The Bacterial Fact of Sawa Lake in Samawa City Southern Iraq

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Abstract

Bacteriological investigation had been performed for samples collected from Sawa lake. The samples included: water samples, sediment samples, crystallized gypsum, which forms the walls of the lake, aquatic plant samples (*Chara* sp.), Zooplankton samples (*Arctodiaptomus salinus*) and fish samples (*Planliza abu*). The bacterial analysis included, heterotrophic bacteria (HB), total coliforms (TCs), fecal coliforms (FCs), halophilic bacteria (*Halococcus* sp. and *Halobacterium* sp.), endophytic bacteria, sulfate and sulphite reducing bacteria, *Clostridium perfringens* and *Vibrio* sp. The results showed that, only HB and halophilic bacteria were presented, while the lake was clear from any types of sulfate and sulphite reducing bacteria and pathogenic bacteria in the winter season.

Key words: Sawa Lake, Halophilic bacteria, Pathogenic bacteria.

Introduction:

Sawa lake is a unique water body of no inflow and outflow, described by the high salinity of the Iraqi inland waters. This lake may be fed by groundwater of the Euphrates and Dammam aquifers through a system of joints, cracks and fissures. Its water level fluctuates during dry and wet seasons. Its water does not dry up because of the equilibrium state between water feed up and evaporation (Jamil, 1977). The water chemistry, which is unique among Iraqi lakes, suggests that, it is probably of relatively recent karstic origin and not a relic of a mid-Holocene origin. It formed over limestone rock. The water in this lake is extremely salty due to heavy evaporation in the searing heat of Mesopotamia, even more than the water in the Arabian Gulf (Al- Quraishi, 2013).

Several studies have been conducted on Sawa Lake to understand the hydrogeological and hydrochemical conditions and the area surround it (Hassan, 2007; Al-Shemari, 2006; Al-Muqdadi, 2003; Bahgat, 1993; Samaan, 1985; Al- Naqash, 1977). No studies were carried out, what so ever, on the presence and distribution of bacteria in Sawa lake, except that of Najum and Hasan (2017) about *Vibrio cholerae*. The samples may reflect the presence or absence of certain bacterial species in this unique ecosystem. The present study represents the first attempt to investigate the occurrence and distribution of bacterial groups in Sawa lake, a little-known wetland in southern Iraq. This made it difficult to compare the results with previous field work and discuss the same field of study. (Najum and Hasan, 2017).

Materials and Method:

Lake description:

Sawa lake is an endorheic basin locates to the west of Al-Samawa city, in Al- Muthanna Province (about 23 km). It lies between longitudes (44° 59 29.01 and 4°5 01 46.61) and Latitudes (31 17 43.10 and 31 19 49.79). The lake has a longitudinal shape with 4.74 km long and a maximum width of 1.77 km isolated by a gypsum barrier with total path of 12.5 km surrounding the lake, and entry of surface water is not available to it. The lake has an elongated shape with a NW-SE trend (Figure 1) (Al-Quraishi, 2013; Awadh, 2016).

Fig.1. Site of Sawa lake (Abed, 2017).

Sampling:

Samples of water, crystallized gypsum, sediments, aquatic plant (*Chara* sp), zooplankton (*Arctodiaptomus salinus*) and fish (*Planliza abu*) were collected from the lake.

A total of 25 water samples were collected from 5 sites along the lake, according to standard methods for examination of water and wastewater (APHA, 2017) into sterile 500 ml sampling bottles. Other samples were collected in sterile plastic bags and placed in a cool box until returns to the laboratory.

Bacteriological analysis:

This method was done to estimate the numbers of bacteria which are presented and, if needed, to find out what sort of bacteria there. The bacteria included are, heterotrophic bacteria (HB), total coliforms (TCs), fecal coliforms (FCs), halophilic bacteria (*Halococcus* sp. and *Halobacterium* sp.), sulfate and sulphite reducing bacteria, *Clostridium perfringens* and *Vibrio* sp.

