Ass. Univ. Bull. Environ. Res. Vol. 16 No. 1 March 2013

AUCES

FUNGI BIODIVERSITY IN THE FRESHWATER ENVIRONMENT OF IRAQ

KHALID A. RASHEED AND KHALID F. HASSAN

***Biotechnology Research Center, Al-Nahrain University**

****Research Center and water laboratories, Ministry of Science and Technology**

ABSTRACT:

The presence of aquatic fungi was studied in the Tigris river in Baghdad in two stations, The first in the surrounding area before Al-Kadhimiya City and the second at Al-Jadriyah bridge, in the winter and summer seasons during 2009-2010 and by means of 14 samples of the river, since been isolate and personification 21 species belonging to eight genera, five genera belong to *Oomycetes* and three types of fungi to *Chytridiomycetes*. The fungus *Saprolegnia* register the highest emergence of 50% followed by fungus Achlya 40%, while *Dictuchus* and *Allomyces* recorded the lowest emergence 0.5%.

INTRODUCTION:

The sewage, industrial and agricultural discharges into rivers lead to the growth of a large number of phytoplankton, zooplankton, plants and animals and increasing concentrations of organic substances in water and to the growth of biodegradable microorganisms such as bacteria and fungi [1].

Surface water contains high levels of organic matter which are used as food for microorganisms. Also, the water temperature may favor the growth of many microorganisms such as bacteria and fungi, which makes the water unfit for human consumption. Fungi play a prominent role in the food chain through the activity together

with other objects to analyze and break down plant and animal residues and convert them to the basic components. Also most of them had advanced enzyme system can break. The materials that make up plant cell walls like cellulose, lignin and fats and its contribution to the analysis of organic pollutants back to the natural components [2&3].

Aaquatic fungi often include members of the class Chytridiomycetes in fresh water detritus on the remains of plants and animals, some of which have economic importance, where they can be parasites to algae, or important economic plants causing several diseases. The member's primitive of class Oomycetes may live in water (water molds), on the remains of animal, plant, and some are parasitic on fish, eggs and aquatic animals. The developed species live as oblige or facultative parasites on the higher plants causing economically important diseases. These fungi are, characterized by the filamentary nature of the wall which consists of cellulose and revise and grow at optimum temperature between 18-20ºC, and they also consume ammonia as a source of nitrogen, and also carbon, sulfur and phosphorus are their basic needs [4&5].

Studies on the presence of fungi in fresh water are few and mostly focused on the ecology of rivers like Diyala River or Shatt al-Arab. Six different species of water fungi were isolated, as *Saprolegnia ferax*, *Achlyaproliferoides*, *Dictuchus sterile* and *A. americana* and *Pythium* spp. from different locations on the Diyala River [6].

Ismail *et al.* [7] studied the taxonomy and ecology of the family Saprolegniaceae, and isolated five species belonging to the genus *Saprolegnia*, while Muhsin *et al.* [8] isolated 11 species belonging *Achlya* from the Shatt al-Arab River. This study aimed at studying the biodiversity of water jungi in the fresh water environment in the Tigris river Iraq.

MATERIALS AND METHODS:

1-Collection of water samples:

Water samples were taken from Tigris River in Baghdad every month during the year 2009-2010, from two selected locations, the first from the area of Al-Taji, near the bridge

Muthanna and represents the entry of the River Tigris to Baghdad and the second from Al-Jadriya. Water samples were collected in 250ml dark sterile bottles. To collect the samples the bottles were opened and closed below the surface of the water depth of 10-20 cm, then transferred directly to the laboratory in a cool box. PH and temperature of the water were recorded immediately.

