Plant Archives Vol. 19, Supplement 2, 2019 pp.2334-2340 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

RELATIONSHIP BETWEEN PHYSICOCHEMICAL PROPERTIES AND MICROBIAL BIODIVERSITY WITH SMALL PROJECT AT WADI EL-NATROUN LAKE, EGYPT

M.S. Aly, M.A. El Aziz, Salah S. Abd El-Ghani and Tamer Gamal Ibrahim Mansour
National Research Centre, Dokki, Cairo, Egypt

Abstract

This study aims at studying microbial diversity in the surrounding area of El-Khadra Lake and its effect on expected agricultural processes to develop this area agriculturally. The soil also was tested for some physical and chemical properties. Soil was divided into 19 profiles to indicate existence of different microhabitats which may be close together. Many microbial techniques were applied for soil analysis to indicate the presence of different microbial activities within different microhabitats. The indication of the microflora in soil showed the dominance of fungi of genus *Aspergillus* sp. Electrical conductivity (EC) and pH of the lake were determined in order to identify microbial distribution within the lake water. Results showed dominance of cyanobacteria in higher EC and pH levels and the Cyanobacteria project was high profitability at that area under study (El-Khadralake, Wadi El-Natroun, Egypt).

Keywords: Microbial biodiversity, physicochemical properties, Wadi El-Natroun, soil analysis and small project.

Introduction

Increasing agricultural production through agricultural and stock development, improving income distribution, and generating employment through the settlement of smallholders and graduates from among the rural population of the over-populated areas of Egypt. Sandy soils have different problems: low fertility and inadequate water retention. Wind erosion, water erosion, drought, loss of irrigation water, salinity and plant nutrients are also expected, El-Hady *et al.* 2009. Aquatic bacteria are classified according to their salt optima for growth as non-halophilic, slightly, moderately or extremely halophilic and halotolerant according to ElSheikh, 1998. Blue-green algae / cyanobacteria are the most common inhabitants of saline-alkaline lakes in different parts of the world (Grant, 2004) as in El-Khadra Lake at Wadi El-Natroun. Although NaCl is the major soluble salt which determines changes in salt osmolarity in the natural environment, it has been shown that other less penetrating solutes like potassium chloride, ammonium and potassium sulphate, potassium and sodium phosphate induce the mechanisms of osmosis in organisms including *Azotobacter chroococcum* and *Klebsiella pneumonia* (Parsons, 1997, Madkour, *et al.* 1990, Miller and Wood, 1996 and Malin and Lapidot, 1996) *Azospirillum brasilense* (Shahaby, 1997) cyanobacteria (Apte and Thomas, 1997). Many species of bacteria respond and adapt hyperosmotic conditions in environment by the intracellular accumulation of low molecular weight organic solutes that are called osmolytes (Csonka and Hanson, 1991). These solutes contribute to positive turgor pressure which is required for cell expansion and growth (Reed *et al.*, 1989).

Therefore, the present study is aiming at evaluation of the relationship between physicochemical properties and microbial biodiversity with profitability calculation of small project at Wadi El-Natrounlake, Egypt.

Materials and Methods

Study Area

Wadi El-Natrun is an elongate, narrow sandy depression, about 50 km long, with an average width of 8km, oriented from south-east to north-west in a NW-SE direction between the latitudes 30° 17` and 30° 33` N and between longitudes 30° 02` and 30° 30` E, situated west of the Nile Delta. Location of Wadi El Natrun on Egypt map and Landsat ETM with image of El-Khadra Lake were showed in Figs (1 and 2).

