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

Yehia A.-G. Mahmoud* and Alaa M. Abou Zeid

Botany Dept., Mycology Research Lab, Faculty of Science, Tanta University, Tanta 31527, Egypt
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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 conferroport, 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 Burullus 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 conferroport, Entophlyctis variabilis, Schizochytrium mangrovei and Thraustochytrium rossii), 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 aquatic 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). Elmaghy et al. (1985) reported five members of cladochytrioid fungi from different streams in upper Egypt and these are Nowakowskiiella 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 Khalil, 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 bio-accumulator 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 (Krumholz 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.

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 ml presterilized beaker and covered with aluminum foil. Sterile organic substances (Pollen grains, cellophane, hemp

*Corresponding author <E-mail: yehiam@dec1.tanta.edu.eg>
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/ml 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.](image-url)
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 ml 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 radio nuclides 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 supernatant solution has been withdrawn and analyzed radiometrically 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:

\[
\text{Uptake percentage} = \left(\frac{A_0 - X}{A_0}\right) \times 100
\]

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, 8.2, 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 conferropol* 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. *Nowakowskkiella 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*, *Allochytrium expansens*, Chy-
Table 1. Zoosporic fungi recovered from 28 collected water samples from four Egyptian lakes

<table>
<thead>
<tr>
<th>Fungi</th>
<th>EdKu</th>
<th>Burullus</th>
<th>Manzala</th>
<th>Qarun</th>
<th>Frequency of occurrence</th>
<th>Occurrence remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allomyces throughout</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>11</td>
<td>H</td>
</tr>
<tr>
<td>A. macrogyrus</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td></td>
</tr>
<tr>
<td>Allochytridum expandens</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td></td>
</tr>
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<td>Aqualinderella fermentans</td>
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<td>0</td>
<td>0</td>
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<tr>
<td>Blastocladiella laevisperma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>L</td>
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<tr>
<td>B. cystogena</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>L</td>
</tr>
<tr>
<td>B. asperosperma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>L</td>
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<tr>
<td>B. emersoni</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>L</td>
</tr>
<tr>
<td>Brevilegiella Keratinophila</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>L</td>
</tr>
<tr>
<td>Schizochytrium mangrovei</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>L</td>
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<tr>
<td>Chytridium confarop</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>H</td>
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<td>Chytriumyces hyalinus</td>
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<td>0</td>
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<td>0</td>
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<td>Cladochytrium replicatum</td>
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<td>0</td>
<td>0</td>
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<td>Leptolegniella exosporus</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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<td>Lagenidium destruenus</td>
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<td>0</td>
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<td>Karlingia dubia</td>
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<td>0</td>
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<td>Nowakowskilla elegans</td>
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<td>0</td>
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<td>N. atkinsi</td>
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<td>3</td>
<td>L</td>
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<tr>
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<td>0</td>
<td>0</td>
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<td>3</td>
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<td>R. sphaerocarpum</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>L</td>
</tr>
<tr>
<td>R. granulosporum</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>L</td>
</tr>
<tr>
<td>R. constantineani</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>L</td>
</tr>
<tr>
<td>Rhizocloesmatium globosum</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>H</td>
</tr>
<tr>
<td>Rhizophylics rosea</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>L</td>
</tr>
<tr>
<td>Thraustochytrium amoeboidum</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>M</td>
</tr>
<tr>
<td>T. rosii</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>L</td>
</tr>
</tbody>
</table>

Number of species collected from each position: 1 1 2 5 4 1 3 4 4 3 3 2 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).
triomes hyalinus, Cladochrytrium replicatum, Nowkows-
kiella elegans, N. akinsii and Rhizophycis rosea were
recorded in the Egyptian soil [Delta region Previously
by Khalil et al. (1995)]. On the other hand, Blastocladia-
lia cystogena, Brevilegniella keratinophila, Leptolegniella exoo-
sporus, Karlingia dubia, Rhizophyдум haynaldii, R. gran-
ulusporum, R. constantineanii and Rizoclosmatium globo-
sum were recorded from water and soil samples obtained
from area of fresh and marine water in Egypt by Mah-

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/ml) where the medium was inoculated with 20 mm
old fungal culture. Samples from the grown medium were
recorded at different time intervals and radiometrically
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. Thraustochytrium 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 radioisotope 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-con-
taminated 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 con-
tamination (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.

Blastocladia cystogena is the most active organism
to uptake Co-60 from radioactive after one week of
its growth. Thraustochytrium rosii came in the second
rank according to its ability to uptake Co-60 from the cul-
ture 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

take to decreased thereafter. However other fungal de-
veloped 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 radioactive 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.

Acknowledgement

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from Radioisotope Department, Atomic energy authority
Egypt, for measuring the radioactivity uptake by fungi.

References

Barr, D. J. S. 1973. Six - Rhizophyдум species (Chytridiales) in
_____. 1975. Morphology and Zoospore discharge in single
_____. 1980. An outline for the reclassification of the Chyrid-
ales, and for a new order, the Spizellomycetales. Bia. 58:
2380-2394.
Booth, T. and Barrett, P. 1971. Occurrence and distribution of
Zooporpic fungi from Devon Island, Canadian Eastern Arctic-
Czeczuga, B., Orłowska, M. and Woronowicz, I. 1997. Fungi in
water reservoirs of the Western Suwalki Lake district and in
some adjacent basins. Rozcztiki. Akademia Medycznej W. Bi-

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

<table>
<thead>
<tr>
<th>Aquatic Fungi</th>
<th>Isotope</th>
<th>% of radioactive uptakes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>First week</td>
</tr>
<tr>
<td>Entophlyctis variabilis</td>
<td>Cs-137</td>
<td>41±3.5*</td>
</tr>
<tr>
<td></td>
<td>Co-60</td>
<td>24±2.0</td>
</tr>
<tr>
<td>Schizochytrium mangrovi</td>
<td>Cs-137</td>
<td>46±3.0</td>
</tr>
<tr>
<td></td>
<td>Co-60</td>
<td>28±2.3</td>
</tr>
<tr>
<td>Brevilegniella keratinophila</td>
<td>Cs-137</td>
<td>33±2.5</td>
</tr>
<tr>
<td></td>
<td>Co-60</td>
<td>31±3.0</td>
</tr>
<tr>
<td>Blastocladia cystogena</td>
<td>Cs-137</td>
<td>32±2.5</td>
</tr>
<tr>
<td></td>
<td>Co-60</td>
<td>35±3.1</td>
</tr>
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<td>Chytridium conferrop</td>
<td>Cs-137</td>
<td>55±4.2</td>
</tr>
<tr>
<td></td>
<td>Co-60</td>
<td>25±1.9</td>
</tr>
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<td>Cs-137</td>
<td>49±3.0</td>
</tr>
<tr>
<td></td>
<td>Co-60</td>
<td>31±2.6</td>
</tr>
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</table>

*Mean ± SD (n = 3).
Zoosporic fungi isolated from Egyptian lakes.

- **Fuller, M. S.** and **Jaworski, A.** 1987. Zoosporic fungi in teaching and research, Southeastern Publishing Corp, USA.