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Monitoring of Free Living Amoeba in Some Drinking Water Treatment Plant in Great Cairo Governorate, Egypt

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ABSTRACT
Background: Free-living amoebae (FLAs) in drinking water exerts an indirect public health hazards as they may harbor pathogenic microorganisms that can escape drinking water treatment processes and reach to end user. The aim of this study was to observe the existence of free-living amoebae through drinking water treatment plants in Great Cairo governorate, Egypt. Water samples were collected from 3 drinking water treatment plants (DWTPs) and filtered through nitrocellulose membranes, then placed on non-nutrient agar with *Escherichia coli* for cultivation of free-living amoebae. Results:The obtained amoebae were morphologically identified and confirmed to genus level. The results revealed that the occurrence of free-living amoebae in intakes and finished water of the examined DWTPs reached 95% and 31%, respectively. The removal percentage of free-living amoebae through different treatment processes reached its highest rate in Rod Elfarag DWTP (100%), followed by Embaba DWTP (63.64%), and reached to (41.67%)in Shubra El-Kheima DWTPs. Almost all the morphologically identified (FLA) strains proved to be related to genus *Acanthamoeba, Naegleria*. **Conclusion**: the presence of free-living amoebae in drinking water exerts an indirect public health hazards as they may harbor pathogenic microorganisms that can escape drinking water treatment processes and reach to end user.

Keywords: Free Living Amoeba, drinking water treatment plants, Greater Cairo.

INTRODUCTION

Free-living amoebae are protozoa found in soil and water. Among them, some are pathogenic and many have been described as potential reservoirs of pathogenic bacteria [(1)]. Free-living amoebae (FLA) are a large diverse group of unicellular organisms in the kingdom protozoa. The presence of FLA in tap water may represent a health risk to both immunocompromised and immunocompetent individuals [(2)]. FLA has been described as reservoirs for several pathogenic bacteria, such as Legionella, Mycobacterium and Chlamydia [(2)]. They can be found in two morphological stages trophozoites and cysts. The trophozoite, or vegetative form, corresponds to the period of metabolic activity of the amoeba with division, feeding, and motility whereas the cyst form corresponds to the dormant phase of amoeba that can resist hostile environmental conditions such as nutrient depletion, osmotic stress, temperature changes, pH variations, and disinfection of water supplies [(3)] **.** Free-living amoebae (FLA) are non-parasitic amoebae, completing their life cycle in the environment without requiring a host organism [(4)]. Free-living amoebae colonize different water systems including drinking water or domestic tap water, which accounts for amoebic keratitis among contact lens wearers who use tap water instead of disinfectant to maintain their lenses [(5)]. Members of only four genera of FLAs are opportunistic pathogens causing infections of the central nervous system, lungs, sinuses and skin, mostly in immunocompromised humans. *Balamuthia* is also associated with disease in immunocompetent children, and *Acanthamoeba spp*. cause a sight-threatening infection, *Acanthamoeba keratitis*, mostly in contact-lens wearers. One heterolobosean amoeba flagellate, *Naegleria fowleri* is dangerously pathogenic for humans [(6)]. Genus Acanthamoeba causes three clinical syndromes amebic keratitis, granulomatous amebic encephalitis and disseminated granulomatous amebic disease (eg, sinus, skin and pulmonary infections) [(7)]. *Acanthamoeba* has impact on the human health is associated with their own pathogenicity. *Acanthamoeba* can also cause central nervous system infections, including gorillas, monkeys, dogs, ovines, bovines, horses, and kangaroos, as well as birds, reptiles, amphibians, and fishes[(8)]. The manner of *Acanthamoeba* movement is similar both at solid substrata and at water-air interface. Adhesion forces developed between *Acanthamoeba* and the water-air interface are greater than gravity, and thus amoebae are transported passively without detachment from the water surface. Disinfection is the main practice for controlling the

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wide variety of pathogenic microorganisms and reducing the level of microbiological contaminants transmitted by waters (recreational, drinking or thermal). Despite various data on sensitivity and resistance of FLA to biocides and increasing health concerns over FLA, there is still a lack of information on the mechanisms of action and efficacy of biocides on amoebae in real systems. A few studies have investigated the effects of chlorine on *Acanthamoeba spp.* trophozoıtes and *Acanthamoeba* cysts [9]. *Naegleria fowleri* causes an acute and fulminating meningoencephalitis in immunocompetent children and young adults. Because only one human case of encephalitis caused by *Sappinia diploidea* is known, generalizations about the organism as an agent of disease are premature. In case of chlorine, few studies have explored the inhibitory efficacy of monochloramine on amoebae. Moreover, all such studies were performed in a buffered liquid medium on a pure culture of amoeba species, not representative of natural ecology of the protozoa [10].

The objective of the present work was to monitoring of FLA in some water treatment plant (study in great Cairo governorate, Egypt) and evaluated the occurrence of potentially pathogenic free-living amoebae in three drinking water treatment facilities and the efficacy of conventional drinking water treatment steps for the removal of free-living amoebae from the produced drinking water.

