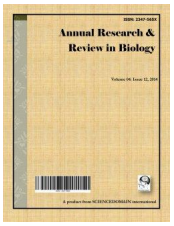

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Micro Flora Associated with Freshwater Medicinal Leeches in Taif City, Saudi Arabia

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Authors' contributions

This work was carried out in collaboration between all authors. Author ABME designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AAI and MFAAA managed the analyses of the study. All authors managed the literature searches and read and approved the final manuscript.

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ABSTRACT

Microflora (Cyanobacteria, Algae and Fungi) associated with the freshwater leech namely, *Limnatis nilotica* were observed for this particular study. The number of catch leeches was higher at Saiysad fresh water bodies (5-28 taxa) than that of Gahdeer Albannat water (1 taxon) throughout the wet season. The Saiysad water contains low oxygen and is highly polluted in comparison to other water bodies. The green algae and diatoms constitute the main bulk of the phytoplankton population of the following two water bodies. Though cyanobacteria in this particular water body of Saiyasad fresh water are much higher in respect to the others, so as the number of leeches are also higherin it respectively. Twenty-one fungal species those are representing 10 genera were collected from the Leech samples (jaws, pharynx and intestine) on the culture media of Potato Dextrose Agar. After th isolation of fungus were done from its carried devices Then also the phytoplanktons show a rapid growth in the respective medium.

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1. INTRODUCTION

Leeches have a wide global distribution in freshwater environments. All bodies of water hold leeches, from huge reservoirs to small park ponds, rivers, lakes and slow-moving streams etc., sometimes lands are also enough covered with the several types of leeches. These terrestrial leeches are mostly found in rain forests around the world. The most preferred habitats for these leeches are -rich organic matter and sometimes they also get attached to the boots and clothes of a human. During dry seasons, most leech species withdraw inside cavities at the bottom of the waters. They can survive even if they lose 90% of their body length by dehydration [1,2,3]. Some leeches are hunters and are prey for other invertebrates like snails, insects and worms, other leeches are highly specialized feeders and only depends upon the eggs of fishes. They use suckers present at anterior and posterior ends of the body and these are important for locomotion**.** Leeches are used for medicinal purposes also, which shows that they are used to reduce blood pressure in humans since ancient times. Today, medicinal leeches are used in Europe mainly for two purposes. The first is the classical blood-letting therapy, which has experienced in various revival cases over the years in the context of traditional revival therapies of natural remedies and traditional curing practices. The second is the production of remedial drugs which contain an extract of medicinal Leeches [4]. Nowadays, leeches bred under sterile laboratory conditions, and are used in medicine. The distribution of leech's indifferent lakes was investigated by Canpolat and Sağlam [5]. On the other side, Demirsoy et al*.* [6] studied seasonal changes of *Hirudo medicinalis* at its different stages of cycles. Linnaeus,1758, first cite the concept of the species in Poyraz and Efteni Lakes. There are several studies present on biology, morphology, ecology and medicinal science that shows usage of *H. medicinalis* of different countries for different clinical purposes. [7,8,9,10,3,11,12,13,14]. *Limnatis nilotica* Savigny, 1822 may enter the body of people in drinking water and leech that sticks to the mucous membrane of the pharynx, nasal cavity, nasopharynx and oesophagus [15,16]. No available literature concerning identification and culturing of leeches in Taif city were conducted , thus the objective of this study was to determine the distribution and density of leech species in two fresh water bodies and report on micro flora (Cyanobacteria, Algae and Fungi) associated on it throughout the study periods show limited references in this regard. In this particular study, the target samples are fresh water leeches along with the microflora, mostly of cyanobacteria, algae and fungi which show growth in the directly proportionate way with the following leeches. In this case, a particular pond water leech has been taken into account i.e. *Limnatis nilotica,* which is a eukaryote belongs from phylum annelid, class *Clitellata*, and of subclass *Hirudinea*.

2. MATERIALS AND METHODS

2.1 Description of the Study Area

The Samples were collected from two freshwater bodies: Saiysad and Ghadeer AL-Banat from 9 to 11 am at wet season (August -November 2017)

Saiysad is upstream of Wadi Wajj; it lies 9 Km northeast of Taif city N 21°18'56.6" E 40°29'01.7". It is 1580 m.a.s.i. It receives the floodwater from Al-Sarawat Mountains. It is running water contains many filamentous algae numerous plants and small fish. Seven water samples were taken from this area and seven baited traps to Leech collections (**Site 1** to **Site 7**) [55].

