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Physicochemical And Microbial Characterization Of Sebkha Ben Ghayadha Tunisia

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Abstract – Nowadays, the problems of rejections constitute an increasingly important danger in the receiving medium. Indeed, water is increasingly affected by organic and mineral contamination and even by the micro-organisms of which some are pathogenic and thus dangerous for the ecosystem. In this context, this study aims to determine the physicochemical characterization (Chemical Oxygen Demand-COD, Total Suspended Solids-TSS, salinity and total nitrogen) and microbiological (mesophilic and thermophilic bacteria, bacteria sensitivity to antibiotics) of the sebkha Ben Ghayadha waters. The results showed COD of 4000 mgO_2/L exceeding the limit fixed by the Tunisian standard TN 106.02 (1989) at 90 mgO_2/L by the Environmental Protection for marine hydrological effluent discharge. However, all the TSS values were under 12 mg/L. Also, total nitrogen (TN) values ranged from 1.9 to 47.8 mg /L,rcorded at a depth of 2,3 and 4 meters, respectively. All the obtained results were consistent with TN 106.02 (1989). The bacteriological analysis based on faecal bacteria counts, showed that all the samples analyzed have loads of fecal bacteria lower than that fixed by TN 106.02 (1989). Bacteria isolated from the waters sebkha showed a high sensitivity to antibiotics suggesting a low virulence.

Keywords: Sebkha, salinity, microbiology, pollution, Ben Ghayadha.

1. Introduction

As their name suggests, wetlands are characterized by their dependence in water. Indeed, their water content mostly discharged from the neighbour sea and land. Several definitions of wetlands have been made by environmental scientists. However, the best definition is that stated at "Ramsar" conference that held in 1971". Wetlands are one of the world's most important environmental assets, containing a disproportionately high number of plant and animal species compared to other areas of the world. Wetlands are vital for human survival and among the world's, most productive environments; cradles of biological diversity that provide the water and productivity upon which countless species of plants and animals depend for survival. Those areas are indispensable for the countless benefits or "ecosystem services". Wetlands exist in every country and in every climatic zone, from the Polar Regions to the tropic ones. They are distributed around the world and cover an area that is 33% larger than the USA (Cowardin, 1979).

In the Mediterranean coastal zone, numerous wetlands are located at low altitudes (Benessaiah, 1997), between the sea and a series of high mountains. This special geomorphology combined with severe erosion involves a torrid sediment transport by rivers flowing into the sea. These physical phenomenons along with the total absence of tides on the coast are the main cause of wetlands training near the coast. Although despite the little Tunisia surface, Tunisia currently has about 256 areas of wetlands. These areas are generally concentrated along the coast, especially in the north where the climate is more humid and heavy rainfall compared to south. Like most Mediterranean countries, Tunisia has lost some of

these wetlands. Right from eighties, number of these environments is drastically decreasing. Because of its presence near Mahdia City Center, the sebkha Ben Ghayadha plays an important role in the urban landscape of the city. Reinstatement in the development plan of Mahdia city would be considered as an essential action to ensure the success of the urban development of the city. Currently, sebkha Ben Ghayadha is causing a host of problems including environment pollution; result of stagnant water during a long period of years, which serves as a large source of larval mosquitoes (Garbaya, 1997). In addition, dense algae and plant covering the sebkha serve as a support for larvae and other insect, even during hot season. Significant degradation of the ecosystem of the sebkha is observed for several years. It is mainly due to the result of urban pollution (discharges of industrial effluents directly into the sebkha and long-term water stagnation inside the sebkha). All the cited condition certainly affects not only the water quality of the sebkha but environment of neighbouring areas.

Generally, sebkhas are considered as particular ecological units with an important role in hydrology and morphology (Bouraoui, 1997). They play an important role in the ecological balance and biodiversity conservation in arid areas. . They are also home to a wide variety of specific plant and animal species of salt and wetland ecosystems. Many migratory birds use it as seasonal parks. sebkhas ecosystems also play a role in the national economy through agriculture, livestock, fishing, salt extraction, tourism and culture. Across the Mediterranean region, natural wetlands are being destroyed and degraded at a rapid rate, with severe consequences for the status of aquatic fauna and flora (Finlayson et al., 1992; Pearce and Crivelli, 1994).

For all these reasons a characterization of the sebkha deserve particular attention.

The objective of this work is ***(i)*** to characterize the physico-chemical water samples and ***(ii)*** to monitor antibiotic resistance of some bacteria isolated from Sebkha Ben Ghayadha as virulency marker.

