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Aquatic Fungi Recovered from Water and Submerged Mud Polluted With Industrial Effluents

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Abstract: Nineteen species were recovered belonging to 9 genera of aquatic fungi in addition to unidentified species of *Aphanomyces, Pythium* and *Saprolegnia*. These species were isolated from surface water and submerged mud samples collected from canal polluted with industrial effluents of Kima factory for fertilizers and River Nile during the period from January to December 1996 at Aswan region, using baiting technique of sesame seeds at 22±1 °C. *Pythium, Nowakowskiella* and *Saprolegnia* contributed the broadest spectra of species. *Pythium* (5 identified in addition to unidentified species), *Nowakowskiella* (4 species), *Saprolegnia* (3 identified and unidentified species), whereas the other aquatic fungal genera of the narrowest spectra of species.

Key words: Aquatic fungi, mud, industrial effluents

Introduction

The occurrence, distribution and seasonal variations of aquatic fungi in relation to water and soil characteristics as well as to the various geographical regions all over the world have been intensively studied for one century (e.g. Willoughby and Rig, 1983 in Spain; El-Hissy, 1994 in Germany; Willoughby, 1962 and Smith *et al*., 1984 in UK; Karling, 1967 and 1968 in New Zealand; Johnson, 1971 in Iceland; Johnson, 1977 in Scandinavia; Sherry, 1984 in Canada; Czeczuga *et al*., 1990 a,b and Czeczuga 1991 a, 1994, 1995a, 1996 in Poland; Cespedes and Castillo, 1982 in Mexico; Fox and Wolf, 1977 and Ulken, 1979 in USA; Rattan *et al*., 1978, 1980 and Al-Saadi *et al*., 1979 in Iraq; Tojo *et al*., 1992 in Japan; Ann and Ko, 1985 in Taiwan; Yung and Stenton, 1964 in Hong Kong; Misra 1982, Gupta and Mehrotra, 1989 and Khulbe 1991 in India Ogbona and Alabi 1991 in Nigeria.

Also, in Egypt several investigation were made dealing with both of regional variations and seasonal fluctuations of aquatic fungi from different fresh water and wetted soil habitats (e.g El-Hissy *et al*., 1994, 1997; El-Hissy and El-Nagdy, 1983 and El-Hissy and Khallil, 1989). Only very limited ivestigations were performed to study the effect of fresh water pollution with sewage effluents (El-Nagdy and Khallil, 1991) and super-phosphate (Khallil and Abdel-Sater 1992). This investigation aims to study the occurrence and distribution of aquatic fungi from water and submerged mud polluted with industrial effluents of Kima factory for fertilizers and River Nile at Aswan region.

Materials and Methods

Collection of Samples: Monthly marginal surface water and submerged mud samples were collected from canal polluted with industrial effluents of Kima factory for fertilizers and from River Nile at Aswan region during January to December 1996. Water samples were collected using sterile glass bottles containing a number of sterile sesame seeds for baiting the fungal propagates. Submerged mud samples were stored at 10-12 °C during experimentation. Five sites were selected along the polluted canal by the industrial effluents of Kima factory for fertilizers and River Nile that located 1km N the point of mixing effluents of polluted canal of Kima factory with the River Nile. These sites and samples (Fig. 1) were named as follows:

S1:Surface water samples (SWS) at the beginning of discharging the effluents of Kima factory in the canal water, S2: SWS at 1km north S1,S3: SWS at 3 km north S1, S4: SWS at the beginning of mixing the effluents of polluted canal of Kima factory with River Nile water and S5: SWS from River Nile after 1km north the point of mixing the effluents of polluted canal of Kima factory with the River Nile water. Submerged mud samples (SMS) were also taken at the same locations of surface water samples and named as: M1, M2, M3, M4 and M5 (Fig. 1).SWS were analyzed for determination of temp.and dissolved oxygen. SWS and SMS were analysed for estimation of pH, total soluble salts, organic matter content, calcium, magnesium, sulphate, nitrate, phosphate and phosphorus.