Ghadeer Al-Banat is located at 9 km southeast of Taif city. A wide stream receives the rainwater from Sarawat and Shafa Sofian Mountains through Wadi Leyah. It is about 1700 m.a.s.i. The water is slightly turbid and almost present all over the year. Its vegetation structure contains algae and *Lemna gibba* clumped on wide areas of the water surface in addition to the submerged plant. The bottom is mainly rocky with some sand and mud. Its water is mostly stagnant with the exception of the flood time and the stream site exposed to limited camping activities. Two water samples were taken from the stagnant area (**Site 8 and 9**), one from the running rainwater (**Site 8**) and sample from the sewage effluent (**Site 9**). Also, two baited traps to Leech collections (Fig. 1) [55].

Leech Captivity: Some leeches were adapted to the laboratory conditions for three months in aerated containers filled with tap water, fed on small fish, and formulated diet, to study the water analysis and micro-flora associated in it (**Site 10**).

Catch Leech and collections: In this study, large tin traps are used to catch leeches. for this purpose some bait are given in the traps (fish head) and pinch the open end of the can closed so that the opening is only one inch wide, sink the can in the water and pull it out in the morning. Mark the spot of the sink which can found later [2]. Leeches collected from fresh water during the wet season (August to November 2017) were placed in plastic containers and transported to the laboratory.

2.2 Physical and Chemical Parameters of Waters in Study Sites

Physical-chemical parameters were carried out according to Standard Methods for Examination of the Water and Wastewater [17]. The electrical conductivity (EC) and pH for each sample were determined. Water analyses including total dissolved salts (TDS; mg L^{-1}) and chlorides (Cl; mg L^{-1}) were analyzed by precipitation by AgCl and titration. Major cations such as sodium (Na), potassium (K), calcium (Ca), and magnesium (Mg; mg L^{-1}) were determined by a flame photometer (Jenway, PFP-7),. Phosphate and nitrate were determined spectrophotometrically by Ascorbate and Chromotropic acid methods.

2.3 Identification of Cyanobacteria and Algae from Fresh Water with or without Leech

Identification of Phytoplankton and cyanobacteria at least to the genus level was carried out using keys and descriptions adapted from [18,19,20,21]. Algal sampling w quantitative to determine the dominance or rareness of each algal taxon with the help of the DAFOR scale (Dominant; Abundant; Frequent; Occasional; Rare).

2.4 Isolation of Fungi Associated

The leech (jaws, pharynx and intestine) were prepared separately under a stereomicroscope, with 20-x magnification, held with sterile gloves and disinfected externally with an alcoholic solution of povidone-iodine accordingly to [22]. The preparation jaws, pharynx and intestine of leech were performed according to techniques described by [23,24], respectively. Each part of leech was inoculated separately (five replicates) on the surface of Potato's Dextrose Agar (PDA) and Sabouraud's Dextrose Agar(SDA) with Chloramphenicol media, then incubated at 30°C for7 days .Repeated sub-culturing were essential to obtain pure cultures. Isolates were characterized according to morphological features, pigmentation of the mycelium and direction of growth of the hypha, whether aerial or lateral, microscopic observation of structures involved in asexual reproduction, e.g. conidia or spores, and in sexual reproduction, and the presence of fruiting bodies. Identification of fungal species w accomplished using appropriate taxonomic techniques, such as those of [25,26,27].

2.5 Data Analysis

In order to detect correlations between Cyanobacteria parameters and environmental data, canonical correspondence analysis (CCA) according to [28] was conducted with species recorded in stands and soil variables using the second matrix [29]. Relationships between leech numbers, cyanobacteria, algae, fungi, parameters water variables were tested using Pearson's simple linear correlation coefficient (r). Variations in species diversity and soil variables in relation to sites were assessed using SPSS software (SPSS, 2011).