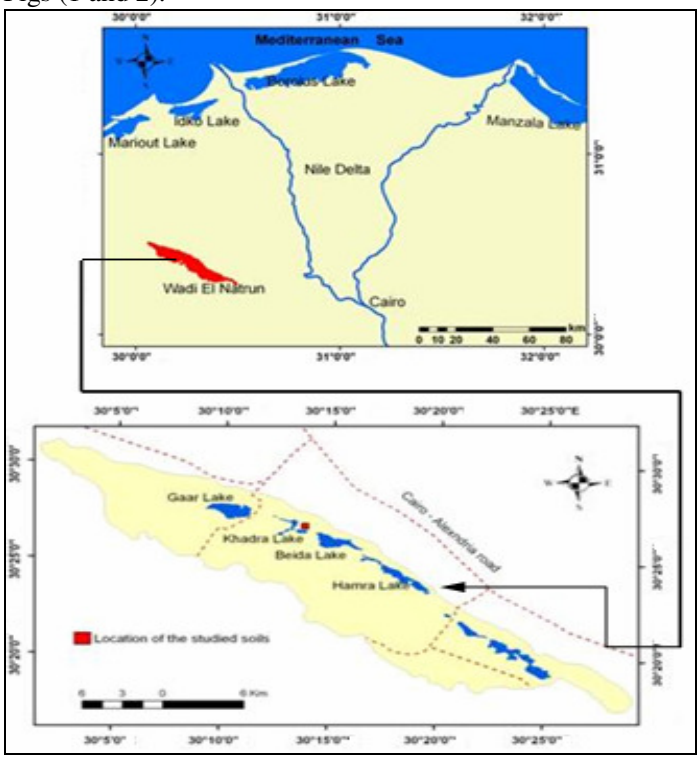

Fig. 1 : Location of Wadi El Natrun on Egypt map and location of studied soils

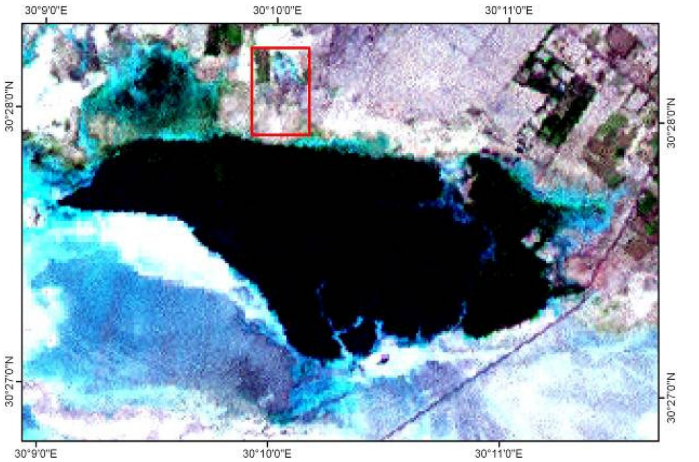

Fig. 1: Landsat ETM+ image of El-Khadra Lake (dark color), location of studied soils is outlined by red color

Microbial isolation from soil samples

Soil was divided into 19 sections, as illustrated in Fig (3 and 4) they are more or less of similar identical, however the depth of water table is varied according to the topography and the wind action as well as the sand rabbles and hammock formations. Soil samples from the studied area were collected. Some physical and chemical properties of studied soil and water in the experimental sites were determined according to (Cottenie, *et al.*, 1982 and Page, *et al.*, 1987).

