

Alkalophily Among Microorganisms Inhabiting Virgin and Cultivated Soils Along Makkah-Al-Taif Road, Saudi Arabia

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Abstract. Alkalophilic microbial contents of cultivated soils were greater than non-cultivated sites, with higher values at altitudes than coastal plain. Alkalophilic bacteria and fungi existed at different magnitudes in all soil samples tested, indicating their wide distribution. Higher fungal population was not necessarily associated with higher counts of alkalophilic fungi. Circular colonies were highly represented among alkalophilic bacteria; ellipticals, chromogenic and mucoid colonies were less common, while actinomycetes were not encountered. Star-shaped bacterial colonies proved to be constituted totally of true alkalophiles. Alkalophilic bacteria were mostly dominated by species of the genus *Bacillus*. *Fusarium* was the highest fungus in frequency of occurrence followed by *Acremonium* and *Aspergillus* spp.

Introduction

Researchers all over the world are spending much labor and time to discover microorganisms with new abilities. Consideration of microorganisms which grow well in alkaline media, alkalophilic microorganisms, has initiated a new aspect of microbiology. They are widely distributed on the earth and they produce new products [1-5]. Most of the work conducted was directed towards systematic microbial and physiological studies [6-9].

Khodair *et al.* [10] began a pioneering survey on the occurrence and density of alkalophilic microorganisms in Saudi Arabian soils, Western region. The present work deals with the occurrence and density of alkalophilic bacteria and fungi in virgin and cultivated soils along Makkah-Al-Taif road, Saudi Arabia, as affected by some prevailing ecological conditions. A further goal was to examine the relationship between distribution of neutrophilic and alkalophilic soil microorganisms and to determine whether estimation of one group could predict the frequency of the other.

Materials and Methods

Four sites were chosen along Makkah-Al-Taif road (in the vicinity of 40° East of the Principal Meridian and 22° N of the Equator). Two sites represent the western coastal plain, while the others represent the Western altitudes of about 1.6 km above sea level. Available information on weather conditions obtained from general directorate of Meteorology (Ministry of Defense and Aviation) show that the coastal plain district is characterized by a long dry annual period (annual rainfall, in 1984-1986, ranging between 38.3 and 123.5 mm fallen in 8 to 10 days) and warm climate (38.6° and 22.6° C for maximum and minimum average annual temperatures, respectively). Western altitudes (sites near Al-Taif) are characterized by a relatively short dry period (annual rainfall ranging between 87.5 and 285 mm in 18 to 41 days) and somewhat cold climate (28.8° & 15.9° C for maximum and minimum average annual temperatures, respectively). Along Makkah-Al-Taif road, there are numerous man-made locations of cultivated areas, which depend on rainfall and underground water for irrigation. Therefore, two sites were chosen in each district for analysis, one site harboring wild plants and the other representing the cultivated fields. In the coastal plain district the first site (25 km from Makkah) represents soil holding wild plant association dominated by *Calotropis procera*, while the second site (60 km from Makkah) exemplifies soil cultivated with *Vicia faba*. Likely, the first site of altitudes (100 km from Makkah) was covered with wild plant association dominated by shrubs of *Juniperus procera*, and in the second site (110 km from Makkah) the soil was holding cultivated trees of *Prunus armeniaca* and *Rosa abyssianica*. The soil in all non-cultivated sites was undisturbed.

Soil sampling

Soil samples were collected according to the technique described by Johnson *et al.* [11, p. 178] at depth of 5-20 cm. Samples were taken at random from each site, brought together into one composite sample which was mixed thoroughly and kept in polyethylene bags.

Soil analysis

Water contents, total water soluble salts, organic matter and pH values were determined in replicate samples according to the techniques by Jackson [12, p. 498].

Microbiological analysis

Dilution plate method by Johnson *et al.* [11, p. 178] was used for counting fungi and both neutrophilic and alkalophilic bacteria. Four agar media were used for counting soil microorganisms considered. For alkalophilic bacteria, the isolation medium was that recommended by Horikoshi and Akiba [13, p. 9]. It consisted of (g/l): glucose, 10; peptone, 5; yeast extract, 5; KH_2PO_4 , 1; $\text{Mg SO}_4 \cdot 7\text{H}_2\text{O}$, 0.2; Na_2CO_3 , 10 and agar, 15. Sodium carbonate solution was sterilized separately to give after mixing with the remaining components a final pH of 10.0 - 10.5. This medium is symbolized in Tables by (HA).

For counting neutrophilic bacteria, the previously mentioned medium (HA) but modified by substituting 0.5g of NaCl for Na₂ CO₃ and symbolized by (MHA) was used. The final pH of this medium was 7.

For fungi, alkaline Sabouraud's agar medium previously recommended by Khodair *et al.* [10] was used for isolation and counting. It is composed of (g/l): glucose, 40; peptone, 10; agar, 20. Separately sterilized Na₂ CO₃ was added to a final concentration of 1% together with chloramphenicol (50 mg/l medium) to suppress bacterial contamination. The final pH of this medium ranged from 10.0 - 10.5. Total soil fungi were counted on Sabouraud's agar medium supplemented with chloramphenicol but devoid of sodium carbonate (final pH 6).

For both neutrophilic and alkalophilic bacteria, incubation of counting plates (triplicate / treatment) was made at 30 ± 1° C for 3 days. Numbers of morphologically distinct bacterial colonies were considered. After counting, all alkalophilic bacterial colonies developed were picked for identification and pure cultured on the same alkaline medium (H A).

For counting total and alkalophilic fungi, incubation of plates (triplicate / treatment) was at 25 ± 1° C for 5 to 7 days. After counting, pure cultures of the developed colonies were prepared and identified to the genus level by microscopic examination and use of approved keys [14, p. 859; 15, p. 290; 16, p. 241; 17, p. 237; 18, p. 364; 19, p. 686; 20, p. 876].

Characteristics of Alkalophilic Bacterial Isolates

It is very difficult to identify and classify all alkalophilic bacterial isolates obtained from counting plates. Therefore, grouping of these isolates according to cell shape, endospore formation and Gram stain was carried out according to the technique previously described by Khodair *et al.* [21] Gram and spore stains were performed on 24 and 72 hrs cultures, respectively.

