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EFFECT OF SOIL SALINITY ON DECOMPOSITION AND HUMIFICATION OF ORGANIC MATTER BY SOME CELLULOLYTIC FUNGI

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SUMMARY

The effects of artificially salinized soil on the activities of five cellulolytic fungi, namely *Alternaria alternata*, *Aspergillus terreus*, *Chaetomium globosum*, *Curvularia lunata* and *Drechslera australiensis*, have been studied under sterilized-soil conditions. The results were also compared with unsterilized treatment. The results reported here indicate that increasing salinity has an inhibitory effect on the microbial activity of soil as reflected by CO_2 evolution, cellulase activity and humification of plant residues. However among the fungi tested *A. terreus* and *C. globosum* produced maximum humification whereas the latter was most tolerant to soil salinity and also had maximum cellulase activity.

Soil salinity and sodicity are the two major problems of agriculture in Pakistan. Many methods including both chemical and organic matter amendments have been recommended for their reclamation (2, 5, 9, 18, 22, 24). In view of the prevalent socioeconomic conditions in Pakistan, Sandhu and Malik (21) proposed a plant-succession scheme, whereby an ecological cycle, starting with a salt-tolerant grass and ending up with an economic crop, is established. For this scheme, a salt-tolerant grass, *Diplachne fusca* (L.) P. Beauv., is used as the primary colonizer which is followed by a relatively less salt-tolerant legume, *Sesbania aculeata* (Wild.) Pers. Green manuring of both these plants and their subsequent decomposition allows release of CO_2 which helps in the solubilization of $CaCO_3$ already present in our soils (9, 10, 12, 18).

Advantages of a reclamation procedure as outlined above are that, in addition to the release of CO_2, the stable organic-matter fractions so vital for the fertility of the soil may be increased and soil structure improved. It is therefore important to study the rates of decomposition of added organic matter and the fungi involved in its mineralization and humification. Previously various fungi involved in the decomposition of organic residues in saline soils had been isolated (11, 12, 13). The object of the present investigation is to study the role played by

individual cellulolytic fungi in the decomposition and humification of plant residues of *S. aculeata* in artificially salinized soils.

MATERIALS AND METHODS

The soil used for this study was a sandy-clay loam collected from the Institute campus that previously had been sown to wheat. Its composition was 58% sand, 26% silt and 16% clay; organic carbon was 0.6% and nitrogen was 0.1%. This soil was artificially salinized by adding a mixture of Na_2SO_4, $CaCl_2$, $MgCl_2$, and NaCl in a ratio of 10:5:1:4 in quantities sufficient to obtain salinity levels of electrical conductivity (SM^{-1}) 0.4, 1.0, 2.0, and 3.0. The salinized soils were given 2 wk to equilibrate after which their electrical conductivity was again measured. For incubation studies the soil was prepared by passing it through a 2-mm sieve. Portions of 100 g of the soil were placed in 250-ml flasks, mixed thoroughly with 2% powdered plant material of *Sesbania aculeata* and brought to 60% water-holding capacity (WHC). This plant material had 43.0% C and 3.8% N. The flasks were closed with rubber stoppers fitted with a glass rod having a small cup at its end. These cups contained 5 ml of 0.5 N NaOH. For sterilized treatments, the flasks containing soil and plant mixture were autoclaved twice for 1 h at intervals of 24 h at 15 psi and 120 C.

Five fungi—*Chaetomium globosum* Kunze ex Fr., *Curvularia lunata* (Wakkar) Boedijn, *Drechslera australiensis* (Bugnicourt) Subram. & Jain, *Alternaria alternata* (Fr.) Keissler, *Aspergillus terreus* Thom—which had been isolated previously from saline soils, were selected for this study. The fungal inoculum was prepared by growing the fungi in Petri dishes containing 20 g acid-washed sand which was moistened with 10 ml of Eggins and Pugh's cellulose medium having following composition: KH_2PO_4, 1.0 g; $(NH_4)_2SO_4$, 0.5 g; KCl, 0.5 g; yeast extract, 0.5 g; $CaCl_2$, 0.1 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; cellulose, 10 g and 1 liter distilled water. Fungi were allowed to grow for 7 da on this sand medium at 30 C. At the end of the incubation period, the sand culture was mixed and 2 g were added to the sterilized soil and mixed well. Duplicate flasks were kept for each fungus and salinity level. Similar flasks containing fertile and nonsterilized soil and amended with 2% *S. aculeata* were kept as controls. All the flasks were incubated at 30 C.

