replicates (1-liter each) from each source.

Greywater samples were collected in acid rinsed, polyethylene containers. In addition, other six replicates (1-liter each) of the tap water were collected from the same sites. Samples were labeled using waterproof markers and transported to the laboratory on ice in insulated containers. The collected water samples from each location were immediately analyzed. The samples were diluted in sterile diluents if necessary, to achieve a countable concentration.

Total coliforms and *E. coli* were detected and enumerated using the Colilert method (APHA, 2005; Pitkanen et al., 2007). One hundred milliliters from a water sample mixed with a Colilert reagent (IDEXX, USA) it is drained into a Quanti-Tray containing 97 wells depending on the accuracy of the result required. After the appropriate incubation period 24 hours at 35ºC, the number of wells positive for total coliforms and *E. coli* are counted. Total coliforms, including *E. coli*, metabolize ortho-nitrophenyl galactopyranoside using the enzyme ß-galactosidase to produce ortho-nitrophenyl, these results in a yellow coloration of the test well. *E. coli* will also metabolize 4-methyl-umbelliferyl glucuronide using the enzyme ß-glucuronidase to produce 4-methyl-umbelliferone which fluoresces under a long wave, 365 nm, and ultraviolet light. Then, results were calculated from relevant MPN probability tables.

Enterococcus sp. was detected and enumerated using the Enterolert method (APHA, 2005; Mannapperuma et al., 2011). One hundred milliliters of water sample mixed with Enterolert reagent (IDEXX, USA) is dispensed into a Quantity-Tray with 97 wells depending upon the resolution of results required. After the appropriate incubation period 24 hours at 42˚C, the numbers of wells positive for *Enterococcus* sp. are counted. *Enterococcus* sp. will metabolize ß-D-glycoside using the enzyme ß-glycosidase to produce 4-methyl-umbelliferyl which fluoresces under long wave (365 nm) ultra violet light. Results are then calculated from relevant MPN probability tables. Assessment of the microbiological quality of the collected greywater depends on the detection of total coliforms, *E. coli* and *Enterococcus* sp. as indicators of pathogenic organisms.

Furthermore, other pathogenic microorganisms were tested for assessment of the microbiological quality of water. *Pseudomonas aeruginosa, Staphylococcus* sp., *Salmonella* and *Shigella* sp. were tested in the greywater samples according to the methods of (APHA, 2005). The pathogenic microorganisms were detected and enumerated by using Cetrimide agar medium (*Pseudomonas* selective agar) for *Pseudomonas aeruginosa*, *Staphylococcus* medium for *Staphylococcus* sp., S.S. agar medium for *Salmonella* and *Shigella* species.

3. Results and discussion

Counts of total coliforms, *E. coli*, *Enterococcus* sp., *Pseudomonas aeruginosa, Staphylococcus* sp., *Salmonella* and *Shigella* as pathogenic microorganisms, were detected in six sources (household, restaurant, mosque, laundry, guest house and sport club). In general, tap water samples did not contain any microbial pathogens under this study. Comparison of the bacterial content of greywater between the different sources, regardless of the sampling periods, is presented in Table 1. On the other hand, in Table 2 is given a comparison of the bacterial content of greywater between sampling periods, regardless of the different sources.

The results indicate that there are variations in counts of bacterial content in greywater samples were due to different sources and sampling periods (Tables 1 and 2). The counts of bacterial content were always higher in restaurant samples, while lower in laundry samples is compared to the other greywater sources (Table 1). On the other hand, data in Table 2 show that the counts of bacterial content were always higher in July samples, while is lower in January samples compared to the other months.

3.1. Total coliforms

The total coliforms count in greywater samples under various sources and sampling periods are graphically drawn in Fig. 1. The results indicated noticeable differences between months covering the four seasons of the year. The total coliforms count ranged between 120 CFU/100 mL (in laundry greywater) and 1598 CFU/100 mL (in the greywater of restaurant). Greywater' samples of July recorded the highest counts in total coliforms compared to the other months.

On the other hand, January samples recorded the lowest counts. This may be due to the high temperature in July appropriate for bacterial propagation compared to January. The variation of total coliforms of greywater samples at different locations indicated wide variability depending on the greywater source. Data generally showed more pollution in the samples from the restaurant, household, gust house and sport club, respectively, while samples from the mosque and laundry were less polluted compared to the other greywater sources.

