

# Biodiversity of Salt-adapted Filamentous Fungi of Red Sea Coast in Upper Egypt

El-Maghraby, O.M.O<sup>1\*</sup>, Youssef, M.S<sup>1</sup>, Marwa AbdeL-Kareem, M<sup>1</sup>, Randa Fathy, A<sup>1</sup>

<sup>1</sup>Department of Botany and Microbiology, Faculty of Science, University of Sohag, Sohag, Egypt

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\*Corresponding author: El-Maghraby, O.M.O

Department of Botany and Microbiology, Faculty of Science, University of Sohag, Sohag, Egypt

## Abstract

Extremophiles are organisms that can thrive under extreme environmental conditions. In this study, isolated and identified fungal genera and their species from Red Sea saline soils in Upper Egypt were carried out. Soil texture, moistures content, organic matter, total dissolved salts and pH value of the soil in addition to some mono- and bi- equivalent elements were analyzed for the samples. 1% glucose-Czapek's agar medium was used for isolation of filamentous fungi. A total of 42 fungal species + 3 varieties of 11 genera were collected and identified that have the ability to survive under extremophilic conditions. Majority of isolated species belonged to genus *Aspergillus* (15 sp. + 2 var. and 32.12% of gross fungal counts) and *Penicillium* (13 sp. and 54.05%), whereas *Acremonium* (1sp. and 4.60%) and *Emericella* (2 sp. +1 var. and 3.35%) were less. Nine species of 7 genera were listed in rare frequency of occurrence ( $\geq 10\%$  of the samples), with very low in counts (collectively, 5.03%). The organic matter content of saline soils had positive effect in flourishing of filamentous fungi with depression in pH value of the soils.

**Keywords:** Soil, Salinity, Extremophiles, Biodiversity, Red Sea.

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## INTRODUCTION

Of saline soils, more than 800 million hectares land in the world is affected by salinity [1]. This amount zaccounts for more than 6 % of the world's total land area, and the saline land extends over all the continents including Africa, Asia, Australasia and America. Saline soils, containing predominantly  $\text{Na}^+$  and  $\text{Cl}^-$ , are widespread globally and affect the production of major crops [2]. Soil salinity, caused by natural of anthropogenic factors, has been recognized as a challenge to cultivation coastal saline soil is widely distributed in Egypt. Soil salinization, either caused by natural factors or anthro- genic activities, has been recognized as a serious problem worldwide, where there is notably in arid and semi-arid regions [3-5]. Salt toxicity has negative effects on soil properties, including high pH, high level of sodium adsorption ratio (SAR) and exchangeable sodium percentage (ESP), poor soil structure and also low water permeability [5-8]. Salt accumulation also has detrimental effects on enzyme activities, microbial and biochemical activities [9, 10], limiting agriculture productivity [11]. Salinity is a stressful factor which reduces microbial communities and has harmful effects on soil organic matter

decomposition and available nutrients uptake. This is similar to the results obtained by [9]. Soil salinity and sodicity are escalating problems worldwide. Accumulation of dispersive cations such as  $\text{Na}^+$  in soil solution and exchange phase has an impact on soil properties. Some effects of salinity and sodicity on physical [12-15], chemical [16-18] and biological [5, 19, 20] properties of soils and also on plant growth [21-24] have been reported in the literature. Excessive amounts of salts have adverse effects on the physical and chemical properties of soil, microbiological processes and on plant growth. The present study aimed for studying: 1- Soil analyses including soil texture, moisture and organic matter contents, pH values, total dissolving elements salts in addition to macro- ( $\text{Ca}^{++}$   $\text{Mg}^{++}$  &  $\text{K}^+$ ) and micro- ( $\text{Fe}^{++}$ ,  $\text{Mn}^{++}$  &  $\text{Zn}^{++}$ ) elements plus sodium ( $\text{Na}^+$ ) as stress element. 2- Filamentous fungi (frequencies and counts) isolated and identified from the soils with special reference to healthy and fertility of the saline soils.

## MATERIALS AND METHODS

A total of 40 samples (~ 500 g) of saline soils were collected (autumn, winter and spring, 2020 & 2021) from rhizosphere of 7 plants in Red Sea Coast, Upper Egypt (Red Sea Governorate) as shown in Table 1.