Recovery of aquatic fungi from water samples: For baiting and recovery of aquatic phycomycetes, the water samples inside bottles containing sesame seeds were poured in Petri-dishes (12 cm in diameter). These plates (5 for each sample) were left overnight at room temperature (20 ± 2 °C) to allow colonization of seeds by fungal zoospores (El-Hissy and Khallil, 1989). After 24 h., the colonized sesame seeds were transferred into other Petri-dishes (10 cm in diameter), which contained sterilized filtered Nile or distilled water and crystalline penicillin (2000 units per liter water) to inhibit bacterial growth (Roberts, 1963). These dishes were examined weekly for indentification. The recovered aquatic fungi were purified on glucose-peptone (GP) agar medium (Willoughby and Pickering, 1977).

Recovery of aquatic fungi from submerged mud samples: The baiting technique was also used for the recovery of aquatic phyomycetes from mud samples (El-Hissy and Abd-Elaah, 1989). 50 g of each mud sample (5 plates for each samples) were introduced into clean sterile Petri-dishes, (12 cm in diameter). The mud sample in each Petri-dish was then flooded with sterile distilled water with the addition of few sterilized sesame seeds. Examination, identification and purification of aquatic fungi were carried out as described for water samples.

Identification of aquatic fungal genera and species: The following references were used for the identification of aquatic fungal genera and species recovered during this investigation: Karling (1977), Rattan *et al*. (1978) and Van Der Plaats-Niterink (1981).

Results and Discussion

Nineteen species (Table 1) belonging to nine genera of aquatic fungi in addition to unidentified isolates of *Aphanomyces*, *Pythium* and *Saprolegnia* were recorded. *Pythium*, *Nowakowskiella* and *Saprolegnia* contributed the broadest spectra of species. *Pythium* was represented by 5 identified in addition to unidentified species which were the commonest. *Nowakowskiella* was represented by 4 identified species of which *N.multispora* was the most prevalent. *Saprolegnia* was represented by 3 identified in addition to unidentified species of which *S.parasitica* was the most dominant. Submerged mud samples were richest in aquatic fungi where 14 identified and 3 unidentified species were obtained whereas only 12 identified and 3 unidentified species were isolated in case of surface water samples.

The majority of the recoverable aquatic fungal genera and species during this investigation were previously recorded in Egypt from the River Nile near Assiut by El-Hissy *et al.* (1982), from Damietta and Rosetta branches of the River Nile (Delta region) by El-Hissy and Khallil (1989), from Egyptian soils (Delta region) by El-Hissy *et al.*, 1994 and from the Nile water near Sohag by Khallil *et al.* (1993).

Away from the River Nile, El-Nagdy and Abdel-Hafez (1990) found that *Allomyces*, *Achlya*, *Dictyuchus*, *Pythium* and *Saprolegnia* were common in water samples collected from some ponds of Kharga Oases.Similar results were also obtained from submerged mud of Aswan High Dam Lake. El-Hissy *et al.* (1997) and El-Hissy (1994), in Germany, found that *Saprolegnia*, *Pythium* and *Aqualinderella* occurred in high incidence in the surface water samples collected from different localities in Tübingen region whereas *Achlya*, *Dictyuchus* and *Pythiopsis* were moderately recovered in the same samples.

The results presented in Table (1), and Fig. 2 show that the richest sites in aquatic fungi isolated from surface water samples were sites 5 and 4 (78 and 67 colonies/ 5 plates, respectively) and these characterized by relatively low or moderate temperature (18-29 °C) and slightly low nitrate concentration (1.13-282.21 mg/L). The poorest site was number one (5 colonies/ 5 plates) which characterized by relatively high temperature (26.1-36.6 °C) and nitrate concentration (19.86-459.77 mg/L). Although there were noticeable variations in the monthly counts of total fungi, the seasonal fluctuations of the recaquatic fungi were of irregular pattern in the most tested sites. The climax of total fungal counts was exhibited in February 1996 in case of S1 and S5 (4 and 15 colonies/5 plates, respectively), in May 1996 in site 2 (6 colonies/5 paltes), in June 1996 in site 4 (17 colonies/5 plates) and during February, May, August and December 1996 in site 3 (5 colonies/5 plates each month). These months have a temperature range between 16.8-31.7°C and pH value between 4.08-9.58 at the different sites.

