

Mycobiota of surface sediments in marshes of Southern Iraq

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Abstract

Twenty sediment samples were taken from ten sites in the southern marshes of Iraq and analyzed for the presence of fungi by three isolation methods. The dilution technique yielded the highest number of genera identified (32 genera). Phenol and acetic acid treated sediments yielded 17 and 16 genera respectively. Phenol treatment method was more selective for ascomycetous fungi yielded the isolation of 12 genera. Sixty seven species assigned to thirty seven genera in addition to sterile mycelia were identified. The isolates were assigned to 43 mitosporic fungi, 20 species of ascomycetes and 4 species of zygomycetes. The most frequent species were in decreasing order: *Aspergillus terreus*, *A.niger*, *Acremonium kiliense*, *Sterile mycelia*, *Graphium putredinis*, *Preussia dispersa*, *A. fumigatus*, *Dichotomomyces ceipii* and *Rhizopus sp.*, our findings were compared with those from similar survey on mycobiota in sediments in several parts of the world.

Key words: Mycobiota, sediments, marshes, Iraq.

1-Introduction.

Marshes and similar aquatic bodies may provide suitable environment in which fungi can grow. However, the low wave action in such habitat may create semi

anaerobic conditions surrounding the surface sediments at the bottom of such aquatic bodies due to oxygen deficiency (Barlocher, 1990; Wong *et al.*,1998). Among several aquatic macrophytes ,

Cyperus, *Phragmites* and *Typha* plants serve as a good substrates for fungi in freshwater marshes (Wong *et al.*,1998)

Fungi play an important role in aquatic ecosystem in the degradation of dead plant materials as well as materials from animal origin such as insect skeleton, fish scales and hair (Wong *et al.*,1998). Moreover, fungi and other microorganisms have the ability to degrade several pollutants including crude oil in the aquatic ecosystem and utilize them as a nutrient sources (Davis and Westlake,1979). They may also metabolize such pollutants to substrates with low harmful effect on the environment (Cerniglia *et al.*,1991; Boonchan *et al.*, 2000;Sutherland, 1992).

Contribution to the knowledge of mycobiota inhabiting aquatic sediments in different parts of the world have been documented by several authors (Bourt and Johnson,1962; El-Wahid *et al.*,1982; Ito and Nakagiri, 1997; Tubaki and Matsuda,1974;Tubaki *et al.*1975; Ueda,1980; Ulfig *et al.*1998). In Iraq, however, previous studies were directed to taxonomic studies describing several novel and interesting species (Abdullah and Abbas,1994a,b,c and d Cannon; *et al.* 1995;Sivanesan *et al.*,1993) as well as some ecological and hygienic studies (Abdullah and Abbas, 2006;Abdullah and Hassan,1995; Abdullah *et al.*2000, 2007).

The present study was directed to obtain better knowledge on the mycobiota inhabiting aquatic sediment in southern marshes of Iraq.

Materials and Methods

Area of study: Southern marshes is the largest wetland areas in Iraq. These include 1: Haur Al-Hammar, 2:The central marshes north of Euphrates and west of Tigris 3:Haur Al-Hawizah. The whole wetlands are located between 29 55 - 32 45 N and 48 25 - 48 30 E. The vegetation is dominated by tall stands of *Phragmites australis*,*Typha angustifolia* , *Cyperus papyrus* and occasionally *Arundo donax*.

Sediment samples: Twenty sediment samples were collected from ten sites in the southern marshes of Iraq during December 2004 to October 2005 (Table 1). The method used for collection of the sediment samples was the same as described previously by Abdullah and Abbas (1994a). The methods used for the estimation of sediment p^H and dissolved oxygen in water as described by APHA (1985). The determination of organic carbon in surface sediment samples was according to El-Wakeel and Riley (1957).

Isolation of fungi: Three isolation techniques were applied: the dilution plate method (Johnson *et al.*,1959),sediment

treatment with 5% acetic acid (Furuya and Naito,1979) and treatment with 2%phenol (Furuya and Naito,1980). Two types of media were used for isolation of fungi potato carrot agar (20g peeled potatoes, 20g carrot, 20g agar, 1L distilled water) and malt extract agar (Himedia,India). Each medium was supplemented with 50ug/ml chloramphenicol (SDI,Iraq) to suppress bacterial growth. Plates for all methods and media were incubated at 25 C in the dark. Single colonies were picked from the plates under a dissecting microscope and transferred to appropriate media to allow fungal development.