Regarding to the fertility and healthy of the soils, some parameters were taken in consideration:-

### 1. Analysis of soil samples

Of soil texture, the pipette method was used for particle-size [25]. Moisture content (M.C.) was determined by drying the soil sample (100 g) in an oven at 105°C for 24h. and the percentage (M.C.%) was calculated according to the equation:

$$\text{M. C. \%} = \frac{W_1 - W_2 \times 100}{W_1}$$

Where,

$W_1$  = initial weight (100 g) of soil

$W_2$  = dry weight of soil

Organic carbon was determined by wet digestion method [26] through oxidation of soil carbon using acidic dichromate reagent. Total dissolving salts were estimated by evaporation of soil solution (1:10) in an oven at 105°C for 24h. and the percentage per dry soil was calculated. The pH-meter (EUTECH instruments pH 510 pH/mV/°C meter) was used for determination of soil pH. The pH was measured potentiometrically in a suspension of 10 g soil in 100 ml sterile dist. water. Cation exchange capacity was calculated as sum of charge equivalents of exchangeable  $K^+$ ,  $Na^+$ ,  $Ca^{++}$ ,  $Mg^{++}$ ,  $Fe^{++}$ ,  $Zn^{++}$  and  $Mn^{++}$  as determined in 5 g soil in 100 ml sterile bi-dist. water by flame atomic absorption spectrophotometer (Perkin Elmer analyst 400 model). Total elemental contents were measured in dilute  $HNO_3$  solutions [27, 28].

### 2. Isolation and identification of soil fungi

Filamentous fungi were isolated by dilution plate method [29] on 1% glucose-Czapek's agar medium ( $NaNO_3$ , 2;  $KH_2PO_4$ , 1;  $MgSO_4 \cdot 7H_2O$ , 0.5;  $KCl$ , 0.5; glucose, 10; agar-agar, 18g; per 1 liter) at  $28 \pm 1^\circ C$ . Isolated, predominant, morphologically distinct colonies were selected, purified by repeated culturing and maintained on glucose-Czapek's agar and 5% NaCl glucose-Czapek's agar slants at  $4^\circ C$ . The isolates were identified based on their colony characteristics and microscopic observations including hyphae and spores morphology [30-39].

## RESULTS

### Soil analyses

The Red Sea soils were subjected to some chemical analyses including: The moisture content (M. C.%) was high in content and estimated 10.3- 24.7 % (mean = 16.28 % M.C.) of which 29 and 11 samples contained  $\geq 20\%$  and  $\leq 20\%$  M.C., respectively. The organic matter (O.M. %) was estimated by 0.002-1.22 % (mean = 0.27 % O.M.), where 37 and 3 samples had  $\geq 1\%$  and 1.02- 10.22% O.M., respectively. The pH value was high and fluctuated between pH 8.15 - 10.45 (mean = 9.62) with regarding 6, 19 and 15 samples had pH 8-9, 9-10 and  $\leq 10$ , respectively. The total dissolving salts ranged between 0.04-2.40 mg/g soil (mean = 0.99) where 3, 7, 14 and 16 samples had 0.025-0.050, 0.050 - 0.100, 0.100 -1.00 and  $\leq 1.00$  mg/g, respectively. The element contents of the soils using the water extracts (di-ionized water) were carried out for estimating the concentrations of mono- ( $Na^+$  and  $K^+$ ) and bi-equivalents ( $Ca^{++}$ ,  $Mg^{++}$ ,  $Fe^{++}$ ,  $Mn^{++}$  and  $Zn^{++}$ ) ions.

