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Potentially pathogenic free-living amoebae in fresh and sea waters from Alexandria, Egypt

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ABSTRACT

A total of 48 samples from two different water bodies;24 from fresh and 24 from marine waters (Alexandria) were collected, filtered, cultured on non-nutrient agar and incubated at 37ºC for the presence of potentially pathogenic FLAs. Amoebae were isolated and identified by means of their morphological characteristics of both trophic and cyst stages. After collection for one year, it was found that freshwater samples had a higher occurrence of FLAs (83.3%) than sea water samples (37.5%). The highest occurrence percentage of FLAs was 100% in freshwater and 83.3% in sea water samples in summer, while their lowest values reached 66.7% and 16.7% in autumn Respectively. Morphological identification of these isolated amoebae revealed the presence of FLAs belonging to 5 genera namely *Acanthamoeba*, *Naegleria*, *Vahlkampfia*, *Vermamoeba* as well as *Vannella*.

INTRODUCTION

Free-living amoebae are ubiquitous Protozoa that can grow and survive without a host, having their natural habitats in the environment for example: air, soil, drinking water, swimming pools and bottled mineral water (**Rivera *et al*., 1981; Gad and Al-Herrawy, 2016**). Humans are continually exposed to these amoebae due to their ubiquitous occurrence in the environment (**Di Filippo *et al*., 2015**). FLAs known to cause disseminated and fatal central nervous system dysfunction which manifests as granulomatous amoebic encephalitis (GAE), pulmonary and kidney infections, nasopharyngeal and cutaneous lesions, primarily in immunocompromised patients **(Marciano-Cabral and Cabral, 2003)**. *Acanthamoeba* spp. cause amoebic keratitis in immunocompetent persons **(Ahearn and Gabriel, 1997; Illingworth and Cook, 1998)**. Another species, *Balamuthia mandrillaris* close relative to *Acanthamoeba*, causes skin and lung infections as well as fatal GAE mostly in healthy children, *Naegleria fowleri* causes a non-opportunistic primary amoebic meningoencephalitis (PAM) in healthy children and young adults **(Gelman *et al*., 2001**). *Sappinia pedata* has also been reported from a brain infection in a healthy man (**Fiester *et al*., 2019**). Further, *Vahlkampfia*, *Vannella* as well as *Vermamoeba* species have been isolated from the eye surface of humans (**Al-Herrawy and Gad, 2017**).

Considering such an emerging worldwide interest in studying soil and fresh water pathogenic FLAs, it is surprising how little is known about sea water pathogenic FLAs. Few studies have so far been carried out on sea water amoebae **(Schaeffer, 1926; Page, 1978; Sawyer 1980 and Sawyer and Lewis, 1987).**

The epidemiology studies on free-living pathogenic amoebae from both fresh and sea water have been conducted all over the world but their spreading in the environment in Egypt is still poorly understood. So, the aim of the present study was to assess the prevalence of potentially pathogenic free-living amoebae for one year from two different water bodies; fresh and sea from two localities in Alexandria.

MATERIALS AND METHODS

Water samples

A total of 48 (24 fresh- and 24 sea water) samples were collected from two different localities in Alexandria. The former was El- Mahmoudiya Canal (Down Muharram Bey Bridge) which represents natural fresh water, while the latter was the beach of Al Mamoura which represents natural sea water. Samples were regularly collected every two weeks for one-year from January to December 2018. Samples were separately collected in sterile autoclavable polypropylene plastic bottles from subsurface water. All samples were transported at ambient temperature to Environmental Parasitology Laboratory, Water Pollution Research Department, National Research Center, Giza where they were processed at the same day of collection.

Cultivation and morphological identification of amoebae

Collected water samples were separately concentrated using the membrane filtration technique as follows: each water sample (One-liter volume) was filtered through a nitrocellulose membrane filter (0.45µm pore size and 47mm in diameter) using a stainless-steel holder, connected with a suction pump. The membrane of each filtered water sample was face to face inverted on the surface of non-nutrient (NN) agar medium seeded with living *E. coli* bacteria and incubated at 30 °C for two weeks with a daily microscopic examination using the inverted microscope (**Health Protection Agency, 2004**). Plates proved to have FLAs were sub-cultured and cloned on new NN agar plates seeded with *E. coli* for morphological examination. All cloned amoebae were evaluated with their morphological criteria of both trophozoite and cyst stages, according to the key represented by **Page (1988**).

