Reprint from

"SOIL ORGANIC MATTER STUDIES"
VOL.II

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 1977

IAEA-SM-211/58

EFFECT OF ORGANIC MATTER AND SALTS ON THE ACTIVITY OF SOME SOIL ENZYMES

A.S. ABDEL-GHAFFAR, M.H.A. EL-SHAKWEER, M.A. BARAKAT
Department of Soil and Water Science,
Faculty of Agriculture,
Alexandria University
and
Soil Salinity Laboratory,
Agriculture Research Center,
Alexandria,
Egypt

Abstract

EFFECT OF ORGANIC MATTER AND SALTS ON THE ACTIVITY OF SOME SOIL ENZYMES.

The activities of dehydrogenase, catalase, protease, cellulase, invertase and amylase, as well as CO_2 evolution, were measured periodically for 28 days using a clay soil with and without the addition of clover straw and NaCl, $CaCl_2$ and Na_2CO_3. The mere addition of the plant material greatly increased the activities of all the soil enzymes tested even in the presence of salts. The only exception to this trend occurred in the $CaCl_2$-straw-treated soil with protease whose activity was lower than in the untreated soil. With $CaCl_2$, dehydrogenase, catalase, protease and amylase activities decreased whereas those of invertase and cellulase increased. With Na_2CO_3, dehydrogenase, catalase and protease activities increased whereas those of invertase and cellulase decreased. Sodium chloride increased only the activities of cellulase and invertase whereas other enzymes were depressed. The simple correlation test between CO_2-C values and the average corresponding activity value for each enzyme showed that dehydrogenase, catalase, protease and amylase had positive and cellulase and invertase had negative correlation coefficients.

INTRODUCTION

Recently, much attention has been devoted to soil enzymology, and a wealth of data have been accumulated [1–4]. Obviously, enzymes in the soil are involved in the decomposition of organic matter and many other chemical transformations in the soil. It is well known that generally the addition of organic matter to soil improves its physical, chemical and biological properties, and that the activities of soil enzymes are usually connected with soil organic matter [4].

In another paper in this Symposium [5] it was demonstrated that salts affected the rate and extent of the decomposition of plant residue added. The decomposition process was favoured by Na_2CO_3 and depressed by other salts tested.

The present work is an attempt to study the effect of Na_2CO_3, NaCl and $CaCl_2$ on the activities of some soil enzymes. Amylase, invertase, cellulase, dehydrogenase, catalase and protease are some of the important enzymes in soil responsible for the rate and course of decomposition of organic materials. Shehata [6], Abd-el-Malek [7] and Shady [8] found that salinity reduced the activity of soil enzymes.

MATERIALS AND METHODS

A top soil (0 – 30 cm) sample was obtained from a fertile field. The soil was clay, and contained 0.53% organic carbon and 0.08% total nitrogen. Its pH was 7.8 (in 1 : 5 H_2O suspension).

The soil sample was air-dried and passed through a 2-mm sieve before using. Powdered clover straw was added to the soil at the 1% rate. The organic carbon and total N contents of this plant residue were 38% and 2.2% respectively. The salts, NaCl, $CaCl_2$ and Na_2CO_3, were used in concentrations equal to 25 meq/100 g soil.

Experiments were conducted at 29 ± 1°C, using 100-g portions of the soil with and without either clover straw or salt or both, and the moisture content adjusted to 60% WHC. Carbon dioxide evolved and the activities of six soil enzymes were determined periodically for 28 days.

The evolved CO_2 was absorbed by NaOH solution and the Na_2CO_3 formed was precipitated as $BaCO_3$. Excess NaOH was titrated with HCl [9]. Results were reported as mg CO_2 – C/100 g soil.

The methods used for determining amylase and invertase activities were those of Ross [10], and cellulase was determined by the method described by Pancholy and Rice [11]. The reducing sugar was determined by Nelson's method and the results were recorded as μmol glucose produced/g soil per 24 h. The titrimetric method outlined by el-Essawi and co-workers [12] was used for determining catalase activity, which was expressed as mmol of H_2O_2 decomposed/g soil per 15 min. Dehydrogenase activity was determined as described by Pancholy and Rice [11], and the results were recorded as μg formazon/g soil per 24 h. Protease activity was determined using gelatin as the substrate and the amount of hyrolysed gelatin was measured spectrophotometrically, using the cupric phosphate reagent [13]. The results were expressed as percentage of gelatin hydrolysed.

