An Ecological Study on the Occurrence and Distribution of Aquatic Fungi in Sarchnar Water Spring within Sulaimani Province/ Kurdistan Region of Iraq

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Abstract

Mycological and ecological studies have been carried out at four stations of Sarchnar water spring: (Sarchnar spring pools, Sarchnar district waste water, Chaq Chaq stream (industrial water) and confluence of these three stations, Selected within Sulaimani Governorate during April to August 2008.Certain physico-chemical parameters of water were perforormed using standard methods. The first investigation of total of twelve aquatic fungus species from 163 isolates was reported.Eleven species were identified in which 8 of them belonging to the family Saproleginaceae including, *Achlya americana, A. apiculata, A. racemosa, Saprolegnia ferax, S. parasitica and S. litoralis and 2 species of Aphanomycese laevis and Aphanomycese stellates,* three an identified species belonging *to each of the familes* Leptomitaceae, Pythiaceae *and* Blastocladiaceae which including *Leptomitus sp*, *Pythium sp* and *Allomyces sp* respectively, and one species of *Chytrid* which belongs to the class Chytridiomycota were recorded. Sarchnar spring pool was the richest station with aquatic fungus species, while Sarchnar district sewage water was the poorest station. Some physico-chemical parameters showing a significant relationship to the fungal occurrence and distributions.

Keywords: aquatic fingi, ecological parameters, occurrence.

Introduction

The knowledge on the aquatic fungi and its relation to physico-chemical parameters in Iraqi water, especially in Kurdistan region is still insufficient, while there are several investigations been carried out on the occurrence and distributions of aquatic fungi in different regions worldwide [1,2,3,4,5]. Fungi are common in the aquatic habitats. They can be found in pools, ponds, lakes, rivers and streams [6, 7]. They colonize leaves, branches, stems of plants, and animal material fallen into water thus contributing to the mineralization of organic matter found in various types of water. Some of them act as parasites of plants, animals and humans, under favorable conditions acquire pathogenic properties, and may be a potential source of infection they were able to grow and cause infection on the muscles of fish and their eggs [8]. Others, as saprobionts, in these fungi play significant role in the aquatic ecosystems. Together with bacteria they break down organic material and help in the recycling of nutrients in nature. The occurrence and distribution of aquatic fungi in relation to water characteristics, includes physical and chemical parameters of the water, as well as to other various geographical regions of the world have been intensively studied [9]. This study conducted to isolation and identification of some aquatic fungi and the relationships between the occurrence of aquatic fungi and some physico-chmical parameters of the water.

Area under Study

- Sarchnar Spring Pool Water-(Station 1)

Sulaimani is the largest town in province and draws the major part of its water supply from the large spring of Sarchnar Fig. (1)

Sarchnar spring located five kilometers to the northwest of Sulaimani at altitude of plateau of about 800 meters above sea levels [10]. The out flow is impounded to form two interconnecting pools, the water from limestone and shale formation. There are 55 water pumps (with different capacities) belong to Sarchnar water treatment project (gaseous chloride type) with a dosing system of $1gm/m^3$of water, which used for disinfecting purposes, all pumps water towards Sulaimani and around. There are vegetation growing around the spring such as *Salix alba*, *Morus alba* and *populas alba.* Level of water decreased as a result of drought year.