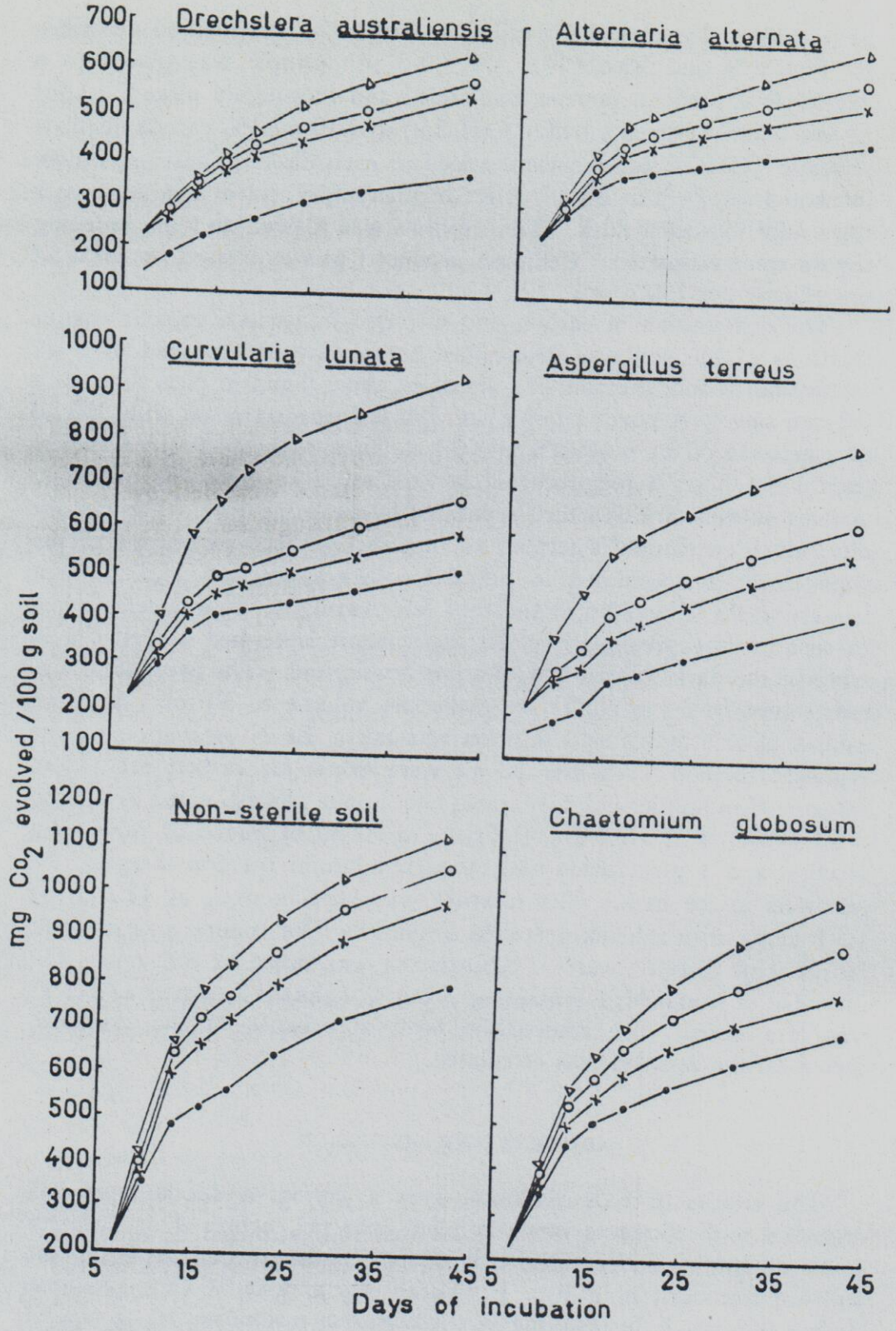
The evolution of CO_2 was estimated titrimetrically after different periods of incubation. After every estimation, fresh 0.5 N NaOH was added to the cups. At different intervals soil samples were taken

aseptically and analyzed for cellulase activity by the method described by Pancholy and Rice (17). A 5.0-g soil sample was placed in a 250-ml flask; 0.5 ml toluene was added and thoroughly mixed. After 15 min 10 ml of acetate buffer (*p*H 5.9) and 10 ml 1% carboxymethylcellulose (CMC) solution were added to each flask. These were then incubated for 24 h at 30 c. After incubation, 50 ml of distilled water were added to each flask. The mixture was filtered and the reducing sugars were estimated. Cellulase produced was expressed in terms of mg glucose per 100 g soil.