MATERIALS AND METHOD

Sampling sites:

Water samples were collected from three drinking water treatment plants (DWTPs) located in Great Cairo governorate, Egypt (Fig.1).

Three DWTPs namely: Shubra El-Kheima, Embaba and Rod Elfarag are working with rapid sand filtration system. Two types of water were collected from the three mentioned DWTPs: raw (inlet) water and finished (outlet) water. Water samples (one-liter volume each) were collected monthly from each of the previously mentioned three DWTPs. The samples were separately collected in one-liter volume autoclavable polypropylene Stoppard containers. Water samples were collected monthly from each water type along one-year period from October 2016 to September 2017 to represent different seasons of the year.

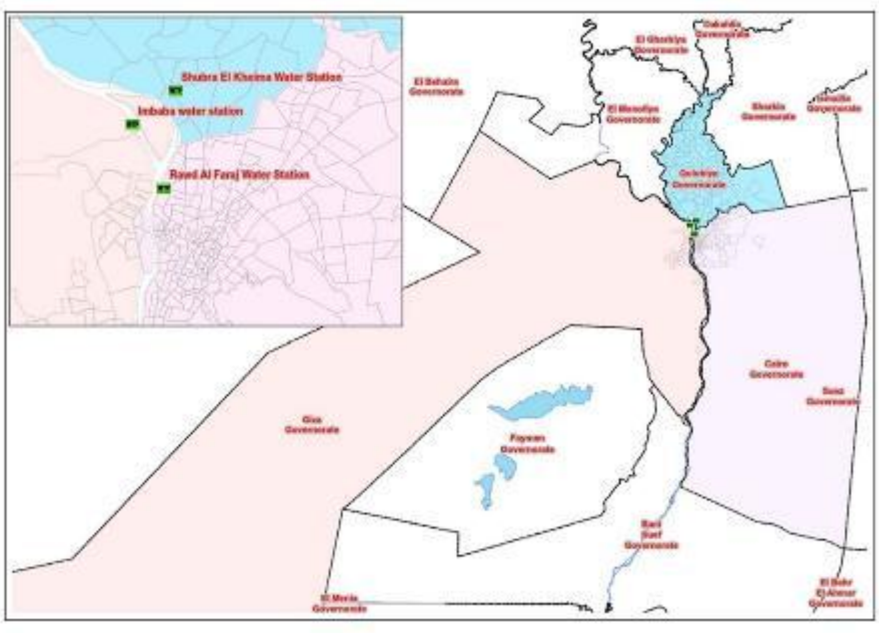

Fig.1. Diagrammatic map for the location of examined drinking water treatment plants in Cairo, governorate.

Concentration, cultivation and isolation of FLAs:

Collected water samples (either raw water or treated water) were separately concentrated using cellulose nitrate membranes (0.45μm pore size and 47mm diameter) using a stainless steel filter holder connected with a suction pump. Filtration was stopped just before drying of the membrane. After filtration process, the membrane was inverted face to face on the surface of a non-nutrient agar (NNA) plate seeded with heat-killed *Escherichia coli*. The plate was wrapped with parafilm and incubated at 37^{o}C to permit growth and multiplication of free-living amoebae existing in water samples (Federation and Association, 2005). Incubated plates were daily examined by inverted microscope (Olympus CXK 41, Japan) for seven days for the presence of any amoebic growth[23].

Morphological identification of isolated FLA:

All cloned amoebae were identified morphologically to the genus level according to the key described by [11]. Isolated members belonging to *Acanthamoeba* were morphologically identified to the species level according to the key of [12]

Statistical analysis

The obtained data were statistically analyzed using Paired T-Test through Minitab statistical program [13].

RESULTS AND DISCUSSION

Occurrence of FLAs in intakes of the examined DWTPs of Great Cairo:

The occurrence of free-living amoebae in water samples collected from the raw water of Shubra El-Kheima DWTP reached (100%), while the occurrence of free-living amoebae in water samples collected from the raw water of Embaba DWTP and Rod Elfarag DWTP reached (91.66%)(Table 1and figures 2 and 3).

Table 1. Occurrence of free-living amoebae in Great Cairo DWTP

Water DWTP	FLA positive Sample on NN agar Finished Water								
	Embaba DWTP			Shobra ElKhima DWTP			Rod El Farag DWTP		
	No of Samples	+ve	%	No of Samples	+ve	%	No of Samples	+ve	%
Raw	12	11	91.66	12	11	91.6i6	12	12	100
Finished	12	4	33.3	12	0	0	12	7	58.3