3. RESULTS AND DISCUSSION

Freshwater *Hirudinea* is one of the most important ecological groups of hydrobionts. Leeches are of scientific interest as an important link in the food chain of aquatic ecosystems, as well as bio-indicators of water pollution [30,31]. Moreover, parasitic leeches are involved in the abundance regulation of host species. *Hirudinea* sp. may be directly related to transmission of bacterial and viral infections [32,33,34,35,36] as well as hematozoa including trematodes, cestodes, and nematodes and parasitic flagellates, which are considered to be pathogenic organisms for aquatic animals. Moreover, ulceration, haemorrhage, and inflammation associated with leech attachment sites weaken the host and may pose an opportunity host to bacterial infections. In the present work, sixty-nine leech individuals were collected from two fresh water bodies that belonging to one species *Limnatis nilotica* according to [6]. Leech density was higher at Saisyad fresh water bodies (5-28 individuals) in that most sites than Gardner Albannat (1) water throughout the wet season using 9 catch leech (Fig. 2, Table 1). The Saisysad water was muddy with a high level of sedimentation in comparison to Gardner Albannat water. In this respect, Demirsoy et al. [6] showed that the number of

leeches in this weight class increases strongly during May and June and reaches a maximum in July. A continuous drop was observed in Rasht after August 2009, associated with low water temperature, reduced amphibian density, and lower vegetation biomass. *Hirudo. oriental* Ulevsky & Trontelj,2005 were observed to hibernate during the cold months. Another condition affecting leech density is the amount of rooted vegetation relative to the volume of water [37]. Small ponds have a large proportion of rooted plants, and the products of their decay lead to a high concentration of humic acids in the water.However, in the present study, the effect of humic acid and its accumulation as a factor affecting the leech community was not measured. Other Laboratory studies showed that the preferred temperature of *H. medicinalis* in a gradient of 7 to 43°C was 21°C, a value midway between the 50 % and 90 % active leeches, as documented in a more recent study [38]. Optimum temperature ranges for growth (22 to 25°C) and breeding (25.5 to 27.5°C) in the laboratory were similar to predicted values for maximum activity in the field. At 39 to 43.5°C, the upper lethal range is remarkably high for this species. These high-temperature requirements have important implications for the survival of *H. medicinalis* in the wild: the leeches were unable to reproduce and survive in many water bodies simply because of the low water temperatures [39].

Dissolved oxygen showed little change during the wet season (4.7 to 6.9 mg l^{-1}). The pH was almost constant at 6.5 to 7.5. However, in Ghadeer Al-|Bannat water, dissolved oxygen ranged from7.0to 7.3 mg $l^{-1,}$ and the pH tended to be on the alkaline side (8.0-8.3). Like other worms, leeches absorb dissolved oxygen through the surface area of their entire body. They are moderately tolerant of pollution and low oxygen conditions. Certain macro invertebrates, like leeches and aquatic worms, thrive in poor quality water. An abundance of these organisms suggests environmental conditions in a body of water have deteriorated. Some of these invertebrates use "snorkels" to access oxygen at the water's surface and are less dependent on dissolved oxygen to breathe [6].

The present study represents a comparative study between the water chemistry and diversity of cyanobacteria and algae of water samples collected from seven sites of Saiysad and two sites of Gadeer Al-Banat during the wet season at Taif (Saudi Arabia). The climate of the studied sites characterized by cold winter and mild hot summer. However, the maximum water temperature at all sites surveyed did not surpass 20.8˚C whereas the minimum temperature was not less than 19.5˚C (Table 1). The response of algal biomass formation at the study sites to water temperature was reflected in retarded or activated algal growth (Table 2). The pH values

Table 1. Leech numbers and some physico-chemicals parameters (mg.L^{-1}) of fresh water at Saiysad and Ghadeer Al-Bannat in Taif city throughout the wet season (August to November, 2017)