The highest fungal population (91 colonies/5 plates in all samples) was recovered from submerged mud sample of M5 at the River Nile. In the different sites, the counts of total fungi did not show any regular seasonal periodicity (Table 1, Fig. 2). However, the highest monthly counts were almost recorded during low or moderate temperature months. The monthly peak of total fungi was attained during November 1996 (19.0 °C) in M5 at the River Nile (15 coloines /5 plates).

Water temperature was the major factor conversily affecting the total counts of aquatic fungal species. This results came in agreement with those obtained by Rattan *et al.* (1980), Smith *et al.* (1984) and Misra (1982). However, Föhnh (1973) and Tomlinson and Williams (1975) proposed a wide range of temperature (14.0-30.0 °C) for adequate fungal growth and reproduction, either in freshwater or in soil in USA.

pH value was negatively correlated with total counts of aquatic fungi in site 1 of Kima drainage canal. These results came in agreement with that obtained by El-Hissy *et al.* (1994); El-Hissy *et al.*, 1992; El-Hissy and Khallil, 1992 and Khallil *et al.*, 1992 in different water and soil habitats.

Dissolved oxygen, showed no clear influence on the occurrence and total counts of aquatic fungi. This is in agreement with the results obtained by Rattan *et al.* (1980) during their studies on some ecological factors affecting seasonal occurrence of aquatic fungi especially species of Saprolegniaceae.

The organic matter content of the tested water and mud samples showed a moderate effect on the occurrence of aquatic fungi. In this respect, El-Hissy and Khallil (1992) reported that the organic matter content of water and mud is a major factor influencing the prevalance of aquatic fungi in El-Ibrahimia canal. In addition, El-Hissy *et al.* (1994) pointed out there was a direct correlation between the organic matter contents and the number of aquatic fungal genera and species.

The content of total soluble salts was influencing the occurrence of aquatic fungi at some tested sites. El-Hissy *et al.*, 1994 and 1997 also reported the occurrence of aquatic fungi from soil samples in Delta region and surface water in Aswan High Dam Lake (Egypt), respectively. El-Hissy and Abd-Elaah (1989) concluded that the content of total soluble salts seems to have no effect on the occurrence of aquatic fungi isolated from soil samples which collected from Upper Egypt. Also, calcium concentration affected the occurrence of aquatic fungi and Misra (1982) also found that calcium plays positive and significant role affecting the frequency percentage of aquatic fungi.

Magnesium has less effect on the total counts of the recovered fungal species. Czeczuga *et al.* (1990 a) found a small number of aquatic fungal species during his study on a site in River Biebrza (Poland) with slightly higher magnesium content.

Sulphate concentration, also affected the occurrence and total counts of fungal species in River Nile samples. Nitrate concentration showed an effect on the total counts of aquatic fungi recovered from water and mud samples of Kima drainage canal and this was in agreement with Czeczuga (1991 b) in some Polish rivers.

Phosphate and phosphorus showed no effect on the total counts of aquatic fungi recovered in this investigation.

Aqualinderella (A. fermentans) was the leading genus in water samples and was of high occurrence (9 months out of 12) at site 5 and moderate (5 , 3 and 5 months) at sites 2 , 3 and 4, respectively while in case of submerged mud samples t conceded its position to *Pythium*. El-Hissy and Khallil (1989) isolated *Aqualinderella* in low to rare occurrence from Delta region and El-Hissy *et al.*, 1994 and 1997 isolated it from Egyptian soil (Delta region) and from mud samples of Aswan High Dam Lake (Egypt), respectively. The highest counts of *Aqualinderella* were recorded during May 1996 from site 4 and site 5, during November 1996, from site 2 and site 4 and during June 1996, from site 3 and site 4 where dissolved oxygen concentration was between 1.2 and 6.69 mg/L.

Coelomomyces (C. dodgei) occupied the second position after *Aqualinderella* in the tested water samples and was recovered in high frequency (6 months) from site 5, in low to moderate incidence (1-4 months) in the other sites and completely missed at site 1. In the submerged mud samples it was of moderate occurrence (4,4,5 and 5 months) at sites 2,3,4 and 5, respectively and completely missed in samples of site 1.