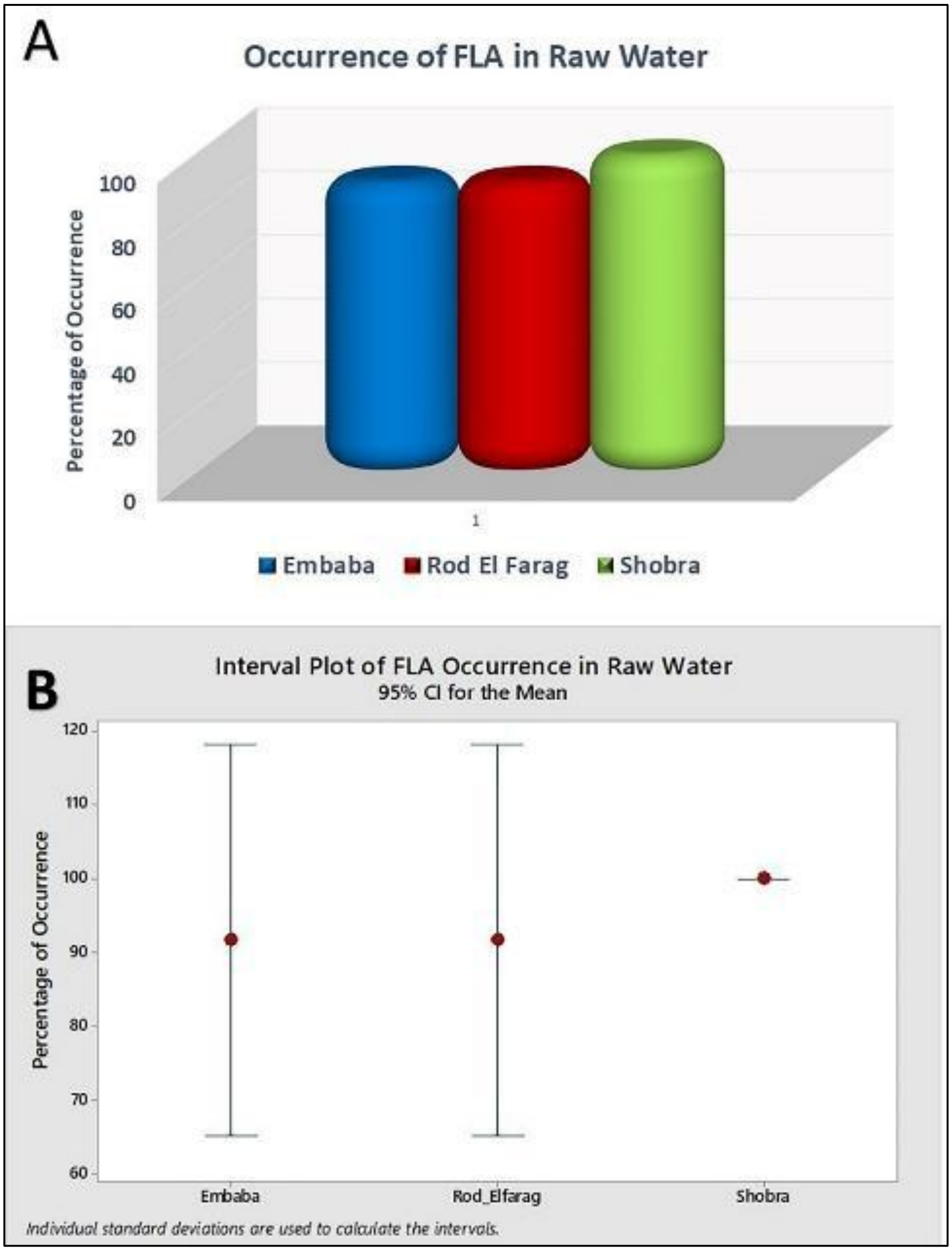

Fig (2): Percentage of occurrence of free-living amoeba in Embaba Rod EL Farag and Shubra El-Kheima (Shobra) DWTPs. where Raw represent the percentage of occurrence in Raw water before treatment.
(A) Column represents Percentage.
(B) Interval plot illustrates both the location and variation in the data. Dots represent the Percentage of occurrence.