RESULTS

Prevalence of FLAs in isolated water samples

Examination of 48 samples (24 freshwater from El- Mahmoudiya Canal and 24 sea water from Al Mamoura beach) after collecting for one year revealed that the highest percentage of FLAs in freshwater samples was 83.3% while, the lowest percentage was recorded in sea water samples (37.5%), based on the morphological criteria of both trophic and cyst stages in NN agar culture **(Chart 1)**.

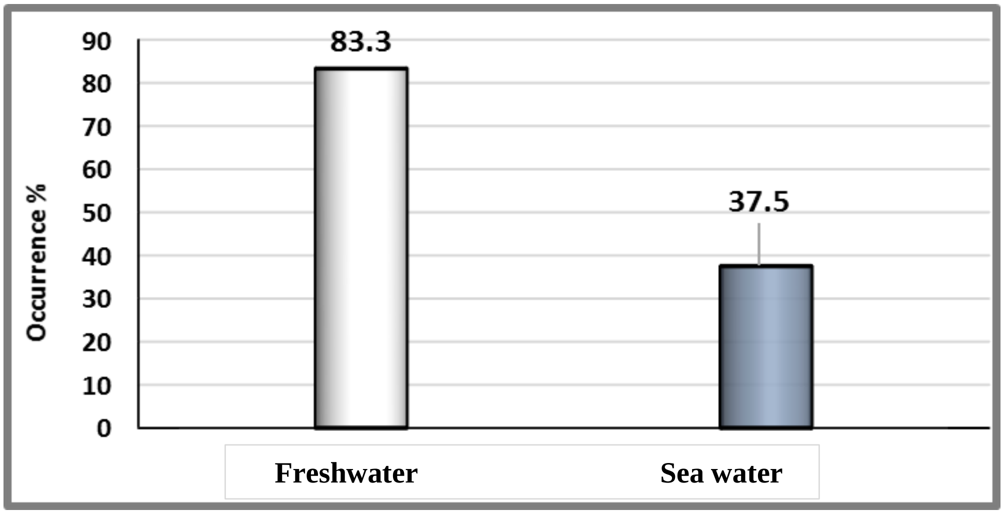


Chart 1: Showing the prevalence of FLAs in isolated water samples.

Monthly occurrence of FLAs in different isolated water samples

The highest occurrence percentage of FLAs in fresh water samples was 100% except those of April and June which decreased to 50% and disappeared completely during December. On the other hand, the highest occurrence percentage of FLAs in sea water samples was 100% in September and July only which declined to 50% in March, April, May, August and November. FLAs were not detected in sea water samples for 5 months: January, February, June, October and December (**Table 1 and Chart 2)**.

The highest occurrence percentage of FLAs in fresh water samples was 100% in both summer and winter and decreased to 66.7% in both spring and autumn. Whereas, the highest occurrence of FLAs in sea water samples was 83.3% in summer and decreased to 33.3% in spring. The lowest occurrence of FLAs in sea water samples was 16.7% in winter, while FLAs were not detected in sea water samples in autumn **(Table 2 and Chart 3)**.

Table 1: Incidence of FLAs in different isolated water samples

Months	Fresh water			Sea water		
	examined	positive	%	examined	positive	%
January	2	2	100	2	0	0
February	2	2	100	2	0	0
March	2	2	100	2	1	50
April	2	1	50	2	1	50
May	2	2	100	2	1	50
June	2	1	50	2	0	0
July	2	2	100	2	2	100
August	2	2	100	2	1	50
September	2	2	100	2	2	100
October	2	2	100	2	0	0
November	2	2	100	2	1	50
December	2	0	0	2	0	0