2-Isolation, purification and diagnosis of water fungi:

Water fungi were isolated using baiting method [9], with sesame seeds *(Sesame indium)* after washing with distilled water and boiling for 20 minutes, samples of water were shaken and poured in the sterile plates of 9 cm diameter with a volume of about 20-25 ml for each plate and 3-5 sterile sesame seeds were added to promote swimming spores for aquatic fungi on germination and growth. The antibiotic chloramphenicol was added (dissolving 250 mg of the antibiotic in 250 ml of distilled water) add 1 ml per plate for the avoidance of bacterial contamination. Plates were incubated at 20°C and examined after 48 hours by light microscope to monitor the growth of undivided threaded fungi. The seeds with appeared growth were washed with distilled water and trans ferried to sterile plates containing distilled water and new sesame seed and 1 ml of the antibiotic and left for 72 hours until swelling of the fungal strands can be separated. Pure cultures of these plates were done by cut a single thread or threads set by glass sterile needles and placed in plates containing sterile distilled water, washed and transferred to another plates containing mineral salt agar, with chloramphenicol and incubated in the incubator for 48 hours to obtain pure colony. A disk of 7 mm diameter was taken from the edge of the colony using sterile piercing cork, placed in the sterile glass plate with sterile distilled water and 1 ml of the antibiotic and the seed of sesame and then plates incubated in the 18-20°C and left to grow with the control a sexual reproduction and sexual use of the light microscope for the purpose of diagnosis based on scientific sources [10-12].

The percentage of appearance of each fungus calculated at each station with the frequency of isolates per species, depending on the total number of fungi in both sites and the total number of isolates, respectively.

3-Culture medium:

Mineral salt agar (Table 1) was used for the development and purification of water fungi [9].

Table 1: Mineral Salt Agar composition

K_2HPO_4 0.7 gm	KH_2PO_4 0.7 gm	$MgSO_4$. $7H_2O$ 0.7 gm	NH_4NO_3 1 gm
NaCl 0.005 gm	Agar 20 gm	Glucose 3 gm	Water 1 L

RESULTS AND DISCUSSION:

Table (2) shows the different water Fungi recorded in 14 samples from both locations of sampling stations of the Tigris River in Baghdad during The seasons of study. 21 species belonging to eight genera of the water fungi were isolated, five genera belonging to (Oomycetes) and three to Chitrsd.

The fewest numbers of fungal speeis were recorded in April and May/2009 and September/2010, repress lnted only by speeis of *Pythium* and *Saprolegnia*.

These two genera are the most common and widespread in the aquatic environment because of its large group of enzymes analyzed Which enable them to living in different environmental conditions and ad aptation of living in conditions of high water temperature [13]. January and February/2010 are more suitable for the living *Achlya*, *Chytridium*, *Leptolegnia*, *Pythiopsis*, *Pythium*, *Allomyces* and *Dictychus* due to low water temperatures of the river 18–20°C [13].

These results were coincided with El-Hissy, *et al.* [14] for the diagnosis and isolation of 18 species of aquatic fungi belonging to 12 genera of different areas of the River Nile, producing 108 fungal colonies of which *Saprolegnia* and *Pythium* are more common fungal genera in these waters.

Table 2:The seasonal occurrence of water fungi in the Tigris River in Baghdad from 2009 to 2010

Fungal species	stations	Year 2009			Year 2010			
		Apr.	May	Oct.	Jan.	Feb.	Mar.	Sept.
Achlya ycologic	K1	-	-	+	+	+	-	-
A. caroliana	K1	-	-	+	+	-	-	-
A. debaryana	K1	-	-	+	+	+	+	-
A. dubia	K1	-	-	+	+	+	+	-
A. flegellata	K1, K2	-	-	+	+	+	+	-
A. klebsiana	K1	-	-	-	+	+	+	-
Achlya spp.	K1, K2	-	-	-	+	+	-	-
Chytridium alba	K2	-	-	-	+	-	+	-
Leptolegnia spp.	K2	-	-	+	+	-	+	-
Pythiopsis spp.	K1	-	-	-	+	-	-	-
Pythium prolifera	K2	+	+	-	+	+	-	-
Pythium spp	K2	+	+	-	+	-	-	-
Saprolegnia anisospora	K1, K2	-	+	+	+	+	-	-
S. diclina	K1	-	-	+	+	+	-	+
Saprolegnia ferax	K1	+	-	-	+	+	+	+
S. parasitica	K1, K2	+	-	-	+	+	+	+
Saprolegnia spp.	K1	+	+	+	+	+	-	+
Allomyces macrogynus	K1	-	-	-	+	+	-	-
Allomyces spp.	K1	-	-	-	+	+	-	-
Dictychus monosporus	K1	-	-	-	+	+	-	-
Dictychus spp.	K1	-	-	-	+	+	-	-