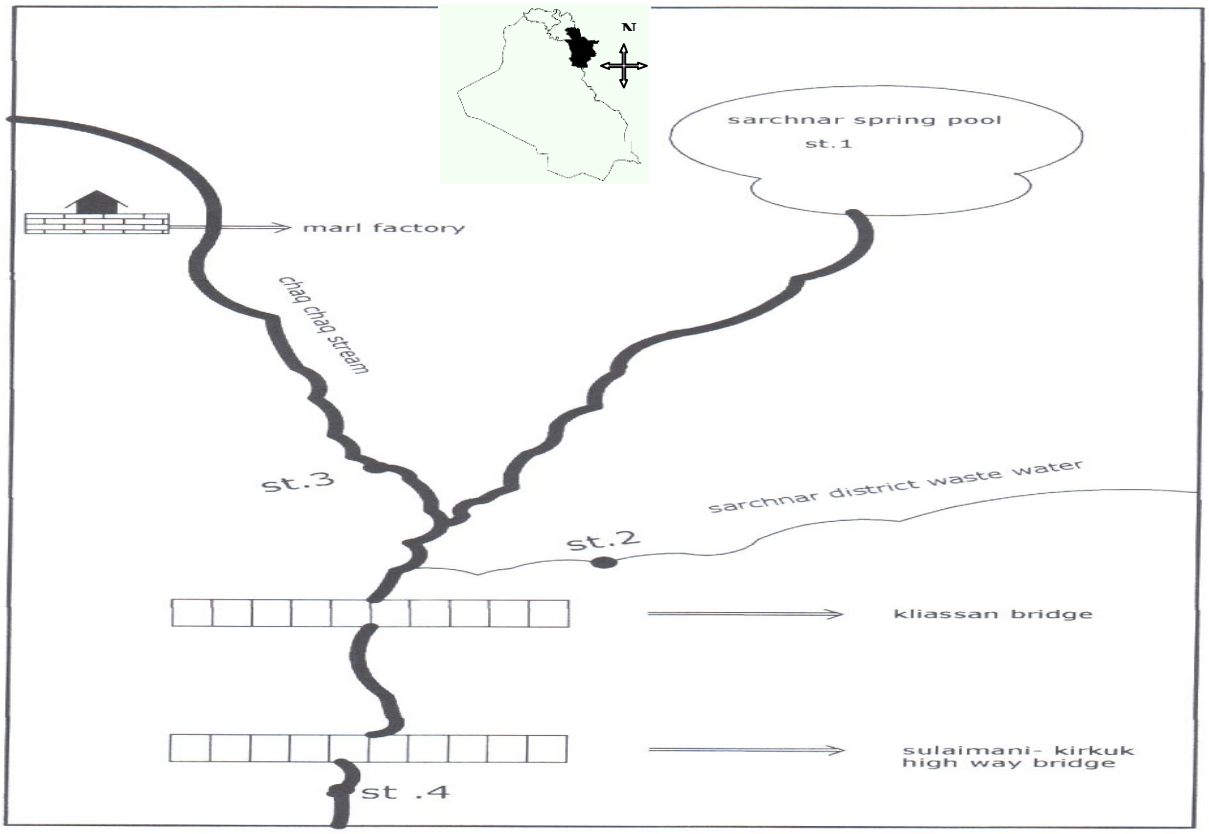

Fig. (1) Schematic digram of the studied area.

- Sarchnar District (Waste Water Stream)-(Station 2)

In this station Sarchnar spring draining to Sarchnar district and kiliassan waste water, the ground of stream consist of pebbles and stones. Stream on both side surrounded by vegetation grown such as Morus alba, Salix alba Rubus sp. and, Ficus carlca.

- Chaq Chaq Stream Water (Industrial Water) - (Station 3)

This stream situated to the northwest of Sarchnar spring area. There are many factories along it, so that industrial water were disposed to the stream, especially carbonate as a result of cutting marl stone, the ground of stream consist of pebbles stones. vegetation growth around the stream are *Morus alba*, *Salix alba*, *Rubus sp.* and *Populus alba*.

- Confluence of Station 1, 2 and 3-(Station4)

This station situated near the high way bridge of Sulamani–Kirkuk, formed as a result of the confluence of fresh, sewage water and industrial water, also the level of water decreased as a result of drought year, the ground consist of pebbles and stones and growing vegetation such as *Morus alba*, *Salix alba, Rubus sp. and Ficus carlca.*

Materials and methods:

Surface water samples usually (10-30cm deep) were monthly collected during April, to Augast, 2008 from each of the four different stations (Sasrchnar spring pools (station 1), Sarchnar district waste water (station 2), Chaq Chaq Stream, (industrial water) (station 3) and confluence of these three stations) (station 4) Fig.(1). The samples for fungi isolation preserved in a cool box when air temperature more than 25°C, the sample bottles were transferred to the laboratory as soon as possible.

Physico-chemical analysis:

Water samples for physico-chemical analysis were collected from four selected stations using a clear polyethylene bottles. Temperature, electrical conductivity, dissolved oxygen and pH were measured in the field using standard methods according to [11, 12], turbidity conducted according to Lind [13], and BOD_5as indicated [14]. Nitrate and nitrite concentration are determined following the methods indicated by [12], and phosphate concentration by [11].