2. Material and methods

2.1. Location of Sebkhat Ben Ghayadha

The sebkha is located in the southern part of the town of Mahdia located in central eastern of Tunisia. It is bordered to the s southeast by the Ksour Essef road which separates it from the sea by a coastal sand dunes and to the northeast by an industrial zone (Figure 1) composed mainly of oil mills and soap factories. In the north, the railway isolated a big portion of the sebkha, where water was collected in pond. To the west, the sebkha is limited by agricultural zones, which is now invaded by agglomerations.

Figure 1. Location of Sebkha Ben Ghayadha

The sebkha is rarely flooded with rainwater (it is estimated that this happens once in 100 years). Its length is about 2400 m and its maximum width is 270 m. The old salt located in the centre of the sebkha is related to sea level Zouila area (APAL/National Employment Agency, 1999). Currently, there is no connection between the sea and the sebkha. However, the sebkha was in restricted communication with the sea via the Zouila channel before it had been isolated from it. This isolation has made sebkha an endoreïqueic depression feeding on water from sea by underground infiltration and subjected to a semi-arid climate with mild winters and hot and dry summer. The annual rainfall is near 300 mm / year. All these characteristics, to which are added the strong urban concentration around the sebkha, increase the

dysfunction of the environment favouring the accumulation of organic and mineral wastes as well as microbial growth.

2.2. Sampling Strategy

The samples were taken over a period of 2 months from March to April 2014. During these months volume of 5L water were taken. Samples were collected in polyethylene bottles filled and sealed without leaving air. Samples for bacteriological analysis were collected in sterile glass bottles. Samples were taken from three different strategic sites and are represented as follows: Site E1 located at the ground with a depth of -4 meters north of the sebkha, site E2 in the middle of the sebkha with a depth of -3 meters and site E3 located at the bottom with a depth of -2 meters south of the sebkha (Figure 2).

Figure 2. Photo illustrator of the geographical points of water sampling
Site E1 located at the ground with a depth of -4 meters north of the Sebkha. Site E2 appointed in the middle of the Sebkha with a depth of -3 meters in the middle of the Sebkha. Site E3 located at the bottom of the Sebkha with a depth of -2 meters south of the Sebkha .

2.3. Parameters analysed

The physicochemical parameters were the COD, total nitrogen and TSS. The parameters such as temperature, pH, salinity and conductivity were measured in situ.

2.3.1. In situ parameters

The temperature is measured using an electrode connected to a multifunction device and expressed in degrees Celsius (°C). The pH is measured at the level of the concerned site by the electrometric method. The electrical conductivity (EC) estimating the amount of salt and ions in the samples is measured using conductivity-meter WTW_LF_196 equipped by an electrode (Type, Tetracon 96-1.5).

2.3.2. Ex situ Parameters

The total nitrogen (TN) was determined by the kjeldhal method (Brenner and Mulvaney, 1982). The chemical oxygen demand (COD) is measured according to 09-23 NT method based on the use of potassium dichromate in the presence of both silver sulphate (Ag_2SO_4) and mercury sulphate (Hg_2SO_4) serving to complex chloride ion. The $K_2Cr_2O_7$ excess is determined by Mohr's salt (ferrous ammonium sulphate). The COD was expressed in mg O_2/L. Total suspended solids (TSS) is determined by fibreglass filtration method. The method consists of filtration of a known water volume by a calibrated glass fibre membrane. After drying the filter at 105 °C for 2 h, the TSS is determined by calculating the difference in weight of the masse residue included in the filter before and after incubation, and results were expressed in g/L.

2.4. Microbiological analysis

The germ count is done by the method of the most probable number (MPN), according to NF T90-413 standards for coliforms and NF T90-413 for streptococci.

2.5. Resistance to antibiotics

The resistance for some strains isolated from water sampled from sebkha Ben Ghayadha is performed according to the agar diffusion method (Soussy et al., 2000). Briefly, a volume of 3 mL from bacterial culture of each strain was applied at surface plates containing trypto-caseine-agar (TSA) previously prepared. Excess culture was removed. The Petri dishes were then dried for 15 minutes at 37 °C. Disks of antibiotics were deposited at surface of inoculated agar medium. The dishes were left for 30 minutes at room temperature. After incubation at 37 °C overnight, the discs surrounding circular zones inhibition correspond to a lack of culture. The diameters of inhibition zones depended only on the sensitivity of the bacteria to the antibiotic in question. More the diameter is important more that the strain is sensible for the antibiotic. Resistance of bacteria to antibiotics was determined according to critical diameters. The following antibiotics presented with 1- and 5-µg disks were considered Gentamicine (15 µg) (GM), Amoxicilline (25µg) (AMX), Ticarcilline (75µg) TIC, Amoxicillin + Clavulanic acid 20 µg /10µg) (AMC), Nalidixic Acid (30 µg) (NA) Fusidic Acid (10 µg) (FA), Amoxicilline (25µg) (AM) and Streptomycine (25µg) (S).