K1: Al-Kadhimiya surrounding area **K2: Al-Jadriyah bridge**
(+) fungal present **(-) fungal absent**

Table (3) shows the percentage occurrence of fungal genera in two stations on the River Tigris in Baghdad and the number of fungal isolates, with the highest rate of appearance of the fungus *Saprolegnia parasitica* by 50%, followed by *Saprolegnia anisospora* by 40% and *Achlya flagellate* by 40% while the lowest percentage of appearance was for Allomyces and Dictychus by 0.5%. The total number of fungal isolates was 83 in 14 sample of water from the Tigris River in Baghdad (Table3).

Table 3: The percentage of recurrence of the fungi recorded in the two stations on the Tigris River during 2009 to 2010

No.	Fungal species	Station (K1)	Station (K2)	No. of Isolation	Fungal appearance (%)
1	*Achlya ycologic*	3	0	3	15
2	*A. caroliana*	2	0	2	10
3	*A. debaryana*	4	0	4	20
4	*A. dubia*	4	0	4	20
5	*A. ycologica*	4	4	8	40
6	*A. klebsiana*	3	0	3	15
7	*Achlya*spp.	2	2	4	20
8	*Chytridium alba*	0	3	3	15
9	*Leptolegnia* spp.	0	3	3	15
10	*Pythiopsis* spp.	3	0	3	15
11	Pythium prolifera	0	5	5	25
12	*Pythium* spp.	0	4	4	20
13	*Saprolegnia anisospora*	4	4	8	40
14	*S. diclina*	4	0	4	20
15	*Saprolegnia ferax*	5	0	5	25
16	*S. parasitica*	5	5	10	50
17	*Saprolegnia* spp.	6	0	6	30
18	*Allomyces macrogynus*	1	0	1	0.5
19	*Allomyces* spp.	1	0	1	0.5
20	*Dictuchus monosporus*	1	0	1	0.5
21	*Dictuchus* spp.	1	0	1	0.5

Results showed that more fungal genera and species have been isolated in the station K1, the region before the Kadhimiya, which represents the entry point of the Tigris to Baghdad, where the pollution resulted from the discharge of waste water and sewage treatment plants, agricultural residues and others, which was dominated by species of Saprolegnia (33 isolates) and Achlya (22 isolates). Species of *Chytridium*, *Leptolegnia* and *Pythium* dominated in the second station (K2) at Al-Jadiriya bridge characterized by high concentrations of organic matter and hydrocarbon. These results jointly adopted with Paliwal and Sati (2009) [15] on their study of the presence of aquatic fungi in the River Kosi in the Himalayas, and its relations with the concentration of organic matter in the water, and the results of Mer, et al [16] in their study about the presence of fungi in water TainiTal in India.

Results of the present study showed scarcity of fungal diversity of the aquatic environment of Iraq in recent years compared with previous finding in year 2002 as reported by Ali *et al.* [17]. More than 14 genera has been isolated, diagnosed and repeatedly appeared in all samples of water obtained from the Tigris River in different seasons, largely due to a continuing seasons of drought and scarcity of rain and low water levels of the Tigris River in recent years, leading to change specifications of the physical and chemical of Iraqi water and therefore on the biodiversity of the aquatic environment.

In the study of Khulbe and Bhargava,[18] for the presence of aquatic fungi in some lakes of India, They found a change of some physical and chemical properties of water which affects the fungal diversity, Christian, *et al.* [19] found that the water fungi had a great sensitivity for the change of aquatic environment specifications and the amount of pollutants discharged to water bodies, and can be considered as evidence vital to the pollution with sulfur, nitrogen and phosphorus compounds, Artz. *et al.*,[20] in their study that society affected by milde in the aquatic environment stated due to change the concentrations of nutrients and dissolved organic matter.

