

**Mycobiota and Pathogenic Bacteria Associated
with Sediments and Water in Al_musharah
Water Project within Missan Province in Iraq**

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Abstract: A total of forty three fungal species were isolated from sedimentation tanks in Al-Musharah water project in Missan province ,southern marsh area of Iraq, the isolated fungi included one new first record species *Allysedium* sp., has been isolated from sedimentation tanks, mentioned new record fungus was described and illustrated and added to Iraqi fungi. Eight fungal species The north is bad.

also were isolated from final product tank. Results revealed that water samples taken from site 1 from Al-Musharah sub district of Missan province was contaminated with faecal coliforms 1,600 colony per 100 ml. and from site 4 within water network of Al-Musharah water project was contaminated with faecal coliforms with 11 colony per 100 ml.

Key words:- Mycobiota, pathogenic bacteria, water project, marsh area, Iraq.

INTRODUCTION:

AL-Musharah water project (with capacity of 200 m³/hr)is the main water facility in Al-Musharah sub district in Iraq Due to high turbidity of Tigris river water and low capacity of water facility, mud accumulates as sediments in the different units of the water project. Tigris river mix with marsh water enriched with different submerged plant debris especially vegetation of reeds(*Phragmites australis*) and Typha(*Typha australis*), the most common submerged plants in Iraqi marshes[1], which push organic matters to the aquatic ecosystem and encourage growth of fungal community .

Many common fungi were isolated from marshes in Iraq, *Aureobasidium pullulans*, *Alternaria alternata*, *Cladosporium cladosporoides* and *Drechslera hawaiiensis*, [2] whereas different fungal species were isolated from sediments of aquatic habitats in Basrah [3], in addition to many other similar regional and international studies [4,5,6].

Chlorine is the disinfectant that most frequently used for water and wastewater treatment. It was used first for industrial applications and to control odor in wastewater in the early 1880s.[7]. Water projects used chlorine to disinfect water from water pathogens(almost *E.coli*, the most pollutant factor of potable water originates from sewage disposals), and fungi consider as one of microorganisms grow in sediments .Sediments consider an ecological niche to fungi, which use carbon sources in submerged plant parts by degradation process to liberate carbon in aquatic ecosystem [8].

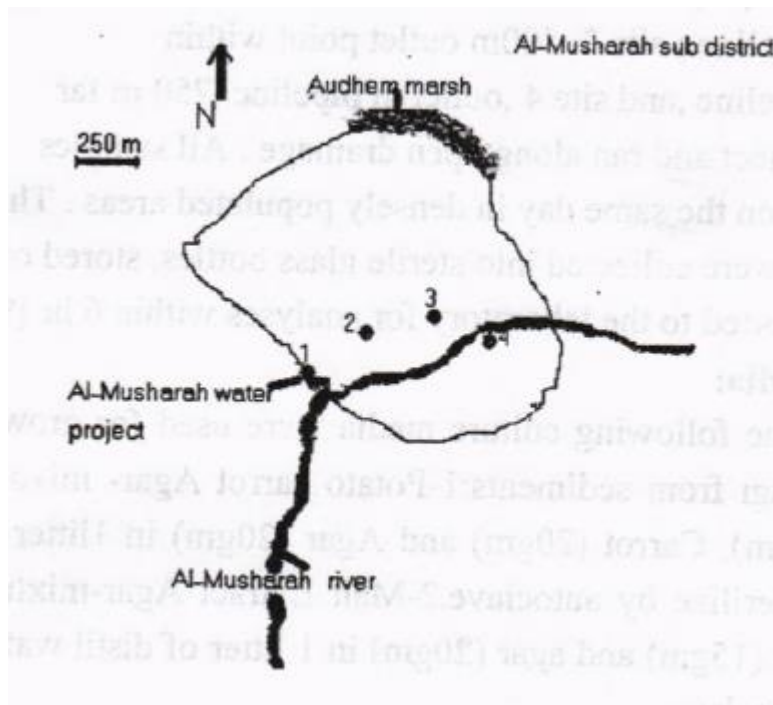
The present study aimed to isolation mycoflora of sediments

and to detect pathogens in water from Al-Musharah water project in Iraq marsh area.