The pH range of alkalophilic bacterial growth was determined using the following buffers: Na H₂ PO₄ - NaOH (pHs 7 and 8.2) and Na₂ CO₃ - Na HCO₃ (pHs 9 and 10). The buffers were separately sterilized before addition in suitable aliquots to HA agar medium devoid of Na₂ CO₃. The carbonate buffer was sterilized by membrane filtration. The final buffer concentration did not exceed 0.2 M. The inoculated plates (triplicate / treatment) were incubated at 30 ± 1° C for 48 hrs, after which the growth was visually detected.

Amylolytic activities of alkalophilic bacterial isolates were also determined qualitatively using starch instead of glucose in HA medium. This medium was buffered to pH 9 which was suitable for development of all alkalophilic bacterial iso-

Table 1. Soil properties of samples collected from different sites along Makkah-Al-Taif road

District	Site* no.	Water content %	Total water soluble salts %	Organic matter %	pH
Coastal plain	I	3.03	0.14	1.19	8.7
	II	12.48	0.41	1.37	9.2
Altitudes	III	11.76	1.36	1.46	8.4
	IV	19.32	1.58	1.62	8.6

*Sites I and III = non-cultivated (natural); sites II and IV = cultivated

Table 2. Counts of neutrophilic and alkalophilic bacteria ($\times 10^6$ per g dry soil) inhabiting soil samples collected from different sites along Makkah-Al-Taif road

District	Site no.*	Neutrophiles** (counts)	Alkalophiles***	
			Counts	% of neutrophiles
Coastal plain	I	8.03	0.03	4.1
	II	13.8	0.86	6.2
Altitudes	III	7.9	0.43	5.5
	IV	9.2	1.19	12.9

*Sites I and III = non-cultivated (natural); sites II and IV = cultivated

**Neutrophiles represent bacterial colonies developed on modified neutral HA medium (MHA)

***Alkalophiles represent bacterial colonies developed on alkaline-buffered HA medium

Table 3. Counts of alkalophilic fungi ($\times 10^5$ per g dry soil) in soil samples collected from different sites along Makkah-Al-Taif road

District	Site*	Total count**	Alkalophilic count***	Percentage of total count %
Coastal plain	I	0.28	0.14	49.82
	II	1.06	0.28	26.04
Altitudes	III	0.99	0.29	29.12
	IV	9.04	2.79	30.83

*Sites I and III = non-cultivated (natural); sites II and IV = cultivated

**Fungal colonies developed on Sabouraud's agar medium devoid of Na_2CO_3 (pH 6)

***Fungal colonies developed on Sabouraud's agar medium supplemented with Na_2CO_3 (pH 10.0–10.5)

lates. Zone of hydrolysis was noticed following addition of iodine solution after 48 hrs incubation at $30 \pm 1^\circ \text{C}$.

Statistical Analysis

The data of Tables 1,2 and 3 were statistically treated according to Bishop [22,p. 232].

Results and Discussion

Soil analysis (Table 1) demonstrated clear differences in the edaphic variables between sites holding wild vegetation (I and III) and cultivated ones (II and IV) when compared at each district. Total water soluble salts were found to be slightly higher in cultivated soils (0.41% & 1.58%) than in soils harboring wild plants (0.14% & 1.36%). Such increase may be due to repeated irrigation with relatively saline underground water. Cultivated soils were also found to contain more organic matter than those covered with relatively sparse wild plants. In the former sites, soils are expected to receive more plant remains. Low moisture content (3%) was observed only in soil sample of site I. The soil moisture content of other samples ranged from 11.76% to 19.32%. All tested soils were alkaline but the pH values, in each district, were higher in cultivated than in non-cultivated soils. This may be due to salts originating from irrigation water, especially sodium salts.

Regarding non-alkalophilic microflora (Tables 2 and 3), high numbers of bacteria (ranging between 7.90 and $13.75 \times 10^6/\text{g}$ soil) and evidently low numbers of fungi (0.28 to $9.04 \times 10^5/\text{g}$ soil) were found. Higher counts of alkalophilic bacteria than alkalophilic fungi per 1 g soil were also observed (Tables 2 and 3).

On statistical bases, correlation relationships revealed positive trends between both alkalophilic and neutrophilic bacteria and all soil factors (pH, organic matter, soluble salts and water content) except for water soluble salts in case of neutrophilic bacteria (Fig. 1). Likely, positive correlations, but at different levels, between both alkalophilic fungi as well as total fungal counts and all soil factors were also evident (Fig. 2).

In terms of count numbers, and in the coastal plain for example, the cultivated site (II) held higher counts of non-alkalophilic bacteria and fungi than that of natural non-cultivated site (I) (13.75×10^6 and 8.03×10^6 for bacteria and 1.06×10^5 and 0.28×10^5 for fungi, respectively). Maximum value of soil water content (12.5%) was reached in site II (cultivated) compared with 3.03% in site I (natural). Similarly, the cultivated soil from the altitudes (site IV) possessed a water content of 19.3% compared with 11.8% in the natural soil of the same district (site III). Likely, the organic matter content in either site II or IV (cultivated) was slightly higher than that prevail-