Fungal Isolation and identification:

Aquatic fungi were isolated by using baiting technique that used by [15, 16, 17]. Sesame seeds *Sesamum indecum* were used as baits. The water samples was poured in Petri dishes (10 cm in diameter) and baited with boiled sesame seeds. For controlling bacterial growth 1.5-2ml of chloramphenicol was added to each plate. Chloramphenicol prepared by dissolving 250mg of antibiotic to 250ml of distilled water. The colonized baits were washed in sterile distilled water and transferred to fresh Petri dishes containing distilled water to which an antibiotic (chloramphenicol) had been added .The seeded plates were incubated at 20±2°C and the baits were examined after every 24 hours and observed for 7 days for any hyphal growth that might appeared by using compound light microscope. For the determination of fungal population, the aquatic fungal species appearing on one plate was counted as one colony. The isolated aquatic fungi were purified on corn meal agar media. The fungi were identified using the following keys: [18, 19, 20, and 21].

Results

Physico-chemical analysis:

Water and air temperature of the studied stations were ranged between 16-33º C and 20-40ºC respectively. Higher turbidity value 170-545 NTU

was recorded in Sarchnar districts, at July 2008 Table (1), Fig. (2). pH was ranged between 7-8.6 in the studied stations. The higher electrical conductivity 2228µS/cm and total dissolved solids 1425mg/l were recorded in St.2 at August 2008 respectively Table (1), Fig. (3).

Sarchnar spring pool was the best aerated station with higher value 8 mg/l, as well as with the lowest Biological oxygen demand value ranged between 1-1.8 mg/l during April and May2008 with pronounced month variation Table(1), Fig.(4).

The range of nitrate concentration was much higher than nitrite in all stations, the reactive phosphate was conducted with a high values ranged between 22.1-48 mg/l in St.4 at July and August 2008 Table (1), Fig. (5).

Table (1)

Physical and chemical parameters in four stations during studied periods April to August 2008 (Mean±SE), maximum and minimum value.

Stations / *Parameters*	*Mean±S.E (Stations*			
	Sarchnar spring pool (St. 1)	***Sarchnar distrct (sewage water) (St. 2)***	***Chaq Chaq stream water (industrial water) (St. 3)***	***The confluence of site 1, 2 and 3 (St. 4)***
Air temperature °C	a 22-38 29±1.41	b 21-40 29.8±1.42	b 20-37 29.8±1.74	b 22-40 30.6±1.42
Water temperature °C	a 16-24 20.8±0.71	b 18-30 24.2±1.06	b 16-31 22.6±1.33	ab 20-33 25±1.03
Turbidity NTU	c 0-0 0±0.00	a 170-545 360.8±27.83	b 0-72 31.94±6.77	d 88-167 135.6±7.03
Total dissolved solids mg /L	a 307.2-339.2 331±3.45	b 174.08-1425 864.8±105.80	c 374.4-947.2 605.95±42.31	d 961.2-1081.6 931.2±33.69
pH (Hydrogen ion concentration)	a 7-7.5 7.3±0.04	b 7.9-8.6 8.32±0.06	b 7.8-8.5 8.12±0.05	b 7.6-8.5 8.12±0.07
Electrical conductivity µS/cm	a 480-548 517.2±5.39	b 272-2228 1345±166.4	d 585-1480 946.8±66.12	c 1080-1705 1455±52.65
Dissolved O_2 mg/L	a 4.2-8 5.94±0.30	d 1.5-3.5 2.56±0.16	c 2.8-6 4.16±0.29	d 1-3 2.48±0.24
Biological oxygen demand mg /L	a 1-1.8 1.34±0.06	b 1.4-3 2.34±0.14	a 1-2 1.66±0.08	a 0.8-3 1.86±0.19
Nitrate mg at N-NO3/L	d 8-14 11.16±0.5	c 20.2-48 38.1±2.46	b 10.2-24 17.96±1.18	a 38-65 50.4±2.23
Nitrite mg at N-NO2/L	b 0.014-0.09 0.03±0.006	ab 0.09-0.26 0.15±0.01	b 0.04-0.17 0.11±0.01	a 0.07-0.65 0.31±0.05
Phosphate mg atP-PO4/L	a 0.9-1.4 1.17±0.03	b 3.37-20.33 10.99±1.36	b 1.83-22.37 13.008±1.80	c 22.11-48 35.07±2.195

