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Ecological Study of an Artificial Saline Lake Ecosystem in Wadi Al-Shatti, Libya

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ABSTRACT

This study provided the first data related to the ecosystem of Ain Al-Mashashiya Lake. It is likely the new apperance of a saline artificial lake formed from agricultural drainage water from surounded local farms, small lakes provide an opportunity to understand intra-ecosystem connectivity and its dynamics. Water samples were collected representing two stations along edges and at the middle of the lake and other samples surrounded soil and vegetation. Lake depth was > 5 meters, the pH was found to be relatively high at edge of the lake (7.46 & 7.44) compared to at middle of the lake (7.38 & 7.32). Highly saline water, i.e., 129.79 & 125.48 dS/m & 126.82 & 133.74 dS/m at edge and middle of the lake respectively. The average level of PO₄ and NO₃ during winter is slightly lower than summer. The water cations abundances were typically Na > K > Ca > Mg, where were for anions, Cl > $SO_4 > HCO_3 > PO_4 > NO_3$. The lake is valuable for biodiversity and provide habitat for many species. Five phytoplankton types have been registered, i.e., Cocconeis, Notholca Pandorina, Oscillatoria, Chlamydomonas. The most common species were Pandorina & Oscillatoria. Soils of the study zone were classified as sandy loam, with a porosity of 44.53%. and saturated hydraulic conductivity (Ks) values was 4×10-3 cm/h and 6.4% of OM content. The soil reaction (pH) was 7.24 and its EC value was 19.42 dS/m that indicated high saline affected soil. The concentrations of Na, Ca & Mg, were as high as 193, 48 & 14.4 mg/kg, respectively, with low NO3 content 0.293 mg/kg. Tamarix (Tamarix aphylla) commonly found along the lake edges. The dry desert climate of the area played a main role in imposing the lake ecosystems.

1 Introduction

Large areas of desert regions are characterized by endorheic drainage and consequently, waters collect in lower topographic areas creating lakes of different sizes and origins. If solute discharge increases, the lake becomes saline. Lakes also provide useful modern analogue environments to interpret fossil sedimentary records (Kumar and Abdullah, 2011). Hot deserts cover between 14 & 20% of the Earth's surface, approximately 19-25 million km². A defining characteristic of a hot desert is aridity. There are arid climates with > 500mm of annual rain that falls in intense events on hard soil or rock, and the water runs off horizontally or evaporates quickly (Rewald *et al.*, 2012). According to the Koppen-Geiger climate classification, deserts are regions with an annual precipitation of less than 250 mm (Spinoni *et al.*, 2015). However, the United Nations Environmental Programme's definition of desert is an annual moisture deficit under normal climatic conditions, where the potential evapotranspiration (PET) is over five times higher than actual precipitation (Huang *et al.*, 2017). Because of these extreme conditions, deserts represent unique eco systems, which support significant plant and animal biodiversity. In fact, there are many different kinds of hot deserts with varying landforms, altitudes and