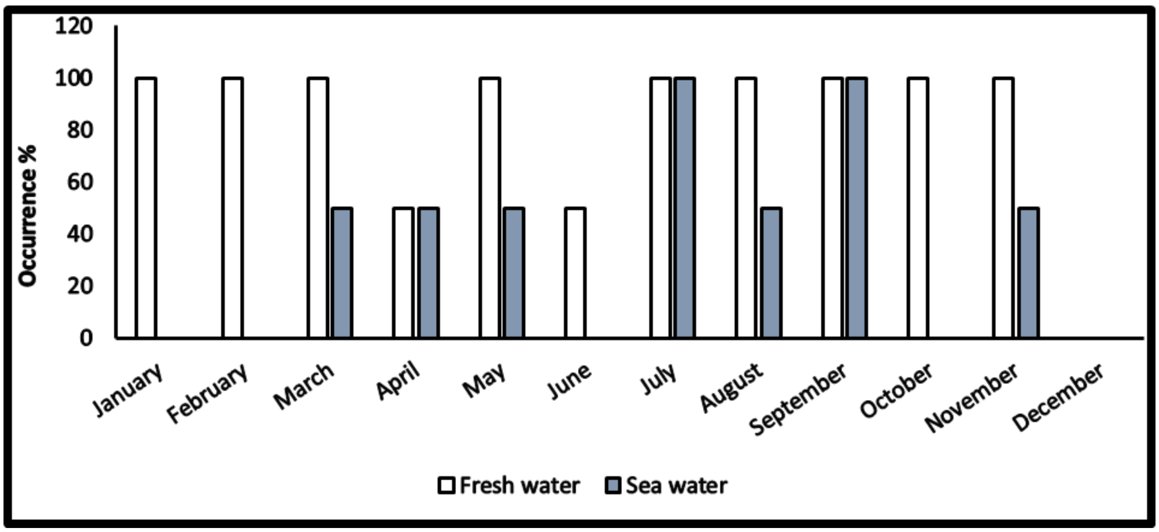


Chart 2: Monthly occurrence of FLAs in different isolated water samples.

Seasonal occurrence of FLAs in isolated water samples

Table 2: Seasonal occurrence of FLAs in different isolated water samples

Seasons	Fresh water		Sea water	
	Examined	Positive	Examined	Positive
Winter January-March	6	6	6	1
Spring April-June	6	4	6	2
summer July- September	6	6	6	5
Autumn October-December	6	4	6	0

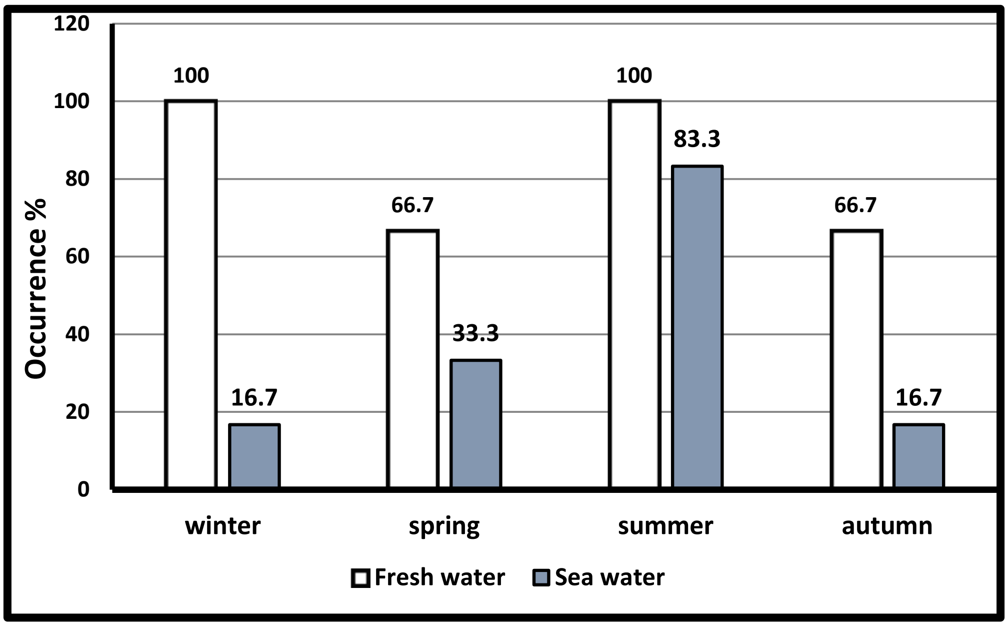


Chart 3: Representing seasonal occurrence of FLAs in isolated water samples.