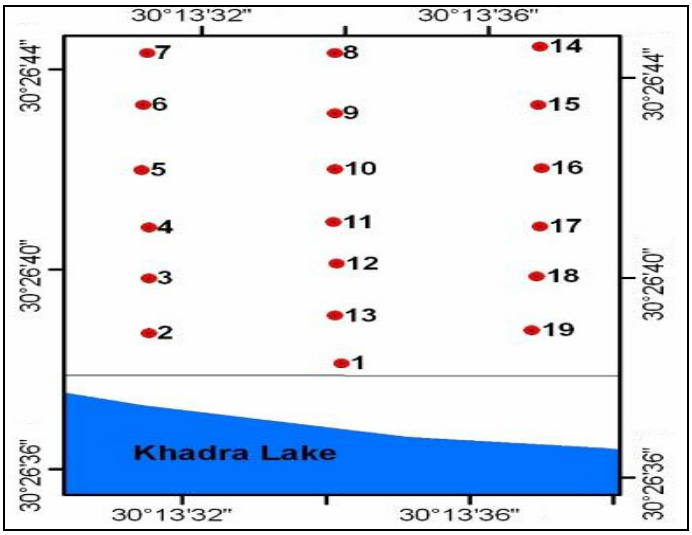

Fig 3: Location of the studied soil profiles

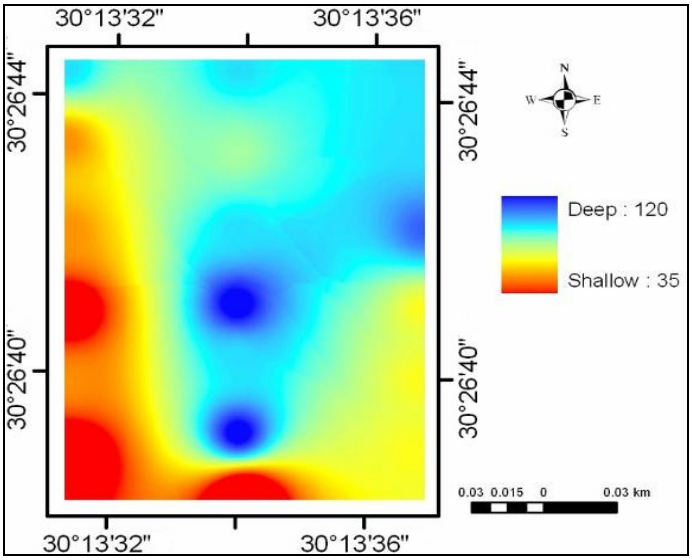

Fig 4: Spatial distribution of soil depth (cm)

In view of the heterogeneity of the soil environment and the consequential existence of different microhabitats which may be close together, the application of most of the currently available microbiological techniques to soil analysis yield data which represent an average or summation of microbial activities within different microhabitats. Table (1) shows a variety of techniques that has been applied to the soil with a view to relating soil condition and performance to microbial activity. The most widely-used direct enumeration procedures are based on the Jones and Mollison technique, where a known amount of soil is suspended in agar, and a standard volume allowed to set on a slide. Suitable staining procedures can be used to distinguish the microbial flora from detritus. Epifluorescence microscopy, where a fluorescent dye is excited by incident illumination from the top, has proved particularly useful, since there is no requirement for the illumination to pass through a thick, relatively opaque specimen.

Most Probable Number procedures (also known as extinction dilution procedures) are based on the concept that the level of dilution of the original sample needed to obtain a negative result in a particular physiological test, or in a particular growth medium, is related to the size of the population in the original sample. The Presumptive Coliform Test is an MPN test, and the technique is useful for the rapid estimation of particular physiological groups, rather than the entire microflora. Substrate utilization studies can also be of use in indirectly enumerating certain groups, and independent, estimations of numbers of nitrifiers in soils have been made in this way. Biomass estimates can be achieved directly by counting and measuring the size of microorganisms in soils, and applying appropriate mass conversion factors for each of the major groups. However, such procedures are extremely laborious and subject to considerable operator error, although this procedure still remains one of the best ways of determining individual biomasses for different morphological groups of microorganisms. The distribution of microorganisms in soil can be determined by preparing soil samples in a variety of ways and examining these by light microscopy or electron microscopy. Growth and activity estimates are often based on changes in microbial numbers or biomass over a period. The rate of colonization of slides or capillaries can also be used to monitor soil activity, and the replica plate procedure has been used extensively in the laboratory. In this procedure, known microorganisms are placed at particular points on the surface of plates of soil, and their growth and spread monitored by periodically replicating from the plate (using a multipoint replicator) on to plates of appropriate nutrient media.

Results and Discussion

The obtained results from studying the surrounding area of El-Khadra Lake for microbial diversity and both some physical and chemical properties were varied due to the sand sheet deposited by the wind action along the past thousands of years, drought and loss of irrigation water and plant nutrients are expected, in agreement with (El-Hady, *et al.*, 2009). The occurrence of bacteria in the lake water and rhizosphere of *Zygophyllum Jumosun* around the lake, which contains total aerobic bacterial count of 285 x 10^6 cfu/l, The grasses grazed by goats supplied the animals faces with proteolytic bacteria in MPN of about 109 x 10^6 and 4 x 10^6 of lypolytic and 0.17 x 10^4 of cellulytic bacteria and negligable amount of azotobacter, in agreement with findings of (Ali, 2003, and Grant 2004).

The geomomorfic formation of the bottom of the whole area is of shale formation depth of these shale is varied due to the sand sheet deposited by the wind action along the past thousands of years. Coarse sand mixed with loam and lime materials with stratified layers are the common morphological features of the soil profile of the area. Thewater table level was quite shallow in some profiles (no. 1, 2, 3, 4, 5and 6). Different micro flora (fungi-bacteria) numbers and repeats were calculated in soil samples by using the mentioned test and the results were reported as follows: a- Fungi: *Aspergillus* sp. 55%, *Penecillium* sp. 34%, *Alternaria* sp. 15 %, *Fusarium* sp. 5%, *Rhizopus* sp. 12% and

Mucor 10%, b- Bacteria: *Bacillus*sp 34%, *Pseudomonas* sp. 25% and *Erwenia* sp. 2.5 %.