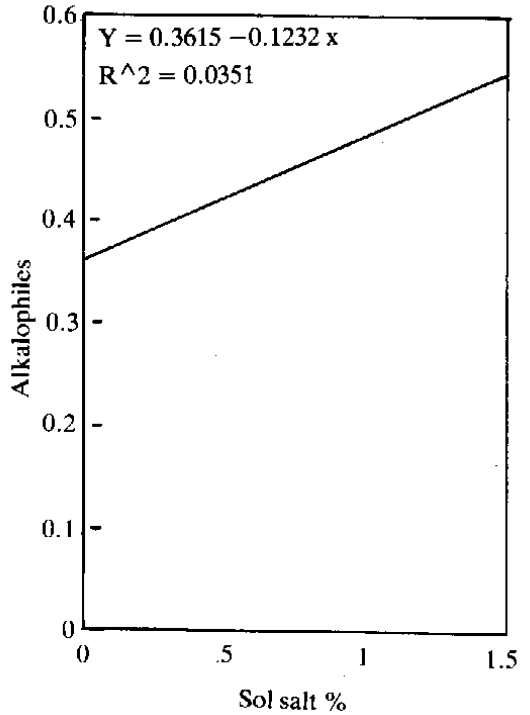
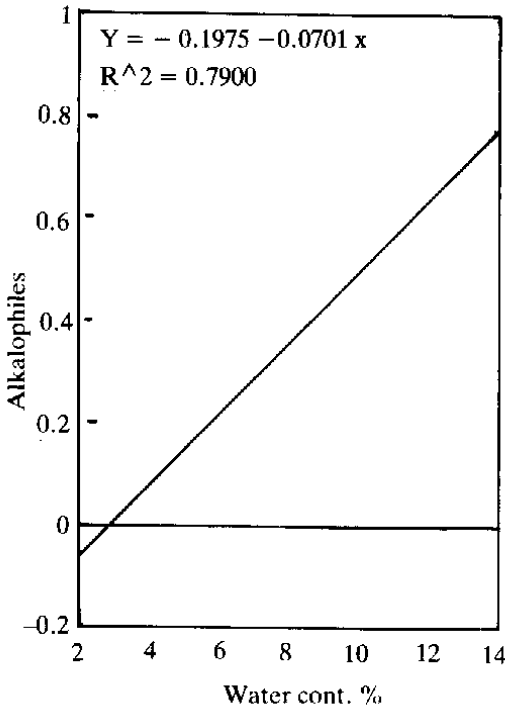
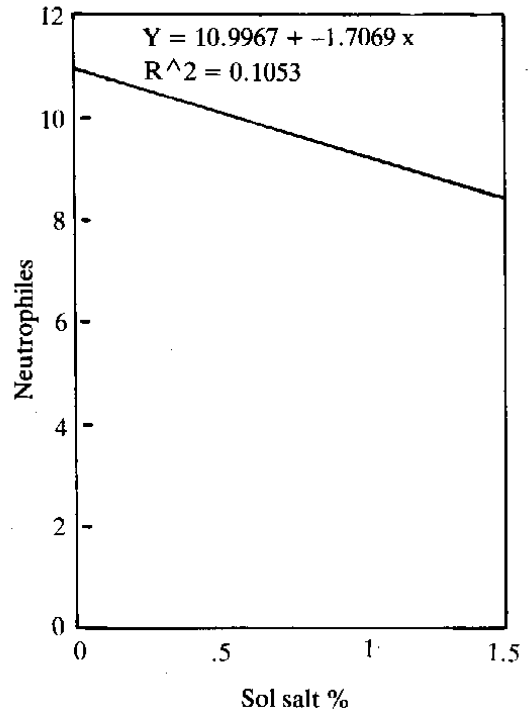
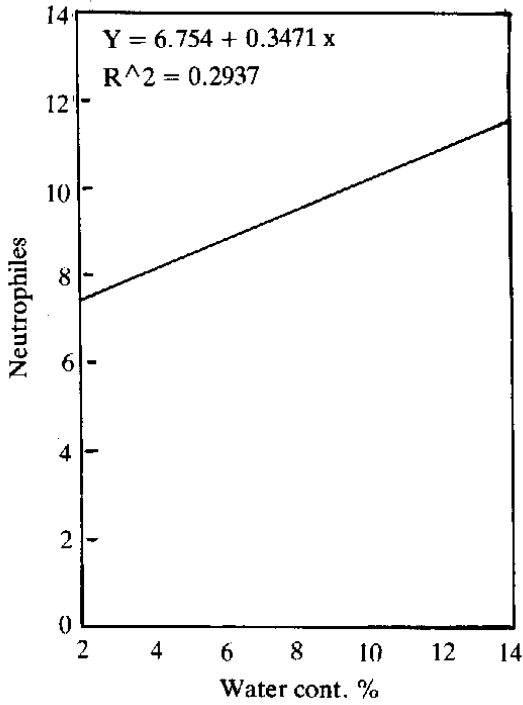


Fig. 1. Regression lines for data in Tables 1 & 2.