Table 1: Collective total counts (colonies / 5 plates in all samples), numbers of cases of isolations (out of 12 months) and frequency of occurrence of aquatic fungal genera and species isolated from surface water and submerged mud samples of canal polluted with industrial effluents of Kima factory for fertilizers and the River Nile during the period from January to December 1996, using baiting technique of sesame seeds at 22 ± 1°C.

Sites	Site 1				Site 2				Site 3				Site 4				Site 5			
Fungi genera and species	Surface water		Mud		Surface water		Mud		Surface water		Mud		Surface water		Mud		Surface water		Mud	
	TC	NCI &OR	TC	NCI &OR	TC	NCI &OR	TC	NCI &OR	TC	NCI &OR	TC	NCI &OR	TC	NCI &OR	TC	NCI &OR	TC	NCI &OR	TC	NCI &OR
Total counts	5		1		26		24		28		47		67		51		78		91	
Aphanomyces	1	1L	0	0	1	1L	0	0	1	1L	3	2L	6	4M	6	3M	8	4M	10	5M
A. astaci	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1L	0	0	1	1L
A. patesnoii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	1L
A. species	1	1L	0	0	1	1L	0	0	1	1L	3	2L	6	4M	5	3M	8	4M	4	4M
Aqualinderlla fermentans	0	0	0	0	13	5M	10	3M	6	3M	12	3M	21	5M	11	3M	20	9H	9	3M
Blastocladiopsis parva	0	0	0	0	3	1L	3	1L	2	1L	5	1L	0	0	0	0	1	1L	2	1L
Ceolomomces dodgei	0	0	0	0	2	1L	7	4M	6	3M	11	4M	10	4M	9	5M	10	6H	14	5M
Dictyuchus sterilis	0	0	0	0	0	0	0	0	0	0	0	0	1	1L	0	0	5	1L	10	4M
Nowakowskiella	0	0	0	0	0	0	0	0	0	0	0	0	3	2L	0	0	4	2L	14	5M
N. elegans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2L
N. elongata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1L	0	0
N. macrospora	0	0	0	0	0	0	0	0	0	0	0	0	3	2L	0	0	3	1L	6	2L
N. multispora	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	2L
Pythium	0	0	1	1L	1	1L	3	1L	8	4M	15	7H	16	5M	13	5M	12	6H	22	9H
P. monospermum	0	0	0	0	0	0	3	1L	4	2L	3	2L	1	1L	0	0	0	0	4	1L
P. papillatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2L	0	0
P. pyrillbum	0	0	0	0	0	0	0	0	4	2L	0	0	1	1L	0	0	0	0	0	0
P. rostratum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1L
P. undulatum	0	0	1	1L	1	1L	0	0	0	0	1	1L	0	0	1	1L	0	0	0	0
Phythium species	0	0	0	0	1	1L	0	0	0	0	11	6H	14	5M	12	4M	10	5M	15	7H
Saprolegnia	0	0	0	0	0	0	0	0	0	0	0	0	8	2L	5	2L	14	3M	10	4M
S. ferax	0	0	0	0	0	0	0	0	0	0	0	0	1	1L	0	0	1	1L	0	0
S. hypogyna	0	0	0	0	1	1L	0	0	0	0	0	0	1	1L	3	2L	1	1L	2	2L
S. parasitica	0	0	0	0	0	0	0	0	0	0	0	0	6	2L	2	1L	11	3M	6	3M
Saprolegnia species	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1L	2	1L
Flagellospora penicillioides	4	1L	0	0	5	1L	1	1L	5	1L	1	1L	2	1L	1	1L	4	1L	0	0
Number of genera	2		1		7		5		6		6		8		6		9		8	
Number of species	1		1		5		5		6		6		10		7		11		13	

TC = Total counts, NCI = Numbers of cases of isolations, O R = Occurrence remark, L = Low occurrence (1-2 months), M = Moderate occurrence (3-5 months) and H = High occurrence (6-12 months).