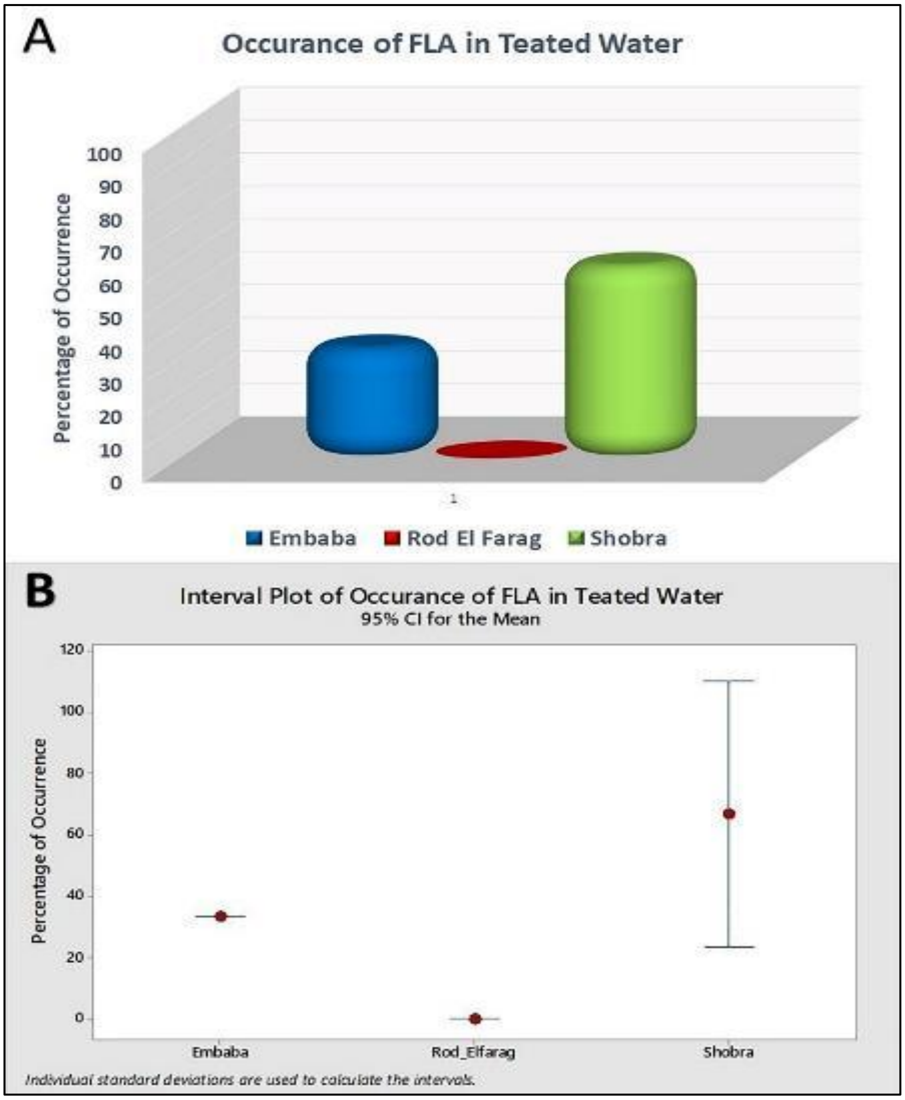

Fig (3): Percentage of occurrence of free-living amoeba in Embaba Rod EL Farag and Shubra El-Kheima (Shobra) DWTPs. Which Raw represent the percentage of occurrence in Raw water before treatment. **(A)** Column represents Percentage. **(B)** Interval plot illustrates both the location and variation in the data. Dots represent the Percentage of occurrence.

On the other hand the occurrence of free-living amoebae in treated water samples of Embaba DWTP reached (33.33%) while the occurrence of free-living amoebae in treated water samples of Shubra El-Kheima DWTP reached (58.33%).The lowest occurrence of free-living amoebae in treated water samples of Rod Elfarag DWTP recorded (0%) respectively (Table 2and figure 3). [(23)] recorded higher occurrences of free-living amoebae in raw water samples from Fayoum governorate and Behera governorates, Egypt (91.7%), (100%). Previous studies revealed that, in the finished water of Damanhur DWTP in Behera governorate Egypt free-living amoebae detected a very lower incidence (25%), (20%)and(4%) in freshwater [(14)]. In other countries of the world, other workers recorded also a lower incidence of free-living amoebae (43.3%) in freshwater samples collected from James River, USA [(15)]. In Bulgaria, [(16)] recorded freshwater amoebae also in a lower percentage (61.1%) than that recorded in the present study that might be attributed to the lower atmospheric temperature in those countries. A higher occurrence of free-living amoebae was recorded in 371 (79%) samples from household distribution systems of Ohio, USA [(17)]. Other studies within the USA and the UK found amoebae in 19% and 48% of households of healthy subjects, respectively. There are factors known to affect the presence of FLAs, such as water source, water treatment method and geographic location, and differences in these across the USA limits the confidence with which these results can be applied to other regions [(18)].

Seasonal variation of FLA in the raw and treated water of examined DWTPs:

By comparing the obtained results from collected data of Embaba, Rod Elfarag and Shubra El-Kheima DWTPs, which represent Great Cairo along different seasons. The results of free-living amoebae in the raw water of Autumn, Winter, Spring and Summer (77.78 %, 100%, 100% and 100% respectively) showed that no significant difference ($p > 0.05$) between them while there were drop of positive sample at autumn season but still not significant ($p > 0.05$) comparing to rest of year. The results of free-living amoebae in the treated water of Autumn, Winter, Spring and Summer (22.23 %, 22.23 %, 33.34% and 55.56% respectively) showed that no significant difference ($p > 0.05$) (Table 2 and Figure 4, 5).

Table 2: Occurrence of free-living amoebae in raw and treated water in selected DWTPs of Great Cairo at different seasons.