3.2. Escherichia coli (E. coli)

Results illustrated in Fig. 2 indicate evident variations between sampling time in *E. coli* counts. As the lowest counts were obtained in greywater samples collected at January regardless of the greywater source. On the other hand variation of *E. coli* counts from the various sources revealed similar trends to the total coliforms data. The restaurant samples gave the highest *E. coli* counts compared to the other sources.

Table 1. Bacterial content of greywater from different sources

Source	*Total Coliforms*	*E. coli*	*Enterococcus sp.*	*Staphylococcus sp.*	*Pseudomonas aeruginosa*	*Salmonella and Shigella* sp.
	CFU/100 mL					
Household	1064	333	197	22	3	42
Restaurant	1255	531	281	34	5	54
Sport club	804	202	146	15	2	26
Laundry	151	24	12	3	0	14
Gust house	843	364	181	18	2	46
Mosque	626	156	118	9	2	22

Table 2. Bacterial content of greywater under different sampling periods

Month	*Total Coliforms*	*E. coli*	*Enterococcus sp.*	*Staphylococcus sp.*	*Pseudomonas aeruginosa*	*Salmonella and Shigella* sp.
	CFU/100 mL					
January	607	199	127	11	2	23
April	865	282	157	15	3	39
July	1044	332	181	21	4	47
October	809	270	149	19	2	35

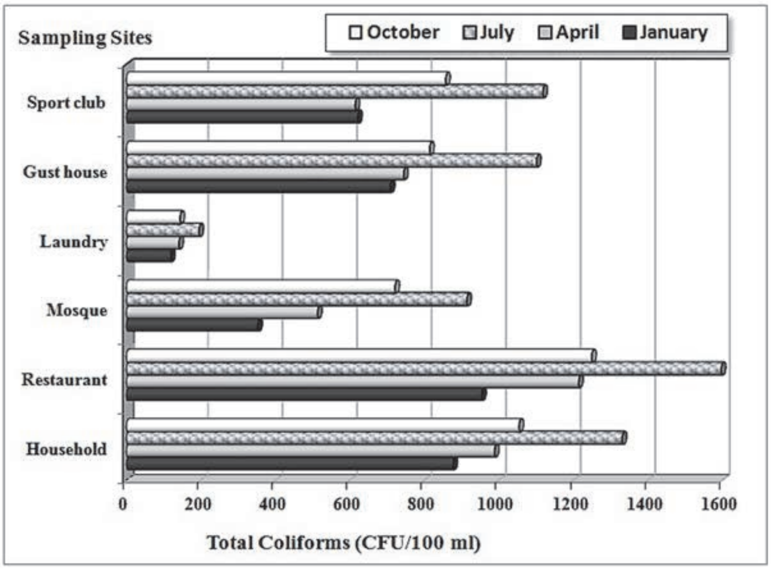

Fig. 1. Total coliforms content in greywater from sampling locations at different periods

3.3. Enterococcus sp.

The data graphically represented in Fig. 3 indicate that all locations showed higher counts of *Enterococcus* sp. over the hot periods of July which may be due to a seasonal dilution factor. On the other hand, temporal fluctuations in *Enterococcus* sp. are shown. The Mean counts of *Enterococcus* sp. was always higher in the restaurant samples followed by household. While the lowest counts of *Enterococcus* sp. were found in laundry, regardless of sampling time.

3.4. Pseudomonas aeruginosa

The data of *Pseudomonas aeruginosa* counts in greywater samples are presented in Fig. 4. The results generally reflected same patterns, of other temporal and locations distribution, however the results reflected comparatively very low counts.

3.5. Staphylococcus sp.

Counts of *Staphylococcus* sp. varied obviously over the entire period of sampling. Higher counts were reported in July samples while lower counts were reported in January samples (Fig. 5). Also the results also reflected differences in *Staphylococcus* sp. counts among the different greywater sources. The lowest counts of *Staphylococcus* sp. were detected in the laundry, mosque and sport club samples, respectively, regardless of sampling time.

3.6. Salmonella and Shigella spp.

Counts of *Salmonella* and *Shigella* spp. also showed relatively lower values in January (Fig. 6).