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life forms (Azizi et al., 2021). Sand dunes, instead rocky plateau, cover approximately 15-20% of deserts or mountain desert landscapes prevail (Rewald et al., 2012). Soil properties effect the degree of aridity and plant productivity. There are soils in so-called edaphic deserts, which are extremely porous and have such low waterholding capacity that the annual precipitation drains through them and rapidly is virtually unavailable for vegetation. Related to water, plant productivity in deserts can also be nutrient limited. For example, nitrogen is a key limiting nutrient in most deserts (Zhang et al., 2021), while phosphorous is the most limiting nutrient in deserts (Rewald et al., 2012). However, in the driest parts there is likely to be insufficient precipitation even for the development of any surface drainage system. Saline lake geochemistry varies over multiple timescales and is the product of a complex system involving precipitation, weathering, groundwater, evaporation, precipitationdissolution reactions, and biological activity. These proximal controls are in turn controlled by the larger climatic, tectonic, and biological contexts in which saline basins form (Carroll and Bohacs, 1999). Small lakes provide an opportunity to understand intra-ecosystem connectivity and its dynamics. However, the information about the ecosystem is important even in the saline lakes that occur from time to time. The main factor that allows saline lakes to form is hydrological closure; that is, evaporation is the primary or only way for water to leave the basin. This condition occurs in many different settings around the world. The sources of water and solutes for saline lake brines are mainly direct precipitation, groundwater or associated surface flow. Groundwater can be derived from the local or regional meteoric system, interstitial water of sediments, or deep basin or hydrothermal fluids (Jones and Deocampo, 2003). In addition, the contributions of human activities, such as agricultural, domestic and industrial input are significant with regard to the physical characteristic and chemical composition of dissolved load of lakes and other water bodies (Khatri and Tyagi, 2015; Amnnah et al., 2020 Masood et al., 2021). The major problem of confined water bodies is usually the pollution from agriculture, industrial sewage and domestic wastewater (Barbosa et al., 2012). Confined water bodies may be regarded as an almost static system. The effects of the human activity on water resources can be divided into four factors: agricultural development; application of agricultural chemicals (nitrogen and pesticide); there are different types of monitoring programs according to their purpose, the most frequently tested parameters are: temperature, pH, turbidity, conductivity, dissolved oxygen, biological oxygen demand (organic matter), solids, nutrients, chlorophyll & coliforms; for in-depth research, metals, toxic substances and biological composition of the lake may also be studied (Bouderbala and Gharbi, 2017). Such information is crucial for developing strategies, programs or technical guidelines for the conservation and sustainable utilization of natural resources, particularly in the hyper-arid southwest region of the country. There is no published study on Ain AlMashashiya Lake, this is the first study conducted at three stations along the lake aiming to throw light on its ecosystem characteristics.

2 Materials and Methods

2.1. Site Description

Wadi Al-shatti is one of the districts of Libya in the south-west part of the country (27°44'6.53 N, 17.01'26°12 E). The area is mostly desert. Wadi Al-shatti is situated within the Murzuq Basin of south-west Libya. The Wadi Al-shatti area is mostly desert. The surface is generally covered by sand. Climatically, the study area is classified as an arid to extremely hyper-arid region. The climate of Wadi Al-Shatti is characterized by an annual mean temperature of 32.3°C, mean maximum temperature of 39.8 °C, mean minimum of 11.2 °C. The study zone has a wide range of temperatures between 0 ^oC in winter at night to 50 ^oC in summer at noon, Present day rainfall throughout the region is less than 20 mm per year on average, and exhibits high inter annual variability (Brooks et al., 2005). The potential evaporation rate is about 2000 mm, and the actual evaporation rate is negligibly less. The mean annual relative humidity ranges from 30 to 40%. The climate belongs to the type III semiarid of the African zonation (Griffiths, 1972). Ain Al-Mashashiya Lake belongs to Wadi Al-Shatti,

An Al-Mashashiya Lake belongs to wad Al-Shatti, located at 27°25'04" N 13°52'49" E, about 10 km from Ain Al-Mashashiya village. According to information obtained from the local citizens, it is newly artificial lake, formed about 8-10 years ago. It is saline lake, oval shape 40 meters long & 10 meters wide. Lake level fluctuations depend upon water input and output balanceand mostly controlled by evaporation. Lake water volume depends on the relative altitude of the watershed boundaries in relationship to the lake bottom and the lake bathymetry, (Figure.1).



Figure (1). Lake View

2.2. Sampling

Water samples were collected at two stations along the lake edge and the middle of the lake. For plankton examination, upper layer water sampling was collected at the same three stations. At each station, one litter of water was preserved with 4% formalin. The samples were transported in the glass cylinders into the laboratory. Field measurements of some parameters were conducted according to Standard Methods (2000), such as water temperature, electrical conductivity using conductivitymeter; pH was measured using pH-meter.