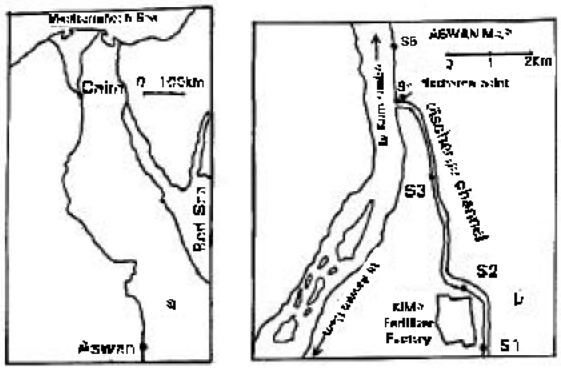

Fig. 1: Maps show the study area and site locations (a: general map of Egypt, b: map shows the drainage canal of Kima factory for fertilizers and River Nile at Aswan district

Coelomomyces was found in water samples during July and August 1996 at site 4 with maximum counts, during September and December 1996, at site 4 and site 5 and during July and November 1996 at site 5 with minimum count. With a maximum count it was recovered from submerged mud samples during November 1996 at site 5 (with a minimum count), during March 1996 at site 2 and site 4, during May 1996 in site 4 and 5 and during November 1996 at site 3 samples. In this respect, Khallil *et al.* (1995) isolated this genus from Egyptian soil (Delta region) with rare occurrence.

Pythium was of high occurrence (6 months) in water samples at site 5; of low to moderate (1-5 months) at sites 2 to 4 and completely disappeared at site 1 samples. This genus was recovered in high frequency (7 and 9 months) from mud samples at site 3 and 5 respectively, but it was of moderate to low incidence (5 to one month) in the other sites. It was occupied the first position in mud samples and the third in water samples. This genus was represented by three identified and one unidentified species in both of water and submerged mud samples, the highest counts of *Pythium* in the investigated water samples were recorded during February 1996 from site 5 and during June and November 1996 from site 4, while in case of mud samples the maximum count was recovered during November 1996 at site 5. In this line, *Pythium* was recovered in high occurrence from the surface water and submerged mud samples in Heath-moor Lake as reported by Höhnk and Bock (1954); from the River Nile water by El-Hissy *et al.* (1982); from mud samples of the River Nile near Assiut (El-Hissy and El- Nagdy, 1983), from Egyptian soil in Upper Egypt (El-Hissy and Abd-Elaah, 1989), from Delta region (El-Hissy and Khallil, 1989); from water samples of some ponds in Kharga Oases by El-Nagdy and Abdel-Hafez (1990); from water and mud samples polluted with industrial effluents of Manquabad superphosphate factory (Khallil and Abdel-Sater 1992); from Egyptian soils at Delta region (El-Hissy *et al.*, 1994) and from mud samples of Aswan High Dam Lake (El-Hissy *et al.*, 1997). In Poland, Czeczuga (1991-1992) isolated *Pythium* from water of Melting snow and Czeczuga and Mazalska (1996) from water of the River Biala. *Pythium* species was the commonest species in water (1-5 months)

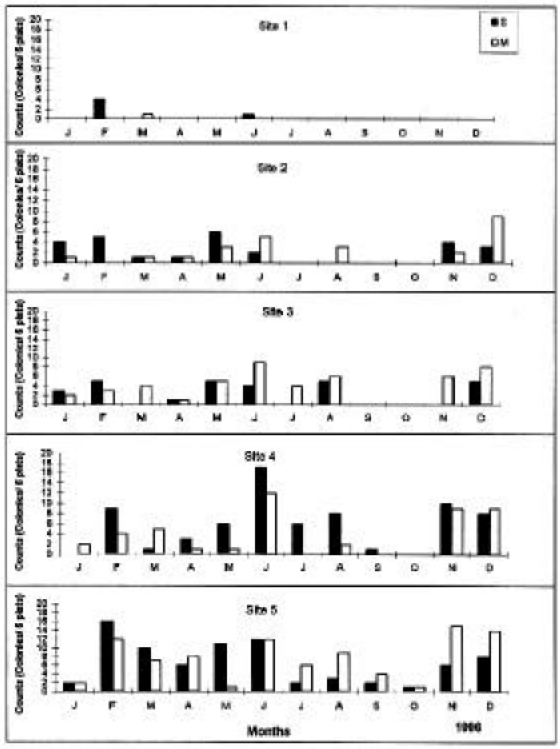

Fig. 2: Monthly total counts of aquatic fungi colonies/5 plates) isolates from surface water (S) and submerged mud (M) samples of canal polluted with industrial effluents of Kima Factory for fertlizers and River Nile during the period from January to December 1996, using baiting technique of sesame seeds at 22±1°C.

and in mud samples (1-7 months).