Incidence of detected FLAs

Examination of FLAs revealed the presence of several amoebae species belonging to 5 different genera: *Acanthamoeba*, *Naegleria*, *Vahlkampfia*, *Vermamoeba* and *Vannella*. The occurrences of these amoebae all over the year indicated that the highest prevalence of FLAs of genus *Acanthamoeba* was recorded in fresh water samples (33.3%) while, the lowest occurrence was recorded in sea water samples (29.2%). Whereas, FLAs belonged to *Vermamoeba* had the same prevalence (4.2%) in both fresh and sea water samples, FLAs belonged to *Naegleria*, *Vannella* and *Vahlkampfia* were 29.2, 12.5 and 12.5%, respectively in only fresh water samples and without any detection of them in sea water samples **(Table 3; Chart 4 & Figures 1-8)**.

Table 3: Incidence of detected FLAs in different water samples

Isolated FLAs	Fresh water	Sea water
	Positive /total examined	Positive /total examined
***Acanthamoeba* sp.**	**(8/24)**	**(7/24)**
***Naegleria* sp.**	**(7/24)**	**(0/24)**
***Vahlkampfia* sp.**	**(3/24)**	**(0/24)**
***Vermamoeba* sp.**	**(1/24)**	**(1/24)**
***Vannella* sp.**	**(3/24)**	**(0/24)**

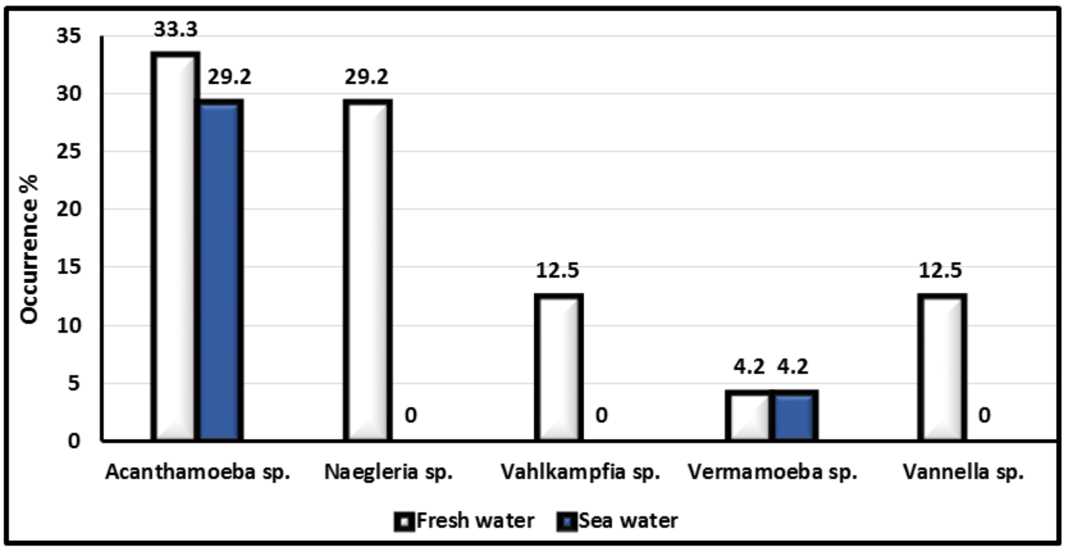
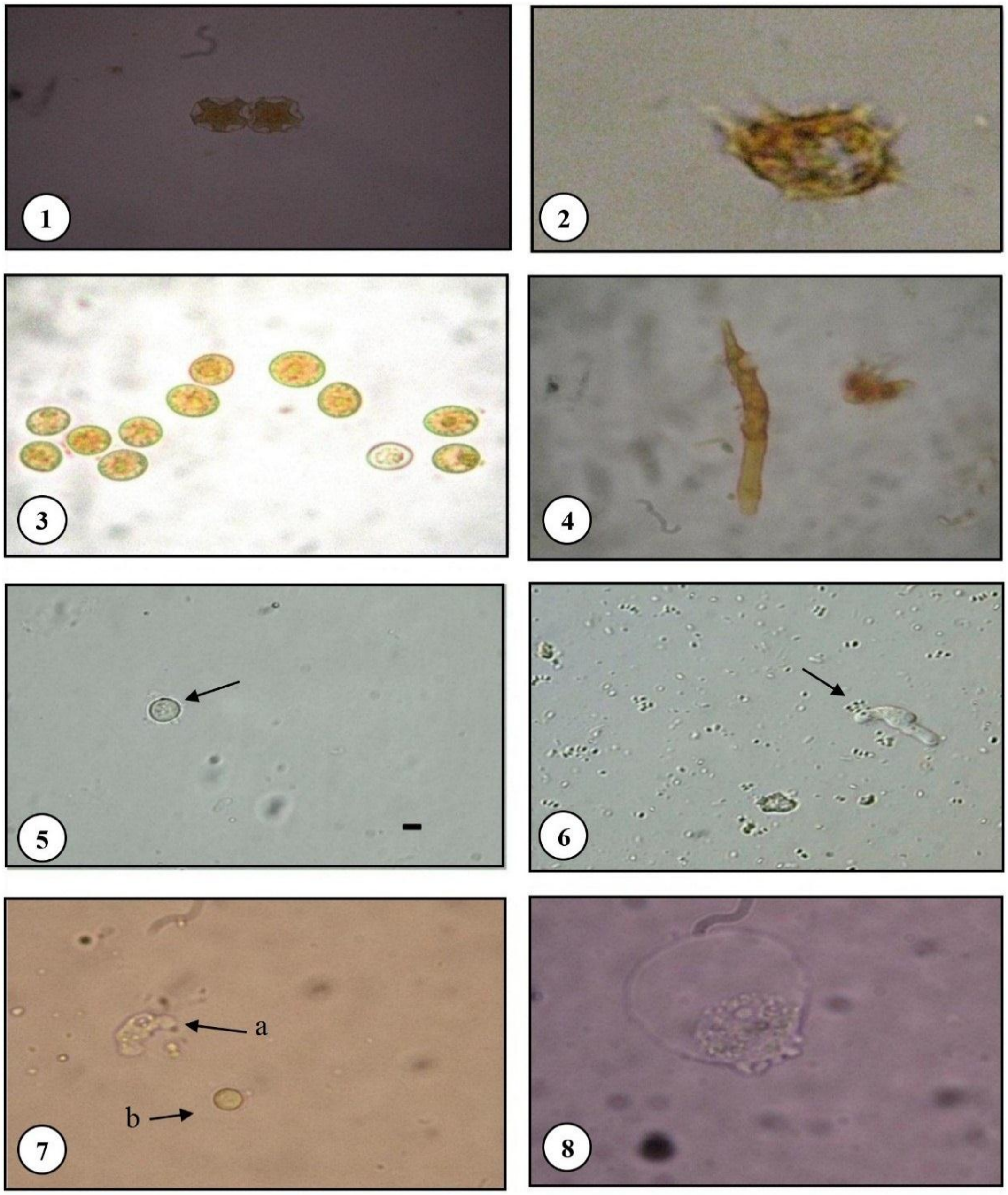