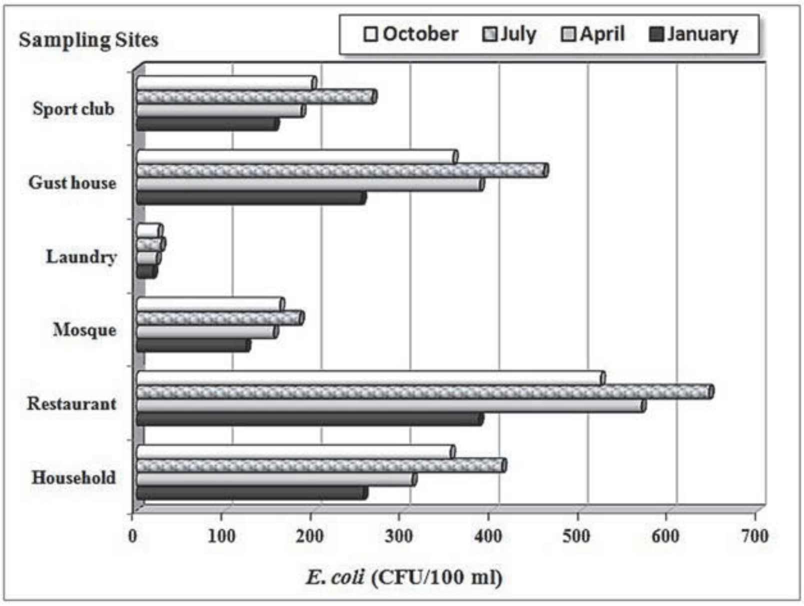

Fig. 2. *E. coli* counts in greywater from sampling locations at different periods

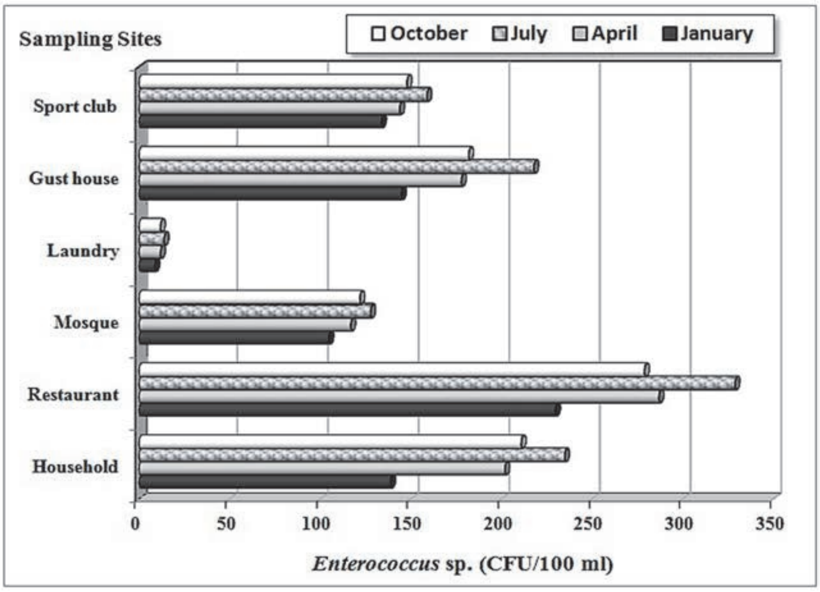

Fig. 3. *Enterococcus* sp. counts in greywater from sampling sites at different periods

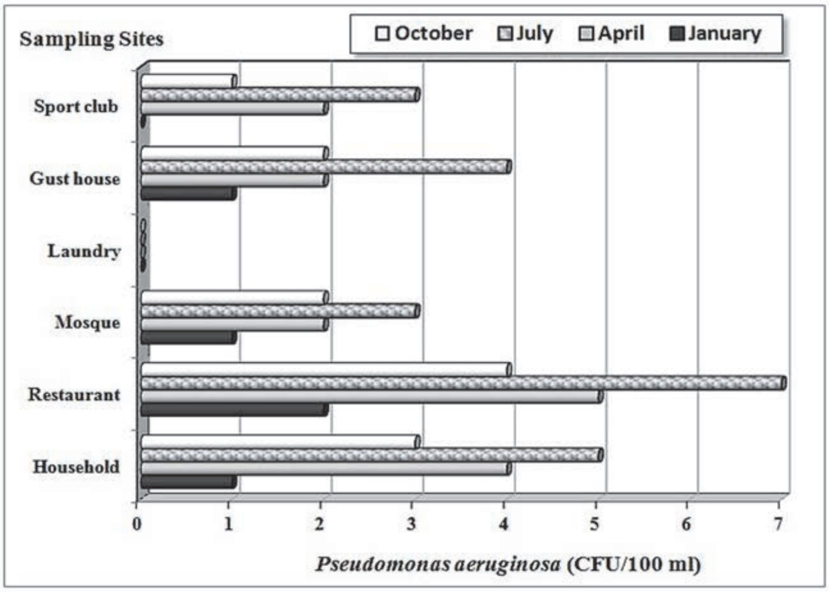

Fig. 4. *Pseudomonas aeruginosa* counts in greywater from sampling sites at different periods

Variation due to locations followed same trends where higher counts were registered in the restaurant followed by gust house and the least counts were recorded in samples of the laundry.