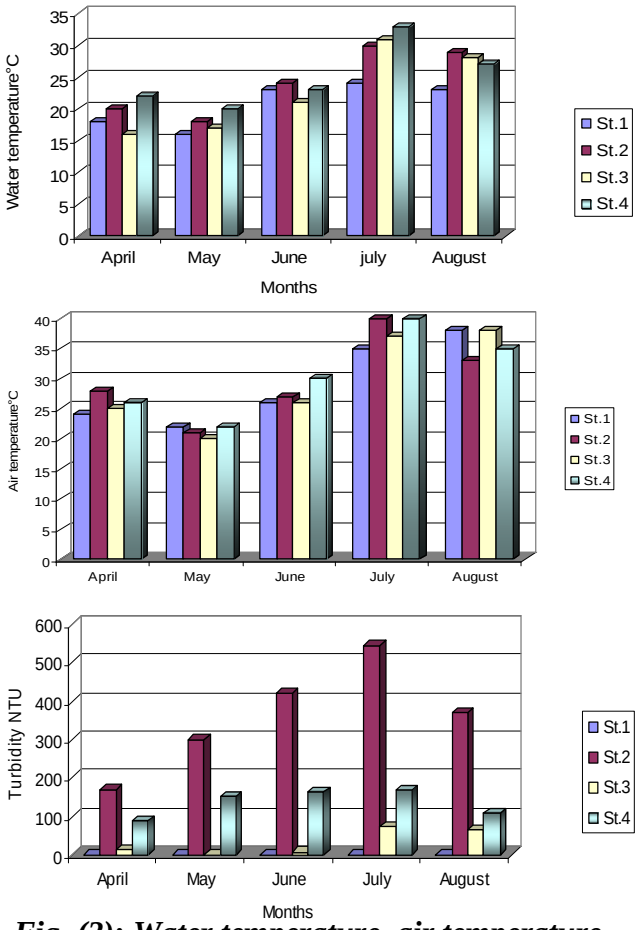

Fig. (2): Water temperature, air temperature and turbidity variation of studied stations during April-August 2008.

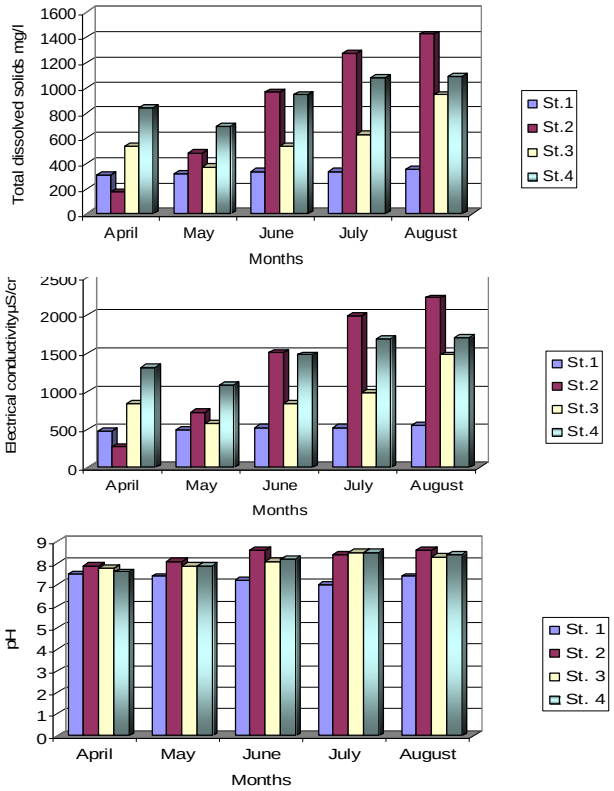

Fig. (3) Total dissolved solids, electrical conductivity and pH variations at studied stations during April-August 2008.