The distribution of carbon and nitrogen in various organic matter fractions of the soil was determined as follows: At the end of 6-wk incubation period, the soil was air dried and ground to pass through a 0.2-mm sieve. A portion (20 g) of this soil was extracted with 200 ml of a mixture of 0.1 N NaOH and 0.1 M Na pyrophosphate in a conical flask for 1 h on a reciprocal shaker. The flasks were left overnight and the supernatant was then separated by centrifugation. The residue after alkali extraction is termed as "humin." A 100-ml portion of the supernatant was acidified to *p*H 2.0 with concentrated H_2SO_4, kept in oven at 90 C for 30 min and then left overnight. It was centrifuged to separate the precipitate. The supernatant is termed as fulvic acid whereas the dark-colored precipitate is humic acid. The precipitate was redissolved in 0.1 N NaOH to make the volume to 50 ml. A 20-ml sample of this humic-acid solution was taken for N estimation by the Kjeldahl method. Another 20 ml were taken for carbon estimation. Organic C in humic acid, fulvic acid and humin was estimated by adding 8 ml H_2SO_4 and 5 ml 2 N $K_2Cr_2O_7$ to 20 ml of humic or fulvic-acid fraction and 1 g air-dried and powdered humin fraction, keeping the reactions in ice bath. The mixture was kept in oven at 110 C for 1.5 h along with a blank prepared similarly. The volume was made to 50 ml with distilled water. Absorbance was noted at 590 nm. Absorbance of a standard containing 5 mg C (glucose) treated as before was also noted. By comparison with absorbance of the standard, carbon in the samples was calculated.

RESULTS AND DISCUSSION

The results of CO_2 evolution over a period of 42 da from soil amended with *Sesbania aculeata* plant material, salinized at different levels of salinity and inoculated with various fungi after sterilizing the soil are summarized in FIG. 1. There was a flush of CO_2 evolution during the first 7 da resulting in the complete neutralization of NaOH


Drechslera australiensis
Alternaria alternata
Curvularia lunata
Aspergillus terreus
Non-sterile soil
Chaetomium globosum
mg Co2 evolved /100 g soil
Days of incubation
700
600
500
400
300
200
100
1000
900
800
700
600
500
400
300
200
100
1200
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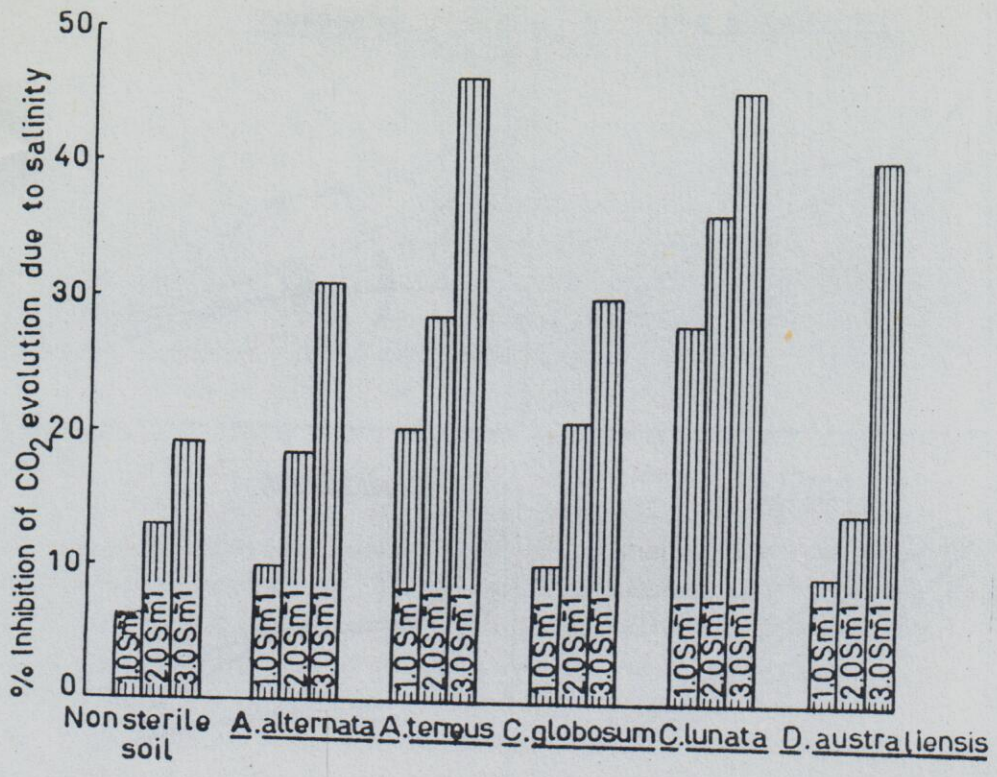