I) Effect of high solute concentration on microbial biodiversity

Electrical conductivity was determined by means of portable conductivity meter. Several classes of salt-tolerant microorganisms are recognized, although such classes are by no means clearly distinct from one another. Microorganisms capable of growth in the absence of salt, but tolerant of varying concentrations are considered to be *halotolerant* and the group includes many yeasts, filamentous fungi, eukaryotic algae, blue-green algae and bacteria. Microorganisms with a specific requirement for salt are designated *halophiles* and there is a number of overlapping subdivisions within this group. As salt concentrations approach saturation (5.2 M), a quite distinctive ecology is generated, and the environment becomes dominated by a few types of microorganisms, usually bacteria of the genera *Halobacterium* (halo bacteria) and *Halococcus* (halococci). The halophilic eukaryotic alga *Dunaliella salina* is also to be commonly found associated with halo bacteria and *halococci* in the most saline environments.

Data of soil analysis indicated that soil salinity is varied from low to moderate to high and very high. This high spatial variability is due to the soil water movement through the soil profile. Consequently, and due to the high evaporation rate of this arid area, salt crust is formed in some places.

Soil salinity is varied from 0.32 ds\m (low) to 9.2 ds\m (very high). Soil pH is in an alkaline range (>8.5) due to the dominance of sodium ions in the soil matrix. Water samples were collected from the water table of some representative profiles (profiles no. 1, 2, 4, 6, 8, 13 and 14).

Table 1: Soil microbial analysis methods used [8]

Aims		Procedures
1. Isolation	(a) Direct	Micromanipulation
		Sieving and dilution
		Pour or spread plates of nutrient media
	(b) Indirect	Enrichment procedures
2. Enumeration	(a) Direct	Counts of microorganisms b microscopy
	(b) Indirect	Viable counting procedures
		Substrate utilization
3. Biomass	(a) Total	Calculations based on counts by microscopy
		ATP
		$CHCl_3$ fumigation
	(b)Specific groups	Calculations based on counts by microscopy
		Assay of group specific substances
4. Distribution	(a) Direct	Microscopy of soil preparations
		Scanning electron microscopy of soil preparations
	(b) Indirect	Buried slides, capillaries, nylon mesh, etc.
5. Growth and activity		Changes in number of biomass
		Soil replica plates
		Colonization of buried slides, capillaries, etc.
		Measurement of O_2 uptake
		Measurement of CO_2 evolution
		Measurement of substrate utilization
		Measurement of product appearance
		Measurement of temperature increase by microcalorimetry

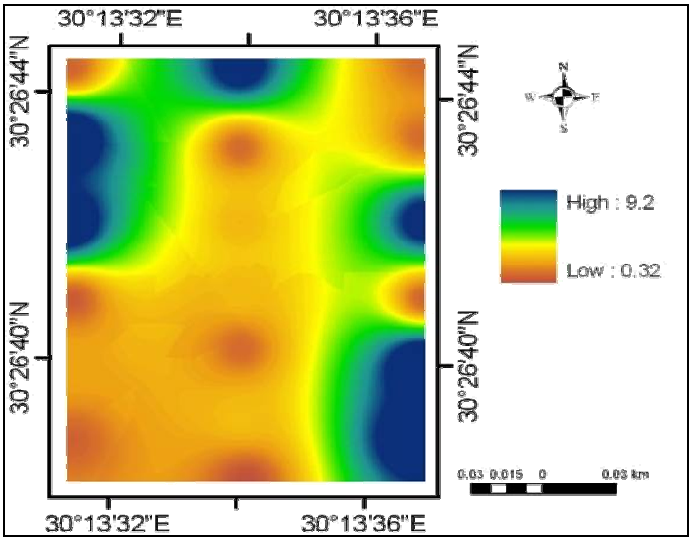

Fig 5: Spatial distribution of soil EC (dS/m)

Data in Table (2) included the water analysis data. All water samples showed high salinity levels, the range of salinity was varied from 2.2 ds\m to 20.2 dS\m. These data indicated that salt accumulation of the surrounding farm lands through the drainage water leaching soil salinity and accumulate it in the shallow water table appears at different depth in the studied area. Soluble salts leached through irrigation practices within the farm do not move out of the soil profile, but accumulate it in the shallow water table appears in most parts of the farm.