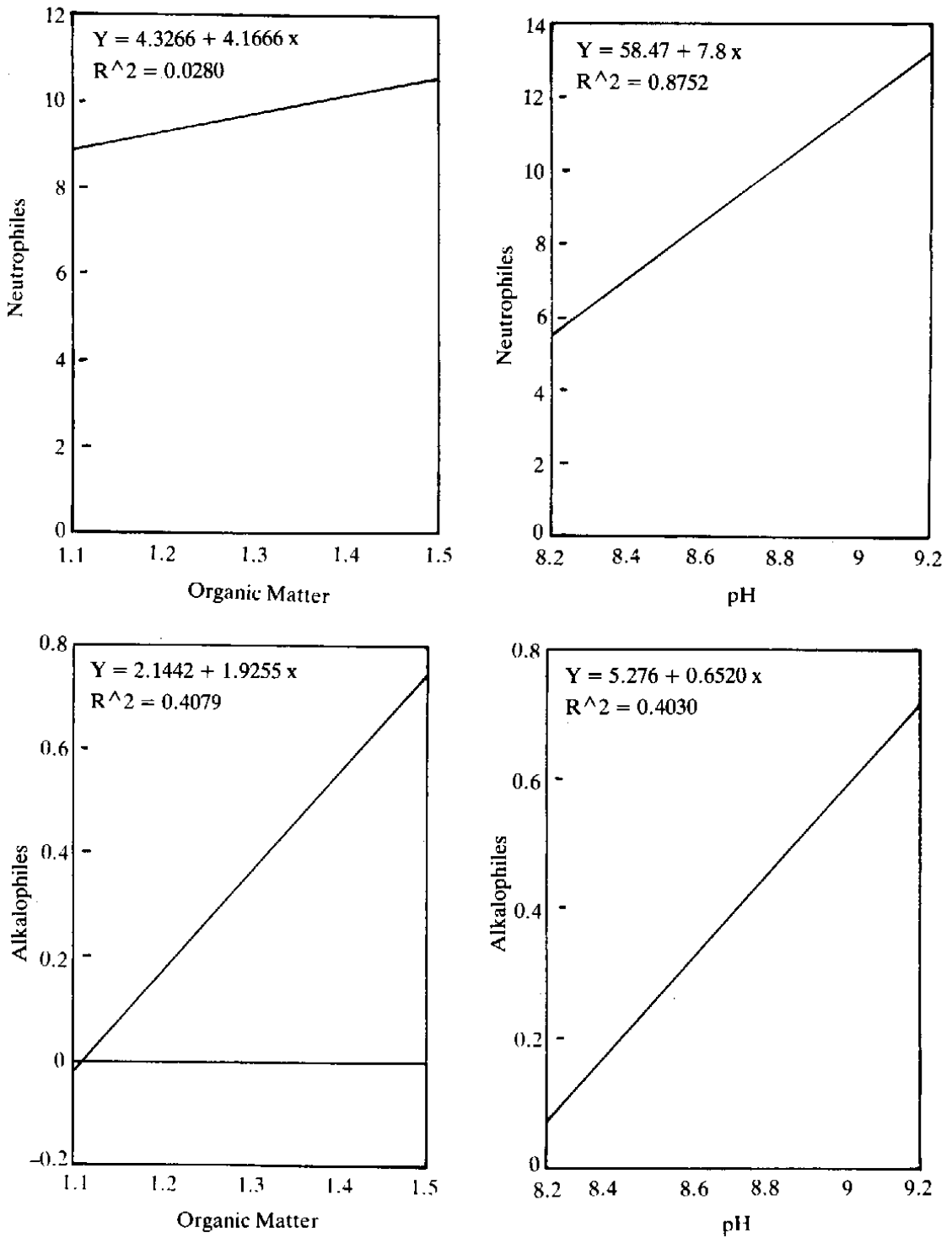


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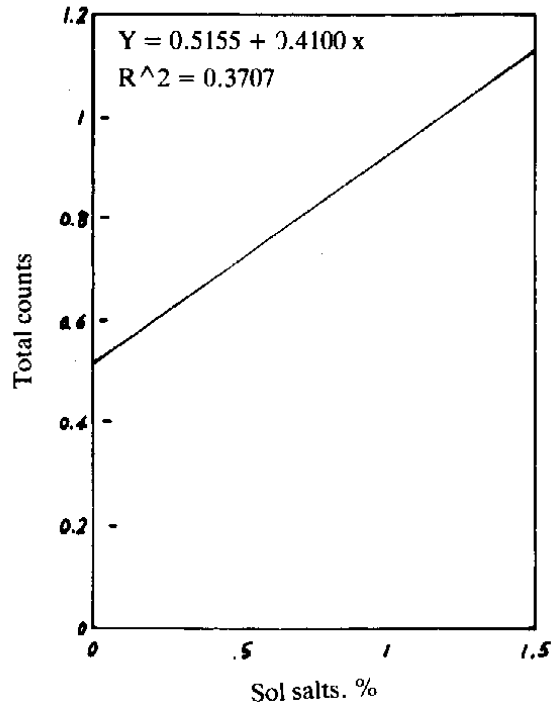
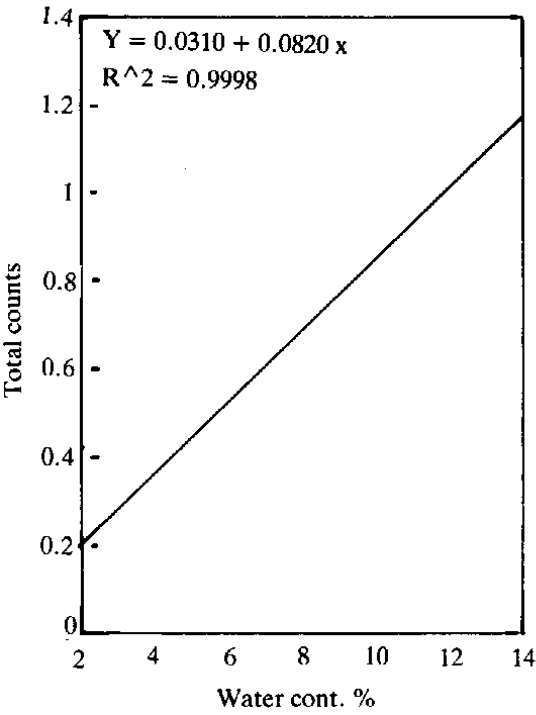
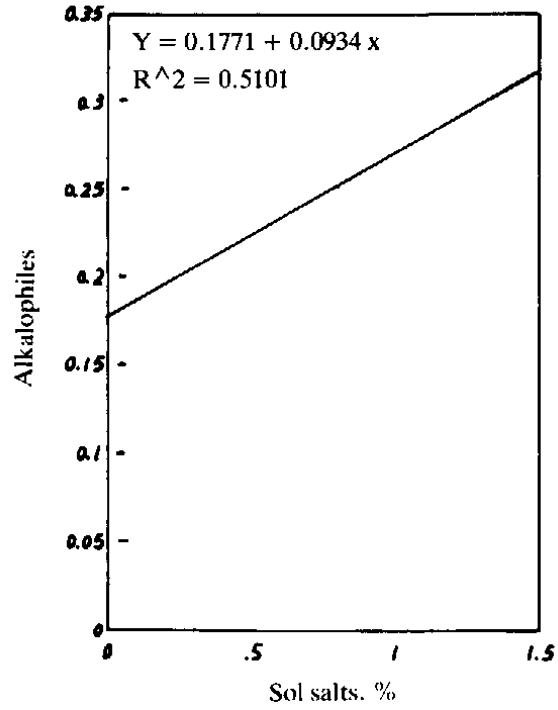
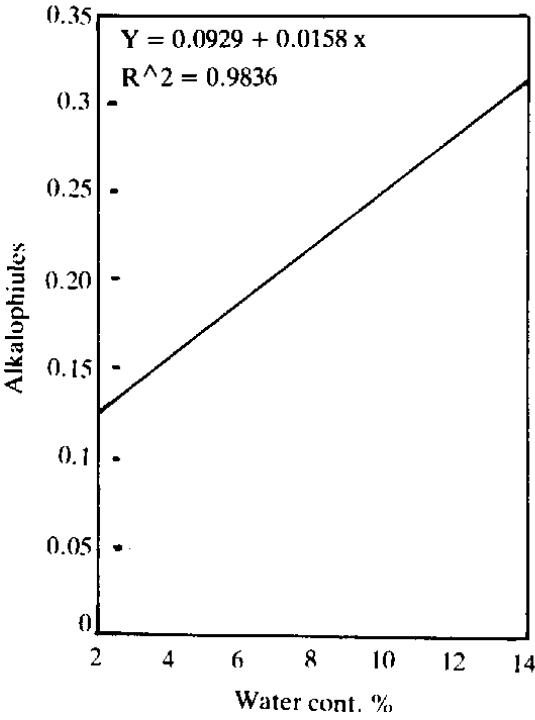


Fig. 2. Regression lines for data in Tables 1 & 3.