General and specific taxonomic references were used for the identification of fungal species (Arx *et al.*1986,1988; Cain,1961; Domsch *et al.*1980; De Hoog and Guarro,1995; Ellis,1971,1976;Horie,1980; Klich and Pitt,1988).

Results and discussion:

Table 1 gives the values of p^H and total carbon percentages in sediments, temperature and dissolved oxygen in water, total fungal colonies count and number of species detected for each sediment sample. The lowest values of p^H ranged from 6.8 - 7.1, the highest were between 7.9 – 8.2, but the most common values ranged between 7.2 – 7.8. Data obtained for p^H values of

surface sediments of Shatt Al-Arab estuary was slightly acidic and the p^H values were ranging from 5- 6.35, except for one station which was slightly alkaline (p^H 7.89) (Abdullah *et al.*,2000).

The percentage of the total carbon content of the 20 sediment samples were varied from relatively low (0.31%) in sample No.14 from Hammar marsh to relatively high value (1.77%) in sample No.4 from Huwizah marsh. Fungal total count of the 20 sediment samples were ranging between 300-2400 CFU/g.dwt. These results indicate a low number of fungal propagules in sediments comparable to numbers in soil of surrounding terrestrial habitat (3.49×10^4 - 5.23×10^4 CFU g/dwt.) and this may be attributed to the semi-anaerobic condition of the sediments. However, data for the total fungal count (467 – 1417 CFU/g.dwt.) obtained by Abdullah *et al.* (2000) for 16 sediment samples from Shatt Al-Arab estuary and North-West Arabian Gulf and data (450 – 2300 CFU /g dwt) by Ito and Nakagiri (1997) for 30 mud samples in Okinawa, Japan showed close values to our findings.

A total of 37genera were isolated and identified by three isolation techniques . These include 4 genera of Zygomycota, 13 genera of Ascomycota , 20 mitosporic genera and unidentified sterile mycelia (5 morphotypes) (Table 2). The generic

composition of the mycobiota in the sediment of southern marshes of Iraq is more or less similar to that reported by Abdullah *et al.* (2000) for surface sediments in Shatt Al-Arab estuary and North-West Arabian Gulf and that reported by Ueda (1980) for river sediments in Nagasaki, Japan and that reported by Bourt and Johnson (1962) for the sediments in the Neuse-Newport estuarine system in coastal North Carolina, USA. There is also similarity with the fungal community inhabiting mud in the tidal zone of Khor Al-Zubair canal, Southern Iraq (Abdullah *et al.*, 2007).

The highest number of genera (32) was obtained by dilution technique, followed by sediment treated with 2% phenol (17 genera) and 5% acetic acid (16 genera). The majority of genera detected only after dilution method were assigned to Zygomycota and mitosporic fungi. The highest number of recovered genera (32) detected by employing the dilution method was probably due to the fact that colonies developing in plates originated from hyphal fragments in addition to were most likely derived from spores (Parkinson and Williams, 1961; Warcup, 1960).

Of the 13 genera of ascomycetes recovered by all methods, 12 genera were isolated only after phenol treatment, followed by 8 genera after acetic acid

treatment. Phenol and acetic acid have been used effectively to increase frequencies of ascomycetes detection from soil or sediments by stimulating ascospores germination or have pasteurization effect on species with thin-walled conidia (Asina and Cain, 1977; Furuya and Naito, 1979, 1980; Ito and Nakagiri, 1997; Ueda, 1980).

Frequency of occurrence for sixty eight fungal species isolated and identified in the present study is presented in Table 3. The isolated species have been divided into four groups according to their percentage of occurrence: H=high frequency (more than 50%), M=moderate frequency (25-less than 50%), L=low frequency (15-less than 25%), VL=very low frequency (below 15%). Three species were isolated with high frequencies (listed in decreasing order): *Aspergillus terreus*, *A.niger*, and *Acremonium kiliense*. The first species occurred in 100% of the sediment samples. The group with moderate frequency of isolation included 6 fungal species and sterile mycelia, *Graphium putredinis*, *Preussia dispersa*, *Aspergillus fumigatus*, *Dichotomomyces ceipii* and *Rhizopus* sp., while that with low frequency was represented by 21 species. The remaining 38 fungal species were isolated with a very low frequency (Table 3). The mere isolation of a fungus from a certain habitat does not

