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Zoosporic Fungi Isolated From Four Egyptian Lakes and the Uptake of Radioactive Waste

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Aquatic fungi from four brackish water lakes; Edku, Burullus and Manzala lakes which are located at the northern region of Egypt and Qarun lake that located in El-Fayoum city are reported in this manuscript. Twenty-nine fungal species which belong to 19 genera of aquatic fungi were recovered from water samples collected from the studied lakes. The most frequently isolated fungal species were *Chytridium conferrop*, *Allomyces throughout* and *Rhizoclosmatium globosum*. *Thraustochytrium amoeboidum* and *Leptolegniella exoosporus* have a moderately occurrence frequency. The maximum fungal count of recovered aquatic fungi was recorded in Burrullus lake followed by EdKu, Manzala and Qarun lakes. This study was extended to test the ability of six selected aquatic fungi (*Brevilegniella keratinophila*, *Blastocladiella cystogena*, *Chytridium conferrop*, *Entophlyctis variabilis*, *Schizochytrium mangrovei* and *Thraustochytrium rosii*), to uptake the radionuclide from their culture medium as a step to biologically treat the waste water or solution with radio-cesium and radio-cobalt. Fifty seven % of Cs-137 and 35% of Co-60 could be removed from liquid waste by the selected aquatic fungi.

KEYWORDS: Aquatic fungi, Lakes, Egypt and Radioactive waste

Zoosporic fungi are found in all kinds of aquatic habitats. Zoosporic fungi are saprophytes or parasites on animals or plants. Zoosporic fungi were commonly isolated from its environments by baiting technique, where baits like pollen grains, insect larvae and cellulose are placed in streams and lakes. Seasonal variations and occurrence of zoosporic fungi in various water areas were studied by several investigators (Khulbe, 1981; Klich and Tiffany, 1985; Misra, 1982; Nasar and Munshi, 1980; Sparrow, 1968). The growth habit and the morphology of these organisms attracted the interest of many researchers and led to intensive studies on their isolation and characterization (Barr, 1975, 1980; Hassan, 1982, 1983). Elnaghy *et al.* (1985) reported five members of cladochytrioid fungi from different streams in upper Egypt and these are *Nowakowskiella delica*, *N. elongata*, *N. granulate*, *N. ramosa* and *Cladochytrium hyalinum*. Previous studies were contributed much to the distribution and seasonal occurrence of zoosporic fungi in various water areas in Egypt (El-Hissy, 1974; El-Hissy *et al.*, 1982; El-Hissy and Khallil, 1989). Czeczuga *et al.* (1997) investigated the mycoflora and the effect of environmental factors of 36 lakes and 4 rivers in the western Suwalki lake district in Poland. They have recorded 109 fungi species. Twenty two species new of the mycoflora of Poland were noted. To our knowledge there has been no research about Egyptian zoosporic fungi inhabiting the Egyptian lakes. Therefore, this study aimed mainly to survey on zoosporic fungi in four lakes and the use of zoospore fungi as a bioaccumulator for radionuclide, in order to get rid of water polluted with radioactive materials. The various applications of radioisotopes in different fields of life produced large amounts of radioactive liquid wastes. Many techniques like evaporation, chemical treatments, dialysis, electrodialysis, ion-exchange (Hang, 1994) and flotation have been applied for the processing of the low and intermediate level liquid wastes. Biological treatments as a recently developed strategy was applied for the treatment of low and intermediate levels of radioactive wastes and also the hazardous waste solutions (Bian *et al.*, 1994; Schmidt and Neumann, 1994). The low running cost and simplicity are the advantages of that technique (Krumpholz and Eder, 1999). No work has been conducted on the possibility of using the aquatic fungi as a water inhabiting organisms to eliminate such radioactive wastes.

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Material and Methods

Samples were collected from eight sites along the lake EdKu, ten sites of the lake Burullus, three sites of Manzala lake and seven sites of Qarun lake. Water samples were placed in sterilized air-tight bottles that were labeled and transported to the laboratory within five hours in a cool container late in 2001.