Table 2 : Water analysis

Profile No.	Water table level (cm)	pH	EC (dS/m)
WT 1	35	8.1	3.6
WT 2	45	8.5	4.94
WT 4	50	8.4	3.8
WT 6	70	9.7	17
WT 8	100	9.95	20.2
WT 13	120	8.4	2.2
WT 14	100	8.7	3.9

Fig. (6) indicated that the microbial distribution according to optimum EC range. The range of EC was varied from 0.41 to 5.5 dS/m. Cyanobacteria optimum EC range is 4.16 – 5.5 as they live in saline and hypersaline environments, but they are also with halophilic bacteria at lower EC levels. Halophilic bacteria grow at lower EC levels (0.41- 4.16), but cannot survive hypersaline conditions like cyanobacteria.

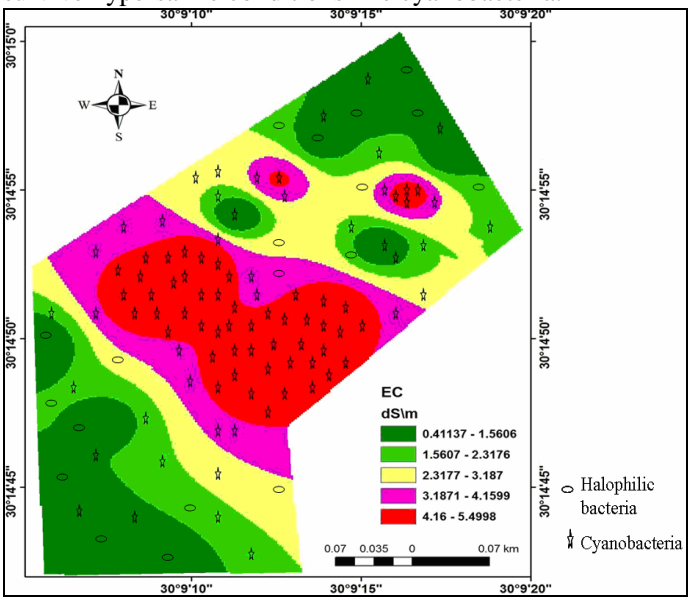

Fig. 6 : Microbial distribution according to EC

II) Effect of pH on microbial biodiversity

Data in Table (3) showed pH value was measured using a Beckman portable pH meter. Very high (>10) and very low (< 3) pH values are inhibitory to most microorganisms, but some are nevertheless to be found growing in environments where pH values approach 0, and indeed at pH values of 11 and above. Table 3 illustrates the range of microorganisms found at extremes of pH.

Fungi tend to dominate moderately acidic environments (pH 3-5) and many common soil fungi such as *Cephalosporium*spp. and*Fusariun-:* spp. are acid-tolerant, although the pH optima of such isolates are closer to neutrality.

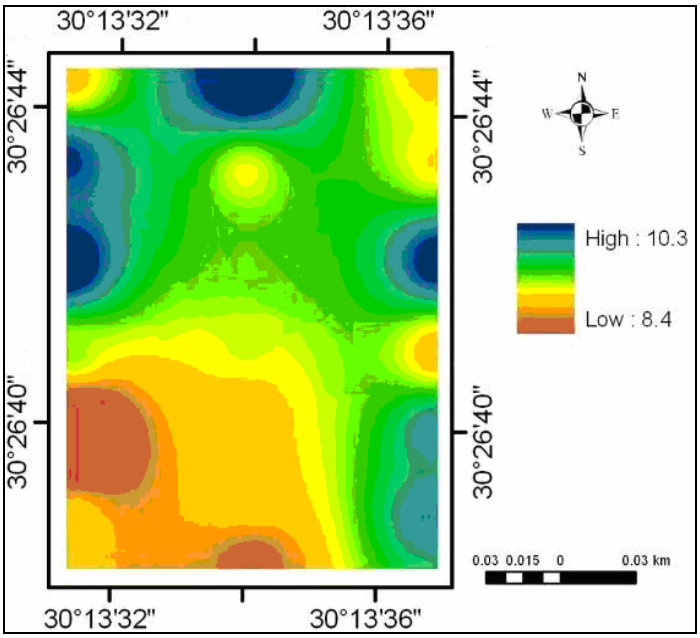

Fig. 7 : Spatial distribution of soil pH

Table 3: Microorganisms capable of growth at extreme pH [8]