2.3. Determination Methods

Titration methods were used for total alkalinity and total hardness and the results from both analyses were expressed as mg/L CaCO₃. Chloride (Cl) determination were done by titration method using Hg (NO₃) (Boyd and Tucker, 1992). Sulphates SO₄, Nitrate (NO₃) and phosphate (PO₄) measurements, which require photometric measurements were done according to the standard procedures (Standerd method ,2000). Calcium and magnesium ions were determined by titrimetric method using E.B.T and Murexid reagents. Na, K, were measured directly using the flame photometer (Franson et al., 1995). Each sample was examined and enumerated via a drop method (APHA, 2005). Various identification tools were used for phytoplankton species identification (Standard Methods 2000, Al-Yamani et al., 2011; Conway, 2012). Soil pH was measured using pH meter in solutions 1:1 ratio (Aishah and Elssaidi., 2019). Texture was analysed using standard pipette-analysis with gravimetric determination (Kettler et al., 2001). EC was measured using conductivity meter (Richards, 1954). Total dissolved solids (TDS) were calculated as (TDS=EC25×0.064), The pycnometer method was used to measure soil particle density (Grossman and Reinsch, 2002). Particle density is expressed as ratio of total mass of soil to their volume (g.cm³). Porosity of soil was calculated according to Anderson and Ingram (1993), using the following equation: **Porosity** (%) = [1-(Bulk)]density/Particle density)] × 100. Hydraulic conductivity (Ks) experiments were performed according to Darcy's Law calculation as described by Misra and Sivonghay (2009). Organic matter (OM) was determined using losson-ignition (LOI) method (ASTM, 2000), using the following equation: %OM= Total C × 1.724. Ca and Mg were extracted using ammonium acetate (pH 7.0). Calcium and magnesium ions were determined by titrimetric method using E.B.T and Murexid reagents (Franson et al., 1995). Cl analyses were done by titration method with Hg (NO₃) ((Standard method ,2000). Phosphorus was determined according to the procedure of Chang and Jackson (1958) and Aishah and Elssaidi, (2019). Sulphates SO₄ and Nitrate (NO₃) measurements, which require photometric measurements, were done according to the standard procedures (Standerd method ,2000). Calcium and magnesium ions were determined by titrimetric method using E.B.T and Murexid reagents. Na, K, were measured directly using the flame

photometer (Franson *et al.*, 1995). Flora of LIBYA and encyclopaedia of Libyan plants were used to identify the vegetation cover around the lake.