Season	FLA positive Sample Percentage on NN agar					
	Raw			Finished		
	No of Samples	+ve	%	No of Samples	+ve	%
Winter	9	7	77.78	9	2	22.23
Spring	9	9	100	9	2	22.23
Summer	9	9	100	9	3	33.34
Autumn	9	9	100	9	5	55.56

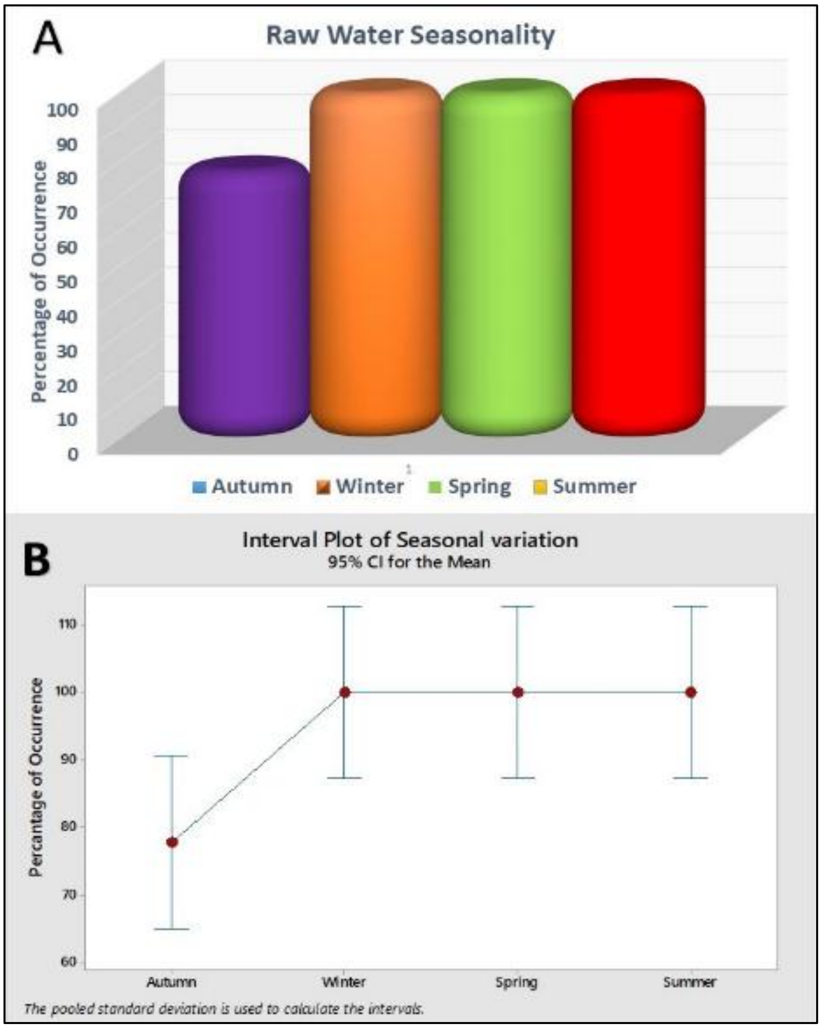

Fig (4): Percentage of occurrence of free-living amoeba in selected Great Ca DWTPs.at different seasons. Where Raw represent the percentage of occurrence in Raw water before treatment. **(A)** Column represents Percentage. **(B)** Interval plot illustrates both the location and variation in the data. Dots represent the Percentage of occurrence.