Isolation of Zoosporic fungi. Zoosporic fungi were recovered from collected water samples by baiting technique where the water samples were transferred into 100 m*l* presterilized beaker and covered with aluminum foil. Sterile organic substances (Pollen grains, cellophane, hemp seeds, snake skin, human hairs, small tree twigs and grass leaves) were added to all beakers as baits. Beakers were kept at 20±2°C, and examined at different time intervals. The colonized baits were then transferred into sterile petri-dishes containing sterile distilled water to which Millipore filter sterilized penicillin G (2 units/m*l* water) was added. The dishes were then incubated at 20±3°C and examined periodically for about 6 weeks where the recovered fungi were identified and purified on glucose-peptone (GP), and glucose yeast extract peptone (GYEP) agar media.

For the identification of aquatic fungal genera and species recovered during this study, the following references were used: Barr (1973, 1975), Booth and Barrett (1971), Sparrow (1950, 1973), Fuller and Jaworski (1987), Canter and Ingold (1984), Youatt *et al.* (1971) and Karling (1968, 1977).

Description of the study lakes. The studied lakes are a shallow brackish water. Edku lake, Burullus lake and Manzala lake are located at the northern of Egypt, along the Mediterranean coast. However, Qarun lake locates in El-Fayoum city at western desert and south to Cairo. Lake Burullus represents the second important Egyptian lake as regards to its total area. The lake is connected with the Mediterranean sea at its northern side through El-Bourg inlet and separated from it by long curving sand barrier. The lake received huge amounts of drainage water through several drains at the southern area. The lake Edku is small than the Burullus and connected with the Mediterranean sea at its northeastern side through Boughaz. It receives