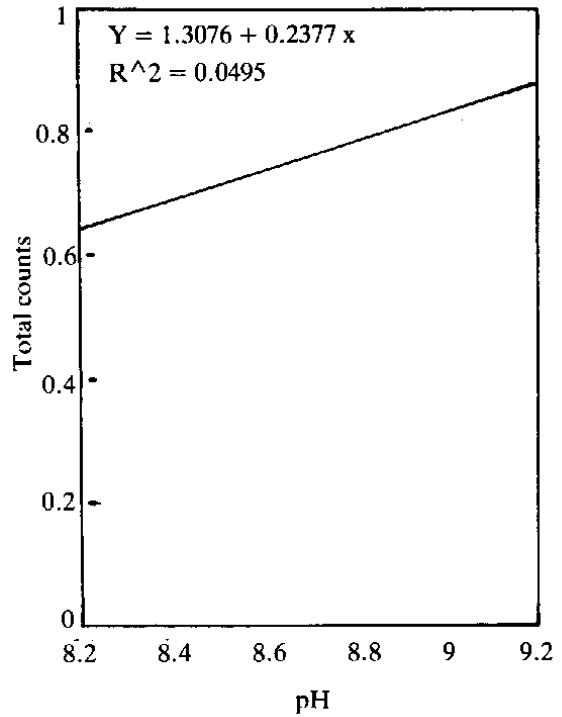
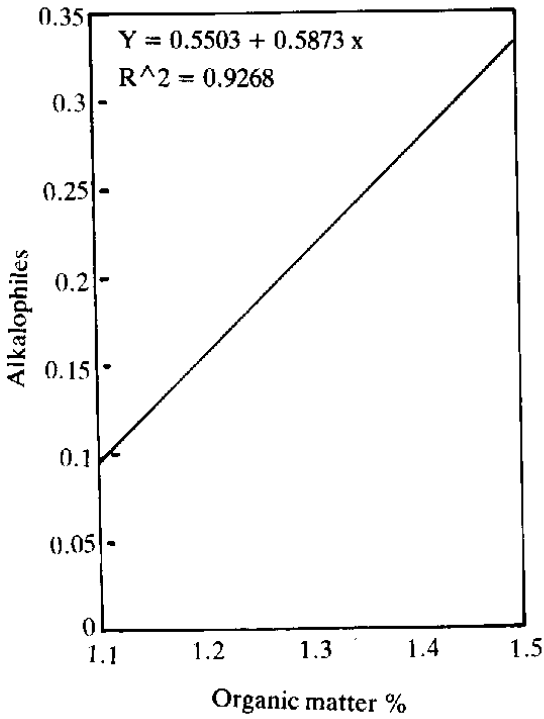
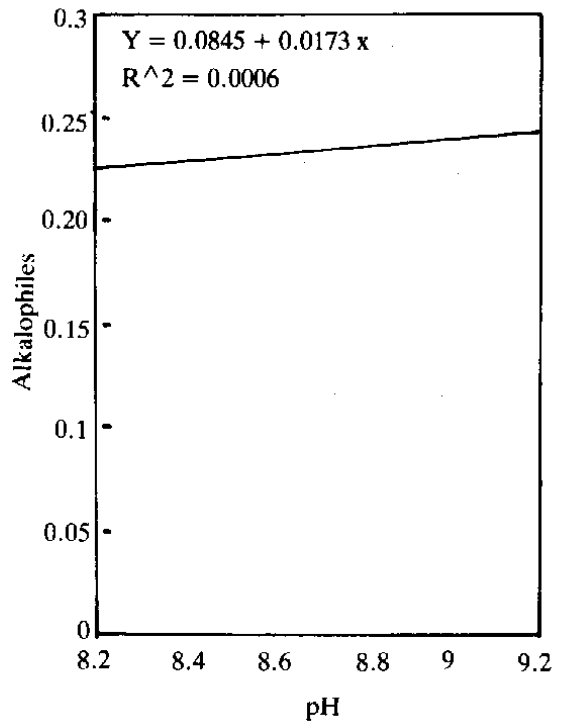
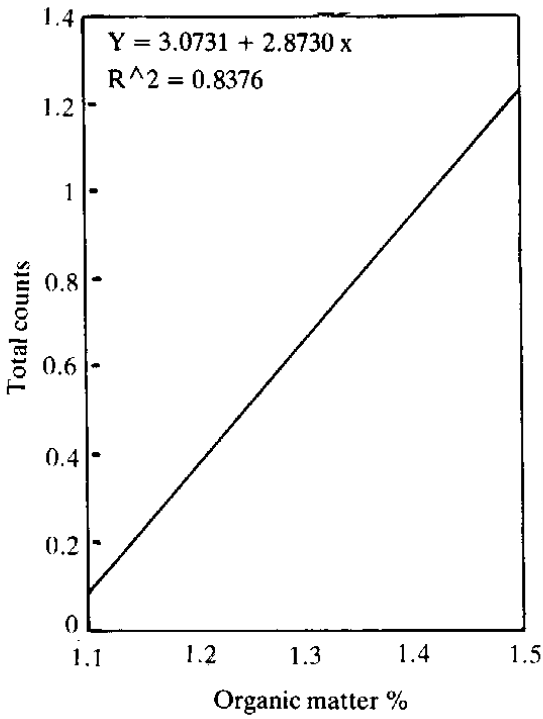


Fig. 2. Cont.

ing in either site I or III (natural), respectively. A similar correlation picture can be reached in soil samples collected from altitudes. A major factor represented in the cultivated soil, is the densely-rooted system of the growing plants where most of the soil may effectively be rhizosphere, while in non-cultivated soils, the wild plants are more widely spaced and the density of roots may be too low to affect most of the soil. Stimulative rhizosphere effects in western region of Saudi Arabia were previously investigated by Khodair *et al.* [21; 23].

The rhizosphere effect on neutrophilic bacteria was more prominent in coastal plain (site II) than in altitudes (site IV), contrary to the response of fungi. Differences in responses may be due to the plants developed and climatic factors; site II, hoding shallow-rooted plants (*Vicia faba*), while site IV harboring deeply rooted trees (*Prunus armeniaca* and *Rosa abyssianica*). Moreover, the former site is characterized by relatively warm climate, while the latter is relatively cold and hence more suitable for fungal development. In agreement with these findings, previous investigators showed that root exudates and their stimulative effects vary with plant species [23], stages of plant growth [21], climatic factors as light intensity and temperature [24; 25] and by soil moisture [26].

Alkalophilic bacteria and fungi existed in all soil samples tested (Tables 2 and 3). Extensive surveys on distribution of heterotrophic bacteria in soils of Western region of Saudi Arabia as well as fungi were previously carried out using neutral media for the former and acidified media for the latter [27;28]. The incidence of alkalophilic microorganisms reached in the present study can be attributed to the use of carbonate-buffered alkaline media (pH 10.0 – 10.5). Alkalophilic microbial contents of cultivated soils (sites II and IV) appeared relatively more than non-cultivated sites, still with higher values at altitudes than sites of the coastal plain. Therefore, it is apparent that both biotic as well as edaphic variables significantly affect the distribution of alkalophiles as was observed with non-alkalophiles. If this observation exists in other habitats, it may be concluded that enumeration of non-alkalophilic bacteria could predict the frequency of alkalophiles, a proposal which needs further investigations.