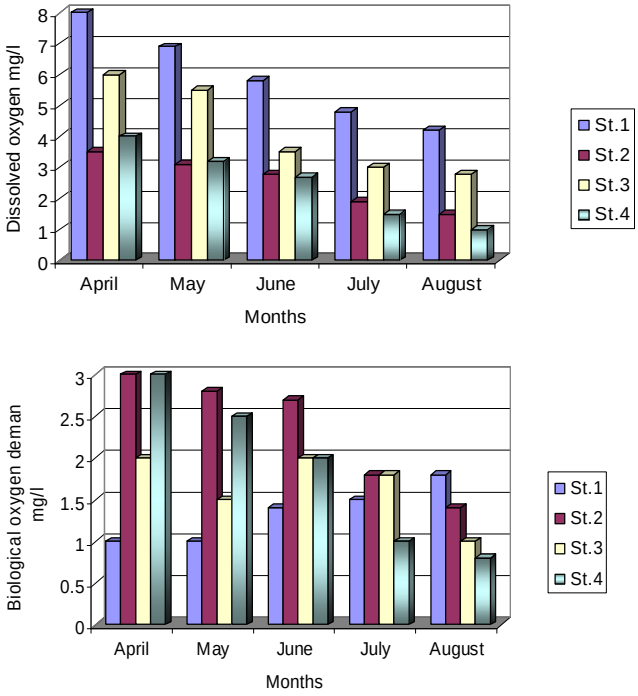

Fig. (4) Dissolved oxygen and biological oxygen demand variation of studied stations during April-August 2008.

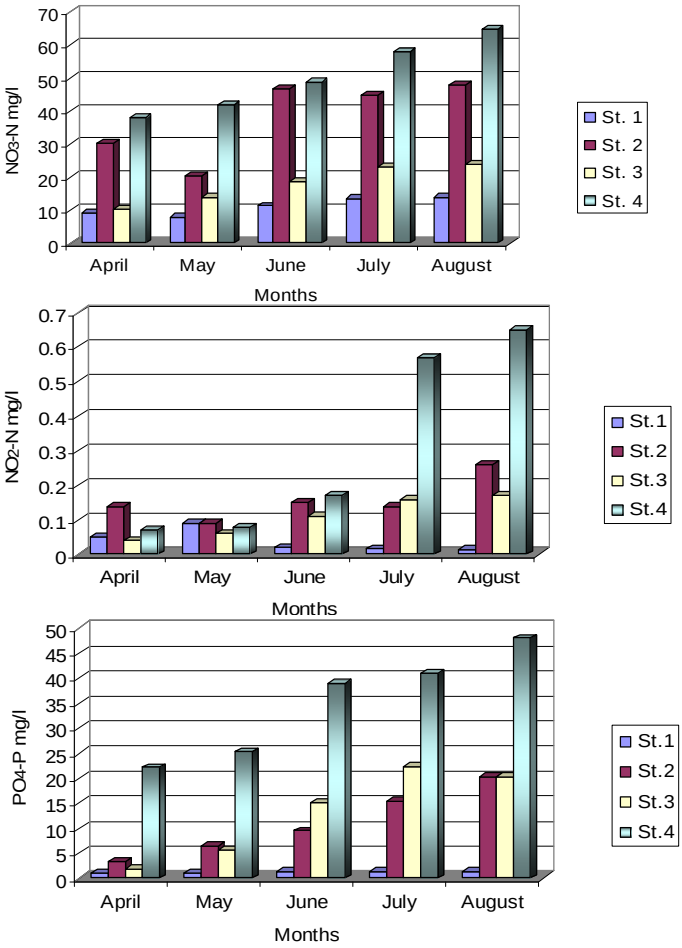

Fig. (5) Variation in Nitrate, nitrite and phosphate concentration of studied stations during April-August 2008.

Occurrence of Aquatic fungi

Twelve genera of aquatic fungi were isolated from four studied stations during April 2008 to August 2008, in which 8 of them belong to the family Saprolegniaceae that includes Saprolegnia, Achyla and Aphanomyces. Only one species belong to each of the families Leptomitaceae, Pythiaceae and Blastcladiaceae that include *Leptomitus*, *Pythium* and *Allomyces* respectively, and one species of *Chytrid* which belong to the class Chytridiomycota were isolated.

The total numbers of fungi that were isolated from all stations reach 163 isolates. The greatest occurrence of isolated fungi located in StNo.1 that contain 77 isolated, followed by 48 isolates in StNo.3, 21 isolates in StNo. 4 and finally 17 isolates from StNo.2 Table (2).