Table (4) and figure (1) shows that the months of January and February were the richest in fungal occurrence, which are characterized by low temperatures. The percentage of similarity between them up to 76.2%. The summer months (April, May and September) were lower than the winter months in the presence and recorded the proportion of similarity of 50%. With decreased rates of similarity for the month of October to 40.8% and March increased by 34.1%. In general, the rate of appearance of the fungal species during the year of the study was a close one, but the two groups formed by a weak similarity was 16.5%. So, it could conclude that the temperature has a direct impact on the distribution and spread of aquatic fungi [13].

Table 4: Half matrix for fungal species distribution during the months of study

	May	October	January	February	March	September
April	**50**	**7.7**	**23.8**	**23.5**	**18.2**	**50**
May		**9.1**	**19.1**	**17.6**	**0**	**14.3**
October			**42.8**	**38.8**	**30.7**	**18.2**
January				**76.2**	**38.1**	**19.1**
February					**33.3**	**25.0**
March						**16.6**

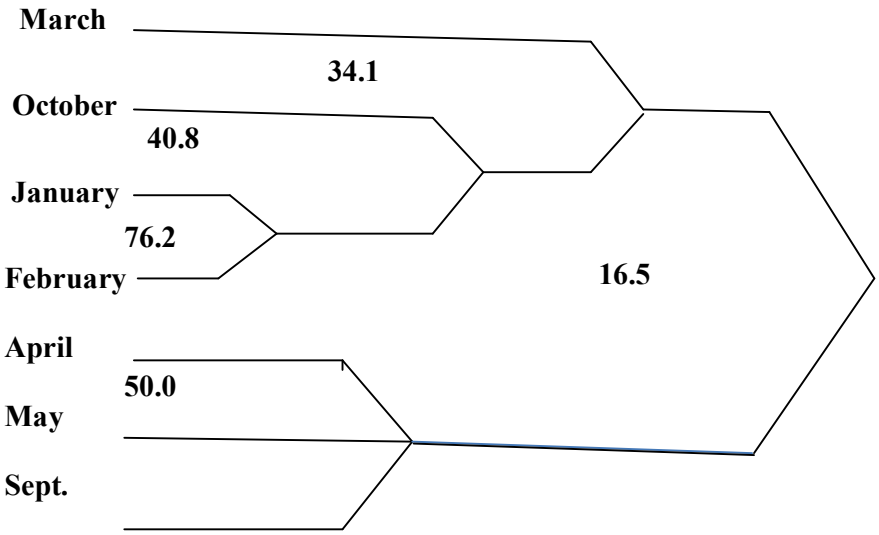

Figure 1: Similarity index for monthly distribution of fungal species during the months study year

REFERENCES:

[1] Whitton, B. A. (2005): River Ecology. Blackwell Scientific Publications, Oxford.

[2] Czeczuga, B. & Woronowicz, L. (2007): Studies on aquatic fungi. Acta Hycological 14 (1): 93-103.

[3] Park, H. C.; Sorenson, W.G. and Davis, R. J. (2006): Aquatic Oomycetes in farm ponds in Bryan County, Oklahoma. Proceed. Oklahoma, Academy of Science. P. 48– 54.

[4] Thompstone, A. & Dix, N.J. (2005): Cellulose activity in the *Saprolegnia ceae*. Trans. Br. Mycol. Soc. 85 (2): 361-366.

[5] Suberkropp, K.F. (2002): Microorganisms and organic matter decomposition. In: river ecology and management: Lessons from the pacific coastal, ecoregion. Naiman , R. J. and Bilby ,R. E., Springer-Verlag , New York , Inc. p: 120-122.

[6] Abd, A. Sh., (1999): Ecological and physiological study on the effect of waste water on some water fungi isolated from river Diyala. MSc Theses, Al Mustansryah University.

[7] Ismail, A. L., S.; Rattan, S. S. & Muhsin, T. M. (1979): Aquatic fungi of Iraqi species of *Saprolegnia*. Hydrobiologia, 65:83-93.