Growth under alkaline conditions (pH > 10)	Growth under acid conditions (pH < 3)
Eukaryotes	
A few genera of fungi including *Aspergillus, Fusarium, Penicillium.*	6-8 genera of fungi including *Saccharomyces, Cephalosporium, Penicillium.*
Diatoms including *Nitzschia*	*Cyanidium* (red algae), Chlorella, *Chlamydomonas, Euglena* (green algae)
Prokaryotes	
A few genera of bacteria including Flarobacterium•, Agrobacterium, Ectothiorhodospira•, Bacillus•.	8-10 genera of bacteria including Thiobacillus*, Metallogenium, Sulfolobus*, Bacillus*, Sulfobacillus*, Thermoplasma*, Thiomicrospira, Leptospirillum.

* Genus contains acidophiles and • Genus contains alkaliphiles

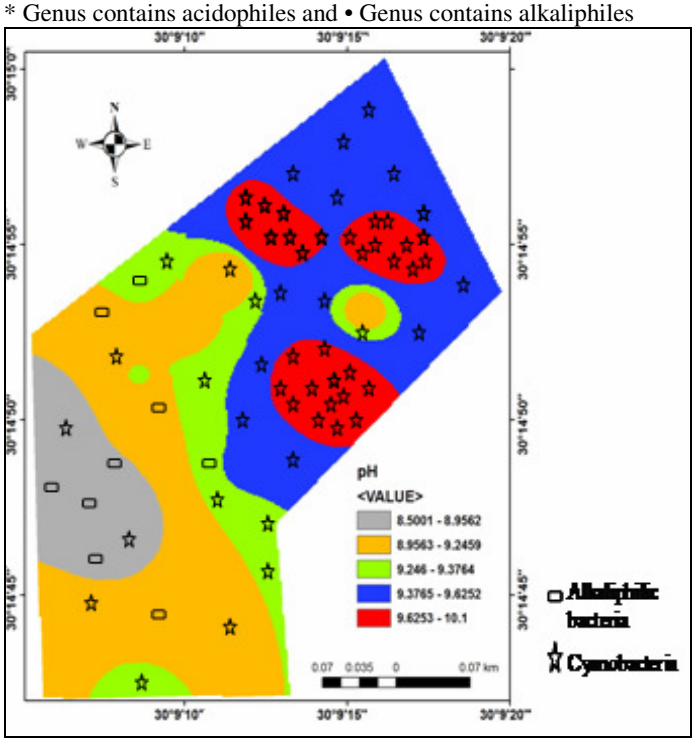

Fig. 8 : Microbial distribution according to pH

The most alkaline naturally occurring environments in the world are soda deserts, which are now uncommon and are in any case in relatively inaccessible areas. The more dilute soda lakes have a characteristic population of blue-green algae, usually *Anabaenopsis arnoldii* and *Spirulina plarensis.* Phototrophic bacteria of the genus *Ectothiorhodospira* also uniquely occupy these lakes, and the more concentrated saline soda lakes harbor populations of characteristic halo bacteria. Many organisms from these lakes appear to be true alkaliphiles, in that they have pH optima about pH 9, and are unable to grow at neutrality. Extremely acid environments are commonly associated with areas of high sulphide concentration and include mine effluents, leach pile effluents, and geothermal springs. The low pH (pH 0-2) is produced by the oxidation of metal sulphides to H_2SO_4 by sulphur bacteria. Not surprisingly, these habitats are dominated by

chemolithotrophic sulphur-oxidizing bacteria, generally of the genus *Thiobacillus*. Many of the *thiobacilli* are acidophiles, in that they have pH optima between 2 and 4 and are unable to grow at neutrality. Acid production by these bacteria can cause serious pollution problems.

The above mentioned figure shows microbial distribution according to optimum pH range. Alkaliphytes can live under high pH values (8.5– 9.4). Cyanobacteria can live at higher pH ranges (9.63 – 10.1), but they can also survive lower pH levels like those of alkaliphilic bacteria.

III) A financial feasibility study for the Cyanobacteria project

First: Data of the study

1- Value of the usufruct project area (200 feddan× 1000 L.E) = 200 thousand pounds.

2- The total area buildings of the project = 1185 c.m

Total cost of building 1185 thousand pounds (the cost of one square meters with finished = one thousand pounds).