Aphanomyces stellatus was the leading species of aquatic fungi which was appeared in high frequency percentag 28.22% and high occurrence percentage 25% *Pythium sp* was of moderate occurrence 15% and frequency percentage of 17.17%,whereas

Achyla americana, Achyla apiculata, Saprolegnia litoralis were of rare occurrence percentage 5% and frequencyof (1.84%), *Allomyces arbuscula* and *Aphanomyces laevis* appears (1.22%and 2.45%), respectivley Table (3).

Table (2)
The frequency percentage of aquatic fungi isolated from studied stations during April 2008 – August 2008.

Stations / Isolates	Station1	Station2	Station3	Station4	Total no. of isolates	% of frequency
Achyla americana	3	-	-	-	3	1.84
A. apiculata	3	-	-	-	3	1.84
A. racemosa	7	-	10	-	17	10.42
Achyla Total	13	-	10	-	23	14.1
Saprolegnia ferax	11	-	5	-	16	9.81
S. litoralis	3	-	-	-	3	1.84
S. parasitica	11	-	-	-	11	6.74
Saprolegnia Total	25	-	5	-	30	18.39
Allomyces arbuscula	-	-	-	2	2	1.22
Allomyces Total	-	-	-	2	2	1.22
Leptomitus lacteus	3	-	4	-	7	4.29
Leptomitus Total	3	-	4	-	7	4.29
Aphanomyces laevis	4	-	-	-	4	2.45
A. stellatus	16	3	18	9	46	28.22
Aphanomyces Total	20	3	18	9	50	30.67
Pythium sp.	16	5	3	4	28	17.17
Pythium ***Total***	16	5	3	4	28	17.17
Chytrid sp.	-	9	8	6	23	14.11
Chytrid Total	-	9	8	6	23	14.11
Total no. of isolates	77	17	48	21	163	99.95
Total no. of species	10	3	6	4	12	

Table (3)
The frequency and occurrence of total fungal isolates from studied stations during April-August 2008.

	Apr	*May*	*Jun*	*Jul*	*Aug*	*Total no. of isolates*	*Frequency %*	*Occurrence*	*Occurrence %*	*Symbol of occurrence*
Achyla americana	-	-	-	-	3	3	1.8	1	5	R
A. apiculata	-	-	3	-	-	3	1.84	1	5	R
A. racemosa	3	10	4	-	-	17	10.4	3	15	M
Saprolegnia ferax	5	-	2	5	4	16	9.81	4	20	H
S. litoralis	-	-	3	-	-	3	1.84	1	5	R
S. parasitica	-	5	2	-	4	11	6.74	3	15	M
Allomyces arbuscula	-	-	2	-	-	2	1.22	1	5	R
Leptomitus lacteus	4	-	3	-	-	7	4.29	2	10	L
Aphanomyces laevis	-	-	-	-	4	4	2.45	1	5	R
A. stellatus	2	13	7	16	8	46	28.2	5	25	H
Pythium sp.	14	-	-	12	2	28	17.1	3	15	M
Chytrid sp.	-	2	7	11	3	23	14.1	4	20	H
Total no. of isolates	28	30	33	44	28	163	99.9			
Total no. of species	5	4	9	4	7	12				

R= rare occurrence 5-9, L = low occurrence 9-14, M = moderately occurrence 15-19, H = high occurrence 20-25

Discussion

The variation of water temperature is affected by air temperature and this was clear through out of this investigation in all studied stations. This phenomenon was observed in other water system in Kurdistan region of Iraq [22, 23]. Temperature is an important factor in an aquatic environment affecting on biological processes [24]. In the present study, water temperature affect the occurrence and distribution of aquatic fungi, the richest number of fungi were of low or moderate temperature 16°C–21°C similar results were reported by [9,25], because at higher temperature sporulation of aquatic fungi decreased [26]. From the results it appears that there is a significant variation of turbdity value between different stations, perhaps because of decreasing water levels in each station which can stir and suspend material from stream bed that increase turbidity value. The pH value of most investigation lies in the alkaline side of neutrality, the obtained value of pH are considered as a usual condition because, generally in Iraq Kurdistan region the pH of water characterized by shift towards the alkaline side of neutrality due to the geological formation of the area which composed mainly of $CaCo_3$ [28] and [29]. In the present study the pH values have a slight effect on aquatic fungi, because they are capable of growing over a wide range of pH between 4.5 – 8.5 [30], nearly the same results in Sarchnar spring and its tributaries obtained that ranged 7.0 – 8.15[31], while both streams of Shaqllawa and Korre have pH value 7.1–8.2 by [32]. Significant variations between electrical conductivity and aquatic fungi investigated.The richest occurrence of aquatic fungi relatively seen in the water samples which characterized with low total dissolved similar results reported in Saudia Arabia [33], in Egypt [34] and in Poland [35]. Dissolved oxygen also play an important role in the population of aquatic fungi in Egypt [9] in Poland [36,37] and in Baghdad [17], depletion in dissolved oxygen may be due to increasing of water temperature [38] or due to oxidative and fermentative degradation of organic mater drained with sewage [39]. BOD_5 is used for determination of biological oxygen demand. BOD_5 ranged between 1-3 mg/l as one knows this value is minimum for water pollution, same results found by [40] in Poland. Low concentration 8 mg at N-NO3/ l of nitrate was recorded in Sarchnare spring pool which may be due to utilize of nitrate by microphytes, while the highest value of nitrate 65 mg at N-NO3/l was recorded in the (confluence of the three stations) at August this result agreed with