3 Results and Discussion

3.1. Lake Water Physicochemical Properties:

As the study provided the first data related to the ecosystem of Ain Al-Mashashiya Lake, there are no data on long-term changes in the lake. In as much as evaporation in excess of precipitation plays such an important role in the development of saline lakes, it is not at all surprising that this lake is to be found in the semiarid or arid regions of the world. However, in the driest parts of the true deserts of the globe, such as the Arabian or Atacama Deserts, there is likely to be insufficient precipitation even for the development of any surface drainage system. The pH was measured for all samples studied over two seasons summer and winter season at the middle and edge of the lake. (Table 1). At edge of the lake, pH found to be 7.46 & 7.44 in summer and winter season respectively, that was found relatively higher than middle of the lake, i.e., 7.38 & 7.32. Detailed analysis describing that the pH values were high in the summer season compared to the values recorder in winter season. However, the analysis also indicates the salinity of this lake was found to be very high, 129.79 & 125.48 dS/m at edge of the lake and 126.82 & 133.74 at middle of the lake in summer and winter season respectively, our result explained this changes in lake water proprieties. Saline inland lakes are often hypersaline (i.e., above the salinity of seawater). As noted, one of the first problems to be noticed in saline lakes studies is the origin of the salts. Due to water unbalance, salinity has gradually exponentially increased in this lake but at different rates. The degree of salinity of lake water indicated that it was brackish all over the lake according to 'Phocaides' classification (Phocaides, 2000). Climate plays a critical role in the water balance of all lakes, the water balance, must be closely controlled by evaporative loss in order to produce elevated salinities. An excess of precipitation will lead to a rise in lake surface area to enable evaporation to accommodate it, hence raising lake levels (Almendinger, 1990). In addition to the average precipitation, inflow has an important impacts on lake chemistry. the water balance highly seasonal or monsoonal systems experience dramatic hydrologic fluctuations, and these can play an important effects on the chemistry of saline lakes. The most important process in this area is agriculture. Currently one of the greatest threats to saline lakes is anthropogenic or secondary salinization as a result of human activity such as industry, agriculture, construction. Lakes from arid zones can dry out during long periods of time, although in most cases, they show a seasonal regime. Phosphorus is considered to be as one of the important nutrients. Dissolved reactive phosphate (PO₄) ranged from 2.16-3.22 mg/L at lake edge, the highest concentration was recorded during summer season. For the middle of the lake, the phosphate ranged from 2.86-2.91 mg/L, the highest concentration was measured during winter season. Overall, average PO₄ levels during winter were slightly lower than summer and the variations during summer are apparently higher than winter. Also, the results showed that the average nitrate levels during summer was slightly higher than winter. NO₃ were ranged from 0.348-0.368 mg/L at edge of the lake, where the highest concentration was recorded in summer season. At middle of the lake, nitrate levels were ranged from 0.319-0.386 mg/L, the highest concentration was recorded during summer season. According to Downing and McCauley, (1992), nutrient sources of the lakes have divergent N:P ratios, ranging from 20 to >200 for precipitation, groundwater and for rural lands and soils were from 10 to <1 for sediments, sewage, urban runoff. According to the results (Table 1), CO3 were not found, while different concentrations of bicarbonate were found in lake water, the HCO₃ concentrations were ranged from 28-36 mg/L at edge of the lake, and were from 20-24 mg/L at the middle of the lake, the highest concentration were measured during summer season. Lake water was found rich in sulphate 52.00-69.00 mg/L as a result of salt accumulation in studied lake basins. SO₄ reduction can occur in the center of the lake. Saline inland lake is often hypersaline. However, there was a clear seasonal pattern for Cl concentrations in the lake. Chloride concentration varied widely throughout the lake, the maximum recorded value was 481 mg/L at lake edge during summer season and the minimum level was 700 mg/L at lake edge during winter season. Increased chloride concentrations can negatively affect water quality. As well as impacting aquatic life, also it can increase the density of the water and affect thermal processes like stratification and mixing (Butcher et al., 2015). Calcium concentrations were ranged between 49-52 mg/L in the edge of the lake and between 67-76 mg/L in the middle, the highest values were during summer season. The concentrations of Mg were high in the lake edges 29-36 mg/L compared to the middle 21.4-26.4 mg/L, the highest values were during summer season. The concentrations of K in the lake water varied considerably, where were high in the edge of the lake 248.49-372.49 mg/L compared to the middle 95.24-169 mg/L, the highest values were during summer season. Potassium is the key component of commonly used potash fertilizer, which is abundant in animal waste. The Na concentrations were varied widely all over the lake in a clear seasonal pattern. The maximum concentration was 557.5 mg/L at the lake middle during winter season and minimum amount was 118.55 mg/L was in the lake edge during summer season. The basic cations abundant concentrations were typically found, Na > K > Ca > Mg, For the concentrations of basic nations were typically Cl>SO₄>HCO₃>PO₄>NO₃. According to Tepe and Mutlu, (2004), the present of sodium and potassium ions may indicate to lake pollution caused by human activities. Sodium is often associated with chloride. It finds its way into lakes from road, fertilizers and human and animal waste. Sodium chloride dissolves readily and increase the solubility and mobility of salt. Calcium and Magnesium ions are both common in natural waters and both are essential elements for all organisms. Ca and Mg, when combined with bicarbonate, carbonate, sulphate and other species, contribute to the hardness of natural waters. The ions composition of lake water could be related to the geology of the area where the studied lake was surrounded by saline soil with a high EC. The salinity arises from differential ion precipitation as water evaporates from the lake. In general terms, CaCO₃ is precipitated first during evaporation and results in relative enrichment of Na, Mg, Cl, and SO₄ in remaining water

Parameters	Units	Lake Edge		Lake mid	
		Summer season	Winter season	Summer season	Winter season
pН	-	7.46	7.44	7.38	7.32
EC	dS/m	129.79	125.48	126.82	133.74
TDS	g/L	83.07	80.31	81.17	85.59
PO ₄	mg/L	3.22	2.16	2.86	2.91
NO ₃	"	0.368	0.348	0.386	0.319
CO ₃	"	-	-	-	-
HCO ₃	"	36.00	28.00	24.00	20.00
SO ₄	"	68.10	69.00	59.40	52.00
Cl-	"	1481.00	700.00	1168.00	1059.00
Ca ⁺⁺	"	52.00	49.00	76.00	67.00
Mg ⁺⁺	"	36.00	29.00	26.40	21.40
K ⁺	"	372.49	248.49	169.00	95.24
Na ⁺	mg/L	118.55	460.00	345.46	557.50

 Table (1). Physicochemical properties of Lake water.