MATERIALS AND METHODS:

A-Sites of sample collection

Samples of water and sediments were collected from 4 sites in Al-Musharah water project in Al-Musharah sub district as shown in draw 1 below:



B-Collection of sediment samples

Water samples were collected from the bottom of sedimentation tanks through gathering water from the lower pipe of the tank and later the collected water filtered by filter paper and the residual of sediment layer on the filter paper was collected after drying by oven under 50 C°.whereas water samples were collected from final product tank after disinfected by chlorination technique by back washing process, through the lower pipe of disinfected water tank, the collected water filtered by filter paper and the residual of sediment layer on the filter

paper was collected after drying by oven under 50C°

C-Collection of water samples:

Water samples (1 L) collected in triplicate for isolation of E. coli and quantitative enumeration of the coliform population (by the multiple-tube fermentation technique) at 4 sites in Al-Musharah water project in Missan province, southern Iraqi marsh area, as follows: site 1, Sedimentation tank of Al- ni Musharah water project; site 2, 250 m outlet point within work distribution pipeline; site 3 500m outlet point within distribution pipeline, and site 4,outlet in pipeline,750 m far from water project and ran along open drainage. All samples were collected on the same day in densely populated areas. The water samples were collected into sterile glass bottles, stored on ice, and transported to the laboratory for analyses within 6 hr [9].

D-Culture media:

For fungi: The following culture media were used for growth of isolated fungi from sediments:1-Potato carrot Agar- mixture of potato (20gm), Carrot (20gm) and Agar (20gm) in 1litter of distil water, sterilize by autoclave.2-Malt Extract Agar-mixture of malt extract (15gm) and agar (20gm) in 1 litter of distil water, sterilize by autoclave.

For water pathogens: In the present technique we used 2 types of culture media, for enumerating coliforms lauryl tryptose (lactose)broth is used in the first (isolation or presumptive)part of the test In the second confirmation part, brilliant green lactose bile (BGLB) broth is used to confirm total coliforms and E.coli medium used to confirm faecal coliforms.

E-Method of fungi isolation: The present study used dilution technique method to isolate fungi from sediments, which summarize by adding 1 gm. of dry sediment to 99 ml. of sterilized distil water in a conical flask with shaking for 10 min.

and drain 1 ml. of the suspension by a sterilized pipette, follow by pouring culture medium on the sample in the Petri dish.

E-Chemical analysis of water samples:

Ph: By using of portable ph-meter Model ((Digital Portable pH meter PW9410)), ph of water samples has been analyzed. and the test was confirmed by Delagua Test Kit from Oxfam.

TDS: Total Dissolved Solids or salinity of water was analyzed by using TDS meter and Electric conductivity meter Model E 202.

Turbidity: Portable turbidity meter Model 304 W from Japan was used for measuring Turbidity in NTU unit, and confirmed by using glass tube for turbidity in Delagua Test Kit from Oxfam.

F- Procedure of the multiple-tube fermentation technique:

Prepared the required number of tubes of culture medium. The volume and strength (single and double) 10 ml. of volumes of single strength medium are appropriate. To prepare 1/10 concentration, pipette 10 ml. of sample into a dilution bottle containing 90 ml. of phosphate-buffer dilution water and later prepare another dilutions (1/100, 1/1000, 1/10000). Pipette the appropriate volumes of sample and diluted sample into the tubes of medium, label the tubes with sample reference number. shake gently to mix the sample with the medium. place the rack with an incubator or water bath under 48 hours at $35 \pm 0.5^{\circ}\text{C}$ or $37 \pm 0.5^{\circ}\text{C}$, after 18 or 24 hours note which tubes shows growth (turbidity and gas production or color change). Record number of positive tubes. return the tubes to the incubator and re-amine after 48 hours, prepare the required number of tubes of confirmation culture medium(BGLB for total coliform and E.coli medium for fecal coliforms). By wire loop transfer an ocular from positive tubes into the confirmation medium if

confirmation of both total and fecal coliforms is required BGLB medium and E.coli medium should be inoculated from each presumptive positive, label tubes and incubate for 48 hours and under $35 \pm 0.5\text{ }^{\circ}\text{C}$ or $37 \pm 0.5\text{ }^{\circ}\text{C}$ to coliform and 24 hours at $44 \pm 0.5\text{ }^{\circ}\text{C}$ for faecal coliforms (E.coli). Record of positive tubes(growth with gas production) and compare the pattern of positive results with a most probable number in table index.

G-Fungal Taxonomy: Isolates of fungi were identified by the author of present research according to fungal keys published in [10],[11],[12],[13],[14],[15],[16],[17].