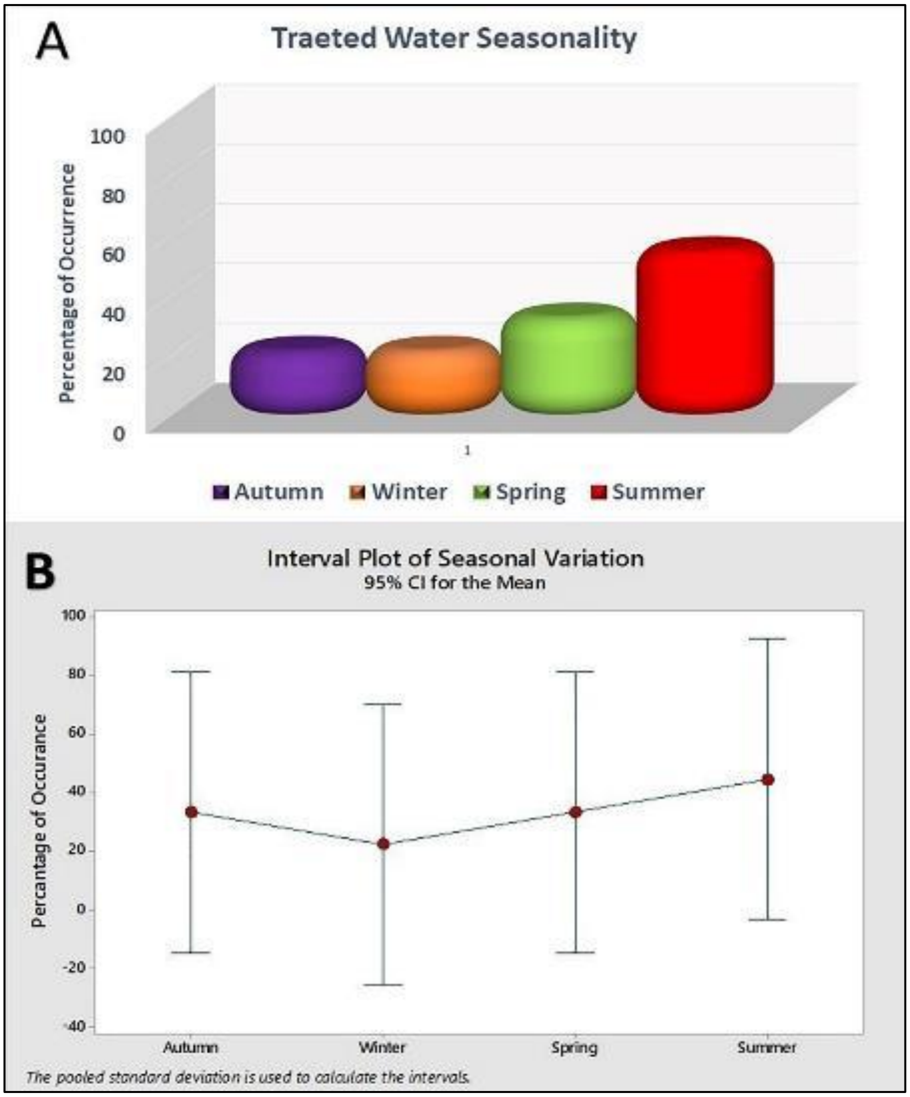

Fig (5): Percentage of occurrence of free-living amoeba in selected Great Ca DWTPs.at different seasons. Which Raw represent the percentage of occurrence in finished water after treatment. **(A)** Column represents percentage. **(B)** Interval plot illustrates both the location and variation in the data. Dots represent the Percentage of occurrence.

These current research results are in harmony with [7]seasons had no significant effect on the prevalence of FLAs in raw water, clearly showed the data collected from selected DWTPs samples which represent Great Cairo along different season of year, they were observed that FLAs prevailed in the warm seasons. Also [19] proved that Prevalence appeared to increase in the summer trial, but without statistical significance (p = 0.096), potentially indicating a seasonal variation. On the other hand [20] showed that seasonal detection rates of *Acanthamoeba* species were the spring (2.9%), summer (32.4%), autumn (2.9%), and winter (8.8%).The greatest percentage of *Acanthamoeba* species was detected during the summer. The results support a previous report that *Acanthamoeba* species were most prevalent in late summer in the aquatic environment [20]. It is possible that water contaminants (such as livestock wastewater and sewage) may be carried into the river after rainfall or riverbed sediment underwent seasonal rainfall in summer that these sediments supported microbial growth [21]. In addition, weather events were found to play a major role in the presence/absence of *Acanthamoeba* species in the river shed, with such changes probably due to resuspension of *Acanthamoeba* species from riverside or riverbed sediment by rainfall or wind action and input from the river shed via run off [20] .The results of [7] were in concordance with the results of the present study. The Present study also showed that the same figure of non-significant between positive samples of DWTPs examined at different season occurred in treated water.

The low percentage of treated water positive samples comparing to raw water were due to efficiency of DWTP treatment. In treated water of rapid sand filtration system DWTP, FLAs were detected in percent 33.3% in each of summer and autumn. In treated water of slow sand filtration system DWTP, the FLAs were recorded only in summer season in percent 33.3%. Statistically, $p = 0.537$, therefore, seasons had no significant effect on the prevalence FLAs in treated water of two different DWTPs. [22]found that free-living amoebae predominated in tap of Greater Cairo in winter (41.7%), followed by summer (25%) while they were evenly distributed in both spring and autumn (16.7% for each).

An increase in *Acanthamoeba*, *Vannella*, and *Naegleria* during the summer has been found in studies of FLA distribution in Oklahoma, Virginia and South Carolina waters [20]. In this investigation, *Acanthamoeba* species were the most prevalent FLAs in the examined water samples. *Acanthamoeba* species were the most widely recognized opportunistic amphizoic protozoa in water[20].

Efficiency of DWTPs for the removal of FLAs:

It was found that the removal percentage of FLA through different treatment processes reached its highest rate in Rod El farag DWTP (100 %) followed by Embaba DWTP (63.64%) then Shubra El-Kheima DWTP (41.67 %) (Table 3 and Figure 6).

Table (3): Eefficiency of DWTPs for the removal of FLA

DWTP	Precentage of Positive Samples		Removal
	Raw	Treated	
Embaba	91.66	33.33	63.64
Rod El Farag	91.667	0	100
Shobra Elkhiema	100	58.33	41.67

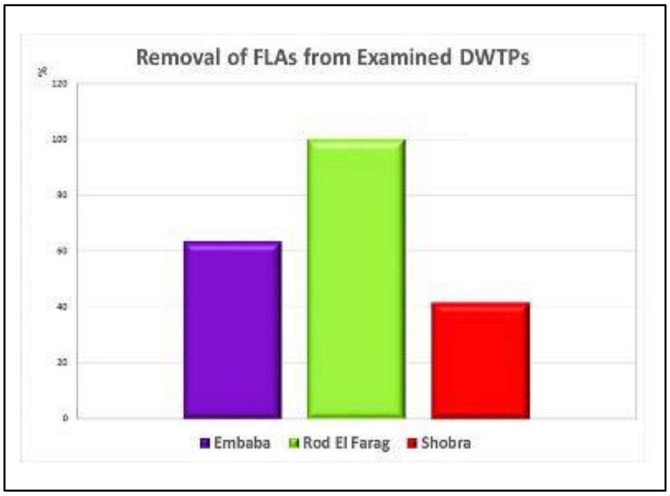

Fig (6): Efficiency of selected Great Cairo DWTPs for the removal of FLAs.