3. Results and Discussion

Currently, there is onlyone oil processing industry disposes the industrial wastes in to southern sebkha. In addition, the presence of old black colour in sebkha border, probably having as sources margins oil was noticed. This may due to the presence of heavy metals, since margin is known by its high level in heavy metal, especially zinc. Sebkha Ben Ghayadha is the natural outlet of the water of Mahdia. During dry periods the groundwater level declined due to its use for agricultural purposes. It follows that the level of water in the sebkha decrease is related to the high evaporation during summer (APAL/ANPE, 1999). According to APAL / ANPE (1999) the succession of periods of flooding (water reaches the surface as a result of the increase of the piezometric level the groundwater and the arrival of the surface flows converging toword the sebkha) and drainage is a seasonal feature of sebkha. Its significant impact on the chemical water and sediment mineral precipitation of calcite, gypsum, iron hydroxide, sulphide oxidation and evaporation of light products such as hydrocarbons and mercury are the main process occurring in the first centimetres of sediment during dry periods. The return of wet periods leads to the re-dissolution of minerals and sulphide formation. The return of water in the sebkha leads to leaching of metals adsorbed that before entering reactions with the sediment remain some times in the water.

3.1. In-situ Physico-chemical parameters

3.1.1. Temperature

Changes in climatic conditions certainly influence significantly the water temperature. This is clearly reflected either in March or in April. Temperature variation of surface sebkha Ben Ghayadha waters were grouped in Table 1. The results showed that the minimum value 17.4 °C was recorded during March. However, the maximum value was recorded in April: 19.7 °C. This gradual increase is certainly a result of an increase in air temperature.

3.1.2. pH

Results grouped in Table 1 showed that the pH values during the range march-April period did not show any variation. The pH average value is about 7.7. Referring to the standard of bathing water fixing the pH between 6.5 and 8.5, it is estimated that the pH of the water of sebkhat Ben Ghayadha is consistent with the Tunisian standard NT 106.02 (1989).

3.1.3. Salinity

Results showed that the salinity of water ranged from 42.5 g /l (mean value of two different sites) recorded in April and a minimum of 29.5 g/l (average value of three stations) recorded in March. This decrease may be due to an inflow of fresh water fed by the rains during this period. This contribution tends to dilute the composition of the water of the sebkha. During the month of April a very significant increase was observed. This increase may be due to evaporation caused by an increase in ambient temperature.

3.1.4. Conductivity

It is well known that the conductivity is closely related to the salinity. During the month of March mean value of three replicates of the water conductivity is 46.2 mS/cm and increase during April to reach a value of 65. 53 mS/cm (mean value of two different sampling stations). The conductivity of the water of sebkha Ben Ghayadha indicates a high concentration of dissolved salts which increases from March to April parallel to the increase in temperature.

Table 1. In situ measurement of physicochemical parameters during the months of March and April.

Month of sampling	Samples nomination	pH	Conductivity (mS/cm)	Salinity (g/l)	Temperature (°C)
March	E1	7.8	43.8	28.3	17.3
	E2	7.6	46.1	29.9	17.4
	E3	7.8	46.1	30.4	17.3
April	E1	7.4	64.7	42.2	19.1
	E2	7.6	66	42.9	19.2
	E3	7.5	67	42.3	19.7

3.2. Ex-situ physicochemical parameters

3.2.1. Total Suspended Solids (TSS)

Monitoring the concentration of suspended solids in the sebkha Ben Ghayadha showed a remarkable decrease. Indeed, TSS concentrations showed in March a value of 6.7 mgL (average value of three repetitions) and recorded 12.4 mg /L in April (Figure 3). Compared to the values fixed by Tunisian standard NT 106.02 (1989) of Environmental Protection to 30 mg/L, values do not exceed the standard.