RESULTS:

43 fungal species were isolated from sediments of water project in marsh area during April 2008, the isolated species belong to Hyphomycetes 33 species, Ascomycetes 6 species and Zygomycetes 4 species. As shown in table 1., in addition to isolation of 8 fungal pieces from final product tank as shown in Table 2.

Table-1 Fungi isolated from sedimentation tanks

No	Fungi
1	Absidia corymbifera (Cohn) Sacc.& Trotter
2	Aphanoascus fulvescens (Cooke) Apinis
3	Alternaria alternata Keissler
4	Alternaria raphani Groves & Skolko
5	Alternaria sp
6	Allysedium sp
7	Aspergills flavus Link & Fries
8	A.fumigatus Fres
9	A.nidulans (Eidam) vuill
10	A.niger van Tieghem
11	A.terreus Thom
12	Aspergillus sp
13	Aureobasidium pullulans (De Bary) Arnaud

14	<i>Bipolaris hawaiiensis</i> (M.B.Ellis) Subram. & Jain
15	<i>Bipolaris spicifera</i> (Bain) v. Arx
16	<i>Chaetomium globosum</i> Kunze & Fries
17	<i>Chaetomium</i> sp
18	<i>Chuppia sarciniferum</i> Deighton
19	<i>Cladosporium cladosporoides</i> (Fresen) de Vries
20	<i>Cladosporium spongiosum</i> Berk. & Curt
21	<i>Cladosporium</i> sp
23	<i>Curvuloia pmniseti</i> (Metra) Boedin
24	<i>Emericella nidulans</i> (Eidam) Vuill
25	<i>Emericella</i> sp
26	<i>Eurotium</i> sp
27	<i>Fusarium oryzae</i> Schlecht
28	<i>Fusarium</i> sp
29	<i>Graphium putredinis</i> (Corda) Hughes
30	<i>Monodictys glauca</i> (Cooke & Harkn) Hughes
31	<i>Mucor</i> sp
32	<i>Penicillium</i> sp
33	<i>Phoma glomerata</i> (Corda) Wollen.&Hochapfel
34	<i>Phoma herbarum</i> Westend
35	<i>Phoma</i> sp
36	<i>Rhizopus stolonifer</i> (Ehrenb: Fr.) Vuill
37	<i>Stachybotrys atra</i> Corda
38	<i>Stachybotrys atra</i> Corda var. <i>microspora</i> Mathur & Sankhla
39	<i>Stachybotrys</i> sp
40	<i>Stemphylium</i> sp
41	<i>Torula herbarum</i> (Pers.) Link from S.F.Gray
42	<i>Trichocladium opacum</i> (Corda) Hughes
43	<i>Ulocladium botrytis</i> Preuss

Table 2- Fungi isolated from final product tank:

No	Fungi
1	<i>Absidia coerlia</i> Bain
2	<i>Alternaria alternata</i> Keissler
3	<i>Aspergillus flavus</i> Link & Fries
4	<i>A. fumigatus</i> Fres
5	<i>A. niger</i> van Tieghem
6	<i>A. terreus</i> Thom
7	<i>Cladosporium cladosporoides</i> (Fresen) de Vries
8	<i>Mucor</i> sp

Description of *Allysidium* sp. as a new record fungus

(Fig. 1)

Colony black, mycelium partly superficial, partly immersed, hypha-branched, septate, dark brown to blackish, smooth, 6-8 μ thick, conidiophores with 1-2 branched, semimacronematous, pale to dark colored, conidiogenous cell monoblastic, conidia in branched chains, spherical to oval, or limoniform, 6-9 μ in diameter, without septa. Fungus isolated from sand filter column of Al-Musharah water project in Iraq. The septate was kept in Basrah University herbarium under the number BSRA 3001 .