Concerning elimination of free-living amoebae during drinking water treatment process in the present work, [23]estimated that other workers in Egypt, recorded that the treatment processes applied to different stages of drinking water treatment production in Damanhour DWTP, Behera governorate, could remove 75% of FLAs present in the inlet water [23]. This record was higher than that of the present study, although all inlet samples of Damanhour DWTP harbored FLAs. The higher removal of FLAs from Rod Elfarag DWTP (100%) was an indication that filtration system is efficient and the treatment steps were efficiently processed.

Since chlorine was introduced into water treatment nearly 80 years ago, it has become almost the only method used for the active disinfection of potable water supplies [10]. In consistent with some studies [23] this predominant position of chlorine has been gained because of its potency and range of effectiveness as a germicide; its ease of application, measurement, control, and economy; its relative freedom from toxic or physiological effects; and its reasonable persistence in waters. It is thought that the difference in susceptibility to chlorine between the two genera (*Naegleria* and *Acanthamoeba*) is more likely to be a consequence of the difference in chemical (especially protein) composition of the cell membranes than of the difference in metabolism.

Recently, it was reported that the Ct values (in mg min/L) required for 2-log reduction of *Acanthamoeba*, *Naegleria* and *Hartmannella* cysts treated with chlorine reached 865, 29 and 156, respectively.

On the other hand, the same organisms this present study clearly showed that usually used chlorine doses (2-7 mg/L) for disinfection of the produced water in drinking water treatment plants were ineffective for killing free living amoebae.

Morphological identification of isolated FLA:

In this study, we examined the presence and concentration of *Acanthamoeba spp*, *Acanthamoeba* can be morphologically recognized namely *A. astronyxis*, *A. royreba*, *A. comandoni*, *A. culbersoni* , *A. quina*, *A. palasteinensis* according to the key of [16].

Table 7: Morphological charcter of isolated *Acanthamoeba* cyst of different species

***Acanthamoeba* isolates**	**Cyst diameter**	**Endocyst**	**Ectocyst**
Acanthamoeba astronyxis	19–22 µm	Stellate	Very delicate, not wrinkled
Acanthamoeba royreba	14–16 µm	Nearly round	Slightly wrinkled
Acanthamoeba comandoni	20–25 µm	Stellate	Delicate, not wrinkled
Acanthamoeba culbertsoni	13.5–15 µm	Not predominantly stellate, nearly rounded with slight angles	Little, slightly wrinkled
Acanthamoeba quina	12–13 µm	Firmly prominent	Noticeably wrinkled
Acanthamoeba palestinensis	17–18 µm	Nearly round with slight angles	Closely encircling endocyst