The recorded numbers of alkalophilic bacteria ranged between 4 and 13% of neutrophilic counts (Table 2). In accordance with these findings, Horikoshi and Akiba [13, p. 10] stated that alkalophilic bacteria, actinomycetes, fungi and yeasts are widely distributed and the number of alkalophilic bacteria found in soil was about 10% to 1% of that of neutrophilic bacteria.

Alkalophilic fungi accounted for 49.82, 26.04, 29.12 and 30.83% of total fungal counts in soil samples from sites I, II, III and IV, respectively (Table 3). Such constitution percentages appeared independent of the magnitude of total fungal counts at any of the sites studied. In other words, a higher fungal population (site IV) was

not necessarily associated with a higher percentage of alkalophilic fungi (30.83%), compared with the lowest fungal density (site I) which comprised the highest percentage of alkalophilic fungi (49.82%). Such observation most probably indicates that other factors (physical or chemical) than fungal density, are responsible for increasing or decreasing the counts of alkalophilic fungi in soil. This interpretation might also lead to the suggestion that alkalophily, in fungi, is most probably facultative rather than obligate.

It was concluded by Campbell [29] that cultural studies of soil microorganisms may be more valuable if bacterial diversity is considered. Such diversity may give an idea of differences in community structure. Therefore, colony characters, cell morphology and staining were taken into consideration in the present study. Comparison between percentages of morphologically distinct colonies (Table 4) developed on HA medium (alkalophiles) and MHA medium (neutrophiles) revealed the following:

Table 4. Percentages of morphologically distinct bacterial colonies appearing on counting plates

*Site no.	Medium	Circulars	Punctiforms	Ellipticals	Star-shaped	Mucilagenous	Chromogenic	Actinomycetes
I	HA	45	29	7	15	4	0	0
	MHA	15	74	7	0	1	3	0
II	HA	31	54	5	5	4	1	0
	MHA	6.2	13.4	2.2	0	78.2	0	0
III	HA	38	6	15	22	14	6	0
	MHA	39	57	3	0	1	0	0
IV	HA	32.7	40.7	5.6	2.8	2.2	15.9	0
	MHA	11.2	28.5	10.8	0	49.1	0.4	0

*Sites I and III = non-cultivated (natural); sites II and IV = cultivated

1. Circulars were more abundant among alkalophiles than neutrophiles in sites I, II and IV. Punctiforms behaved similarly in sites II and IV, but were more abundant among neutrophiles in sites I and III.

2. Ellipticals, chromogenic and mucoid colonies (except neutrophiles of sites II and IV) were poorly represented, while actinomycetes were not encountered.

3. Star-shaped bacterial colonies were only recorded on the alkaline medium (HA) with values ranging between 2.8 and 22.0%. This may indicate that star-shaped bacterial colonies were obligate alkalophiles since they were unable to develop on a neutral medium.

The above findings could be clarified by investigating cell morphology and staining of alkalophiles developed on HA medium. Table 5 shows that alkalophiles were mostly dominated by species of the genus *Bacillus* (Gram positive, spore-forming, aerobic and / or facultative anaerobic rods). The dominance of spore-forming alkalophilic bacteria may indicate that alkalophilic bacteria are permanently represented in soils even under extreme conditions. Pseudomonads (unidentified Gram negative, non-spore-formers, aerobic short rods), the genus *Micrococcus* (Gram positive, too small in size) and Gram positive asporogenous aerobic rods were also isolated from all tested soil samples but at relatively moderate frequency. In densely plant rooted soils (sites II and IV), Gram positive asporogenous rods and small sized micrococci were abundant, with a concomitant drop in the genus *Bacillus*. This indicates that bacterial identities gave more precise evidences for the effects of rhizosphere as compared to results of total counts.

Species belonging to 16 genera of soil-inhabiting fungi in addition to *Mycelia sterilia* were developed on alkaline (pH 10.0 – 10.5) counting plates (Table 6). The isolates include filamentous and yeast fungi. Thirteen genera of the recorded fungi were recovered from the cultivated site at altitude (IV), where the highest numbers of alkalophilic fungi were observed (Table 3). In contrary, the expected rhizosphere effect in the coastal plain (site II) was reflected by increased fungal colonies of only *Fusarium* and *Acremonium* spp. which developed on counting plates. Although, densities of *Fusarium* spp. greatly increased in the cultivated site of the coastal plain, they decreased in the respective site at altitude (II and IV, respectively). This may indicate the selective stimulative rhizosphere effect exerted by different plant species.

Among all alkalophilic fungi, *Fusarium* was the only genus of high frequency of occurrence, being isolated from all sites examined. Moderate frequency of occurrence of fungal species developed on alkaline medium (10.0 – 10.5) was represented by *Aspergillus*, *Geotrichum*, *Trichophyton* and *Acremonium*, being recovered from only two sites out of four. The remaining 11 genera plus *Mycelia sterilia* were of low occurrence (one case of isolation). According to their relative densities they can be arranged descendingly as, *Scopulariopsis*, *Paecilomyces*, *Sepedonium*, *Monilia*, *Humicola*, *Cunninghamella*, *Penicillium*, *Dactylaria*, *Aureobasidium*, *Beauveria*, *Oidiodendron* and *Mycelia sterilia*. Whether high and moderate frequencies of occurrence of fungi originally inhabiting alkaline soils and isolated on alkaline media are indicative of their level of alkalophily, is a relation which needs further studies.