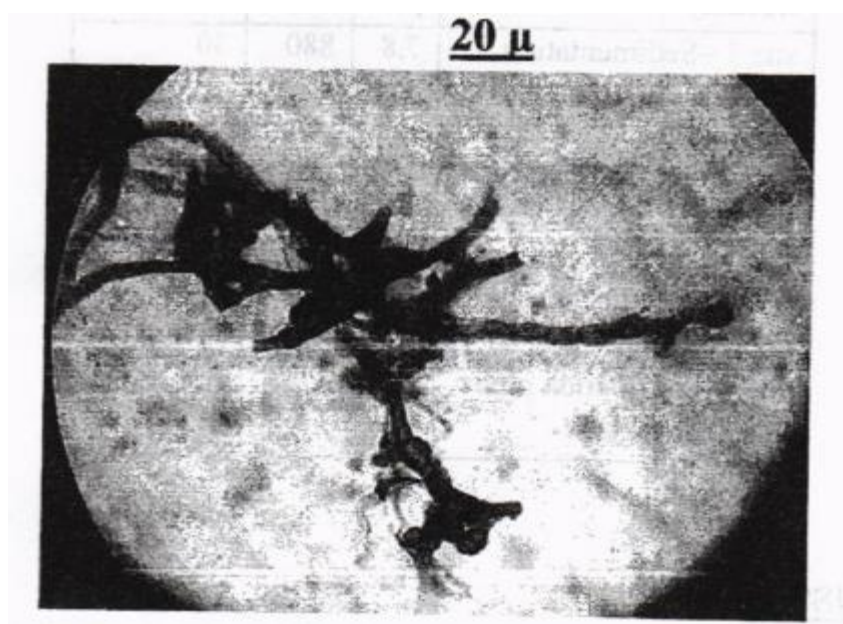


Fig. 1 Picture of Allysidium sp.

Table 3 - Bacteriological analysis of water samples.

Locality	Most probable number of total coliform per 100ml	Most probable number of faecal coliform per 100ml
Site 1 - Sedimentation tank (Raw water)**	1,600	1,600
Site 2 - Distribution pipe (Treated water)	ND*	ND
Site 3 - Distribution pipe (Treated water)	ND	ND
Site 4 - Distribution pipe (Treated water)	22	11

*ND = not detected

**Mean of three samples.

Table 4 - Chemical and physical analysis of water samples

Locality	pH	TDS	Turbidity
Site 1 - Sedimentation tank (Raw water)	7.8	880	30
Site 2 - Distribution pipe (Treated water)	7.7	870	<5
Site 3 - Distribution pipe (Treated water)	7.7	870	<5
Site 4 - Distribution pipe (Treated water)	7.7	870	<5

DISCUSSION:

Sediments occupy nutrients, especially organic matter, which encourages microorganisms growth [18]. Al-Musharah water project intake of water from high turbidity Tigris River (30 NTU), under low capacity of marsh area water facilities, sediment layers precipitated at the bottom of sediment tanks and final product tank, leads to more fungal growth and development as shown in Table 1, especially *Aspergillus* species due to their ability to survive under various conditions [19]. Table 2 revealed isolation of 8 species of fungi from the final product tank, compared with 43 fungi isolated from the sedimentation tank, which confirms the influence of chlorine in the disinfection process .

In the present study, we found possible water samples to be contaminated with coliform and fecal coliform bacteria due to leakage of the water network of a marsh community in Al-Musharah sub-district as a result of old pipes and networks, which facilitate the entrance of sewage and wastewater from the sewage open canals system into water leakage pipes, as shown in Table 3

, which revealed high concentrations of coliforms and faecal coliforms (1,600 and 1,600, respectively) at site 1, whereas the concentration of faecal coliforms in potable water taken from the water network at site 4 exceeded the standard permissible limits recommended by various regulatory bodies for drinking water (11 colony per 100 ml), which indicated contamination of the distribution pipe line with water pathogens.

CONCLUSIONS:

Sediments consider an ecological niche to fungi, use carbon sources by degradation process, and contribute to mitigation of the aquatic environment; however, chlorine contributes to reducing mycobiota in water. However, the presence of leakage in potable water pipe lines may pose health risks to marsh dwellers using the domestic water supply for drinking and relying on the old water network.

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عزل البكتيريا المرضية المصاحبة للرسوبيات في

مشروع ماء المشرح في محافظة ميسان

د. حسين النصراوي

د. غانم حسين مجيد

هيئة التعليم التقني

الخلاصة: دراسة عزل البكتيريا الحالية ٤٣ نوعاً من البكتيريا من خزان الترسيب وأنواع من البكتيريا من خزان
التصفية النهائي في محطة تصفية المياه على جانب المشرح في محافظة ميسان وقد تم وصف وتصوير نوع
Allysedium sp كتسجيل جديد في العراق .

وتبين من الدراسة تلوث المياه في ناحية المشرح في محافظة ميسان ببكتريا القولون حيث تأكد وجود تركيز
١٦٠٠ مستعمرة في ١٠٠ مل في موقع ١ في حين تبين ان عينات المياه المأخوذة من موقع ٤ ضمن شبكة
التوزيع في محطة التصفية كانت ملوثة ببكتريا القولون بتركيز ١١ مستعمرة في ١٠٠ مل من الماء.