Of mono-equivalent ions, sodium ions ( $Na^+$ ) had the highest counts amongst the cations of the soil tested and ranged between 28,000-3990,000 (mean= 1163050)  $\mu g/g \times 10^{-3}$ . Whereas, potassium ions ( $K^+$ ) had the second mono-equivalent cation, in general,  $K^+$  ions were very low compared with  $Na^+$  and varied between 12.4 - 500 (mean = 59.17)  $\mu g/g \times 10^{-3}$ . Of bi-equivalent ions, five bi-equivalent ions were calculated in the soil extracts of the soils under investigation. The cations (ions) were classified into macro-elements ( $Ca^{++}$  and  $Mg^{++}$ ) and micro-elements ( $Fe^{++}$ ,  $Mn^{++}$  and  $Zn^{++}$ ) based on their utilization by the plants. The two macro-elements were calcium ( $Ca^{++}$ ) and magnesium ( $Mg^{++}$ ), where  $Ca^{++}$  had the higher counts in the soils and fluctuated between 7.5 - 2760 (mean = 519.54)  $\mu g/g \times 10^{-3}$ . Whereas,  $Mg^{++}$  ions were very less in counts compared with  $Ca^{++}$  and ranged between 4.6-390 (mean = 63.61)  $\mu g/g \times 10^{-3}$ . The three micro-elements (trace elements) ions ( $Fe^{++}$ ,  $Mn^{++}$  and  $Zn^{++}$ ) were subjected for quantities of analyses.  $Fe^{++}$  had the best counts and ranged between 0.110-1.163 (mean = 0.264)  $\mu g/g \times 10^{-3}$ . Manganese ( $Mn^{++}$ ) and Zinc ( $Zn^{++}$ ) contents in soil were very low compared with iron ( $Fe^{++}$ ) ions.  $Zn^{++}$  was detected in all samples varied between 0.015-0.230 (mean = 0.039)  $\mu g/g \times 10^{-3}$  in bi-ionized dist.  $H_2O$  extracts of the soils. Whereas,  $Mn^{++}$  had the lowest in quantity and estimated by 0-1.287 (mean = 0.072)  $\mu g/g \times 10^{-3}$  with completely disappeared in 9 samples of the tested soils (Table 1).

**Table 1: Collection of saline soils (40 samples) from the rhizosphere of dominant plants in Upper Egypt and their moisture contents (M.C. %) with some chemical analyses of soil including organic matter contents (O.M%), pH values ( pH ), total dissolved salts (T.D.S mg/g) and some ions of elements  $\mu\text{g/g} \times 10^{-3}$  including mono-equivalent ( $\text{Na}^+$  and  $\text{K}^+$ ) and bi- equivalent ( $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ ,  $\text{Fe}^{++}$ ,  $\text{Zn}^{++}$  and  $\text{Mn}^{++}$ )**