Parameter's	Saiysad							Ghadeer Al-Banat		Leech captivity
Sites	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**
Leech numbers	**0**	**5**	**0**	**13**	**11**	**28**	**1**	**1**	**0**	**10**
Water Temp.	19.7	19.5	19.7	19.9	20.2	20.1	19.7	20.8	20.7	20.2
PH	7.3	7.7	7.9	7.1	6.9	7.1	7.5	8.1	8.1	7.1
Dissolved oxygen	6.6	5.3	6.9	5.6	6.3	4.7	5.1	7.0	7.3	7.6
Cl	11.2	10.8	11.7	11.3	9.8	9.4	9.6	7.5	9.7	9.8
E.C (m.moh,s^{-1})	550	501	497	440	430	490	480	501	550	615
T.D.S	0.6	0.6	0.5	0.58	0.57	0.6	0.6	1.7	1.8	1.0
Ca^{++}	7.2	7.0	7.5	7.9	7.3	7.7	6.9	9.3	8.7	7.0
Mg^{++}	3.1	3.1	3.0	3.7	3.5	3.2	3.3	3.5	4.3	3.1
NO_3	11.5	9.7	9.9	11.1	10.8	10.7	11.0	7.9	7.7	9.8
PO_4	2.1	2.3	3.0	3.9	3.4	3.1	2.8	3.0	3.2	1.8
Na^+	35.1	30.5	32.5	35.7	30.7	29.7	30.1	33.8	35.9	29.8
K^+	2.9	3.1	3.7	3.1	2.9	3.7	3.1	5.6	5.8	2.9

of the collected waters were on the alkaline side in Gadeer Al-Banat and may be neutral in Saiysad during the study period; it was attributed to the excessive growth of algae that could resultin increased photosynthetic activity**.** Roland [40] showed that the pH values of water habitats usually support the growth of the certain algal group. The results here in obtained enabled us to make some general comments on the water chemistry and algae. The range and average of nitrate in the water samples were 7.7 to 11.5 mg.l^{-1} The range of some chemical analysis (mg/L) of water samples collected during the study period were: phosphorus (2.1-3.9), chloride (7.5-11.7), calcium (7.0-9.3), magnesium (3.1-4.3), sodium (29.7-35.9), and potassium (2.9-5.8) mg .l-1. The Cyanobacteria, as well as the algal diversity of the study sites, generally increased in Gadeer Al Banaat than in Saiysad sites. The main environmental factors influencing microalgal growth and chemical composition are light, nutrients, temperature and pH [41].

In the present work, a comparison of the algal populations was made for the ten study sites including a number of species of the relative amounts of the various algal groups. Green algae where the highly abundant group in all study sites; this contributes also to the greatest number of species. Bacillariophyta were the least frequent (Tables 3-4). The data presented here and those obtained by other researches clearly indicated that the inhibited and stimulated the growth of certain algal groups or species might be one aspect of the role played by water chemistry in modifying algal population and growth activities in water habitats [42,43]. In this respect, Stewart and Wetzel [44] recorded that green and blue-green algae constitute the main bulk of the phytoplankton population of temperate lakes during summer.

Twenty-Three species of cyanobacteria were identified from the collected water samples. These species were most dominant in Gahdeer Al Banaat in comparison to Saiysad sites that may be reflecting a number of Leeches collection with positive correlations in these sites. Concerning the green algae in the study sites, 16 species were identified throughout the study periods. Some green algae species were more dominant in Saiysad in comparison to Gahdeer Al Banaat sites, that may be reflected a number of Leeches collection with negative correlations in these sites. With respect to Bacillariophyta, eight species were identified throughout the study periods, of which two species only were identified in Gardner Al Banaat sites, and no species in Leech Captivity. These algae were more sensitive to pollution than other algal groups. It worthy to mention that no algal species of euglenophytes and pyrrophytes were identified in all sites during for the study periods. In conclusion, largely the weathering of rocks and the seasonal pattern of rainfall [45] determine this type of water body, the whole ecosystem including water chemistry (e.g. pH, conductivity). This climatic and chemical status provides a reasonable explanation for the frequent occurrence of cosmopolitan taxa capable of tolerating wide environmental fluctuations that attributed the rarity of endemic taxa of such shallow lakes to the scarcity of long periods of stability.