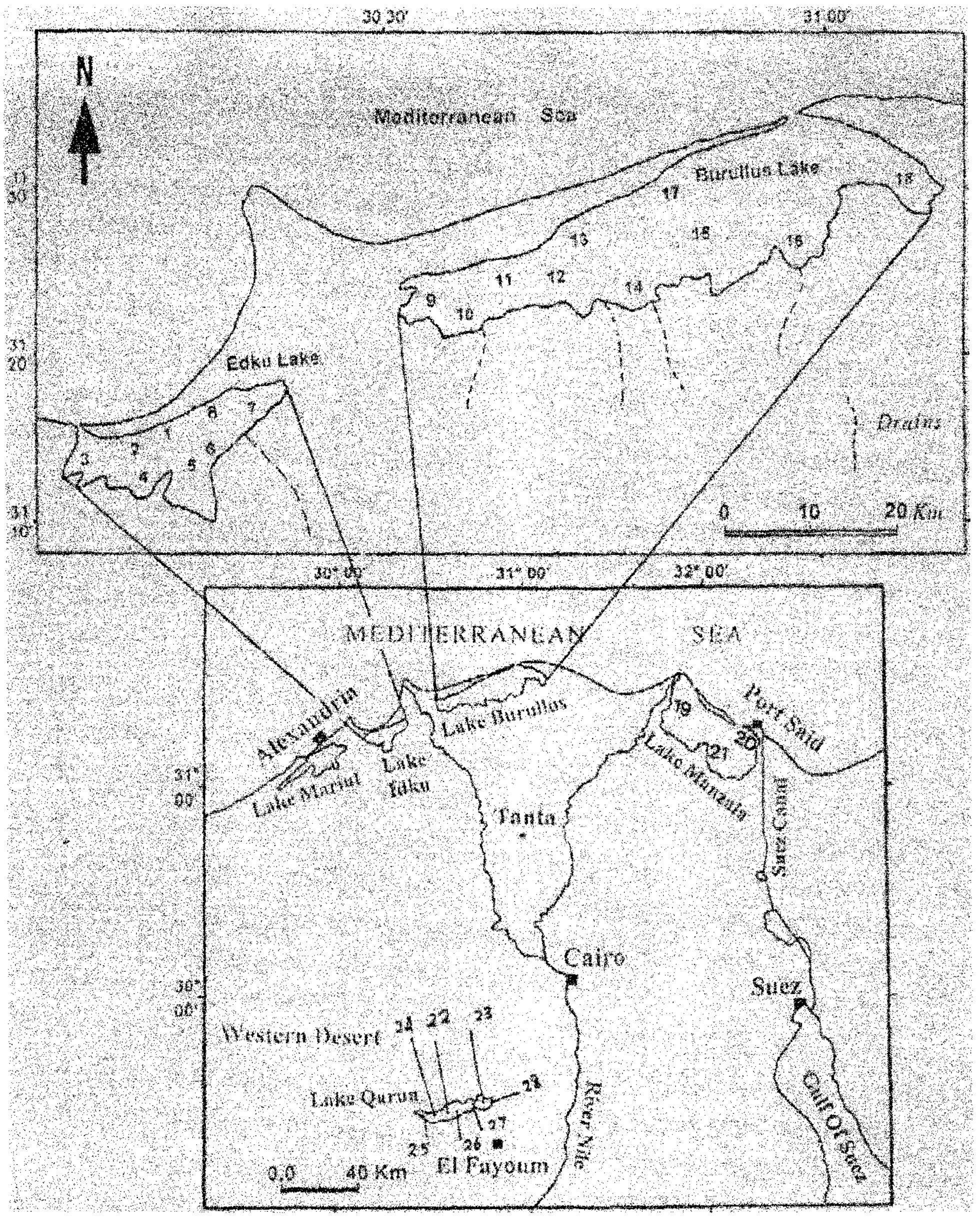

Fig. 1. Sampling sites in the studied lakes.

drainage water through drains in the southern area. Both Burullus and Edku lakes are located in the zone of seasonal tropical climate with rainy and dry season.

Lake Manzala occupies the northeastern corner of the Nile delta between Mediterranean sea and Suez Canal. The lake is presumed to have resulted from the accumulation of the Nile flood water; before the construction of the high dam in the low lying land which it occupies. Widespread land reclamation and establishment of fish farms have resulted in major reduction in the area of the lake and its marshlands. This lake receives untreated and/or primary treated waste water. On the other hand, lake Qarun is the only enclosed saline lake among the inland lakes of Egypt. The lake receives agricultural drainage water and sewage through two main drains, El-Batts and El-Wadi in addition to eleven other small drains. The distribution of lake stations in the studied lakes are shown in Fig. 1.

Biological treatment of radioactive waste water contaminated with Cs-137 and Co-60 by six selected aquatic fungi. Six selected aquatic fungal organisms that have been isolated in a pure form were grown in a glucose yeast extract peptone (glucose, 20 gm; yeast extract, 5 gm; peptone 10 gm per 1 *l* of dist. Water). Before the inoculation, 50 m*l* of the growing medium was autoclaved. Mycelial disc about 20 mm in diameter from 14 days old fungal culture was placed into the sterile medium. Radioactive waste solution containing about 170 Bq/ml labelled with carrier free Cs-137 and Co-60 radionuclides was added to the previously prepared culture and then left at room temperature for incubation. After a definite incubation period an aliquot volume of the supernal solution has been withdrawn and analyzed radiometerically using Multichannel Analyzer PCA-A purchased by Oxford instrument Inc. USA. To evaluate the efficiency of the biological process the radioactivities uptake by the tested organism was calculated according to the following equation:

$$\text{Uptake percentage} = [(A_0 - X)/A_0] \times 100$$

where A_0 = the initial activity added,
X=the radioactivity remaining in the solution.

Results and Discussion

Zoosporic fungi. The aquatic fungi of the studied lakes were relatively different. During the investigation for the aquatic fungi in the collected water samples at the sampling time, the pH value for Burullus, Edku, Qarun and Manzala was 7.8~8.3, 8.3, 7.53~8.59 and 6.87~7.98, range of respectively. On the other hand, the lake salinity was between 1.4% to 2.3% for Burullus lake and about 2.1% for water collected from Edku lake. However, the salinity of water samples collected from Qarun and Manzala was 15~30% and 0.16~3.70%, respectively.

Twenty-nine species which belong to 19 genera of aquatic fungi were recovered from water samples collected from the studied lakes (Table 1). *Chytridium conferrop* is the most frequently isolated aquatic fungus where it has 11 occurrence frequency. Then, *Allomyces throughout* recorded the second of occurrence frequency, and *Rhizoclosmatium globosum* is the third highly occurred aquatic organism from the studied lakes. *Thraustochytrium amoeboidum* and *Leptolegniella exoosporus* have moderately occurrence frequency. The number of the fungi recovered during this study from each lake is too low comparing to the number of fungi recorded from 36 lakes and 4 rivers in the Western Sunwalki lake district, Poland (Czeczuga *et al.*, 1997). They have recorded 109 fungi species.