mean that such a fungus has a certain activity in that habitat, species showed low and very low isolation occurrence at the present study are well known either soil inhabiting fungi or associated with aquatic macrophytes (Domsch *et al.* 1980; Apinis *et al.* 1972; Pugh and Mulder, 1971). Abdullah *et al.* (2000) reported that *Phoma* spp., *Aspergillus flavus*, *A.niger*, *Penicillium* spp., *Mortierella parvispora*, *A.fumigatus*, *Dichotomomyces ceipii*, *Chaetomium atrobruneum*, *Acremonium kiliense*, *Pseudoeurotium multiporum* and *Talaromyces flavus* were the most frequent species isolated from aquatic sediments of Shatt Al-Arab estuary and North-West Arabian Gulf. Abdullah and Abbas (2006) reported *Aspergillus niger*, *A.terreus* *A.fumigatus* among the species isolated with high frequency from surface sediments of Shatt Al-Arab and its creeks at Basrah. Moustafa and Sharkas (1982) reported that *A.fumigatus*, *A.niger*, *A.nidulans*, *A.flavus* and *Stachybotrys atra* were the dominant species in tidal mud flats of Kuwait. Ito and Nakagiri (1997) recorded *Phoma* spp., *Acremonium* spp., *Penicillium purpurogenium*, *Aspergillus terreus* and *Talaromyces flavus* to be the most frequent species in mangrove mud in Okinawa, Japan, while Abdullah *et al.* (2007) found that *A.niger*, *A.terreus*, *Stachybotrys atra*, *A.flavus*, *A.fumigatus*, *Alternaria alternata*,

Rhizopus stolonifer, *Penicillium glabrum* as the most frequent fungi in tidal zone of Khawr Al-Zubair canal, southern Iraq.

The high frequencies of isolation displayed by the above fungi in our study and in similar studies carried out in other parts of the world led to the suggestion by several authors (Abdullah *et al.*, 2000; El-Wahid *et al.*, 1982; Moustafa and Sharkas, 1982; Bourt and Johnson, 1962; Ito and Nakagiri, 1997) that these species may live in active form in the mud and they expect that these species may contribute to the microbial activity in mud. Al-Dossari *et al.* (2001) reported the capability of *Aspergillus terreus* and *Acremonium killense* strains isolated from surface sediments of Shatt Al-Arab river in degradation of a mixture of five polycyclic aromatic hydrocarbons in laboratory. More recently, Al-Dossari (2008) reported high degradative ability displayed by isolates of *Aspergillus niger*, *A.terreus*, *Paecilomyces* sp., and *Acremonium* sp., isolated from sediments of southern marshes of Iraq against crude oil *in vitro*.

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Table 1. Some chemical and physical characteristics of sediments and water samples, total fungal count, number of species in each sample ,site and date of collection.

Sample No.	carbon	C	Sediment PH mg/L	Water %total	CFU/g Temp.	No.of D.O	Site	Date	species
1	8	0.75	16	12	400	7	Central marshes (Hurushin)	23.12.2004	
2	8.2	0.83	16.2	11.7	500	9	Central marshes (AL-Fartoos)	23.12.2004	
3	7.9	0.68	15.9	11	325	8	Central marshes (Um AL-Shweich)	23.12.2004	
4	7.7	1.77	15.5	12.8	600	8	Huwizah marsh (Um Al- Warred)	23.12.004	
5	7.6	0.97	15	12.6	550	7	Huwizah marsh (Al-Turaba)	23.12.2004	
6	7.8	0.74	16	12.2	725	12	Central marshes (AL-Fuhud)	23.12.2004	
7	7.6	0.62	19	10	1600	11	Central marshes (Al-Fartoos)	10.3.2005	
8	7.5	0.48	18.5	10.6	825	14	Central marshes (Al-Fuhud)	10.3.2005	
9	7.8	0.71	19.8	9.9	1550	11	Hammar marsh (Al-Burka)	10.3.2005	
10	7.4	0.49	26	9	855	8	Hammar marsh (Al-Dubbon)	10.5.2005	
11	7.3	0.49	26	8.8	2400	19	Hammar marsh (Al-Dubbon)	10.5.2005	
12	7.2	0.56	27	8.5	625	10	Central marshes (Um Shewaich)	10.5.2005	
13	7.4	0.59	27.3	10	965	19	Central marshes (Al-Hurushin)	10.5.2005	
14	6.8	0.31	31	7	450	6	Hammar marsh (Al-Burka)	25.7.2005	
15	6.9	0.46	33	6.5	300	11	Hammar marsh (Al-Mushab)	25.7.2005	
16	7.5	0.69	27	8	2400	16	Huwaiza marsh (Um Al-Warred)	29.9.2005	
17	7.3	0.57	27.8	7	500	8	Hammar marsh (North Rumaila)	29.9.2005	
18	7.2	0.93	25.8	10.2	876	13	Huwizah marsh (Al-Turaba)	15.10.2005	
19	7.1	0.89	26	9.5	755	15	Hammar marsh (A-Mushab)	15.10.2005	
20	8	0.69	25.9	9.7	750	7	Hammar marsh (North Rumaila)	6.10.2005	