Concerning to fungal species associated with fresh water medicinal leeches, twenty-one fungal species representing 10 genera were collected from Leech samples (jaws, pharynx and intestine) on Potato Dextrose Chloramphenicol Agar (20 species and 9 genera) and Sabouraoud's Dextrose Chloramphenicol Agar (14 species and 8 genera) at 30°C for 7 days (Tables 5 and 6). Twenty fungal species representing nine genera were isolated from 10 Leech sample (jaws, pharynx and intestine) on Potato Dextrose Chloramphenicol Agar at 28±2°C (Table 5). *Yeast* was the most common genus that recovered in the high-frequency occurrence of the samples and total fungi. It was represented by three species. *Aspergillus* occupied the second place in the number of cases of isolation that recovered in a high frequency of occurrence of samples and total fungi. It was represented by six species were (*A. candidus, A. flavus, A. nidulans, A. niger, A. terreus and A. Versicolor*). *Penicillium* occupied the third place in the number of cases of isolation. It was represented by three species namely, *P. brevicompactum, P. chrysogenum and P. citrinum*. While *Fusarium (F. oxysporum and F. Solani), Cladosporium cladosporiosis, Geotrichum candidum, Cochliobolus lunatus*, *Rhodotorula rubra* and *Trichoderma viride* were isolated in rare frequency occurrence of the samples and total fungi, respectively.

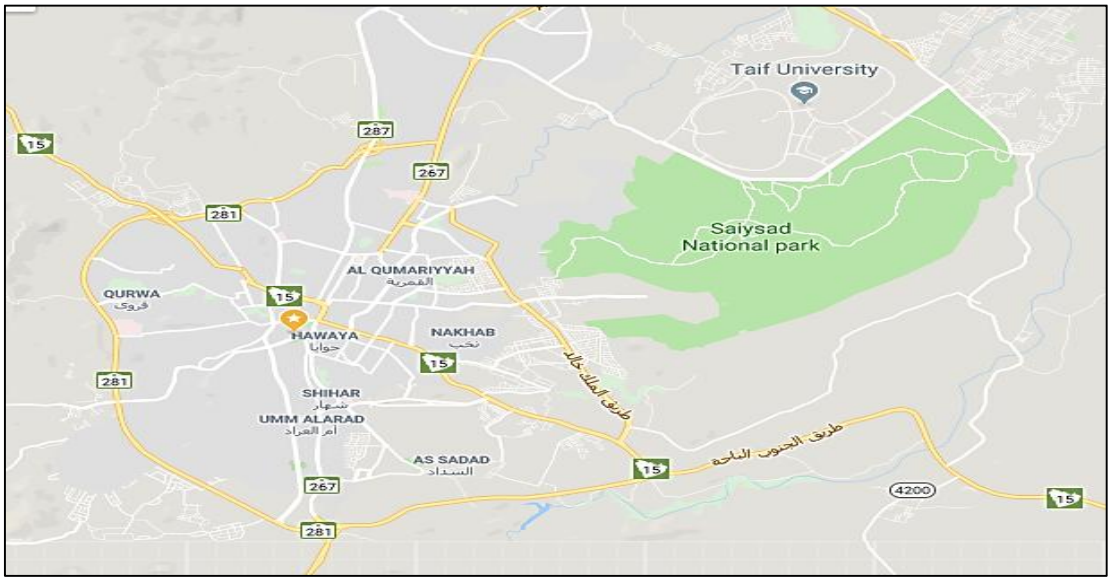

Fig. 1. Google map showing sampling sites in Taif-KSA

Also, fourteen fungal species representing eight genera were isolated from 10 Leech sample (jaws, pharynx and intestine) on Sabouraoud's Dextrose Chloramphenicol Agar medium at 28±2°C (Table 6). Similarly, *Yeast* was the most common genus that recovered in the high-frequency occurrence of the samples and total fungi. It was represented by four species. *Geotrichum candidum* occupied the second place in the number of cases of isolation and recovered in a high frequency of occurrence of samples and total fungi. *Rhodotorula rubra* occupied the third place in the number of cases of isolation. *Aspergillus* was isolated in the moderate frequency of occurrence and was represented by 3 species (*A. nidulans, A. niger*, and *A. terreus*). *Penicillium* was isolated in a low frequency of occurrence. It was represented by two species namely, P. *chrysogenum* and *P. purpurgenum*. While *Fusarium oxysporum*, *Cladosporium cladosporiosis, Alternaria alternata* and *Trichoderma viride* were isolated in the rare frequency of occurrence.It is worthy to mention that, some fungi were isolated only on Potato dextrose chloramphenicol agar, which includes *Aspergillus candidus*, *A. flavus*, *A. versicolor*,