All reported data represent the average of three replicates.

RESULTS AND DISCUSSION

The addition of clover straw caused striking and remarkable increases in the activities of the six soil enzymes tested regardless of the salt added, as shown in Tables I–VI. Similar results have been reported by other workers [1, 2, 6–8, 11, 14, 15]. The only exception was the very low protease activity in the $CaCl_2$-treated soil (Table III). Pancholy and Rice [11] stated that the type of organic matter added to the soil determined the activity of the soil enzymes, but they did not find any correlation between soil enzymatic activity and amount of organic matter.

Generally, the activities of dehydrogenase (Table I) and catalase (Table II) were highest in the soils incubated for 4 days, and then decreased gradually. Other enzymes (protease, cellulase, invertase and amylase), particularly cellulase, showed a gradual increase throughout the experimental period (28 d). This trend was found in nearly all treatments.

The addition of NaCl, $CaCl_2$ and Na_2CO_3 (25 meq/100 g soil) to the soil treated with 1% clover straw caused different changes in the activities of the six soil enzymes under investigation. The extent and magnitude of the salt effect on soil enzyme activity varied with incubation time, type of salt and enzyme. Similar findings have been reported by Egyptian workers [6–8].

Dehydrogenase activity values are shown in Table I. They were highest in the presence of Na_2CO_3 and lowest with $CaCl_2$. Upon incubation of the soil-straw mixture for 4 days, the amounts of formazan formed, in ug/g soil per 24 h, were 242.7, 240.7, 95.8 and 268.7 for the salt-free soil and soils treated with NaCl, $CaCl_2$ and Na_2CO_3 respectively. These data indicate that $CaCl_2$ inhibited and $NaCO_3$ stimulated dehydrogenase activity. Sodium chloride slightly depressed the formation of formazan.

Catalase activity showed a trend similar to dehydrogenase activity (Table II). The amount of H_2O_2 decomposed by the soil-straw mixture that had been incubated for 4 days was 1.7 mmol/g soil per 15 min. The corresponding values in the presence of NaCl, $CaCl_2$ and Na_2CO_3 were 1.5, 1.3 and 1.8 respectively. The catalase activity was depressed more by $CaCl_2$ than by NaCl and it was increased by Na_2CO_3. It is of interest to note that catalase activity of the untreated soil (no salts or clover straw) did not change during the experimental period.

TABLE I. EFFECT OF SALTS ON DEHYDROGENASE ACTIVITY

Figures are in μg formazan (TPF)/g soil per 24 h

Days of incubation	Untreated soil	Soil treated with clover straw and			
		None	NaCl	$CaCl_2$	Na_2CO_3
0	43.9	214.8	242.7	160.9	258.0
4	57.2	242.7	240.7	95.8	268.7
12	23.9	228.1	89.1	99.1	234.8
20	16.0	63.8	69.2	57.9	147.0
28	18.0	61.2	93.1	63.2	131.0

TABLE II. EFFECT OF SALTS ON CATALASE ACTIVITY

Figures are in mmol H_2O_2 decomposed/g soil per 15 min

Days of incubation	Untreated soil	Soil treated with clover straw and			
		None	NaCl	$CaCl_2$	Na_2CO_3
0	0.8	0.8	0.6	0.6	0.8
4	0.8	1.7	1.5	1.3	1.8
12	0.8	1.6	1.4	1.3	1.8
20	0.8	1.4	1.3	1.1	1.4
28	0.8	1.2	1.4	1.2	1.4

TABLE III. EFFECT OF SALTS ON PROTEASE ACTIVITY

Figures are in percentage of gelatin hydrolysed

Days of incubation	Untreated soil	Soil treated with clover straw and			
		None	NaCl	$CaCl_2$	Na_2CO_3
0	0.3	0.7	0.7	0.3	0.7
4	0.5	5.5	5.5	0.3	20.5
12	0.5	10.3	8.9	0.3	21.1
20	0.8	10.8	10.9	0.3	21.2
28	1.1	10.7	10.1	0.5	21.8

Table III shows the activity of protease, expressed as percentage gelatin hydrolysed under experimental conditions. After 28 days' incubation, Na_2CO_3 increased greatly the protease activity whereas $CaCl_2$ severely depressed it. The $CaCl_2$-treated soil had 25 times less protease activity than the salt-free soil. With Na_2CO_3, proteolysis was about twice as high as in the control soil and about 73 times as high as in the $CaCl_2$-treated soil. Protease activity of the $CaCl_2$-treated soil did not change with the addition of clover straw or during the 28 days of incubation. The ability of the soil to hydrolyse gelatin was slightly depressed by NaCl.