FIG. 2. Percentage reduction in CO_2 evolution by various fungi due to salinity.

in the cups. However the rate of CO_2 evolution decreased with increase of salinity in all the treatments. Maximum CO_2 was evolved in the case of nonsterile soil amended with plant material. Among the sterilized soils inoculated with various fungi, maximum CO_2 evolved with *Chaetomium globosum*. Next in the decreasing order were *Curvularia lunata, Aspergillus terreus, Alternaria alternata* and *Drechslera australiensis*. Percentage inhibition of CO_2 evolution due to increasing salinity levels as compared to the fertile soil (0.4 Sm^{-1}) was calculated and is summarized in FIG. 2. Minimum inhibition of CO_2 evolution was observed in the case of nonsterile soil. Among the sterilized soils, those containing *Alternaria alternata* and *Chaetomium globosum* had a relatively low percentage inhibition of CO_2 evolution ranging from 10–11% at 1.0 Sm^{-1}; 18–20% at 2.0 Sm^{-1} and 30% at 3.0 Sm^{-1}; with *Drechslera australiensis* lower inhibition equivalent to 9.6% at 1.0 Sm^{-1} and 14.4% at 2.20 Sm^{-1} was observed but at 3.0 Sm^{-1} the percentage inhibition

FIG. 1. Evolution of CO_2 from nonsterile soil and sterilized soil inoculated with various fungi at different soil-salinity levels and amended with 2% material of *Sesbania aculeata*. Legend for FIGS. 1 and 3: —△— EC 0.4 Sm^{-1}; —○— EC 1.0 Sm^{-1}; —X— ES 2.0 Sm^{-1}; —●— EC 3.0 Sm^{-1}.