Fig. 2. Freshwater leech *Limnatis nilotica* collected from the study sites

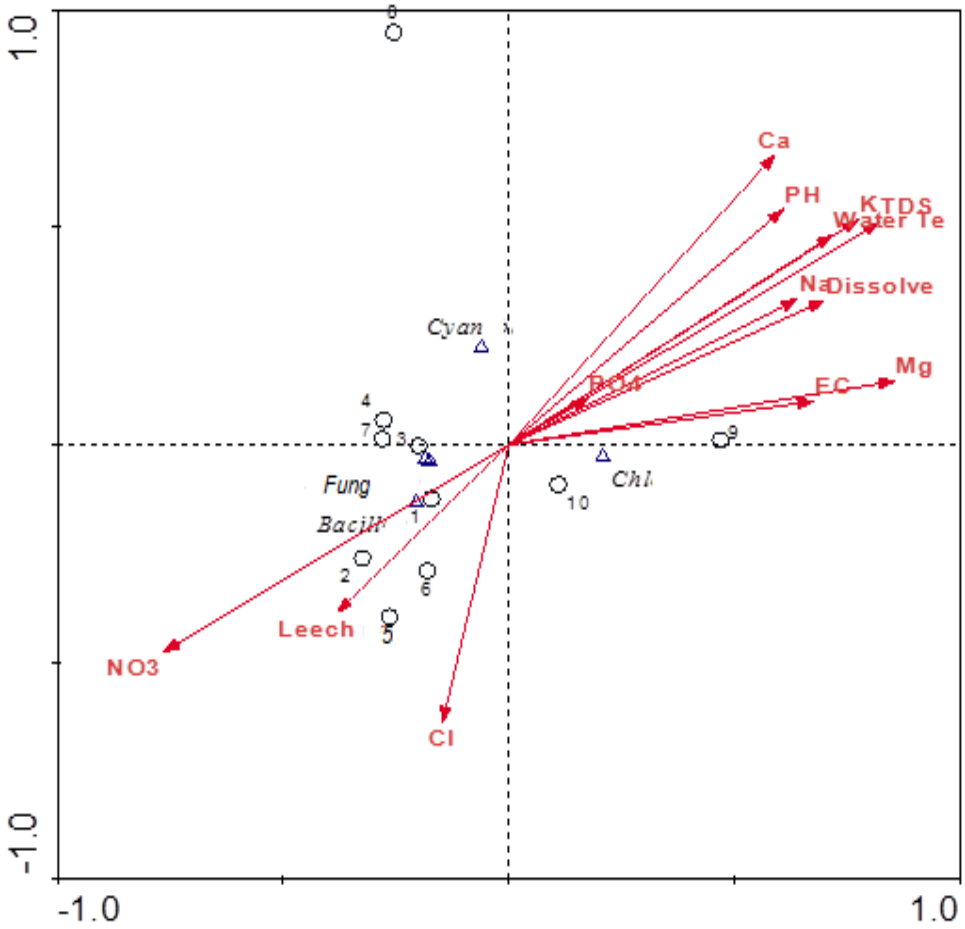

Fig. 3. CCA Biplot ordination of the leech, cyanobacteria, algae, and fungi species with environmental variables. Chl. Chlorophyta, Cyn. Cyanobacteria, Bacil. Bacillariophyta, Fun. Fungi

Cladosporium cladosporiosis, *Cochliobolus lunatus*, *Fusarium solani*, *Penicillium brevicompactum*, and *P. citrinum*. While *Alternaria alternate, Penicillium purpurgenum* and *yeast d* were isolated only on Sabouraoud's dextrose chloramphenicol agar medium. Other authors in both people with fungal skin colonisation and candidosis [46,47,48,49,50] found the same data of these fungal strains most frequently. In this respect, Litwinowicz and Blaszkowska [51] isolated 152 strains of yeast-like fungi and yeasts belonging to 14 species and 3 genera were identified from *Hirudo verbena* Carena,1820. Moreover, some cases of chromoblastomycosis caused by *Fonsecaea* species after wild leech bites have been reported [52,53]. Khalil et al. [54] stated that twenty-two species belonging to 11 genera of zoosporic fungi were collected from 10 freshwater sites and from five species of leeches.

The application of CCA on the cyanobacteria, green algae, diatoms and fungi communities and environmental variables indicated that diatom and fungi species, nitrate, chlorides and dissolved oxygen are correlated positively with total number leeches, while others are negatively correlated with chlorides, pH, EC, green algae and cyanobacteria. On the other hand, the correlation analysis between leech and associated micro flora community variables indicated a highly positive correlation (Fig. 3).