that of [31]. Minimum value of phosphate was recorded in St 1 0.9mg at P-PO4/L where as maximum value was recorded in St 4, 48 mg atP-PO4/l. High concentration of phosphate can connected to the excessive use of fertilizers in the area which less to increase of phosphate concentration in ground water, also sewage loads with soap and detergents are major source of phosphorus in aquatic system as same that 0f [29]. The highest occurrence of aquatic fungi in this study appeared in Sarchnar spring pools St 1 which correlated to high dissolved oxygen concentration and suitable water temperature when compared to other stations , followed by St 3 which was polluted by industrial effluent also aeration is best. However, the two last stations St 2 and St 4 showed a low percentage of aquatic fungi, which may due to the water being more polluted, as they contain low concentrations of dissolved oxygen. The same result was achieved by [17]. The best season for growing aquatic fungi is autumn because of suitable range of temperature [5], while the worst being in summer because of higher rivalry from plants and eating the fungi by aquatic animals [41]. However there are some occurrences in other months, as the high occurrence of aquatic fungi in this investigation is *Aphanomyces stellatus* that appeared in high (H) occurrence reached %25 that grow in all months from all four stations.

Conclusion

The hydrogen ion concentration of water is alkaline, Sarchnar spring have less alkalinity than other stations.m Only Sarchnar springs have best aeration. Sarchnar spring and Chaq Chaq stream contain high numbers of aquatic fungi than the other two stations.

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الخلاصة

اجريت دراسة فطرية وبيئية لاربعة محطات مياه اختيرت ضمن محافظة السليمانية (بركة تجمع مياه سرجنار و مياه الفضلات المنزلية المجاورة لمنطقة سرجنار و جدول جق جق و تجمع المناطق الثلاث السابقة). خلال خمسة اشهر من نيسان 2008 و لغاية آب 2008.

تمت دراسة بعض الصفات الفيزيائية والكيميائية للماء باستعمال الطرق القياسية. تم تسجيل 12 جنس من مجموع 163 عزلة، باستعمال طريقة المصائد (الطعوم) لعزل الفطريات المائية.

تم تشخيص احدى عشر نوعا فكان ثمانية منها تعود الى عائلة Saprolegnaiceae وتتضمن*Achlya americana* و *A. apiculata* و*A. racemosa* و *Saprolegnia ferax* و*S.litoralis* و *S . parasitica* ونوعين من *Aphanomycese laevis* و *Aphanomycese stellates*, وثلاثة انواع لم يتم تشخيصها والتي تعود الى كل من العوائل:

Leptomitaceae, و Pythiaceae و Blastocladiaceae والتي تتضمن الانواع: *Leptomitus*, *Pythium* *Allomyces* على التوالي و نوع واحد من الكتريدات والتي تعود الى صنف Chytridiomycota، واضهرت نتائج هذه الدراسة بان حوض نبع ماء سرجنار اغنى محطة في تواجد الفطريات المائية، بينما محطة مياه المجاري في منطقة سرجنار كانت الافقر في تواجد الفطريات المائية. تبينت بان هنالك علاقة معنوية توضح تاثير بعض الصفات الفيزوكيميائية على تواجد وتوزيع الفطريات المائية.