Sample No.	Place of collection	Latin name of the plant	M.C. %	O.M %	pH	T.D.S mg/g	Element $\mu\text{g/g} \times 10^{-3}$						
							$\text{Na}^+$	$\text{K}^+$	$\text{Ca}^{++}$	$\text{Mg}^{++}$	$\text{Fe}^{++}$	$\text{Mn}^{++}$	$\text{Zn}^{++}$
1	South of Quseir	<i>Avicennia marina</i>	24.7	0.034	8.82	0.069	28000	25.6	23.8	32	0.165	0.009	0.021
2	South of Quseir	<i>Avicennia marina</i>	23.04	0.024	8.15	0.040	70000	34.8	26.4	50.2	0.138	0.007	0.015
3	South of Quseir	<i>Avicennia marina</i>	20.5	0.026	9.56	0.660	36000	60.5	22.6	70.5	0.172	0	0.042
4	South of Quseir	<i>Avicennia marina</i>	20.5	0.014	9.25	0.455	2830000	79.7	86.1	117	0.203	0.009	0.034
5	Quseir	<i>Avicennia marina</i>	16.5	0.006	9.85	0.129	1060000	12.4	10.5	8.7	0.110	0.003	0.019
6	Wadi-El-Gemal	<i>Avicennia marina</i>	20.1	0.030	9.77	0.822	82000	20.4	11.4	9.5	0.220	0	0.23
7	Wadi-El-Gemal	<i>Avicennia marina</i>	20.7	0.062	9.68	0.505	1100000	18.4	30.3	28.4	0.162	0.004	0.016
8	Marsa Alaem	<i>Avicennia marina</i>	20.03	0.024	9.57	0.990	920000	22	14.5	39.1	0.246	0.004	0.022
9	Quseir	<i>Tamarix nilotica</i>	22.6	0.020	9.05	2.00	520000	22.6	17.5	32.3	0.16	0	0.041
10	Marsa Alaem	<i>Zygophyllu m album</i>	10.74	0.052	10.01	1.75	610000	30.4	18.5	61.6	0.160	0.013	0.029
11	Wadi-El-Gemal	<i>Imperata cylindrical</i>	19.3	0.002	9.00	0.026	870000	16.2	29.1	18.8	0.167	0.003	0.023
12	Wadi-El-Gemal	<i>Artemisia herba-alba</i>	11.7	0.082	8.99	0.009	640000	16	7.5	4.6	1.163	0.032	0.027
13	Hurghada	<i>Tamarix nilotica</i>	16.3	0.012	8.36	1.15	2460000	83.3	1980	40	0.256	0.191	0.057
14	Hurghada	<i>Nitraria billardierei</i>	16.9	0.038	10.07	1.22	610000	490	2760	160	0.268	0.720	0.056
15	Hurghada	<i>Zygophyllu m album</i>	12.2	0.002	10.30	1.00	3990000	65.3	11.9	68	0.673	0.007	0.033
16	Marsa Alaem	<i>Tamarix nilotica</i>	16.4	0.020	10.13	2.15	990000	500	646	390	0.519	1.287	0.092
17	Marsa Alaem	<i>Zygophyllu m album</i>	12.1	0.020	9.80	1.31	720000	86.5	2475	80.5	0.260	0.025	0.036
18	Wadi-El-Gemal	<i>Phragmites australis</i>	11.7	0.020	10.38	1.77	1100000	41	146.1	88	0.221	0.002	0.025
19	Marsa Alaem	<i>Tamarix nilotica</i>	10.67	0.024	10.39	0.07	2940000	50.2	185.7	101.8	0.172	0.005	0.026
20	Wadi-El-Gemal	<i>Phragmites australis</i>	19.27	0.012	10.14	1.95	930000	40.5	154	89.5	0.180	0.004	0.035
21	Wadi-El-Gemal	<i>Zygophyllu m album</i>	16.87	0.010	10.15	2.40	2080000	48.8	2750	20	0.297	0.039	0.044
22	Wadi-El-Gemal	<i>Imperata cylindrical</i>	12.65	0.070	10.45	1.10	1530000	21	373.5	41.6	0.185	0.038	0.031
23	Wadi-El-Gemal	<i>Artemisia herba-alba</i>	10.55	0.018	10.36	1.00	1460000	30.2	82.7	40.3	0.824	0.013	0.035
24	Wadi-El-Gemal	<i>Artemisia herba-alba</i>	10.30	0.028	10.30	1.36	850000	34	171.5	70.2	0.249	0.004	0.025
25	South of Hurghada	<i>Nitraria billardierei</i>	13.59	0.16	9.02	0.885	1460000	21.7	237.5	49.8	0.178	0	0.025
26	South of Hurghada	<i>Nitraria billardierei</i>	11.32	0.24	9.78	0.077	710000	49.4	199	63.5	0.421	0.007	0.028
27	South of Hurghada	<i>Nitraria billardierei</i>	14.33	0.54	9.83	0.069	1700000	23	2520	24.4	0.205	0.001	0.034
28	South of Hurghada	<i>Tamarix nilotica</i>	11.04	0.40	10.14	0.050	3570000	22.6	276	49.8	0.214	0	0.028
29	South of Hurghada	<i>Zygophyllu m album</i>	12.19	0.44	8.76	0.775	1770000	27.5	556	31.4	0.191	0.002	0.042
30	South of Safaga	<i>Avicennia marina</i>	20.18	0.98	9.82	4.00	1360000	18	237	28.2	0.204	0.003	0.026
31	South of Safaga	<i>Nitraria billardierei</i>	11.65	0.58	10.40	1.026	1620000	27.7	40.9	32.1	0.185	0	0.020
32	South of Safaga	<i>Avicennia marina</i>	18.01	0.36	10.13	0.665	890000	21.2	30.6	30	0.148	0.007	0.046
33	South of Safaga	<i>Avicennia marina</i>	18.40	0.38	9.08	1.280	599000	22	18.9	19.8	0.265	0.004	0.055

34	South of Safaga	<i>Zygophyllum album</i>	13.5	0.58	10.38	0.954	466000	20.1	2760	35	0.207	0.003	0.032
35	South of Safaga	<i>Phragmites australis</i>	18.59	0.40	9.00	0.108	2390000	60.5	91.1	66	0.219	0	0.024
36	South of Quseir	<i>Tamarix nilotica</i>	11.85	1.02	9.09	1.00	542000	40.3	260.3	110	0.186	0.386	0.042
37	South of Quseir	<i>Avicennia marina</i>	23.5	1.02	9.31	2.20	689000	34.5	199.6	89	0.166	0.032	0.028
38	South of Quseir	<i>Phragmites australis</i>	21.87	1.22	9.07	1.63	90000	48	182.5	101.2	0.250	0.013	0.037
39	South of Quseir	<i>Avicennia marina</i>	18.36	0.92	9.70	0.974	120000	30.2	532	90.3	0.138	0	0.048
40	South of Quseir	<i>Zygophyllum album</i>	16.51	0.98	8.80	0.094	80000	50.1	605.5	61.2	0.194	0	0.030
Average (mean)			10.3-24.7 (16.28)	0.002-1.22 (0.271)	8.15-10.45 (9.62)	0.04-2.40 (0.99)	28000-399000 (1163050)	12.4-500 (16.933)	7.5-2760 (144.985)	4.6-390 (10.595)	0.110-1.163 (0.512)	0-1.287 (0.076)	0.015-0.230 (0.034)