3.2.2. Chemical Oxygen Demand (COD)

Following the Tunisian standard NT 106.02 (1989) of Protection of the Environment, the COD value is 90 mg O2/l while the values of COD recorded in the sebkha showed a remarkable rise. It is recorded in March a value of COD equal to 3550 mg O2/l. However, in April this value increased to reach 4050 mg O2/l (Figure 3). This increase may be due to poor ventilation of sebkha based to climate change and may be at the source of bed odours.

3.2.3. Dosage of total nitrogen

The results of total nitrogen of Ben Ghayadha sebkha showed insignificant variability recorded for the month of March and April (Table 2). It can be noted that the site (3) recorded relatively high total nitrogen content (47.8 at March and 47.6 at April) compared to other sites (1 and 2) which respectively record an average value of 1.9 and 7.7. The high value may be a result of a dense release of pollutants at the southern part of the sebkha inducing an eutrophication.

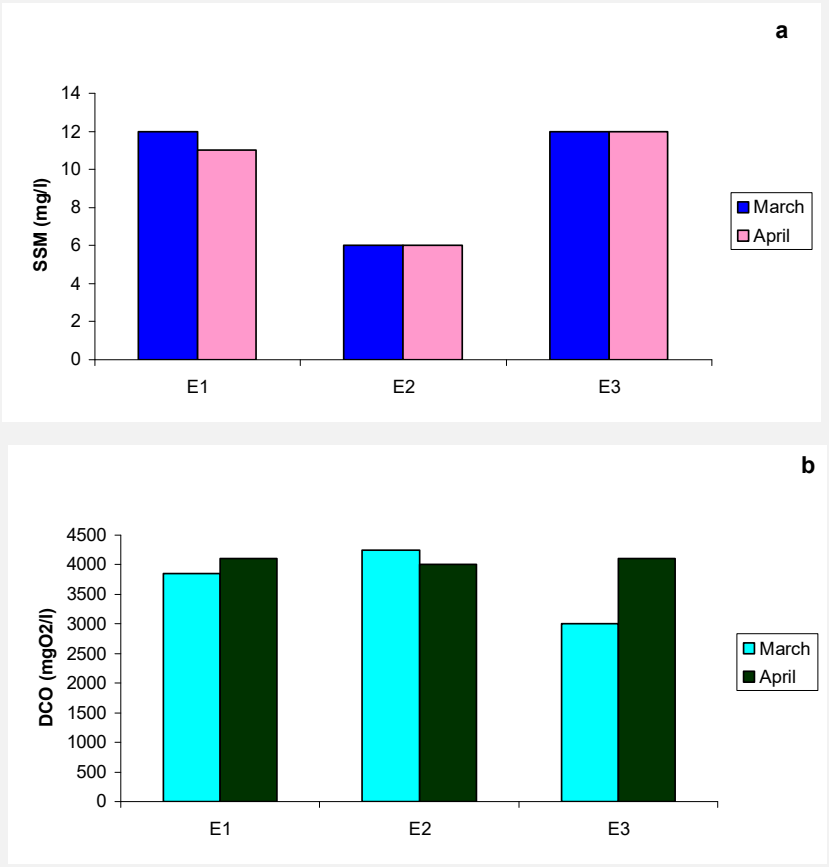

Figure 3. Changes in levels of solid suspended matter (SSM)(A) and COD (B) of water samples collected from the Sebkhat Ben Ghayadha during the months of March and April

Table 2. Variation of total nitrogen Sebkha Ben Ghayadha during the month of March.
Sampling points NT mg/l

	Month	
Sample	March	April
	NT (mg/L)	
E1	1.9	1.9
E2	7.6	7.8
E3	47.8	47.6

3.3. Bacteriological analysis

3.3.1. Thermophilic and mesophilic Bacteria

The results of microbiological analysis of waters sebkhat Ben Ghayadha showed that the concentrations of fecal coliforms, fecal streptococci presented low and stable values during the period of the study (Table 3). Faecal streptococci required are 1000 MPN/100ml and the value required for fecal coliforms is 2000 MPN/ml. Also, results showed that the water of sebkha Ben Ghayadha is poor in mesophilic and thermophilic bacteria. This may be due to the high salinity of the sebkha which may inhibit the growth of most bacteria.

Table 3. Variation of the most probable number (MPN) of fecal coliforms, fecal streptococci, mesophilic and thermophilic bacteria

Month Sampling	Fecal coliform	Fecal streptococcus	Mesophilic bacteria	Thermophilic bacteria
	Bacteria/100 ml			
March	3	4	43	0
April	220	0.8	65	172

3.3.2. Antimicrobial

The majority of strains showed sensitivity to antibiotics (Figure 4).