In the lake Edku twenty-one fungal species were collected from the eight sampling sites. *Nowakowskiella elegans*, *Schizochytrium mangrovei* and *Thraustochytrium amoeboidum* were recovered once from sites 1, 2 and 3, respectively (Table 1). On the other hand five fungal species were isolated from site four. Thirty-three fungal species were recovered from different water samples collected from Burullus lake. A closer view on the sampling sites show that it is characterized by a lower salinity value (1.2‰) and pH value of 8.3, that is nearly similar to other stations of Edku lake. Five fungal species were recovered from sites 18 and four species from sites 9 and 12. *Allomyces throughout*, *Chytridium Conferrop* and *Rhizoclosmatium globosum* were isolated from large number of sites within the Burllus lake (Table 1).

Fifteen fungal species belonging to thirteen fungal genera were recovered from the Manzala lake. Eight, seven and six fungal species were recovered from sites 19, 20 and 21 of the Manzala lake respectively. *Leptolegniella keratinophilum* was isolated from all sites of Manzala lake.

In the lake Qarun fourteen fungal species belonging to eleven fungal genera were recorded from different sampling sites within Qarun lake. *Allomyces throughout* was recorded in five sites out of seven sampling sites in Qarun lake. Whereas, *Aqualinderella fermentans* was recovered from four sampling sites.Qarun lake has the highest salinity level (15~30%), which might explain the disappearing of some aquatic fungal species that isolated from other stations. Also, there are three fungal species that recorded in Qarun lake only which are *Aqualinderella fermentans*, *Blastocladiella cystogena* and *B. lavisperma*. It seems quite clear that the availability of organic matter, pH value, the water salinity and the water temperature play important role in the existence and propagation of aquatic fungi in different studied lake.

Allomyces macrogynus, *Allochytridum expandens*, *Chy-*

Table 1. Zoosporic fungi recovered from 28 collected water samples from four Egyptian lakes

Lakes	EdKu								Burullus										Manzala			Qarun							Frequency of occurrence	Occurrence remarks
Fungi / Sample sites	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28		
Allormyces throughout	0	0	0	+	0	0	0	0	0	0	+	+	+	+	+	0	0	0	0	0	0	0	+	+	+	+	0	+	11	H
A. macrogynus	0	0	0	0	+	0	0	0	0	+	0	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	4	L
Allochytridum expandens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	0	0	0	0	0	0	0	0	0	0	3	L
Aqualinderella fermentans	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	+	+	+	4	L
Blastocladiella laevisperma	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	+	0	0	2	L
B. cystogena	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	+	2	L
B. asperosperma	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	2	L
B. emersonii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	+	0	0	0	0	0	0	0	2	L
Brevilegniella Keratinophila	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	0	0	0	0	+	0	0	3	L
Schizochytrium mangrovei	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	+	0	0	3	L
Chytridium conferrop	0	0	+	+	+	0	+	0	+	+	+	0	0	+	+	+	+	0	0	+	0	0	0	0	0	0	+	0	13	H
Chytriomyces hyalinus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	1	L
Cladochytrium replicatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	1	L
Leptolegniella exoosporus	0	0	0	+	0	0	0	0	+	0	0	0	0	0	0	0	0	+	0	0	0	+	0	0	+	+	0	0	6	M
Lagenidium destruens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	1	L
Leptolegniella keratinophilum	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	+	+	+	0	0	0	0	0	0	0	3	L
Monoblepharella taylori	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	3	L
Entophlyctis variabilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	+	0	0	0	0	0	0	0	2	L
Karlingia dubia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	1	L
Nowakowskiella elegans	+	0	+	+	0	0	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	L
N. atkinsii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	0	0	0	0	3	L
Rhizophydium haynaldii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	0	0	0	0	3	L
R. sphaerocarpum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	+	0	0	0	0	2	L
R. granulosporum	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	+	+	0	0	4	L
R. constantineani	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	L
Rhizoclosmatium globosum	0	0	0	+	+	0	0	+	0	0	0	+	0	+	+	+	+	+	0	0	+	0	0	0	0	0	0	0	10	H
Rhizophlyctics rosea	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	+	0	0	0	0	0	0	0	0	0	1	L
Thraustochytrium amoeboidum	0	0	0	0	+	+	0	+	0	0	0	0	0	0	0	0	0	0	+	0	+	0	0	0	+	+	0	0	7	M
T. rosii	0	0	0	0	0	0	0	0	0	+	+	0	+	0	0	0	0	+	0	0	0	0	0	0	0	0	0	0	4	L
Number of species collected from each position	1	1	2	5	4	1	3	4	4	3	3	4	2	3	3	3	3	5	8	7	6	5	3	6	5	8	2	3		
Total fungal count	21								33										21			32								