3.2. Microscopic Biodiversity of the Lake

Phytoplankton quantitative samples were collected by vertical net (55 mm mesh diameter). Samples were immediately preserved in 4% neutral formalin. The tabulated results are the mean of the three replicant values. The major groups of phytoplankton were subjected to detailed microscopic analysis and identification using the following identification tools used by (Al-Yamani et al., 2011; Conway, 2012). The results of this study (Table 2) showed low Algal biodiversity compared to other aquatic environments and were in line with (Oren, 2005). that could be related to high water salinity and anthropogenic impact. Five types have been registered, which are Cocconeis, Notholca Pandorina, Oscillatoria, Chlamydomonas, the most common species found were Pandorina & Oscillatoria. Lower phytoplankton taxa richness could be due to the lake's recent origin, small size, and high level of salinity. Weather conditions, physical factors, nutrient availability and also geographical location play important roles determining phytoplankton successions in the urban lakes. Higher pH average during summer compared to winter was due to the growth of aquatic organisms flourishes in worm weather, where favoured pH values ranging from 6.5-8.5 (Chapman, 1996). The phytoplankton succession might have specific patterns under weather conditions. The dry desert climate on the study area played a main role in imposing the lake ecosystems, given the high temperatures and little rainfall, this make the effects of human activities on biodiversity in this region much more dangerous. Saline lakes are important environmental features, with significant geochemical impacts on ecology, water resources, and deferent activity around the world. The lake is valuable for biodiversity conservation as they can provide habitat for many species and highly increasing local biodiversity of the surrounded area.

Table (2). Zooplankton in the Lake.

Cocconeis		
Kingdom	Animalia	
Class	Bacillariophyceae	
Order	Achnanthales	
Family	Cocconeidaceae	
Genus	Cocconeis	
Notholca Kingdom Phylum Class Order Family Genus	Animalia Rotifera Monogononta Polima Brachionidae Notholca	

Pandorina

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Kingdom	Animalia
Phylum	Chlorophyta
Class	Chlorophyceae
Order	Chlamydomonadales
Family	Volvocaceae
Genus	Pandorina
Oscillatoria	
Kingdom	Animalia
Phylum	Cyanobacteria
Class	Cyanophyceae
Order	Oscillatoriales
Family	Oscillatoriaceae
Genus	Oscillatoria
Chlamydomonas	
Kingdom	Animalia
Phylum	Chlorophyta
Class	Chlorophyceae
Order	Chlamydomonadales
Family	Chlamydomonadaceae
Genus	Chlamydomonas

3.3. Vegetation Cover

Tamarix (Tamarix aphylla) is found along watercourses in arid areas. It is a very resistant tree to saline and alkaline soils, distributed at the east and north side of the lake because it is tolerates very high salinity, drought and high temperatures. Saline tolerance is achieved in plants by various cellular and physiological mechanisms Tamarix tree is a sand dune stabilizer. It has the stamina, living and reproduction around highly saline water, temperature strikes and a dry desert climate. The present situation of biodiversity in this area is very critical and it is reflected by the certain natural ecosystems, However, detailed ecological and floristic accounts remain very scarce particularly for the study ears. However, saline lakes around the world are important nesting, feeding and staging areas for all types of water birds. Tamarix aphylla is found along watercourses in arid areas. It is very resistant to saline and alkaline soils.

3.4. Soil Physicochemical Properties

Soil properties influence the degree of aridity and thus plant productivity. Soil within the study zone, is classified as sandy loam soil and the most important characteristic is that it contains a small percentage of clay mineral 0.9%, and carbonite (CO₃) 0% (Table 3). The porosity of tested soil was 44.53%, which means that the soil is well aerated and permeable. The porosity is an important physical attribute in reproducing the field condition of soils in the laboratory. Saturated hydraulic conductivity (K_s) value of tested soil was 4×10^{-3} cm/h, it is within the usual values of such soils (Hussien and Fayyadh, 2014). The K_s determines the capacity of a soil