TABLE IV. EFFECT OF SALTS ON CELLULASE ACTIVITY

Figures are in μmol glucose/100 g soil per 24 h

Days of incubation	Untreated soil	Soil treated with clover straw and			
		None	NaCl	$CaCl_2$	Na_2CO_3
0	9.8	170.3	181.4	192.1	145.1
4	10.5	183.6	188.6	268.6	168.9
12	9.8	192.6	247.3	312.5	193.1
20	10.3	198.3	281.6	335.3	201.6
28	9.4	210.9	338.1	392.4	234.9

TABLE V. EFFECT OF SALTS ON INVERTASE ACTIVITY

Figures are in μmol glucose/g soil per 24 h

Days of incubation	Untreated soil	Soil treated with clover straw and			
		None	NaCl	$CaCl_2$	Na_2CO_3
0	48.1	69.1	66.3	63.1	44.1
4	38.5	60.9	71.2	88.4	26.9
12	30.2	64.3	77.3	79.4	42.7
20	32.7	66.9	79.6	100.7	43.5
28	32.7	73.2	81.3	103.7	46.1

TABLE VI. EFFECT OF SALTS ON AMYLASE ACTIVITY

Figures are in μmol glucose/g soil per 24 h

Days of incubation	Untreated soil	Soil treated with clover straw and			
		None	NaCl	$CaCl_2$	Na_2CO_3
0	1.9	9.3	7.3	7.8	10.6
4	2.7	19.7	18.2	16.4	17.4
12	2.2	19.6	18.8	16.7	17.8
20	3.7	19.6	18.8	16.6	18.1
28	6.5	19.3	18.9	16.8	18.4

Cellulase activity was greatly increased by the addition of clover straw, and increased more in the presence of NaCl and $CaCl_2$ (Table IV). Generally the activity in the clover-straw-treated soil was 20–40 times higher than in the untreated soil. The activity of cellulase was slightly affected by Na_2CO_3. In all treatments, the activity was highest in the soil incubated for 28 days.

Invertase activity generally showed trends similar to cellulase (Table V). Invertase was stimulated by NaCl and $CaCl_2$ but definitely depressed by Na_2CO_3 compared with the control (soil treated with clover straw). Also, maximum invertase activity was attained after incubation of the soil for 28 days.

TABLE VII. CORRELATION COEFFICIENT (r) BETWEEN ENZYME ACTIVITIES AND CO_2-C EVOLVED FROM SOIL TREATED WITH CLOVER STRAW

Enzyme	Enzyme activity with treatment[a]				r
	Control	NaCl	$CaCl_2$	Na_2CO_3	
Dehydrogenase	162.1	147.0	95.4	207.9	+0.96
Catalase	1.3	1.2	1.1	1.4	+0.95
Protease	7.6	7.1	0.3	17.1	+0.91
Cellulase	191.1	247.4	304.2	188.7	-0.97
Invertase	66.9	75.1	87.1	40.7	-0.94
Amylase	17.5	16.4	14.9	16.5	+0.46
Cumulative CO_2-C mg/100 g soil per 28 d	91.1	66.3	46.7	103.2	–

[a] Enzyme activities are expressed as in previous tables. Each figure in the table represents the average activity (5 incubation periods X 3 replicates) of the enzyme.

It is of interest to note that high invertase and cellulase activities are associated with low dehydrogenase and catalase activities and vice versa. Also, Skujins [1] reported a similar association between invertase and catalase.

The values for amylase activity are presented in Table VI. Addition of clover straw increased the amylase activity five times or more. The salts (NaCl, $CaCl_2$ and Na_2CO_3), especially $CaCl_2$, lowered the activity of amylase but the activity was consistently much higher than in the untreated soil.