### Mycoflora of saline soils

Forty samples of the soils were examined for isolation of filamentous fungi on 1% glucose-Czapek's agar at 28°C and identification to species level on 1% glucose- and/or 1% glucose-Czapek's agar supplemented by 5% NaCl. The gross fungal count was fluctuated between zero to 45 colonies/mg dry soils (7 samples were free from filamentous fungi) totally, 28.64 colonies in the soils tested (Table 2).

A total of 42 fungal species + 3 varieties of 11 genera were identified. *Aspergillus* was quite the most dominant based on frequency (77.5 % of the samples) and less in count (32.12 % of gross count). The genus was represented by 15 species + 2 varieties, of which *A. niger* and *A. fumigatus* were superior in counts (collectively, 23.9 % of total aspergilli and 7.68% of gross count). The *Aspergillus* species, *A. flavus*, *A. versicolor*, *A. carbonarius*, *A. terreus*, *A. ambiguus* and *A. ficuum* were low in frequencies (12.5 – 22.5% of the samples) and variable counts with promotion of *A. versicolor* (23.9% and 7.68%, respectively). Whereas, other *Aspergillus* species were identified in low frequencies (2.5 - 10% of the sample) with collectively count, 15.65% of aspergilli and 5.03% of gross fungi.

*Penicillium* (13 species) was isolated and identified from saline soils tested (47.5% of the samples) represented 54.05 % of gross count, *P. funiculosum* was the superior of *Penicillium* count (86.56 % of penicillia and 46.79% of gross fungal count) with moderate frequency (30% of the samples). Whereas, two *Penicillium* (*P. duclauxii* and *P. variable*) were observed in low counts (collectively, 6.98 % of penicillia

and 3.77% of gross fungal counts) and low in frequencies (each, 15 % of the samples). The remaining *Penicillium* species were rare in frequencies (2.5 – 10% of the samples) with variable counts (collectively, 6.46% of penicillia and 3.49% of gross fungal count).

*Acremonium* (*A. strictum*) had the third place based on frequency and count (25% of the samples and 4.61% of gross count). Whereas, *Emericella* occupied the fourth place according to the occurrence (15 % of the samples) and relatively low count (3.35% of gross counts). Of the genus 2 species + 1 variety were isolated in rare frequencies and low counts (2.5 – 10% of the samples and 16.67 – 58.33% of total *Emericella*).

Regarding of the rare frequency of occurrence ( $\geq 10$  % of the samples) of saline soil, 9 species of 7 genera were listed. Of the previous genera, 2 and 5 genera were represented by 2 and 1 species, respectively collectively accounting 5.03% of gross fungal count. *Trichoderma* had the best count (1.68 % of gross count). The Dematiaceous Hyphomycetes e.g *Torula*, *Stachybotrys* and *Cladosporium* (each, 1 species) in addition to *Eurotium* (2 species) as ascospore-forming fungi was rare in frequencies and counts. Sterile mycelia (white) was also rare in frequencies and counts (5% of the samples and 0.56 % of gross fungal count) as shown in Table 2.

It is worthy to mention, six samples of saline soils had the highest organic matter contents (0.98 – 1.22 mg/g soil) with lowering of pH values (pH: 8.8 – 9.82) and flourishing the mycoflora (0.24 – 1.04 colonies/mg dry soils).