to transmit water and as such is an essential soil physical property that affects soil-plant-water relations and processes. It is one of the most variable soil properties because it is associated with soil texture and structure but is also affected by many other factors such as topography, vegetation, land use, climate and so on (Deb and Shukla, 2012). Accordingly, an accurate knowledge of the values of the Ks is a prerequisite for initiating an efficient water management scheme. Tested soil contains organic matter (OM) 6.4%. Desert soil in dry conditions often shows low levels of organic matter. The dry hot climate plays a vital role in determining the amount of organic material because the area is poor in vegetation cover. High temperatures affect all soil biological, physical and chemical reactions because high temperatures encourage the rapid disintegration and disappearance of organic residues from the soil. The overall soil pH tends to be slightly alkaline reaction 7.24. Moreover, due to a high degree of drought conditions and high evaporation rates the total dissolved salts in tested soil was 12.43 g/L and EC was 19.42 dS/m indicating high-saline soil. Due to high evaporation rates, desert soil is affected by accumulated several salt ions. The concentrations of Na and K were 193 & 38.6 mg/kg, respectively; while were for Ca and Mg were 48 & 14.4 mg/kg, respectively. However, the amount of Na, Ca and Mg in the surrounding soil were high. The NO₃ content of the tested soil was 0.293 mg/kg, which is considerably low. The primary purpose of NO₃ is to work as a source of nitrogen for the nutrition and growth of plants as well as soil microorganism. Next to nitrogen, phosphorus (P) is the second most important macronutrient as an essential plant nutrient (Srinivasan et al., 2012). The available phosphorus content of the desert soil was 39.65 mg/kg, and lake can sometimes be directly related to nearby agricultural activities as agricultural lands typically receive significant amounts of fertilizers and phosphorus) and plant protection products; high concentrations of specific chemicals in streams (Royer et al. 2006, Zobrist & Reichert, 2006). Soils with pH values between 6 and 7.5 are ideal for Pavailability. Climatic and site conditions, such as rainfall and temperature, and moisture and soil aeration (oxygen levels), and salinity affect the rate of P mineralization from organic matter decomposition.

Table (3).	Physicochemical	properties of soil.
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Soil Texture	Sandy loam	sand% 68.75 Silt% 30.35 clay% 0.9
Porosity	%	44.53%
Saturated hydraulic conductivity (K_s)	cm/h	4×10 ⁻³
OM	%	6.4%
pH	-	7.24
EC	dS/m	19.42
TDS	g/L	12.43
PO ₄	mg/kg	39.65
NO ₃	"	0.293
CO ₃	"	-
HCO ₃	"	12.0
SO ₄	"	56.6
Cl-	"	149.0
Ca++	"	48.0
Mg ⁺⁺	"	14.4
K ⁺	"	38.6
Na ⁺	mg/kg	193.0

Finally, it could be concluded that irrigation with drainage water discharge from agricultural fields into depressions is a specific cause of artificial lake origin in the arid areas. The lake is mainly storage body. Unfortunately, saline lakes have been perceived as being unimportant, of less utility, and less abundant than fresh waters. Due to its geographical location and geological background, the water properties is deeply influenced by climate. The lake is unique ecosystem, and the seasonal differences were noted. The results indicate that the

water quality of this desert lake could be affected by agricultural cultivation especially in the middle. The lake is mostly saline. The lake was characterized by the high salinity that results mainly from the small catchment area and high evaporation and temperature. The chemical composition of lake water in natural ecosystem is related to geological and lithological structure, topography, climatic conditions, vegetation and soil properties. However, soil properties play an important role in soil hydrology and influence the degree of aridity and plant growth. We hope that our results can be used as the starting point for the long-time monitoring of changes in the lake ecosystem, which provide an important contribution to our knowledge of arid area lakes' response to climate variability.

4 Conclusions

Small lakes provide an opportunity to understand intraecosystem connectivity and its dynamics. This is the first data linked to the ecosystem of Ain Al-Mashashiya Lake. it is likely the new case of a saline artificial lake formed from agricultural drainage water from local farms. Lake depth was >5 meters. The chemical composition of lake water in natural ecosystem is related to geological and lithological structure, topography, climatic conditions, vegetation and soil properties. The average of PO₄ and NO₃ level during winter is slightly lower than summer. The water cations found abundance were typically Na > K > Ca > Mg, while were for basic anions, $Cl > SO_4 > HCO_3 > PO_4 > NO_3$. The lake is valuable for biodiversity and provide habitat for many species, five phytoplankton types have been registered, The most common species were (Pandorina & Oscillatoria). Soils of the study zone is classified as sandy loam, with EC 19.42 dS/m indicating high -saline affected soil.. Tamarix (Tamarix aphylla) commonly found along the lake. The area dry desert climate played a main role in imposing the lake ecosystems.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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