0 absent + isolated, H = High (isolated 10-13); M = Moderate (isolated 8-6); L = Low (isolated 1-5).

triomyces hyalinus, *Cladochytrium replicatum*, *Nowkowskiella elegans*, *N. atkinsii* and *Rhizophlyctis rosea* were recorded in the Egyptian soil [Delta region Previously by Khallil *et al.* (1995)]. On the other hand, *Blastocladiella cystogena*, *Brevilegniella keratinophila*, *Leptolegniella exoosporus*, *Karlingia dubia*, *Rhizophydium haynaldii*, *R. granulosporum*, *R. constantineani* and *Rizoclosmatium globosum* were recorded from water and soil samples obtained from area of fresh and marine water in Egypt by Mahmoud (1991).

Biological treatment of Cs-137 and Co-60 radioactive waste water. The biouptake of radionuclides as an approach to treat radioactive waste water is depending on the ability of different microorganisms and also on the conditions under which the microorganisms are grown. The selected aquatic fungi were growing in glucose yeast extract peptone containing radioactive waste solution (~170 Bq/m*l*) where the medium was inoculated with 20 mm old fungal culture. Samples from the grown medium were collected at different time intervals and radiometerically analyzed and the data obtained is shown in Table 2. The maximum radioactive uptake of radioactive waste of Cs-137 was achieved by *Chytridium conferrop* where it takes 57% of the radioactivity. The efficiency of Cs-137 uptake by selected aquatic fungi is depending upon the fungal species and the incubation time. *Thrautochytrium rosii* scored the second level of efficiency in uptake of Cs-137 after one week of incubation. The capacity of a quatic organisms in uptake of radioactive waste is becoming more or less stable after the second week of incubation with the radiowaste except for some fungal species. We attribute the radioactive waste uptake by fungal organism to its different physiological pathways which link with dyes formation like melanin. Laboratory studies on yeast and fungal biomass have shown an effective uptake of uranium, leading to the biological treatment of metal-contaminated effluents (Mclean *et al.*, 1998). Actually, the mechanism of radioactive metal bioaccumulation are poorly understood in microorganisms. Melanin is known to be form in non-lichenized fungal hyphae as a response to a wide range of environmental stresses including metal contamination (Gadd, 1993). We therefore hypothesized that the ability of the aquatic fungi to form melanin during their growth may have a relationship to their ability to uptake more or less from the radioactive waste solution.

Blastocladiella cystogena is the most active organism to uptake Co-60 from radioactive waste after one week of its growth. *Thraustochytrium rosii* came in the second rank according to its ability to uptake Co-60 from the culture medium but after two weeks of its growth (Table 2). The ability of the selected aquatic fungi to uptake the Co-60 or Cs-137 was varied, where some fungi performed a maximum uptake after one week of growth and its ability

Table 2. Ability of six selected aquatic fungi to uptake of Cs-173 and Co-60 as radioactive waste (170 Bq/m*l*) after one, two and three weeks as incubation time

Aquatic Fungi	Isotope	% of radioactive uptakes		
		First week	Second week	Third week
Entophlyctis variabilis	Cs-137	41±3.5*	30±1.0	27±2.2
	Co-60	24±2.0	22±2.0	20±1.5
Schizochytrium mangrovi	Cs-137	46±3.0	33±1.8	23±2.1
	Co-60	28±2.3	23±2.0	25±1.8
Brevilegniella keratinophila	Cs-137	33±2.5	48±3.0	21±2.0
	Co-60	31±3.0	25±2.2	22±2.4
Blastocladiella cystogena	Cs-137	32±2.5	39±3.0	17±1.8
	Co-60	35±3.1	21±2.0	20±1.0
Chytridium conferrop	Cs-137	55±4.2	57±4.2	45±4.0
	Co-60	25±1.9	31±3.5	23±2.0
Thraustochytrium rosii	Cs-137	49±3.0	36±4.0	36±3.2
	Co-60	31±2.6	34±3.2	30±2.9

*Mean ± SD (n = 3).

uptake to decreased thereafter. However other fungi developed more efficiency during the second week of growth and decreased there after.

Further study should be done to determine in a more details about the mechanisms of radioative accumulations and the enzymes involved in these pathways. But we emphasis here that adding the aquatic fungi and some essential nutrients for their growth to the water area highly polluted with radioactive wastes will be effective to get ride of the water contamination.

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