Regarding the effect of initial pH of the medium on the growth of bacterial isolates (Table 5), results show that they can be differentiated into 2 groups. The first group grew only on highly alkaline media (pH 9.0 & 10.0), thus defined as obligate alkalophiles [13;29]. The second group could grow on neutral and slightly alkaline

Table 5. Total percentages of bacterial genera and groups appearing on alkaline HA medium, in addition to percentages of their obligate alkalophilic isolates

Site no.	<i>Bacillus</i> spp.		Pseudomonads		Gr + ve Asporogenous rods		Gr + ve <i>Micrococcus</i>	
	Total	Obligate isolates	Total		Total	Obligate isolates	Total	Obligate isolates
I	45.4	10	22.7		18.2	0	13.6	0
II	28.9	45.4	23.7		28.9	0	18.4	0
III	23.6	61.9	27.3		6.1	50	3	0
IV	10.7	33.3	14.3		28.6	0	46.4	15.4

*Sites I and III = non-cultivated (natural); sites II and IV = cultivated

Table 6. Genus range, relative density (as % of total count at each site) and frequency of occurrence of alkalophilic fungi in soil samples collected from different sites along Makkah-Al-Talf road

Genus	*Relative densities at sites				No. of cases of isolation	**Frequency of occurrence
	I	II	III	IV		
Fusarium	9.52	78.08	57.14	4.05	4	H
Aspergillus	52.38	-	-	12.16	2	M
Geotrichum	2.38	-	-	6.76	2	M
Trichophyton	7.14	-	42.86	-	2	M
Acremonium	-	21.92	-	1.35	2	M
Scopulariopsis	-	-	-	25.68	1	L
Paecilomyces	-	-	-	13.51	1	L
Sepedonium	-	-	-	12.16	1	L
Monilia	9.52	-	-	-	1	L
Humicola	-	-	-	8.11	1	L
Cunninghamella	-	-	-	6.76	1	L
Dactylaria	-	-	-	4.05	1	L
Penicillium	4.76	-	-	-	1	L
Aureobasidium	-	-	-	2.7	1	L
Beauveria	-	-	-	1.35	1	L
Oidiodendron	-	-	-	1.35	1	L
Sterile mycelium	14.29	-	-	-	1	L

*Sites I and III = non-cultivated (natural); sites II and IV = cultivated

**Occurrence remarks: H = High (3-4 cases); M = Medium (2 cases); L = Low (1 case)

media (pH 7.0 and 8.2) and most of them showed relatively poor growth at pH 9, but all of them could not grow on highly alkaline medium (pH 10.0), thus distinguished as alkaline tolerant [29] or facultative alkalophiles [30]. The obligate alkalophiles, in the present study, were indexed by the genus *Bacillus* inhabiting all tested sites, Gram positive asporogenous rods in site III and the genus *Micrococcus* in site IV. Therefore, it can be concluded that alkalophilic bacteria inhabiting the tested soil samples are dominated by facultative alkalophiles. Obligate alkalophilic bacteria will survive only at high soil pH values in specific microhabitats where they can flourish at such high pH as 10.0 or more. Conversely, facultative alkalophiles have a wide tolerance to pH variations and thus may be expected to represent a permanent part of soil populations. Previously described facultative and obligate alkalophilic bacteria include species of the genera *Bacillus* [31], *Micrococcus* [6], *Pseudomonas* [32], *Closteridium* and *Flavobacterium* [33], *Arthrobacter* [34] and Corynform bacteria [35].

The amylolytic activity of alkalophilic isolates of the present study was investigated (unpublished data). All members of the genus *Bacillus* and low proportion of the Gram positive asporogenous rods (about 20%) produced amylase(s). Therefore, alkalophilic microorganisms can be included in the definition of active soil microorganisms.

Studies in microbial ecology, such as the present one, is a necessary step towards better understanding of the behavior, growth, density and distribution of alkalophiles as affected by different ecological factors in their natural habitats, the soil, especially in arid lands (Saudi Arabia). Successful isolations, no doubt, will lead to the discovery of microorganisms with new abilities (DNA). New novel products, enzymes, antibiotics and others have been found and others will be discovered in the future. Therefore, the importance of the "alkaline world" lies in the fact that it can be used as a new DNA source for genetic engineering.

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References

- [1] Horikoshi, K. "Production of Alkaline Enzymes by Alkalophilic Microorganisms. Part I. Alkaline Protease Produced by *Bacillus* No. 221." *Agric. Biol. Chem.*, 35 (1971), 1407-1414.
- [2] Yamamoto, M.; Tanaka, Y. and Horikoshi, K. "Alkaline Amylases of Alkalophilic Bacteria." *Agric. Biol. Chem.*, 36 (1972), 1819-1823
- [3] Sunaga, T.; Akiba, T. and Horikoshi, K. "Production of Penicillinase by an Alkalophilic *Bacillus*." *Agric. Biol. Chem.*, 40 (1976), 1363-1367.
- [4] Sunaga, T.; Akiba, T. and Horikoshi, K. "Separation and Properties of Penicillinase of an Alkalophilic *Bacillus*." *Agric. Biol. Chem.*, 43 (1979), 477-480.
- [5] Kobayashi, Y. and Horikoshi, K. "Production of Extracellular Polyamine Oxidase by *Penicillium* sp. No. PO-1." *Agric. Biol. Chem.*, 45 (1981), 2943-2945.