**Table 2: Total count (TC) of fungal genera and species isolated from saline soils (40 samples), number of cases of isolation (NCI) and occurrence remark (OR) on 1% glucose-Czapek's agar at 28 ± 1°C**

Type of soil Genera & species	Saline (sandy) soil	
	TC	NCI & OR
<i>Aspergillus</i>	<b>9.20</b>	<b>31 H</b>
<i>A. niger</i> Van Tieghem	1.12	14 M
<i>A. fumigatus</i> Fresenius	1.08	10 M

<i>A. flavus</i> Link	0.64	9 L
<i>A. versicolor</i> (Vuillemin) Tiraboschi	2.12	9 L
<i>A. carbonarius</i> (Bainier) Thom	0.96	7 L
<i>A. terreus</i> Thom	0.76	7 L
<i>A. ambiguus</i> Sappa	0.76	5 L
<i>A. ficuum</i> (Reich.) Hennings	0.32	5 L
<i>A. terreus</i> var. <i>africanus</i> Fennell & Raper	0.36	4 R
<i>A. sydowii</i> (Bainier & Sartory) Thom & Church	0.20	3 R
<i>A. tamaritii</i> Kita	0.28	3 R
<i>A. clavatus</i> Desmazieres	0.16	2 R
<i>A. aegyptiacus</i> Moubasher & Moustafa	0.04	1 R
<i>A. candidus</i> Link	0.12	1 R
<i>A. ochraceus</i> Wilhelm	0.04	1 R
<i>A. terreus</i> var. <i>aureus</i> Thom & Raper	0.16	1 R
<i>A. ustus</i> (Bainier) Thom & Church	0.08	1 R
<b>Penicillium</b>	<b>15.48</b>	<b>19 M</b>
<i>P. funiculosum</i> Thom	13.40	12 M
<i>P. duclauxii</i> Delacroix	0.44	6 L
<i>P. variabile</i> Sopp	0.64	6 L
<i>P. citrinum</i> Thom	0.16	4 R
<i>P. lanosum</i> Westling	0.32	3 R
<i>P. steckii</i> Zaleski	0.12	2 R
<i>P. javanicum</i> Van Beyma	0.04	1 R
<i>P. lilacinum</i> Thom	0.16	1 R
<i>P. miczynskii</i> K.M. Zalessky	0.04	1 R
<i>P. piscarium</i> Westling	0.04	1 R
<i>P. resticulosum</i> Birkinshaw, Raistrick & G. Smith	0.04	1 R
<i>P. simplicissimum</i> (Oudem.) Thom	0.04	1 R
<i>P. verruculosum</i> Peyronel	0.04	1 R
<b>Acremonium strictum</b> W. Gams	<b>1.32</b>	<b>10 M</b>
<b>Emmericella</b>	<b>0.96</b>	<b>6 L</b>
<i>E. varicolor</i> Thom & Raper	0.56	4 R
<i>E. nidulans</i> Eidam	0.24	1 R
<i>E. nidulans</i> var. <i>lata</i> Thom & Raper	0.16	1 R
<b>Trichoderma</b>	<b>0.48</b>	<b>4 R</b>
<i>T. hamatum</i> (Bonorden) Bainier	0.24	2 R
<i>T. koningii</i> Oudemans	0.24	2 R
<b>Stachybotrys albipes</b> Berkeley & Broome	<b>0.20</b>	<b>3 R</b>
<b>Torula fici</b> Crous	<b>0.32</b>	<b>3 R</b>
<b>Circinella umbellata</b> Van Tieghem & La Monnier	<b>0.08</b>	<b>2 R</b>
<b>Cladosporium herbarum</b> (Persoon) Link	<b>0.04</b>	<b>1 R</b>
<b>Eurotium</b>	<b>0.28</b>	<b>1 R</b>
<i>E. amstelodami</i> Thom & Church	0.20	1 R
<i>E. chevalieri</i> Mangin	0.08	1 R
<b>Scopulariopsis brevicaulis</b> (Saccardo) Bainier	<b>0.04</b>	<b>1 R</b>
<b>Sterile mycelia</b> (S.m. white)	<b>0.16</b>	<b>2 R</b>
<b>Gross total count</b>	<b>28.64</b>	
<b>No. of genera and species</b>	<b>42 sp. + 3 var. of 11 genera</b>	
<b>Percentage of infected samples</b>	<b>82.5 %</b>	

**Occurrence Remark (OR):**

H: High occurrence, 20-40 samples (50-100% of the samples).

M: Moderated occurrence, 10-19 samples (25-47.5% of the samples).

L: Low occurrence, 5-9 samples (12.5-22.5% of the samples).

R: Rare occurrence, 1-4 samples (2.5-10% of the samples).