Water Samples:

Water samples were subjected to bacteriological analysis.The membrane filtration technique (APHA, 2017) was used by passing 100 ml through 0.45 µ Millipore WCN type filters (Whatman Corp., Japan). The membranes were placed onto the following media: nutrient agar plus 10% sodium chloride (NA + 10% NaCl) for HB (Brisou *et al*., 1974). MacConkey agar at 35 °C and 44.5 °C for TCs and FCs respectively. Differential Reinforced Clostridial Medium (DRCM) for sulfate and sulphite reducing bacteria and *C. perfringens*, which incubated anaerobically in an anaerobic jar using either Oxoid anaerobic gas generating kits (Code No. BR 38) or Al-Razi anaerobic gas kits (B. No. 41317) for 2-3 weeks at 37 °C. Thiosulfate Citrate Bile Salts Sucrose agar (TCBS) for *Vibrio* sp. at 37 °C and Gibbons

medium (GB) plus NaCl to give final concentrations of 10% and 15% selective media for halophilic bacteria at 25 °C for 48 h. (Brisou *et al.*, 1974).

Sediment Samples:

Samples of sediments were collected from the margins and middle of the lake, in addition to samples of crystallized gypsum, which form the walls of the lake.

One gram of each sample was suspended in 99 ml of sterile distilled water and shaken vigorously for 5 min. The liquid was serially decimal diluted supernatant in sterile distilled water of 10^{-1} – 10^{-6} and was plated on NA + 10% NaCl, MA at 35 °C and 44.5 °C, DRCM, TCBS and GM + 10% and 15% NaCl.

Aquatic Plant Samples:

The genus *Chara* is the only kind lives in Sawa lake. The stalks were stripped of leaves and dissected into segments which cultured with leaves in nutrient agar to isolating HB. On the other hand, segments of stalk and leaves were macerated using a sterilized glass homogenizer (Brand- Germany) after washing with ethanol at 70% for 5 min and a final washed with sterile distilled water up 5-8 times. The extracts were then serially diluted with sterile distilled water and plated in NA+ NaCl and TCBS. The plates were incubated at 25 °C for 5-6 days to detect any endophytic bacteria, while, TCBS plates were incubated at 37 °C for 24 h.

Zooplankton Samples:

Arctodiaptomus salinus was identified as the main genus in Sawa Lake. It was dissected under stereo microscope; the mouth and stomach were cultured in NA+ NaCl and TCBS to recover HB and *Vibrio* sp respectively.

Fish Samples:

Planliza abu was the main genus lives in Sawa Lake. Smears from the skin under scales, gills, muscles, kidney, liver and stomach were cultured in NA+ NaCl, MacConkey agar and TCBS for the determination of HB, total coliforms and *Vibrio* sp. respectively.

Results and Discussion:

Table (1) indicates the presence of heterotrophic bacteria and halophilic bacteria (other than *Halococcus* and *Halobacterium*) in water, crystallized gypsum and sediments. The benefit of isolating halophilic bacteria, including *Halococcus* and *Halobacterium* is their uses as a skin care products and can be exploited in the food industry, bioremediation and pharmaceuticals (NCBI, 2007; Bonete *et al*., 2015; Russell and Nelson, 2016; Gontia-Mishra *et al*., 2017; Jung *et al*., 2017).

Table 1. Bacterial contents (CFU / 100 ml) in water, crystallization gypsum and sediments in Sawa lake.

Sample source	HB	TC	FC	SRB	SRC	*Clostridium perfringens*	*Vibrio*	Halophilic	
								Aerobic	Anaerobic
Water	4.5 X 10^2	Nil	Nil	Nil	Nil	Nil	Nil	1.0 X 10^3	1.7 X 10^2
Gypsum	1.5 X 10^2	Nil	Nil	Nil	Nil	Nil	Nil	2.2 X 10^3	2.0 X 10^2
Sediment	2.7 X 10^2	Nil	Nil	Nil	Nil	Nil	Nil	6.5 X10^3	3.9 X 10^3

Other groups of bacteria were not found. In contrast Jamil (1977) suggested that, the formation of the crystallized gypsum of the walls may be related to sulphate reducing bacteria with algae, which covering the rocks to supply the necessary energy and oxygen in the crystallization process. Meanwhile, we couldn't isolate this bacterium in the present study.

Similar results were obtained from the aquatic plant, zooplankton, and fish (Table 2). The main reason to investigate the bacterial contents in these living organisms is that, at low temperature some bacteria, especially *Vibrio* sp. attach to zooplankton, plants and fish, and so cann't be detected in the water during the winter months (Sochard *et al*., 1979; Nalin, 1976; Huq *et al*., 1983; Islam *et al*., 1994). However Najum and Hasan (2017) isolated *Vibrio* sp. during March- December, 2016.