Chart 4: Representing the incidence of detected of FLAs in different water samples.



Figs. (1-8): Photomicrographs of the isolated free living amoebae**:**
Figs. (1&2): *Acanthamoeba* sp. (cyst and trophozoite, respectively)**;**
Figs. (3&4): *Vermamoeba* sp. (cyst and trophozoite, respectively)**;**
Figs. (5&6): *Vahlkampfia* sp. (cyst and trophozoite, repictevely)**;**
Fig. (7):*Naegleria* sp. (a) trophozoite and (b) cyst**;**
Fig. (8): *Vannella* sp. trophozoite. Bar = 10 µm**.**

DISCUSSION

Few studies were conducted reporting the detection and existence of FLAs in sea water in Egypt **(Salahuldeen, 2017)**. The current results showed that the prevalence of free-living amoebae in fresh and sea water for one year was 83.3% and 37.5%, respectively. These results may indicate that FLAs were generally common in water samples, with salinity 29.44g / l. Salinity tolerance would also seem relevant and useful for distinguishing between ‘freshwater / soil’ amoebae and ‘marine’ amoebae **(Mbugua, 2008)**. Several studies recorded that naked amoebae showed different responses to salinity although most seemed to grow over wide ranges **(Mbugua, 2008)**. Additionally, FLAs can resist treatment with copper-silver, chlorine, chlorine dioxide, ozone and monochloramine **(Thomas *et al*., 2004)**. Such resistance of FLAs is due to their cyst walls which contain cellulose that forms a physical barrier against chlorine **(Thomas *et al*., 2010)**. **Storey *et al*. (2004)** reported that some amoebae cysts can survive chlorine concentrations as high as 100 mg/L for 10 minutes. Moreover, it was reported that water heating enhances the proliferation of thermophilic strains **(Griffin, 1972)**. Being cyst-formers, many amoebae are able to survive for long periods under extreme conditions of temperature **(Dimmick *et al*., 1979; Lundholm, 1982)**.