**DISCUSSION**

Fungi are very successful inhabitants of soils, due to their high plasticity and their capacity to adopt various forms in response to adverse or un-favorable conditions [40]. Due to their ability to produce a wide variety of extracellular enzymes, ability to break down all kinds of organic matter, decomposing soil components and by their regulating the balance of carbon and different nutrients [41]. Fungi convert dead organic



matter into biomass, organic plus amino acids and carbon dioxide (Frac, 2018). The diversity and activity of fungi is regulated by various biotic (plant and other organisms) and abiotic (soil pH, moisture, salinity, structure and temperature) factors [42, 43]. Fungi can be found in almost every environments and can live in wide range of abiotic factors [44], therefore the present study was designed to throw light on soil analyses, and mycoflora of saline soils (Red Sea Coast) in Upper Egypt.

### 1. Soil analyses

Regarding the results obtained, the saline soils were sandy in texture, moderate to high moisture contents (tidal waves of Red Sea), alkaline pH and moderate concentration of total dissolving salts with very low organic matter contents. The elements in the soil samples had relatively high concentrations of macro-elements ( $K^+$ ,  $Ca^{++}$  &  $Mg^{++}$ ) in addition to  $Fe^{++}$  with low contents of  $Mn^{++}$  and  $Zn^{++}$  as micro-elements whereas,  $Na^+$  as stress element was very high in most samples tested. Based on soil analyses with correlation the micro-organisms (bacteria and fungi) of the previous literatures in this aspect, soil health and the closely related terms of soil quality and fertility, is considered as one of the most important characteristics of soil ecosystems. The integrated approach to soil health assumes that soil is a living system and soil health results from the interaction between different processes and properties, with a strong effect on the activity of soil microbiota [45]. The colonization of land by plants appears to have coincided with the appearance of mycorrhiza- like fungi. Over evolutionary time, fungi have maintained their prominent rate in the formation of mycorrhizal associations. In addition, however they have been able to occupy other terrestrial niches of which the decomposition of recalcitrant organic matter is perhaps the most remarkable [46]. Plant roots exude substantial amounts of low molecular weight organic compounds such as amino acids, sugars and organic acids, resulting in increased microbial population and activity [47-50]. During the evolution of terrestrial microbial life, fungi become the major decomposers of recalcitrant organic matter. Bacteria on the other hand have been able to maintain a significant role in the degradation of simple substances [46].

The soils had sharply alkaline pH values (8.15 – 10.45), where 15/40 sample (37.5%) had pH = 10 or more. The variation in soil pH is related to parent material, rainfall, topography and organic matter content of the soil [51]. In this respect, The correct pH is crucial for the healthy plant growth and its effects on the amount of nutrient available [52]. Also, soil pH is considered as one of the most essential factors influencing plant up take of trace elements [53]. Based on the results obtained concerning total dissolving salts (TDS) and sodium ions ( $Na^+$ ) concentrations in the soils tested, the soil had the high concentration of TDS (mean= 0.99 mg dry soil) as well as  $Na^+$  concentration (mean= 1162050  $\mu g/g \times 10^{-3}$ ). According to the previous studies concerning the salinity

and saline-alkaline soils, soil salinity is one of the key factors that threatens plant existence worldwide and is a major challenge to sustain crop production and soil quality. It limited research on there's pones of microbial communities and enzyme activities under soil amendments application of saline-alkaline soils [54]. Agriculture land derived from saline-alkaline soils will not have high plant growth and productivity unless they are ameliorated by using the appropriate agronomic and amendments practices [55]. Soil enzyme activity play a key role in nutrients recycling making them accessible to plants and micro-organisms. [56] Soil micro-organisms are considered to be one of the vital factors for evaluating soil quality and the application of soil amendments increasing the enzyme activity, which leads to a higher yield [57-59]. Also, regulations of the microbial community, composition and function involve a pH-dependent mechanism [56]. Concerning the available micro-elements contents, three elements ( $Fe^{++}$ ,  $Mn^{++}$  &  $Zn^{++}$ ) were estimated, where the iron ions have the best counts in the soils tested. In this respect, iron (Fe) is one of the most studied and important element in mineral nutrition of the plants [60].

### 2. Soil mycoflora

The research has been carried out to add new information on the biodiversity of salt-adapted filamentous fungi. Data obtained concerning abiotic factors persuasively proved that this type of soil suffering from several problems including low organic matter and low water activity ( $w_a$ ) with high salinity and high alkaline of soil (saline-alkaline soils) as extremophile habitat. These extremophiles are ubiquitously present in natural ecological niches such as salt lakes, inland seas, evaporation ponds of seawater, saltersn, glaciers, the arctic and ant arctic regions ... ect [61].