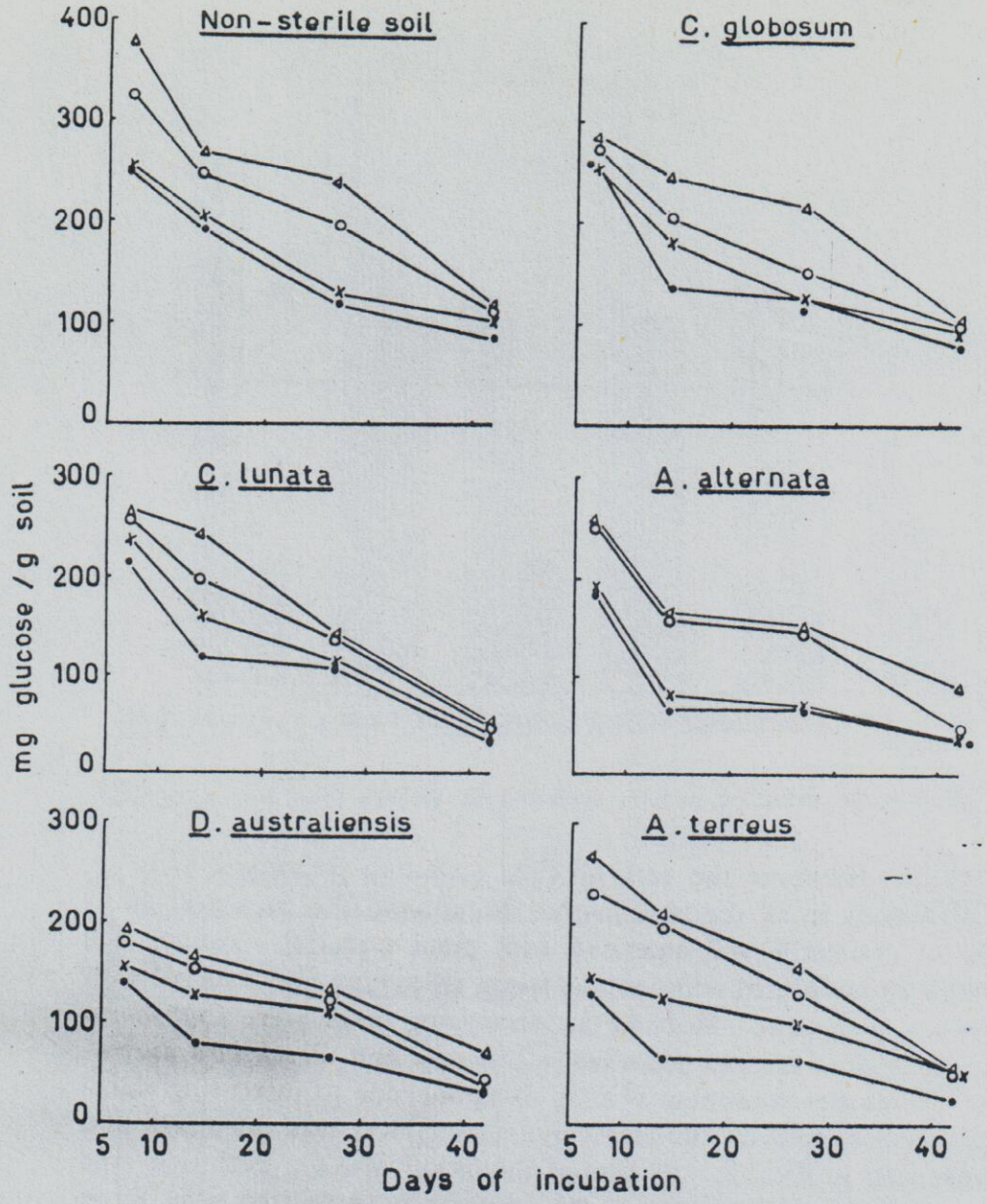

FIG. 3. Cellulase activity in terms of mg glucose/g soil from nonsterile soil and sterilized soil inoculated with various fungi at different salinity levels and amended with 2% material of *Sesbania aculeata*.

increased to 42.0%. *Aspergillus terreus* and *Curvularia lunata* had higher inhibition percentage at each of the three salinity levels; *C. lunata* showing the maximum inhibition of CO_2 evolution with the increase in salinity. If the salt tolerance of the tested fungi is compared with rates of CO_2 evolution *Chaetomium globosum* stands out both for

its relative salt tolerance and maximum CO_2 evolution among the fungi tested.

The results of soil cellulase activity after 7, 14, 27 and 42 da of incubation are summarized in FIG. 3. These results also followed the general pattern of CO_2 evolution. Maximum cellulase in all the treatments was observed after 7 da. A sharp decline in its production was observed after 14 da. This decline was more pronounced in the case of nonsterile soil and where *C. globosum* and *Alternaria alternata* were inoculated into sterilized soil. A steady decrease in cellulase activity was observed after 14 da of incubation. As regards the effect of salinity, in all the treatments there was a decrease in cellulase activity with increase in salinity levels. Among the fungi inoculated, *C. globosum* had the highest cellulase activity. In all cases, the effect of salinity was most pronounced between 7 and 14 da of incubation whereas after 42 da the differences narrowed. The decrease in cellulase activity during incubation can be attributed, in addition to the salinity effect, to the exhaustion of substrate and production of glucose in quantities sufficient to inhibit its production. Mandels and Reese (15), while reviewing the literature related to inhibition of cellulases, indicated the inhibitory effect of small quantities of glucose on cellulase production.