The obtained results revealed that greywater' samples of July month (28-38°C) recorded the highest counts of tested microorganisms while decreased in January month (5-15°C) samples compared to the other months. This is because the temperature in the range from 20°C to 40°C is the optimum temperature for most microbes to grow. At this temperature all aspects of the cell metabolism function at their optimum values, the cell is able to rapidly increase in size, divide and their growth rate is at its maximum value. On the other hand, at low temperatures molecules move slower, enzymes cannot mediate in chemical reactions, and eventually the viscosity of the cell interior brings all activity to stop.

From the above mentioned results, it could be noticed that, restaurant, household, guest house and sport club samples always contained higher counts of bacterial content compared to the other samples (laundry and mosque). The microbial characteristics of greywater vary greatly among different sources owing to the various living habits, personal hygiene of the users in addition to other factors such as dishwashing patterns, cleaning products used, laundering practices, and bathing. Typical greywater total coliforms values were reported in range 10^2-10^3 CFU/100mL, for laundry and bath greywater, respectively (Siegrist, 1977), while total coliforms values for house with two adults and one child amounted to 1.9 X 10^8 CFU/100mL (Casanova et al., 2001).

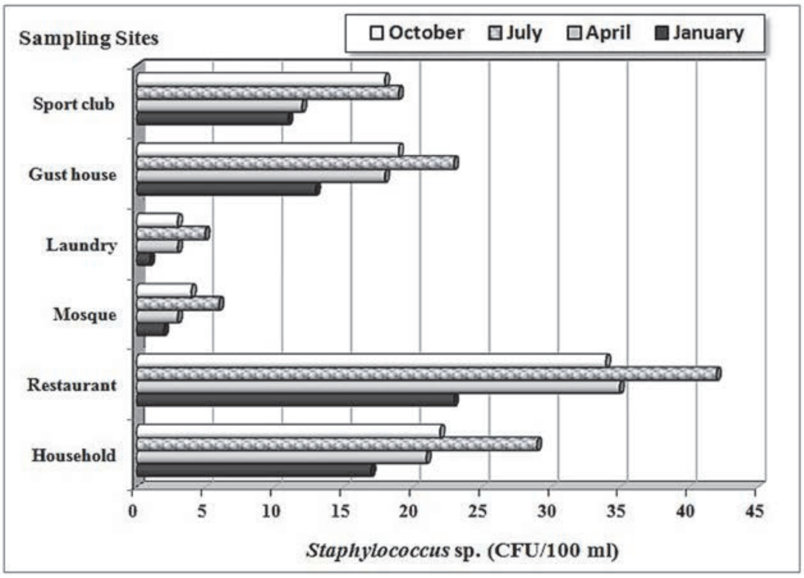

Fig. 5. *Staphylococcus* sp. counts in greywater from sampling sites at different periods