Table 2. Diversity of cyanobacteria species of fresh water at Saiysed and Ghadeer Al-Bannat (with or without leeches) in Taif city throughout the wet season (August to November 2017)

Algal taxa	Saiysad							Ghadeer Al-Banat		Leech captivity	Occurrence remarks
Sites	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**%**
Anacystis sp.	+		+				+	+	+		50
Calothrix artcha fermy						+		+	+		30
C. braunii Bornet & flahault	+							+	+		30
Chrococcus subnarius (Hansgirg) Kovacik			+	+				+			30
C.turjidus (Kutz) Naeg		+					+	+	+		40
Gloeocapsa psismagma (Breebisson) komarer					+			+	+		30
Lyngbya biebliana Claus				+					+		20
Nostoc carneum Agardh								+			10
N. microscopicum Carmichael							+	+			20
N. piscinale kutz		+			+			+			30
N. pruniforme C.AAgardh								+		+	20
Oscillatoria subbrevis Schmidle	+			+				+			30
Phormidium californicum Drouet						+		+		+	30
Ph. formosum (Bory ex Gomont) Anagnostidis		+					+	+			30
Ph. tergestinum (Kutzing)			+					+	+		30
Ph. pachydermaticum Fremy				+					+		20
Pseudoanabaena balatonica Scherffel et Kol									+		10
P. papilloterminat (Kiselev)Kukk								+	+		20
P. starmachii Anagnosstidis.	+						+		+		30
Schizothrix radius Komare				+				+	+		30
S. semiglobosa Geitler								+	+		20
Scytonema coactil Montagne			+					+	+		30
Spirulina nodosa Schiller	+						+		+		30
Total number of species	**5**	**3**	**4**	**5**	**2**	**2**	**6**	**17**	**15**	**2**	

Table 3. Diversity of chlorophyta species of fresh water at Saiysad and Ghadeer Al-Bannat (with or without leeches) in Taif city throughout the wet season (August to November 2017)

Algal Taxa	Saiysed							Ghadeer Al-Banat		Leech captivity	Occurrence remarks
Sites	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**%**
Ankistrodesmus falcatus (Corda) Ralfs							+	+	+	+	40
Chlamydomonas sp	+	+	+	+	+	+					60
Chlorella acuminate Gern	+			+			+				30
C. pyrenoidosa (Zeitl) Lund	+		+		+	+	+			+	60
C. vulgaris Beijer	+	+			+					+	40
Chlorococcum humicola (Nag.)	+	+	+		+			+	+	+	70
C. hypnosporum Starr	+	+		+	+	+	+	+	+	+	90
C. infusionum (Schrank) Menegh		+				+				+	30
C. minutum Starr				+							10
C. oleofaciens Trainor& Bold	+		+		+		+		+	+	60
Dictyosphaerium pulchellum Wood					+	+	+	+	+	+	60
Micractinium sp	+		+			+			+	+	50
Oocystis borgei Snow		+									10
Pandorina morum Bory							+				10
Scenedesmus qudricuda Klebs	+	+	+	+	+	+	+		+	+	100
Tetraspora gelatinosa (Wahlb.) gardh	+									+	20
Total number of species	**10**	**6**	**6**	**5**	**9**	**8**	**6**	**4**	**97**	**12**	

Table 4. Diversity of *Bacillariophyta* species of fresh water at Saiysad and Ghadeer Al-Bannat (with or without leeches) in Taif city throughout the wet season (August to November 2017)

Algal Taxa	Saiysad							Ghadeer Al-Banat		Leech captivity	Occurrence remarks
Sites	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**%**
Achnanthus lanceolata Breb					+	+	+				30
A. hauckiana Grunow	+	+			+			+	+		50
Cyclotella menegheniana (Kg.) (Kütz)				+			+		+		30
Dentecola teinuis Kutz	+		+		+	+	+				60
Fragilaria pinnata Malham Tarn	+	+			+						30