Aphanomyces was of moderate to low occurrence in surface water samples (4 to one month from all samples at all sites) and mud samples (2-5 months from 3 sites). The highest collective populations of *Aphanomyces* were recorded from surface and submerged mud samples at site 5. Its highest count was at site 4 from water samples during June 1996 and at site 5 from mud samples during December 1996. This genus was represented by *Aphanomyces* species in water samples and by *A. astaci, A. Patesonii* in addition to undientified species. *Aphanomyces* was previously isolated by El-Hissy *et al*. (1994) from Egyptian soil (Delta region); El-Hissy *et al*. (1997) from mud samples of Aswan High Dam Lake (Egypt).

Saprolegnia was of moderate to low occurrence in both water and mud samples. It was isolated from sites 2,4 and 5 (1,2 3 months out of 12, respectively) in case of water samples while in submerged mud, it was isolated from sites 4 and 5 (2 and 4 months, respectively). Its highest collective total count was at site 5 in both water and mud samples. It was represented by *Saprolegnia hypogena, S. parasitica* and *S.* species which isolated from water and mud samples and *S. ferax* which isolated only from water samples. These species were isolated only during winter and spring seasons (from January to April, 1996). The isolated species were reported to be common in different water and soil habitats in Egypt (Khallil *et al*., 1993; El-Hissy and Abd-Elaah 1989 and Khallil and Abdel-Sater 1992).

Blastocladiopsis (B. parva) was identified in the surface water and submerged mud samples in low occurrence (one month). The highest collective and monthly counts were in sites 2 and 3 during May 1996 from water and mud samples, respectively. El-Hissy *et al*., 1997 isolated, this genus, from Egyptian soil (Delta region) and from Aswan high Dam lake, respectively. In addition, Czeczuga 1994, 1995a,b; and Czeczuga *et al*., 1996 isolated it from fresh water in Poland.

Nowakowskiella was of low incidence (two months) in water (sites 4 and 5) and in moderate incidence (5 months) in mud samples (sites 5). It was represented by *N. macrospora* in water and mud samples. *Nowakowskiella elongata* was isolated only from water samples and *N. elegans* and *N. multispora* were isolated form mud samples. These results were in accordance with those obtained by El-Hissy *et al*. (1994) who identified 11 species of *Nowakowskiella* from Egyptian soil (Delta region).

Dictyuchus (D. sterilis) was of low occurrence in water samples (one month in sites 4 and 5) with total count one and 5 colonies/5 plates during February and June 1996, respectively. Temperature during these months were 19.8 °C in site 4 and 19.5 °C in site 5. This genus was of moderate occurrence in mud samples at site 5 only with collective count 10 colonies/5 plates in all samples. These results are in agreement with those obtained by Ziegler (1958) who found *Dictyuchus* althrough the year in soil and water samples in Florida. Dayal and Tandon (1963) found that the maximum production of *Dictyuchus sterilis* takes place during the months of August to March. Similar results were also obtained El-Hissy and Khallil (1992) and Khallil *et al*. (1992) who found that the richest periods in *Dictyuchus* were those of low or moderate temperature months during their work on water, soil and mud samples. In contrast, El-Hissy and Khallil (1989) found that the richest seasons in *Dictyuchus* populations were summer and spring and the poorest was autumn in water samples from Delta region (Egypt), they, also, found *D. sterilis* yielded its maximal count in summer.

In this study *Flagellospora penicillioides* (a member of aquatic Hyphomycetes) appeared interminttenly at all sites but in low occurrence between one and two months. This fungus flourished during February 1996 in water samples and during January 1996 in mud samples, TSS between 19.1-29.2 °C were 219-6734 mg/L. This species was also dominated in the Nile water near Sohag with highest number of colonies being recorded in November, 1990 (Khallil *et al*., 1993). In Lithuania, Markovskaja (1996) recorded this species in former Soviet Military forestries in saline water at 10-18 °C.

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