On one side, the present study showed that highly percentage of FLAs was 83.3% in fresh water samples from El- Mahmoudiya Canal (Down Muharram Bey Bridge) more than that previously recorded in Egypt **(Al-Herrawy *et al*., 2013),** they found the highly percentage of FLAs isolated from Nile water, too was 56.0%. In Virginia, USA, **Ettinger *et al*. (2003)** reported 43.3% FLAs in freshwater samples collected from the James River. In Taiwan, **Hsu *et al*. (2014**) detected 14.3% FLAs (*Acanthamoeba* and *Naegleria*) from fresh water samples. In Bulgaria, **Tsvetkova *et al*. (2004)** detected 52 out of 75 (69.33%) positive samples for FLAs natural fresh water samples, in addition to 31 out of 33 (93.93%) FLAs from rivers water samples. They also found 14 out of 18 (77.77%) positive samples for FLAs from lacks water samples, 4 out of 10 (40%) positive for FLAs from springs water samples and 28 out of 46 (60.86%) positive samples from artificial freshwater reservoirs. The difference in detection rates of FLAs in different countries and localities may be influenced by geographical conditions and water sources **(De Jonckheere, 2011; Kao *et al*., 2013)**.

On the other side, the current study showed that the occurrence of FLAs was 37.5% in 24 sea water (Mediterranean Sea) samples. While, **Mbugua, (2008)** detected 66.7% FLAs from 24 Mediterranean Sea samples in Egypt, too.

The current investigation showed that the incidence of *Acanthamoeba* spp. in different fresh and sea water samples were higher than that of *Naegleria* species. This finding was in accordance with that obtained from Malaysia **(Init *et al*., 2010)** and Taiwan **(Tzeng *et al*., 2013),** where *Acanthamoeba* spp. had a higher prevalence compared to *Naegleria* spices in fresh water. The reason underlying the much lower prevalence rates of *Naegleria* spices might be due to the considerably weaker survivability of the *Naegleria* spices cyst, which do not survive beyond 6 months. Also, the relatively low prevalence of *Naegleria* spices in the examined water samples might be associated with the higher sensitivity of their cysts to chlorination **(De Jonckheere and Van De Voorde, 1976)**. While, *Acanthamoeba* spp. cysts can live for more than 20 years **(Mazur *et al*., 1995)**. **Init *et al*. (2010**) suggested that the thick double-walled cysts of *Acanthamoeba* spp. are more resistant, thus remain viable in the dry-hot areas whereas, *Naegleria* spices cyst are fragile and susceptible to desiccation, thus they prefer watery or moist areas for growth and proliferation.

The present work revealed that FLAs were detected in fresh water samples over the year, except December, while, in sea water samples, FLAs were not detected in 5 months: January, February, June, October and December. However, FLAs were more prevalent in both fresh water and sea water in summer months. **Salahuldeen (2017)** detected FLAs in sea water samples in Egypt over the year except 3 months only: November, December and January, although they were more prevalent in summer**.** Several studies **(Hoffmann and Michel, 2001; Tsvetkova *et al*., 2004; Muchesa *et al*., 2015)** documented that the density and diversity of FLAs were influenced by warmer seasons of the year.

In the current study, *Vermamoeba* species was detected in both sea and fresh water samples. However, **Salahuldeen (2017)**, detected *Vermamoeba* species in sea water samples only in Egypt, while **Ettinger *et al*. (2003)** isolated *Vermamoeba* species from James River near Williamsburg, Virginia, USA.

Vermamoeba species was detected in the present investigation in 4.2% (1/24) from fresh water samples, whereas, **Hsu *et al*. (2014)** in Taiwan detected 9.2% (6/65) of *Vermamoeba vermiformis* from Puzih River. The present results showed that *Acanthamoeba* spp. were the most prevalent amoebae in both fresh and sea water samples. *Acanthamoeba* spp. were the only amoebae that could survive in high saline environments such as seawater and ocean **(De Jonckheere, 1987&1991).**

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