Algal Taxa	Saiysad							Ghadeer Al-Banat		Leech captivity	Occurrence remarks
Sites	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**%**
Hantzschia amphioxys (Her.) Grun. Cl	+	+	+		+						40
Navicula sp	+	+		+	+	+	+				60
Nitzschia linears (Ag)w.smith	+	+	+	+	+	+	+				70
Total number of species	**6**	**5**	**3**	**3**	**7**	**4**	**5**	**1**	**2**	**0**	

Table 5. Fungal species isolated from leech (jaws, pharynx and intestine) of fresh water at Saiysad and Ghadeer Al-Bannat in Taif city throughout wet season (August to November 2017), on PDA medium at 28°C

Genus/Species	Saiysad							Ghadeer Al-Banat		Leech captivity	Occurrence remarks
Sites	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**%**
Aspergillus candidus	-	-	-	-	+	-	+	+	+	-	40
A. flavus	+	+	-	-	-	-	+	-	-	+	40
A. nidulans	-	-	-	+	-	-	-	-	-	-	10
A. niger	+	+	+	+	+	-	-	-	-	+	60
A. terreus var. terreus		+	-	-	-	+	-	-	-	-	20
A. versicolor	-	-	+	-	-	-	+	-	-	-	20
Cladosporium cladosporioide	+	-	-	-	-	+	-	-	-	-	20
Cochliobolus lunatus	-	-	-	-	+	-	-	-	-	-	10
Fusarium oxysporum	-	+	-	-	-	-	+	+	-	-	30
F. solani	-	-	-	-	+	-	-	-	-	-	10
Geotrichum candidum	+	+	+	+	+	+	-	-	+	-	70
Penicillium brevicompactum	+	-	-	+	-	+	-	-	-	-	30
P. chrysogenum	-	+	-	-	-	-	+	-	-	-	20
P. citrinum	-	-	-	-	+	-	-	+	-	-	20
Rhodotorula rubra	-	-	-	+	-	+	-	+	-	-	30
Trichoderma viride	-	-	-	-	-+	-	-	-	-	-	10
Yeast a	-	+	+	+	+	+	+	-	+	+	80
Y. b	+	+	+	+	+	+	+	+	+	-	90
Y. c	+	-	+	-	+	+	+	+	-	+	70
Total number of species	**7**	**8**	**6**	**7**	**10**	**7**	**8**	**7**	**4**	**4**	

Table 6. Fungal species isolated from leech (jaws, pharynx and intestine) of fresh water at Saiysad and Ghadeer Al-Bannat in Taif city throughout wet season (August to November 2017), on SDA medium at 28°C

Genus/Species	Saiysad							Ghadeer Al-Banat		Leech captivity	Occurrence remarks
Sites	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**%**
Alternaria alternata	-	-	-	-	+	-	-	-	-	-	10
Aspergillus nidulans	-	-	-	+	-	-	-	-	-	-	10
A. niger	+	+	-	-	-	-	-	-	-	+	30
A. terreus var. terreus		+	-	-	-	-	-	-	-	-	10
Fusarium oxysporum	-	-	-	-	+			-	-	-	10
Geotrichum candidum	+	+	+	+	+	+	-	-	+	+	80
Penicillium chrysogenum	-	+	-	-	-	-	+	-	-	-	20
P. purpurgenum	-	+	-	-	-	-	-	-	-	-	10
Rhodotorula rubra	-	+	-	+	+	-	+	+	-	-	50
Trichoderma viride	-	-	-	-	+	-	-	+	-	-	20
Yeast a	+	+	+	+	+	+	+	+	+	+	100
Y. b	+	+	+	-	+	+	+	+	+	-	80
Y. c	+	+	+	+	+	+	+	+	+	+	100
Y. d	+	+	-	+	-	+	+	+	-	+	70
Total number of species	6	10	4	6	8	5	6	6	4	5	

4. CONCLUSION

Investigations revealed that the animal used to drink water from a nearby pond that consumes rainwater are prone to cause severe infection by the following leeches. The owner of the present case was educated not to allow the other animals to drink water from the infected pond. As microbial contamination of leeches poses a risk of transmission of pathogens to humans, contact with non-sterile leeches may cause a potential hazard to human health either with the resources of contamination like cyanobacteria or pathogenic fungi, which implies that this leech can act as a vector of these potential human pathogens. Therefore, education and awareness increasing of traditional herders about contaminated water and natural springs are necessary at this concernwhere as reventive measures should be taken in this regard carefully.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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