Table 2. Isolation % of fungal genera by different isolation techniques.

Fungal genera	Isolation %	Dilution method	Acetic acid treatment	Phenol treatment
<i>Absidia</i>	5	-	-	
<i>Acremonium</i>	65	10	-	
<i>Alternaria</i>	25	-	-	
<i>Aspergillus</i>	100	15	20	
<i>Byssochlamys</i>	-	10	5	
<i>Chaetomium</i>	-	5	15	
<i>Chrysosporium</i>	10	-	-	
<i>Cladophialophora</i>	5	-	-	
<i>Cladosporium</i>	15	-	5	
<i>Curvularia</i>	15	-	-	
<i>Dichotomomyces</i>	15	5	5	
<i>Emericella</i>	5	-	10	
<i>Eurotium</i>	5	-	10	
<i>Fusarium</i>	20	-	-	
<i>Gillmanila</i>	20	-	-	
<i>Graphium</i>	30	10	-	
<i>Humicola</i>	20	-	-	
<i>Microascus</i>	-	15	5	
<i>Monodictys</i>	5	-	-	
<i>Mortierella</i>	5	-	-	
<i>Mucor</i>	15	-	-	
<i>Neosartorya</i>	10	10	5	
<i>Paecilomyces</i>	30	10	-	
<i>Penicillium</i>	40	5	5	
<i>Phoma</i>	15	5	-	
<i>Preussia</i>	5	25	15	
<i>Pseudoallescheria</i>	10	-	5	
<i>Pseudoeurotium</i>	-	15	-	
<i>Rhizopus</i>	25	-	-	
<i>Sordaria</i>	-	10	5	
<i>Stachybotrys</i>	20	-	-	
<i>Sterile mycelia</i>	35	10	5	
<i>Talaromyces</i>	25	-	20	
<i>Thielavia</i>	-	-	10	
<i>Trichoderma</i>	20	5	5	
<i>Trichurus</i>	10	-	-	
<i>Ulocladium</i>	20	-	-	
<i>Verticillium</i>	10	-	-	

Table 3. List of fungal species, their percentage of occurrence and frequency class

Fungal species	Occurrence%	Frequency class
<i>Absidia corymbifera</i> (Cohn)sacc. and Trotter	5	VL
<i>Acremonium curvulum</i> W.Gams	10	VL
<i>A.kiliense</i> Curtz	50	H
<i>A.reseogriseum</i> (Saksena)W.Gams	5	VL
<i>Acremonium</i> sp.	10	VL
<i>Alternaria alternata</i> Keissler	15	L
<i>A.chlamydospora</i> Mouchacca	10	VL
<i>Aspergillus candidus</i> Link	10	VI
<i>A.clavatus</i> Desmazieres	5	VL
<i>A.flavus</i> Link	20	L
<i>A.fumigatus</i> Fresenius	25	M
<i>A.niger</i> Tieghem	55	H
<i>A.terreus</i> Thom	100	H
<i>A.versicolor</i> (Vuill.)Triqboschi	10	VL
<i>A.wentii</i> Wehmer	5	VL
<i>Byssochlamys nivea</i> Westling	15	L
<i>Chaetomium atrobruneum</i> Ames	15	L
<i>C.globosum</i> Kunze	5	VL
<i>Chrysosporium merdarium</i> Ames	5	VL
<i>Chrysosporium</i> sp.	5	VL
<i>Cladophialophora bantiana</i> (Sacc.)de Hoog et al.	5	VL
<i>Cladosporium herbarum</i> (Pers.)Link ex Gray	15	L
<i>C.spongiuseum</i> Berk. and Curt.	5	VL
<i>Curvularia lunata</i> (Walker)Boedijn	15	L
<i>Dichotomomyces ceipii</i> (Milko)Scott	25	M
<i>Emericella nidulans</i> var. <i>nidulans</i> (Eidam)Vill.	10	VL
<i>Eurotium cristatus</i> Raper and Fennel	15	L
<i>Fusarium moniliforme</i> Sheld	5	VL
<i>F.oxysporum</i> Schelcht.:Fr.	10	VL
<i>Fusarium</i> sp.	5	L
<i>Gillmanila humicola</i> Barron	20	L
<i>Graphium putredinis</i> (Corda)Hughes	30	M
<i>Humicola grisea</i> Traaen	20	L
<i>Microascus cinereus</i> (Emile Weil and Gaudin)Curz	5	VL
<i>M.trigonosporus</i> Emmons and Ddoge	15	L
<i>Monodictys</i> sp.	5	VL
<i>Mortierella parvispora</i> Linnem	15	L
<i>Mucor hiemalis</i> Wehmer	15	L
<i>Neosartorya</i> sp.	15	L
<i>Paecilomyces variotti</i> Bain	15	L
<i>Paecilomyces</i> sp.1	20	L
<i>Paecilomyces</i> sp.2	10	VL
<i>Paecilomyces</i> sp.3	5	VL