3- Cost of the plant fence with a length of 2200 meter = 2200 seedlings of the sedition plant × (including the cultivation) = 110 thousand pounds.

4- Cost of the wire fence with a length of 1850 meter with a height of 2.5 meter = 1850 × one hundred pounds (linear meter) – 1850 thousand pounds.

5- Construction: (20 basins each area 150 squared meter pipes and pumps and (service road).

a- Cost of basins = 20 basin with its inclusions × 50 thousand pounds = one million pounds.

b- Cost of the road = 600 thousand pounds.

c- Machines: (moss-dryer and laboratory equipment).

d- Cost of moss-dryer = 75 thousand pounds.

e- Cost of laboratory equipment = 30 thousand pounds (consumed for 10 years – purchase is required twice during the life of the project).

f- Living furniture & supplies = 100 thousand pounds.

g- Expenses of establishing and publishing = 200 thousand pounds.

h- Working capital: includes labors, electricity costs and fuel.

6- Monthly wages and salaries = 60 thousand pounds.

7- Value of energy required and fuel per month = 10 thousand pounds.

8- Annual general and administrative expenses: include the social insurance (26% of the value of wages and salaries = 187.2 thousand pounds) and general expenses reach about 30 thousand pounds.

9- Annual value of maintenance and spare party = 0.005% of the value of machines, buildings fences and the road (2045 thousand pounds) = 1025 thousand pounds.

10- Cash reserves for the project = (0.05 of the investment costs = 177.75 thousand pounds (divided by 20 years).

Second: Feasibility study

A) The investment costs of the project = 3.555 million pounds.

Table 4: The investment costs of the project

Statement	Value in thousand pounds
Buildings	1185
Fences	295
Construction	1600
Machines, equipment and furniture	205
Working capital (month production cycle)	70
General and administrative expenses	200
Total	3555

B) Typical annual costs of the project (starting from the fourth year).

Table 5: Typical annual costs of the project

Statement	Value in thousand pounds
Usufruct	200.0
Workers	720.0*
Energy & fuel	60.0
Annual (general and administrative expenses)	217.2**
Annual value of maintenance and spare parts	5.45
Cash reserve	8.9
Total	1211.55

(*) wages and salaries in the first three years of the project reach about 200 thousand pounds.
(**) annual general and administrative expenses in the first three years of the project reach about 60 thousand pounds.

Table 6 : Annual revenues of the project in thousand pounds

Year	A mount of dried moss in ton	Price for ton thousand pounds	Value in thousand pounds
1-3	--	--	--
4	16.25	80.0	1300
5	22.5	80.0	1800

6	26.25	80.0	2100
7-8	32.5	80.0	2600
9-13	32.5	96.0	3120
14-18	32.5	115.2	3744
19-20	32.5	138.2	4492.8

Table 7: Depreciation assets of the project in pound

Statement*	% of depreciation	Value of original	Premium of depreciation	Depreciated value	Scarp value
Buildings	5	1185000	47400	948000	237000
Wire fences	5	185000	7400	148000	37000
Moss-dryer	5	75000	3000	60000	15000
Furniture and living supplies	5	100000	4000	80000	20000
Total	--	--	--	--	309000

*Assuming depreciation for each asset with a life span of the project for not calculating a table of replacement and renovation.

Table 8: Net cash flow of the project during its assumed life

Statement	0	1-3	4	5	6	7-8	9-13	14-18	* 19-20
In flows	0	0	1300	1800	2100	3600	3120	3744	4492.8
Investment costs	3555	--	--	--	--	--	30	--	--
Operating costs	0	534.35	1211.55	1211.55	1211.55	1211.55	1211.55	1211.55	1211.55
Total out flows	3555	534.35	1211.55	1211.55	1211.55	1211.55	1211.55	1211.55	1211.55
Net cash flow	-3555	-534.35	88.45	588.45	888.45	1388.45	1878.45	2532.45	3281.25

* adding value of scarp for the last year when calculating the internal rate of return.

Table 9 : Net flows for project by using appropriate discount rates during the life of the project (the implementation period in one year).