At the end of the 6-wk incubation period, the distribution of carbon and nitrogen in various organic-matter fractions of the soil was determined and is summarized in TABLE I. Maximum humification took place in nonsterile soil as it had maximum carbon and nitrogen in the humic-acid fraction. Similarly among the inoculated soils, *C. globosum* produced maximum carbon and nitrogen in humic acid, comparable to that of nonsterile soil. Corresponding amounts of carbon were observed in *Aspergillus terreus*-treated soils. The effect of salinity on humification was quite evident in the case of *Alternaria alternata* where humic-acid carbon decreased from 1.62 to 0.65 mg/g soil with increase in salinity. Minimum humification took place in the case of *Drechslera australiensis*. It also had the lowest rate of carbon mineralization.

It is evident from the results of humification and carbon mineralization, that with the increase in CO_2 evolution, humification also increases. However, in the case of *A. terreus,* humification is relatively more than carbon mineralization. During a separate investigation this fungus, when cultured on asparagine-glucose medium, produced "humic-acid"-like substances which could be precipitated on acidification of the culture filtrate. There is evidence in the literature (3, 16) that fungi can synthesize polyphenols which polymerize to form humic-acid-like substances.

TABLE I

DISTRIBUTION OF CARBON AND NITROGEN IN DIFFERENT ORGANIC-MATTER FRACTIONS IN INOCULATED AND UNINOCULATED SOIL (MG/G SOIL) AFTER INCUBATION FOR 42 DA

Fungus and treatments[a]	Humic acid		Fulvic acid		Humin	
	C	N	C	N	C	N
Alternaria alternata						
St. 1	1.62	0.21	0.56	0.07	6.32	0.32
St. 2	0.95	0.18	0.92	0.11	6.63	0.33
St. 3	0.82	0.17	0.93	0.07	7.00	0.41
St. 4	0.65	0.17	0.92	0.05	7.68	0.43
Aspergillus terreus						
St. 1	1.75	0.23	0.43	0.05	6.32	0.32
St. 2	1.66	0.21	0.49	0.04	6.35	0.38
St. 3	1.65	0.21	0.35	0.04	6.72	0.40
St. 4	1.05	0.19	0.90	0.03	7.30	0.43
Chaetomium globosum						
St. 1	1.81	0.25	0.44	0.03	5.50	0.26
St. 2	1.75	0.25	0.50	0.03	5.75	0.31
St. 3	1.62	0.24	0.38	0.02	6.00	0.33
St. 4	1.62	0.24	0.19	0.02	6.44	0.40
Curvularia lunata						
St. 1	1.75	0.21	0.50	0.08	6.00	0.20
St. 2	1.68	0.21	0.32	0.04	6.50	0.38
St. 3	1.62	0.21	0.38	0.05	6.75	0.39
St. 4	0.85	0.17	0.90	0.08	7.00	0.38
Drechslera australiensis						
St. 1	0.95	0.19	0.98	0.05	7.32	0.41
St. 2	0.85	0.18	0.90	0.06	8.75	0.49
St. 3	0.85	0.18	0.86	0.04	7.79	0.45
St. 4	0.53	0.17	0.72	0.05	8.25	0.45
NS 1	1.87	0.28	0.56	0.03	5.32	0.23
NS 2	1.87	0.28	0.38	0.02	5.50	0.29
NS 3	1.85	0.26	0.40	0.03	6.00	0.30
NS 4	1.62	0.25	0.44	0.03	6.56	0.34

[a] St. = sterilized soil; NS = nonsterile soil; 1, 2, 3, 4 = salinity levels at E.C. 0.4 Sm^{-1}, 1.0 Sm^{-1}, 2.0 Sm^{-1}, and 3.0 Sm^{-1}, respectively.

In the present investigation the effect of soil salinity on the activities of celluloytic fungi has been studied. Previously much has been written regarding fungal tolerance of high salt environment especially by marine organisms (5). Tresner and Hayes (23) studied the NaCl tolerance of terrestrial fungi by including the salt in the culture medium. Similar studies have also been done by Kulik (6) and Rai and Agarwal (19, 20), but there have been no reports regarding the effect of salts on

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The results reported here indicate that increasing salinity has an inhibitory effect on the microbial activity of soil as reflected by CO_2 evolution, cellulase activity and humification of plant residues. However, this effect varied with different fungi. Under natural conditions where these fungi grow with numerous other microorganisms, information on their competitive saprophytic ability should be of interest. In a previous study (14), *C. globosum* was found to be the most successful colonizer of tissues of *Diplachne fusca* buried in saline soil. Similar studies with other fungi may reveal interesting results.

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