<i>Penicillium</i> sp.1	20	L
<i>Penicillium</i> sp.2	10	VL
<i>Penicillium</i> sp.3	10	VL
<i>Phoma</i> sp.1	10	VL
<i>Phoma</i> sp.2	10	VL
<i>Preussia dispersa</i> (Clum)Cain	30	M
<i>P.nigra</i> (Routien)Cain	5	VL
<i>Pseudallescheria ellipsoideum</i> (Arx and Fassativa) McGinnis	15	L
<i>Pseudoeurotium multisporum</i> (Saitoet)Stolk	15	L
<i>Rhizopus</i> sp.	25	M
<i>Sordaria fimicola</i> (Rob.)Ces. and Denot	5	VL
<i>Sordaria</i> sp.	5	VL
<i>Stachybotrys atra</i> Corda	20	L
<i>S.oenanthes</i> Ellis	5	VL
Sterile mycelia	35	M
<i>Talaromyces flavus</i> (Klocker)Stolk and Samson	10	VL
<i>T.stipitatus</i> (Thom)Benjamin	20	L
<i>T.trachyspermus</i> (Shear)Stolk and Samson	5	VL
<i>Thielavia terricola</i> (Gilman and Abbott)Emmons	10	VL
<i>Trichoderma harzianum</i> Rifai	5	VL
<i>Trichoderma</i> sp.1	10	VL
<i>Trichoderma</i> sp.2	5	VL
<i>Trichurus spiralis</i> Hasselbring	10	VL
<i>Ulocladium chlamydosprum</i> Mouchacca	20	L
<i>Verticillium</i> sp.	10	VL

VL: Very low, L: Low, M: Medium, H: High

المجموعة الفطرية في الرسوبيات السطحية لاهوار جنوب العراق

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الخلاصة

جمعت عشرون عينة من الرسوبيات السطحية من مواقع مختلفة في اهورار جنوب العراق وتم عزل الفطريات منها باستخدام ثلاث تقنيات مختلفة . عزل 32جنسا باستخدام تقنية التخافيف و16جنسا بمعاملة الرسوبيات بحامض الخليك تركيز 5% فضلا عن 16 جنسا باستخدام المعاملة بالفينول تركيز 2% . اظهرت المعاملة بالفينول اكثر انتقائية لعزل الفطريات الكيسية . تم عزل وتشخيص 67نوعا تعود الى 37جنسا . شخّصت العزلات على انها تعود الى 41نوعا من الفطريات الناقصة و20نوعا من الفطريات الكيسية و4انواع من الفطريات اللااحية ونوعين من الفطريات البكتيدية فضلا عن عزل (5) شكل من الخيوط العقيمة . اكثر الانواع ترددا وبترتيب متناقص على التوالي *Aspergillus terreus*, *A.niger*, *Acremonium kiliense*, *sterile mycelia*, *Graphium putredinis*, *Preussia dispersa*, *A.fumigatus*, *Dichotomomyces ceipii*, and *Rhizopus sp.* تم مناقشة نتائجنا مع نتائج مسوحات اجريت على فطريات الرسوبيات السطحية لبيئات مائية في مناطق مختلفة من العالم.