The correlation coefficients between CO_2-C evolved in the case of clover-straw-amended soil and the corresponding activity values for each enzyme are shown in Table VII. It is evident that dehydrogenase, catalase, protease and amylase had positive whereas cellulase and invertase had negative correlation coefficients.

These data indicate that $CaCl_2$ depressed the activity of the enzymes that were positively correlated with CO_2-C evolution, and enhanced the enzymes that were negatively correlated with it. Na_2CO_3 behaved quite contrary to $CaCl_2$, at least with respect to dehydrogenase, catalase, protease and invertase. NaCl increased the activity of cellulase and invertase but it was not as effective as $CaCl_2$.

REFERENCES

[1] SKUJINS, J. "Enzymes in soils", Soil Biochemistry 1 (McLAREN, A.D., PETERSON, C.H., Eds), Marcel Dekker, New York (1967) 371.

[2] KUPREVICH, V.F., SHCHERBAKOVA, T.A., "Comparative enzymatic activity in diverse types of soil", Soil Biochemistry 2 (McLAREN, A.D., SKUJINS, J., Eds), Marcel Dekker, New York (1971).

[3] GALASTYAN, A.Sh., Enzymatic activity of soils, Geoderma 12(1974) 43.

[4] KISS, S., et al., Biological significance of enzymes accumulated in soil, Adv. Agron. 27 (1975) 25.

[5] EL-SHAKWEER, M.H.A., GOMAH, A.M., BARAKAT, M.A., ABDEL-GHAFFAR, A.S., "Effects of salts on decomposition of plant residues", these Proceedings, IAEA-SM-211/22, Vol.I.

[6] SHEHATA, S.M., Evaluation of some Biological Tests as Parameters for Microbial Activities related to Soil Fertility, Ph.D Thesis, Cairo University (1972).

[7] ABD-EL-MALEK, S.M., Enzymatic Activities in Soils as Indicators of Fertility, MSc Thesis, Cairo University (1973).
[8] SHADY, M.A.M., Studies on some Microbiological Enzymes in Soils, PhD Thesis, Ain-Shams University (1975).
[9] BLACK, C.A. (Ed.), Methods of Soil Analysis, Part 2, Am. Soc. Agronomy, Madison (1965).
[10] ROSS, D.J., A survey of activities of enzymes hydrolysing sucrose and starch in soils under pasture, J. Soil Sci. 17 (1966) 1.
[11] PANCHOLY, S.K., RICE, E.L., Soil enzymes in relation to old field succession: Amylase, cellulase, invertase, dehydrogenase and urease, Soil Sci. Soc. Am., Proc. 37 (1973) 47.
[12] EL-ESSAWI, T.M., et al., Preliminary studies on some soil enzymes Alex. J. Agric. Res. 21 (1973) 125.
[13] SKUJINS, J., Dehydrogenase: An indicator of biological activities in arid soils, Bull Ecol. Res. Comm. (Stockholm) 17 (1973) 235.
[14] KHAN, S.U., Enzymatic activity in gray wooded soil as influenced by cropping systems and fertilizers, Soil Biol. Biochem. 2 (1970) 137.
[15] EL-ESSAWI, T.M., Studies on Soil Enzymes with Special Reference to Soil Phosphatase (Methylparathion Hydrolase), PhD Thesis, Alexandria University (1972).

DISCUSSION

W. ROCHUS: The activity of the different enzymes depends on the pH. If you add Na_2CO_3, you are perhaps working nearer the pH optimum. Could this be a reason for your results?

A.S. ABDEL-GHAFFAR: In all treatments, every enzyme was tested with a buffer solution of recommended pH. In the paper on the effect of salts on the decomposition of plant residues [5], it was seen that Na_2CO_3 and $CaCO_3$ promoted the decomposition process whereas $MgCO_3$ depressed it.

R.A. SOBULO: Do you think the activity of the enzymes is due to the anion or the cation of the salts added to the system?

A.S. ABDEL-GHAFFAR: I cannot say that it is the cation or the anion; probably it is both.

K.A. MALIK: Did you estimate cellulase activity at higher concentrations of salts, especially NaCl? We found increased activity of cellulase at lower concentrations of NaCl.

A.S. ABDEL-GHAFFAR: No, I did not test the activity of cellulase in the presence of salt concentrations higher than 25 meq/100 g soil.