Years	Inflow	Discount rate 15%	Present value of the inflows	Outflows	Present value of the outflows	Net present value	Discount rate 25%	Present value of the inflow	Present value of outflows	Net present value
0	0.0	1.0	0.0	3555	3555	-3555	1.0	0.0	3555	-3555
1-3	0	2.283	0.0	534.5	1219.9	-1219.9	1.952	0.0	1043	-1043
4	1300	0.572	743.6	1211.55	693	50.6	0.41	533	496.7	36.3
5	1800	0.497	894.6	1211.55	602.1	292.5	0.33	594	399.8	194.2
6	2100	0.432	907.2	1211.55	523.4	383.3	0.26	546	315	231
7-8	2600	0.703	1827.8	1211.55	851.7	976.1	0.38	988	460.4	527.6
9-13	3120	1.095	3416.4	1211.55	1359.5	2089.8	0.45	1404	558.7	858.8
14-18	3744	1.274	4769.9	1211.55	1543.5	3222.4	0.15	561.6	181.7	379.9
19	4492.8	0.07	314.5	1211.55	84.8	229.7	0.014	62.9	17	45.9
20	4801.8	0.061	292.9	1211.55	73.9	219	0.011	52.8	13.3	39.5
Total	--	--	13166.9	--	10506.9	2660	--	4742.3	7040.7	-2298.4

Conclusion

Finally, results showed dominance of cyanobacteria in higher EC and pH levels and the Cyanobacteria project was high profitability at that area under study (El-Khadralake, Wadi El-Natroun, Egypt).

References

Aly, M.S. (2003). "Microbial diversity of El-Khadralake". J. Agric. Sci., Mansoura Univ., 28(2): 1061- 1066.

Apte, S.K. and Thomas, J. (1997). Possible amelioration of coastal soil salinity using halotolerant nitrogen-fixing cyanobacteria". Plant Soil, 189(2): 205-211

Cottenie, A.; Verloo, M.; Kiekens, L.; Velgh, G. and Camerlynch, R. (1982). Chemical analysis of plants and soils, Lab, Anal Agrochem. State Univ., Ghent Belgium, 63.

Csonka, L.N. and Hanson, A.D. (1991). Procaryoticosmo regulation: genetics and physiology. Am. Rev. Microbiology, 45: 569-606

El-Hady, O.A.; Abd El-Kader, A.A. and Shafi, A.M. (2009). Physico-bio-chemical properties of sandy soil conditioned with acrylamide hydrogels after cucumber plantation. Australian Journal of Basic and Applied Sciences, 3(4): 3145-3151.

El-Sheikh, E.A.E. (1998). Effect of salt on rizoba and bradyrhizobia. Review, Annl. Applied Biology, 123(3): 507-524.

Grant, W.D. (2004). Half lifetime in soda lakes, (Introductory Chapter). In Halophilic Microorganisms (Eds. A. Ventosa), 17-22.

Grant, W.D. and Long, P.E. (1981). Environmental microbiology. 1st Ed, East Kilbride, Scotland, Thomson Litho Ltd.

Madkour, M.A., Smith, L.T. and Smith, G.M. (1990). Preferential osmolyte accumulation: A mechanism of osmotic stress adaptation in diazotrophic bacteria. Applied Environmental Microbiology, 56: 2876-2881.

Malin, G. and Lapidot, A. (1996). Induction of synthesis of tetrahydropyrimidine derivatives in streptomyces strains and their effect on *EcherichiaColi* in response to osmotic and heat stress, J. bacterial, 178(2): 258-395.

Miller, K.J. and Wood, J.M. (1996). Osmoadaptation by rhizosphere bacteria, Ann. Rev, Microbial, 50: 101-136.

Page, A.L.; Miller, R.H. and Keeney, D.R. (1982). Methods of soil analysis, Part2, Chemical and micro- biological properties. 2ed. USA, Amer. Soc. Agron. Madison, W.I.,

Parsons, T.; Takahashi, M. and Hargrave, B. (1997). Biological oceanographic processes, 2nd ed. PC'rgamol1 Press, Oxford.

Reed, R.H.; Borowitzka, L.J.; Mackay, M.A.; Chudek, J.A.; Foster, R.; War, S.R.C.; Moore, D.J. and Stewart, W.D.P. (1989). Organic solutes accumulation in osmotically stressed cyanobacteria. Mcrobial. Rev, 39: 51-56.

Shahaby, A.F. (1997). "Osmoregulation *Azospirillum brasilense* : Production of glutamic acid and prolin response to stress", J. Agric. Science. Mansoura University, 22(12): 4817-4833.