[8] Muhsin, T. M.; Rattan, S. S. & Ismail, A. L. S. (1984): Aquatic fungi of Iraqi species of *Achlya*. Sydowia, Ann. Mycol. 37: 224-237.

[9] Jones, E. B. G. (1971): Aquatic fungi. In: Both, C., (Methods in Microbiology) Vol. (4), 2nd ed. Academic press, New York, 795 pp.

[10] Cocker, W.C. (1965): The *Saprolegniaceae* with notes other water molds. Univ. N.C. Press, Chapel Hill, North Carolina, 201 pp.

[11] Seymour, R. L. (1970): The genus *Saprolegnia*. Verlag Von J. Cramer, Germany, 124 pp.

[12] Alexopoulos, C. J.; Mims, C. W.; Blackwell, (1996): Introductory Mycology. 4th ed. John Wiley & Sons, Inc. New York.

[13] Bozena, K. and Alicja K. (2004): Aquatic fungi and fungus-like organisms isolated from surface waters situated near Białystok in Podlasie Province of Poland using the insect *Notonecta glauca* as bait. Poland Mycologia Balcanica, 1: 117–123.

[14] El-Hissy, F. T.; Khallil, A. M. and El-Nagdy, M. A. (1991): Mycoflora of water pooles in the vicinity of some ancient pharonic temples in upper Egypt. J. Islamic Academy of Sciences, 4(4): 293-296.

[15] Paliwal, P. C. and Sati, S. C. (2009): Distribution of aquatic fungi in relation to physico-chemical factors of Kosi River in Kumaun Himalaya. Nature and Science, 7(3): 1545-0740.

[16] Mer, G. S.; Sati, S. C. and Khulbe, R. D. (2008): Occurrence, distribution and seasonal periodicity of some aquatic fungi of Sat Tal (Naini Tal), India. Hydrobiologia 76: 200-205.

[17] Ali, E. N., Zainal, B., Hassan, K. F. and Sabti, H. A. (2005): The seasonal presence of aquatic fungi in the Tigris River during 2002. University of Baghdad. Umm Salamah Journal of Science page nos- 2 (2).

[18] Khulbe, R. D. and Bhargava, K. S. (2005): Distribution and seasonal periodicity of watermolds of in some lakes of Nainital Hills, India. Hydrobiologia. 54: 67-72.

[19] Christian, M.; Wurzbacher1, F. B. and Grossart, H.P. (2010): Fungi in lake ecosystems. Aquat Microb Ecol. 59: 125–149.

[20] Artz. R.; Anderson, I.; Chapman, S.; Hagn, A.; Schloter, M.; Potts, J. and Campbell, C. (2007): Changes in Fungal Community Composition in Response to Vegetational Succession during the Natural Regeneration of Cutover Peat lands. Microb Ecol. 54: 508–522.

دليل تنوع الفطريات المائية في بيئة المياه العذبة العراقية

خالد عباس رشيد، خالد فالح حسن*

مركز بحوث التقنيات الاحيائية – جامعة النهرين – العراق

*** وزارة العلوم والتكنولوجيا – دائرة بحوث وتكنولوجيا البيئة والمياه – مركز بحوث ومختبرات المياه – العراق**

تمت دراسة تواجد الفطريات المائية لبيئة نهر دجلة بمدينة بغداد في محطتين، الأولى عند منطقة المحيط قبل الكاظمية، والثانية عند جسر الجادرية في موسمي الشتاء والصيف خلال عامي ٢٠٠٩-٢٠١٠ ، وبواقع ١٤ عينة من مياه النهر. تم عزل وتشخيص ٢١ نوعاً تابعاً إلى ثمانية أجناس فطرية، وبواقع خمسة أجناس تعود للفطريات البيضية وثلاثة إلى الفطريات الكتريدية. سجل الفطر *Saprolegnia* **أعلى نسبة ظهور ٥٠% يليه الفطر** *Achlya* **٤٠%، فيما سجل الفطرين** *Dictuchus* **و** *Allomyces* **أقل نسبة ظهور بلغت ٠,٥%.**