Some researchers (Hallman *et al*., 1997; Kobayashi and Palumbo 2000; Zinniel *et al*., 2002; Ryan *et al*., 2007; Santoyo *et al*., 2016) isolated endophytic bacteria from surface plant tissue or extracted from internal plant tissue. In the present study, we couldn't isolate any type of endophytic bacteria or any types of pathogenic bacteria, and this is may be related to the reason that, Sawa Lake water is highly saline (over saturation) and the dominant cation- anion was Mg- Cl and this property differ from sea water (Maulood and Al-Mousawi, 1989; Hassan, 2007), and this may have affected the microorganisms living in the lake.

Sulfate was found in seawater, sediment, or water rich in decaying organic material. Sulfate-reducing microorganisms are common in anaerobic environments where they aid in the degradation of organic materials (Dexter Dyer, 2003).

In spite of the high concentration of sulfate in Sawa lake water (Radi and Allawi, 2017; Farhan and Hussin, 2016; Hassan, 2007; Al- Quraishi, 2013), but we couldn't isolate any type of sulfate reducing bacteria and this is may be related to the fact that, the hypersaline ecosystems are generally inhabited by a limited variety of life forms. When the salt concentration level is about 10% a few vertebrates can tolerate. Above this level, only invertebrates such as zooplankton, algae and bacteria have been reported (Ollivier *et al.,* 1994). In the present study, in addition to the zooplankton and heterotrophic bacteria *Chara* were isolated from the Lake, and this is may not be enough for the growth of sulfate reducing bacteria.

Because Sawa Lake is the only water body available for the city of Samawa and its surroundings, and according to the present study it can be exploited for tourism.

Table 2. Bacterial contents in aquatic plant, zooplankton and fish from Sawa lake.

Sample source	HB	TC	Endophytic bacteria	*Vibrio*
***Chara*: Leaf**	**2.0 X 102**	**ND**	**Nil**	**Nil**
Stem	**1.6 X 102**	**ND**	**Nil**	**Nil**
***Arct. salinus:* Mouth part**	**2.3 X 101**	**ND**	**-**	**Nil**
Stomach	**3.6 X 101**	**ND**	**-**	**Nil**
***Planliza abu*: Skin**	**8.0**	**ND**	**-**	**Nil**
Gills	**5.0**	**ND**	**-**	**Nil**
Muscle	**Nil**	**ND**	**-**	**Nil**
Liver	**Nil**	**ND**	**-**	**Nil**
Kidney	**Nil**	**ND**	**-**	**Nil**
Stomach		**ND**	**-**	**Nil**

HB: Heterotrophic Bacteria; TC: Total Colifirm; FC: Fecal Coliform; SRB: Sulphate Reducing Bacteria; SRC: Sulphite Reducing Clostridia; Nil: No Growth; ND: Note Done.

Conclusion:

Sawa lake represents a unique, natural closed water body suitable for development of tourism in southern Iraq. As results shown that only HB and halophillic bacteria were presented, while there were no SRB,SRC, or pathogenic bacteria. Further detailed microbiological studies of the lake are recommended.

Conflict of interests:

The authors have not declared any conflict of interests.

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الواقع البكتيري لبحيرة ساوة في مدينة السماوة جنوبي العراق

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الملخص

نفذت هذه الدراسة للتحري البكتيري من العينات التي جمعت من بحيرة ساوة. شملت العينات كلاً من عينات الماء، والترسبات، والجبس المتبلور المأخوذ من جدران البحيرة، وعينات من النباتات المائية (*Chara* sp.)، والهائمات الحيوانية (*Arctodiaptomus salinus*) وعينات أسماك (*Planliza abu*). وقد شمل التحليل البكتيري فحص أعداد كل من البكتيريا متغايرة التغذية Heterotrophic bacteria (HB) ، وبكتيريا القولون الكلية total coliforms (TCs)، بوكتيريا القولون البرازية fecal coliforms (FCs)، والبكتيريا المحبة للملوحة halophilic bacteria (*Halococcus* sp. و *Halobacterium* sp.)، وبكتيريا النبيت الداخلي endophytic bacteria، والبكتيريا المختزلة للكبريتات والكبريتيت sulfate and sulphite reducing bacteria، إضافة الى دراسة النوعين *Clostridium perfringens* و *Vibrio* sp. . أظهرت النتائج احتواء العينات المفحوصة على كل من البكتيريا متغايرة التغذية، والبكتيريا المحبة للملوحة فقط، بينما خلت البحيرة من تواجد البكتيريا المختزلة للكبريتات، والكبريتيت، والبكتيريا الممرضة خلال فصل الشتاء.

الكلمات المفتاحية: بحيرة ساوة، البكتيريا المحبة للملوحة، البكتيريا الممرضة.