- [6] Akiba, T. and Horikoshi, K. "Identification and Growth Characteristics of Galactosidase-Producing Microorganisms." *Agric. Biol. Chem.*, 40 (1976), 1845-1849.
- [7] Ikura, Y. and Horikoshi, K. "Isolation and Some Properties of Alkalophilic Bacteria Utilizing Rayon Waste." *Agric. Biol. Chem.*, 41 (1977), 1373-1377.
- [8] Kobayashi, Y. and Horikoshi, K. "Identification and Growth Characteristics of Alkalophilic *Corynebacterium* sp. which Produces NAD(P)-Dependent Maltose Dehydrogenase and Glucose Dehydrogenase." *Agric. Biol. Chem.*, 44 (1980), 41-47.
- [9] Horikoshi, K.; Nakao, M.; Jurono, Y. and Sashihara, N. "Cellulases of An Alkalophilic *Bacillus* Strain Isolated from Soil." *Can. J. Microbiol.*, 30 (1984), 774-779.
- [10] Khodair, A.A.; Ramadani, A.S. and Aggab, A.M. "Occurrence and Density of Alkalophilic Bacteria and Fungi in Saline Soils of Makkah District, Saudi Arabia." *Arab Gulf J. Scient. Res.*, 9 (1991), 119-132.
- [11] Johnson, L.; Curl, E.; Bond, J. and Fribourg, H. *Methods for Studying Soil Microflora-Plant Disease Relationships*. Minneapolis: Burgess Publishing Company, 1960.
- [12] Jackson, M.L. *Soil Chemical Analysis*. London: Constable and Co. Ltd., 1958.
- [13] Horikoshi, K. and Akiba, T. *Alkalophilic Microorganisms, A New Microbial World*. New York: Springer-Verlag, 1982.
- [14] Domsch, K.H., Games, W. and Anderson, T.H. *Compendium of Soil Fungi*. London: Academic Press, 1980.
- [15] Domsch, K.H. and Games, W. *Fungi in Agricultural Soils*. London: Longman Group Limited, 1972.
- [16] Barnett, H.L. and Hunter, B.B. *Illustrated Genera of Imperfect Fungi*. Minneapolis: Burgess Publishing Company, 1972.
- [17] Booth, C. *The Genus Fusarium*. Kew, Surrey, England: Commonwealth Mycological Institute, 1971.
- [18] Barron, G.L. *The Genera of Phycomycetes from Soil*. Baltimore: The Williams and Wilkins Company, 1968.
- [19] Raper, K.B. and Fennel, D.L. *The Genus Aspergillus*. Baltimore: The Williams and Wilkins Company, 1965.
- [20] Raper, K.B. and Thom, C. *A Manual of the Penicillia*. Baltimore: The Williams and Wilkins Company, 1949.
- [21] Khodair, A.A.; Ramadani, A.S. and Aggab, A.M. "Microbiological Studies in Desert Soil of the Western Region of Saudi Arabia. II. Variable Roles of Flowering and Fruiting of *Zygophyllum simplex*, L. Mostly Assessed by Fungal and Bacterial Intensities in its Root Region Masked by a General Stimulatory Rhizosphere Effect." *Fac. of Ed. J. Umm Al-Qura Univ.*, 8 (1982), 109-129.
- [22] Bishop, O.N. *Statistics for Biology*. London: Longman Group Limited, 1983.
- [23] Khodair, A.A.; Ramadani, A.S. and Aggab, A.M. "Microbiological Studies in Desert Soil of the Western Region of Saudi Arabia. I. Development of Fungi and Bacteria in the Rhizosphere of Some Wild Annual Plants in Relation to their Stages of Growth." *Fac. of Ed. J. Umm Al-Qura Univ.*, 7 (1981), 57-71.
- [24] Rovira, A.D. "Root Excretion in Relation to the Rhizosphere Effect. IV. The Influence of Plant Specis, Age of Plant, Light, Temperature and Calcium Nutrition on Exudation." *Plant and Soil*, 11 (1959), 53-64.
- [25] Hale, M.G.; Fay, C.L. and Shay, F.J. "Factors Affecting Root Exudation." *Advanced Agron.*, 23 (1971), 89-109.
- [26] Ivanov, V.P.; Yacobsen, G.A. and Fomenko, B.S. "Effect of Soil Moisture on Metabolism and Root Excretion." *Fiziol. Rast.*, 2 (1964), 630-637.
- [27] Khodair, A.A. and Aggab, A.M. "Yeast and Filamentous Fungi on the Surfaces of Aerial Parts of *Euphorbia cyparissioides*, *Solanum incanum* and *Lycium schawii* Wild Perennials Growing in a *Juniperus procera* Association at About 2 km Altitude in Saudi Arabia." *Fac. of Ed. J. Umm Al-Qura Univ.*, 8 (1982), 119-134.

- [28] Khodair, A.A. and Ramadani, A.S. "Rainfall Washing Effect on Bacteria and Fungi and Their Recolonization on Aerial Surfaces of Some Wild Plants in Saudi Arabia." *J. Coll. Sci. King Saud Univ.*, 15 (1984), 365-377.
- [29] Campbell, R. *Microbial Ecology*. Oxford, UK: Blackwell Scientific Publications, 1983.
- [30] Guffanti, A.A.; Finkelthal, O.; Hicks, D.B.; Falk, L.; Sidhu, A.; Carro, A. and Krulwich, T.A. "Isolation and Characterization of New Facultatively Alkalophilic Strains of *Bacillus* Species." *J. Bacteriol.*, 176 (1986), 766-773.
- [31] Koyama, N.; Takinishi, H. and Nosoh, Y. "A Possible Relation of Membrane Proteins to the Alkalostability of A Facultatively Alkalophilic *Bacillus*." *FEMS Microbiol. Lett.*, 16 (1983), 213-216.
- [32] Hale, E.M. "Isolation of Alkalophilic Bacteria from Alkaline Reservoir Used in An Industrial Process." *Proceedings of the 77th Annual Meeting of the American Society of Microbiology*, Abstracts, 150 (1977).
- [33] Souza, K.A.; Deal, P.H.; Mack, H.M. and Turnbull, C.E. "Growth and Reproduction of Microorganisms Under Extremely Alkaline Conditions." *Appl. Microbiol.*, 28 (1974), 1066-1068.
- [34] Shima, M.; Onishi, S.; Mizumori, S.; Kato, N. and Sakazawa, C. "Degradation of 4-Chlorobenzoate by Facultatively Alkalophilic *Arthrobacter* sp. Strain SB8." *Appl. Environ. Microbiol.*, 55 (1989), 478-482.
- [35] Souza, K.A. and Deal, P.H. "Characterization of a Novel Extremely Alkalophilic Bacterium." *J. Gen. Microbiol.* 101 (1977), 103-109.

مدى حب القلوية بين الكائنات الدقيقة المستوطنة للتربة المزروعة وغير المزروعة على امتداد طريق مكة - الطائف بالمملكة العربية السعودية

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ملخص البحث . أظهرت الدراسة أن الكائنات الدقيقة المحبة للقلوية كانت أعلى تعداداً في التربة المزروعة عنها في التربة غير المزروعة، مع ارتفاع القيم في تربة المرتفعات عنه في تربة السهل الساحلي . ووجدت البكتريا والفطريات المحبة للقلوية في جميع عينات التربة المدروسة، مما يدل على سعة انتشارها . ولم يكن التعداد الفطري الكلي المرتفع في عينات بعينها من التربة مقترناً بالضرورة بتعداد عالٍ من الفطريات المحبة للقلوية . وكانت المستعمرات المستديرة أكثر تمثيلاً للبكتريا المحبة للقلوية، بينما المستعمرات البيضاوية والملونة والهلامية أقل تمثيلاً، أما الأكتينومايستات فكانت غائبة . وثبت أن كل المستعمرات البكتيرية النجمية الشكل مكونة من بكتريا محبة للقلوية حقيقة . واتضح من النتائج أن البكتريا المحبة للقلوية قد سادت فيها الأنواع التابعة لجنس الباسيلاس، بينما كانت الفيوزاريوم هي الفطرة الأكثر تواجداً يليها أنواع الأكريمونيام والأسبرجيللس .