The extremophiles are organisms than can thrive under extreme environmental conditions. There are many types of extremophiles, which require different growth conditions and habitats to grow, among them are halophilic and halotolerant microbes. These microbes are reported to grow in habitats of high salinity regions including the sea, sediments, lakes mines, plant and soil. They need high carbon source and salt concentration to achieve maximum tolerable condition for their survival. High salinity survival and tolerance of these microbes are mechanized due to their osmotic and ionic stress which are regulated through their genetic expression of enzymes, proteins, cell wall compositions and transporters [62].Also, extremophilic microbes survive under extreme ecosystems such as hot, cold, salty, sandy, highly acidic or alkaline habitat, due to their physiological and metabolic activities [63]. In the same respect, the microbes that inhabit high-salt environments are adapted to high levels of ions as well as to low water activity ( $W_a$ ) and as described as halophilic or halotolerant. Halophiles are basically salt-loving organisms that inhabit hypersaline environments [64].

Also, Oren [64] reported that microbial life at high salt concentration are phylogenetic and metabolic diversity. An operative definition of halophilic fungi according to their relationship to salt, halophilic fungi are defined as those fungi that can cultured *in vitro* at a salt concentration of 3M and can be isolated from natural habitats of salinity above 1.7 M [65]. More, previously used the term (halophilic) for all organisms that require some level of salt for growth including concentration around 35 g/L (3.5% salts) as found in sea water [63].

A total of 42 species in addition to 3 varieties of 11 genera were isolated and identified from Red Sea Coast in Upper Egypt (~ 600km Northern Orbit of Cancer) on isolated medium, 1% glucose -Czapek's agar. Based on the previous studies, the solar salterns, dead sea, arid desert, sobka soil and terrestrial habitats of mud had been explored for their biodiversity studies [37, 66-68]. *Aspergillus* (15 sp. + 2 var.) and *Penicillium* (13 sp.) were the superior in number of isolated species and frequencies (77.5% and 47.5% of the samples, respectively). The two genera were collectively counted 86.42 % of gross fungal counts. Recently (2017-2021), halophilic fungi around the world from different habitats proved that *Aspergillus* (~8 species) was the superior genus [69-73] followed by *Penicillium* (2 species) occupied the second place [72].

*Acremonium* (*A. strictum*) and *Emericella* (*E. varicolor*, *E. nidulans* and *E. nidulans* var. *Lata*) were detected in moderate and low frequencies (25% and 15% of the samples, collectively, 4.67% of gross count). In this respect, the two genera (secondary metabolites producers e.g. antibiotics and mycotoxins, respectively) were detected in moderate or low frequencies from Egyptian desert [66, 74] and cultivated [37, 75] soils. Also, *Acremonium* was widely detected from tropical and sub-tropical regions including saline soils. Whereas, *Emericella* (ascospore forming fungi) was more dominant in tropical regions of the world [37, 74].

The remaining fungal genera and their species (9 sp. of 7 gen.) were rare in frequencies ( $\geq 10\%$  of the samples) with very low in counts (5.02% of gross count). Based on the previous literature, concerning halophilic fungi of the world (2017-2020). *Aspergillus glaucus* "*Eurotium herbariorum*" group, *A. amstelodami* and *A. chevalieri* in addition to some species of *A. glaucus* (under the species level) were listed [70, 76, 77], *Scopulariopsis* species and *Cladosporium* species [69]. Finally, some halophilic fungi (e.g. *Gymnoascus halophilus*, *Magnusella marinae*, *Microascus trigonasporus*, *Paraneria triangularis* and *Yarrowia lipolytica*) were isolated from different sources [69, 72, 78-81] were not detected in this study.

The organic matter content plays an important role in flourishing the soil mycoflora with lowering in pH value of the soil [74, 75].

## CONCLUSION

Of extremophilic soils (Red Sea Coast), *Aspergillus* and *Penicillium* were the superior in frequencies and counts, whereas other filamentous fungi including ascospore forming, protecting, Hyphomyces and Mucorales were limited except *Acremonium strictum*. Also, organic matter content is available factor in flourishing of mycoflora with depression of pH values of saline soils.

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