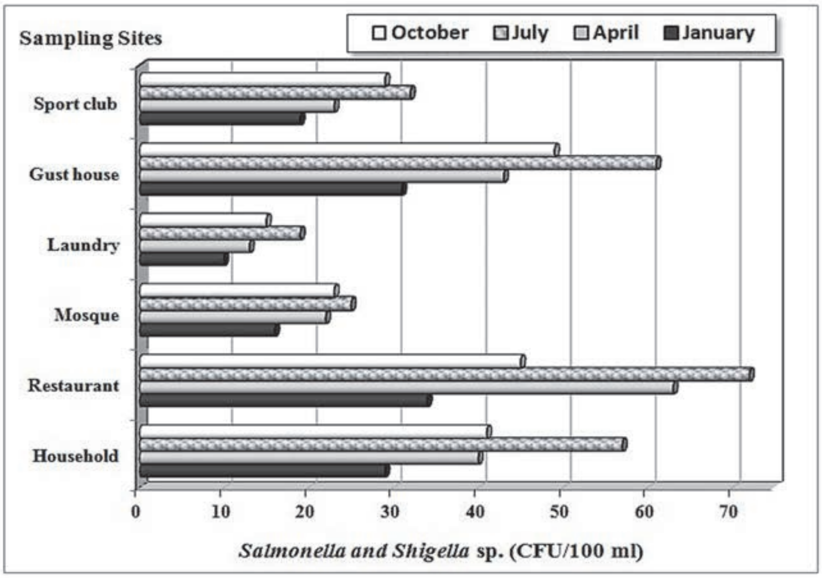

Fig. 6. *Salmonella* and *Shigella* spp. counts in greywater from sampling sites at different periods

The microbial load of greywater is related to the presence of fecal contamination. The main hazard from greywater comes from fecal cross-contamination (Ottosson and Stenstrom, 2003).

The health risks associated with the application of greywater especially in the agriculture sector are considered to be lesser comparing to those for the use of wastewater. Where, greywater generally has lower concentrations of microbial pathogens than wastewater, but it may still contain some pathogens, which are come into the greywater from various sources such as washing babies' diapers, laundry, personal hygiene (WHO, 2006).

Generally, the most used indicators for pollution in greywater is the presence of coliforms bacteria, whereas the *E. coli* are mostly of fecal origin and it is considered as the most reliable indicator than other coliforms groups. At the same time, toilet wastes are not included in the greywater, so fecal contamination is too limited and due to certain activities, such as childcare, showering and washing contaminated laundry i.e. diapers (Bodnar et al., 2014; Chaillou et al., 2011; Jefferson et al., 2004; Katukiza et al., 2015; O'Toole et al., 2012). However, the present study revealed that laundry greywater contains lower concentration of total coliforms and *E. coli* when compared to other sources. This may be due to the type of laundry selected for this study which is a commercial huge laundry usually does not receive the kind of cloths which are not contaminated (i.e. diapers).

Faecal contamination is measured by the use of common indicator organisms, such as coliforms and *Enterococcus* sp. Wastewater generated from bathtubs, showers and hand basins is considered to be the least contaminated type of greywater. According to WHO (2006), thermo tolerant coliforms concentrations have been assessed in shower and bath water to be in the range of 10^2 to 10^5 CFU/100 mL. On contrary, the present study reported relatively higher counts. Also, report of WHO (2006) mentioned that more than ten percent of the world's population consumes foods produced by irrigation with wastewater. This percent will be mostly higher in low-income countries with arid and semi-arid climates. Generally, the population increases are the main reason which forces for further demand on other water resources. In addition, the production of wastewater will increase as an outcome of continued urbanization. Thus both wastewater and greywater are considered a major challenge in the overall management of water resources.

Among comparison of total coliforms and *E. coli* of greywater from the different sources in Mansoura city and international standards, reveals that the greywater from most of the locations is polluted to the extent that it is not acceptable for use on home garden before treatment according to WHO guidelines (WHO, 2006).

USEPA and USAID have recommended strict guidelines for reuse of treated greywater. Where total coliform allowed in treated greywater for irrigation depends on the type of crop grown. For restricted irrigation (include crops that eaten uncooked) no detectable fecal coliform bacteria are allowed in 100 mL, but for unrestricted irrigation of fodder crops, the guideline limit is < 200 CFU/100 mL (US EPA, 2004).

Referring to the above agency's guidelines, the study data shows that greywater samples exceed these values in some locations. So, greywater should be keep away from direct human contact unless the greywater is disinfected and it treatment.

Therefore, we recommend using some treatment processes in this study such as microfiltration system which is effective enough to remove the low load microbes from some greywater sources tested such as laundry and mosque. On the other hand, we preferred selecting coagulation/flocculation system as it is more efficient in high pathogen removal from some greywater sources tested such as restaurant, household, gust house and sport club. The treated greywater can be reused and recycled in different purposes such as for gardening, flushing toilets, car washing, firefighting, and industrial purposes.

4. Conclusions

From the microbiological point of view, it is reasonable to conclude that greywater quality in Mansoura city varies among sources and it has to be treated before reuse. Greywater from restaurant the highest risk (531 CFU of *E. coli*/100 mL), while greywater from laundry the lowest risk (24 CFU of *E. coli*/100 mL). However, greywater from the bathroom and laundry are safe and can be used for toilet flushing. The reuse of greywater on-site should be encouraged with the awareness of risk.

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