Figure 4. Photo of lyses inhibition of the strain S2 by AMC, NA, S, AM and FA antibiotics
Gentamicine (GM), **Amoxicilline (AMX),** Ticarcilline **(TIC)**, Amoxicilline + acide clavulanique (AMC), Acide nalidixique (NA) Acide fusidique (FA), Amoxicilline (AM), Streptomycine (S

In fact, strains S1 , S2, S3 and S4 have shown sensitivity to each of the following antibiotics GM, AMC, NA, S, C, FA, AMX, TIC and PSC while the remaining strains are more or less resistant to antibiotics such as S5 and S6 strain (Table 4).

Table 4. Antibiotypy test results applied to the strains isolated from Sebkha

Antibiotics	Strains					
	S1	S2	S3	S4	S5	S6
GM	+	-	++	+	+	+
AMC	±	++	++	+++	±	±
NA	+	++	++	+	-	-
S	++	++	++	+	+	+
AM	-	+++	+	++	-	-
C	+++	-	++	-	-	-
FA	++	+	+	+	-	-
AMX	+++	+	-	-	-	-
TIC	+++	+	+	++	++	-
CFP	+	++	ND	±	+	+

(-): No lysis , (+ + +): lysis = diameter 2 cm , (+ +): lysis = 1cm diameter , (+): diameter = 0.5 cm lysis .Gentamicine (GM), **Amoxicilline (AMX),** Ticarcilline **TIC**, Amoxicilline+acide clavulanique (AMC), Acide nalidixique (NA) Acide fusidique (FA), Amoxicilline (AM), Streptomycine (S)

4. Conclusion

The present work is the first one which considered physicochemical and microbiological study of sebkhat Ben Ghayadha located near Mahdia city. It highlighted the current state of the sebkha and could be an interesting tool to elucidate the origin of evil existing around the sebkha odorous. This work lead to the following conclusions:

Water temperature showed a gradual rise throughout our study period. North to south sebekha Ben Ghayadha, all samples analyzed have shown a small basic trend, as they were characterized by high salinity. The total nitrogen values at different sites of the sebkha varied related to the studied samples. Indeed, the nitrogen content is very high in southern sebkha, so promoting eutrophication, whereas for other sites nitrogen concentrations are low in nitrogen content. The suspended solid water content is variable at different locations analyzed. Generally, the water sebkha Ben Ghayadha is poor in mesophilic and thermophilic bacteria. Also, fecal load of these waters remained inferior to the Tunisian standards NT 106.02 (1989). Bacteria isolated from the sebkha show a high sensitivity to antibiotics suggesting a low virulence. Further microbiological studies following the anaerobic bacteria responsible for bad odours deserve to be developed.

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5. References

Benessaiah 1997 Socio-economic aspects in the preservation and development of wetlands Mediterraneans , management and conservation of wetlands Tunisian , page 24-25.

Bouraoui S 1997 Legal, Management and Conservation of Tunisian wetlands, page 39.

Brenner JM, Mulvaney CS 1982. Nitrogen total. In: Black CA (ed) Methods in Soil Analysis: Agronomy., pp 595–624.

Cowardin. 1979. Classification of Wetlands and Deepwater Habitats of United-States? The Canadian Naturalist, page 85.

Finlayson, C.M., Hollis, G.E., Davis, T.J. (Eds.), 1992. Managing Mediterranean Wetlands and their Birds. Proc. Symp., Grado, Italy 1991. IWRB Special Publications, No. 20.

Garbaya M 1997 Some guidelines for sustainable management of wetlands management and conservation Tunisian wetlands, page 54-55.

Pearce, F., Crivelli, A.J., 1994. Characteristics of Mediterranean Wet- lands. MedWet publications, No.1. Tour du Valat.

Ramsar 1971 Manual Ramsar Convention, 4th edition , page 7. Wetland values and functions. World Wetlands Day. Made in Iran. February 1971.

Soussy, C. J., G. Carret, J. D. Cavallo, H. Chardon, C. Chidiac, P. Choutet, P. Courvalin, H. Dabernat, H. Drugeon, L. Dubreuil, F. Goldstein, V. Jarlier, R. Leclercq, M. H. Nicolas Chanoine, A. Philippon, C. Quentin, B. Rouveix, and J. Sirot. 2000. Comité de l'Antibiogramme de la Société Française de Microbiologie. Communiqué 2000-2001. Pathol.Biol. 48:832-871.