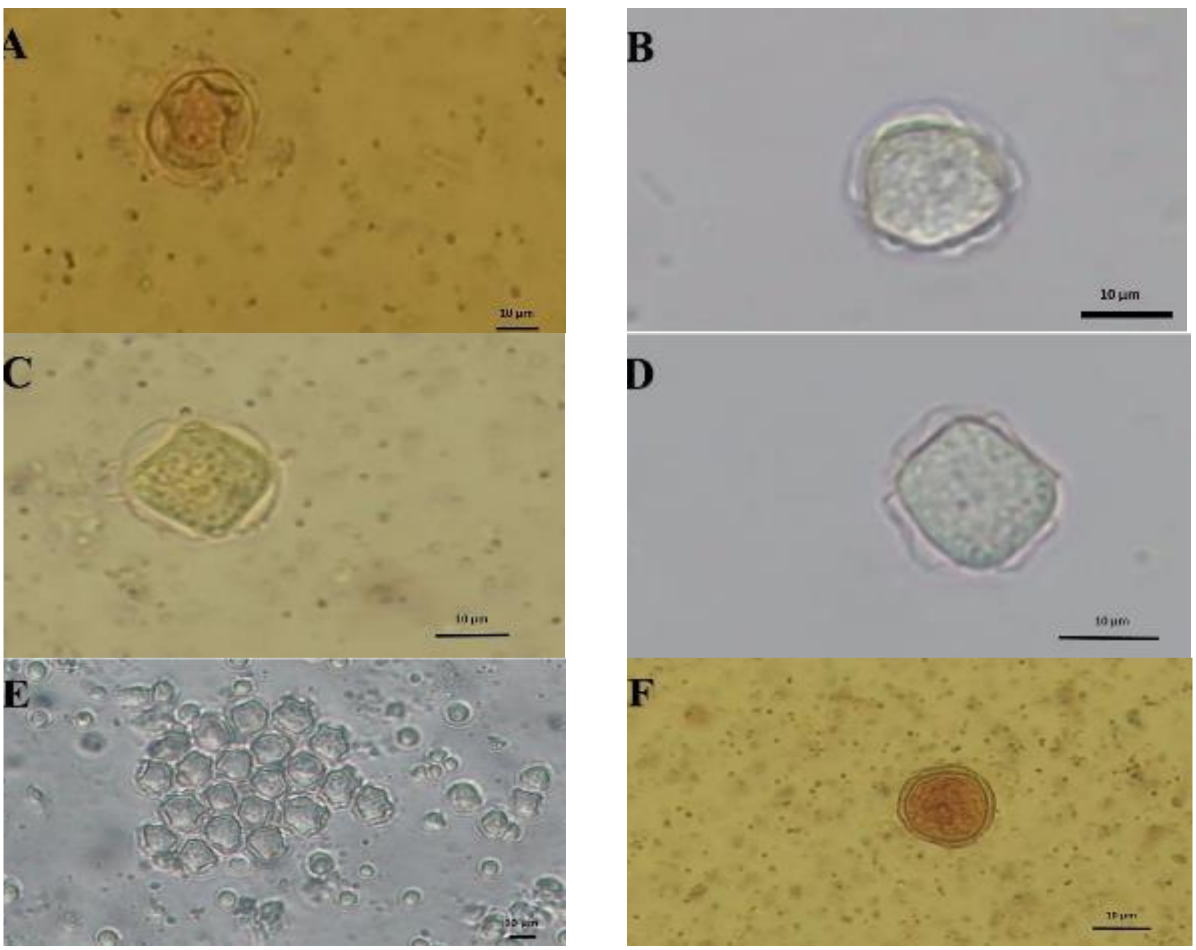

Figure 7: Photomicrograph showed different *Acanthamoeba* cyst morphology A; *Acanthamoeba astronyxis* , B; *Acanthamoeba royreba*, C; *Acanthamoeba comandoni*, D; *Acanthamoeba culbertsoni* , E; *Acanthamoeba quina* , F; *Acanthamoeba palestinensis*. B,D and E unstained. A, C and F stained with Lugol's iodine.

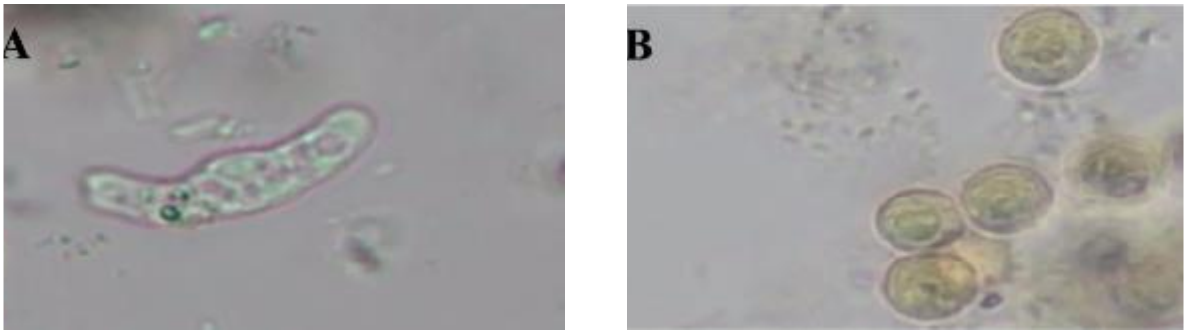

Figure 8: Photomicrograph showed A: Trophozoite (unstained) B: Cyst of *Naeglaria sp.* (stained with Lugol's iodine).

[24] photographed morphologically different species of environmental isolates of *Acanthamoeba* but they did not name them. They had described the mature (resting) cyst previously, as it was spherical with a double wall, an outer exocyst that was continuous over the entire surface of the cyst, and an inner endocyst that was discontinuous in an area referred to as the ostiole. A layer of refractile granules was embedded in the margin of the cytoplasm [12]. The morphologic cyst characters of a new species of Acanthamoeba isolated from a freshwater fish in Korea and tentatively named Korean isolate YM-4 were similar to those of *A. culbertsoni* and *A. royreba*, which were previously designated as Acanthamoeba group III. This new species was identified and signed *Acanthamoeba sohi* [25]. Trophozoites of Naegleria were long slender or oval measuring from 12-25μm in length and 10-20μm in width. The nucleus had a distinct nuclear membrane and a centrally located prominent nucleolus. The cyst was spherical with a round, smooth double wall and measured 8-15 μm in diameter. At the level of ordinary microscopy, it was clear that cysts of different *Naegleria* species were too similar morphologically to be distinguished from each other. Other workers in Egypt were in concordance with the results of the present study they recorded occurrence of *Naegleria* species in tap water samples [22].

CONCLUSION

Acanthamoeba were the most prevalent in treated water. The presence of potentially pathogenic *Acanthamoeba* species and *Naegleria* in drinking water may lead to disorders for the users. Moreover, the presence of other free-living amoebae in drinking water exerts an indirect public health hazards as they may harbor pathogenic microorganisms that can escape drinking water treatment processes and reach to end users. Our data clearly showed that the removal of FLAs was significantly effective through drinking water treatment process by selected Great Cairo DWTPs. In addition, data proved that season difference not affect presence of FLAs in raw water or treated water.

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