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THE MODE OF SALT INHIBITION ON THE DECOMPOSITION OF CLOVER  
STRAW ADDED TO A CLAY SOIL

By

M.H.A. El-Shakweer

Department of Soil & Water Sciences, Faculty of Agriculture  
in Fargum, Cairo University.

ABSTRACT

The mode of salt inhibition on the decomposition of clover straw added to a clay soil was studied at  $29 \pm 1^\circ\text{C}$ . The salts added were  $\text{NaCl}$ , at a rate of 25 mg./100 g. soil. The evolved  $\text{CO}_2$  was determined periodically for 28 days.

Michaelis-Menten equation was found valid with respect to clover straw organic carbon but invalid with respect to soil + clover straw organic carbon as substrates. The control,  $\text{NaCl}$  and  $\text{CaCl}_2$  treatments showed the same  $V_{\text{max}}$  but had different  $K_i$  values (336, 545 and 943, respectively) indicating competitive inhibition. The dissociation constant ( $K_i$ ) due to salts was 41.2 and 15.8 m.mole/100 g. soil for  $\text{NaCl}$  and  $\text{CaCl}_2$  treatments respectively.

INTRODUCTION

Plant residues added to soils undergo decomposition by micro-organisms. This process is associated with the evolution of  $\text{CO}_2$  which is used to measure the rate and extent of organic matter decomposition.

Mathematical expressions of the decomposability of organic matter added to soils under humid and semi-humid climatic conditions had been deduced (Broadbent and Norman, 1946; Broadbent and Bartholomew, 1948; Lees and Porteous, 1950; Jenkinson, 1963 and Russell, 1964). However, no attempt has been devoted to that of arid and semi-arid environments especially salt-affected soils.

As a continuation of a previous work (El-Shakweer, 1976 and El-Shakweer et al., 1977); this work aimed to clarify mathematically, the mode of salt inhibition on the decomposition of clover straw added to a clay soil.

MATERIALS AND METHODS

A surface alluvial soil sample (0-30 cm) was leached with distilled water until freed from salts, air-dried, passed through a 2 mm-sieve and thoroughly mixed. The soil was chemically (Black et al., 1965) and was found to contain 5272 ppm organic carbon, 751 ppm total nitrogen and 8.2% total carbonates. The pH of the saturated soil extract was 7.8 and EC was 0.8 mhos/cm at  $25^\circ\text{C}$ .

Mature clover plants were air-dried and ground to pass through a 60-mesh sieve. Organic nitrogen and carbon contents were determined according to the methods described by Jackson (1962) and were found 2.17% and 15.3% respectively.

Salt solutions of NaCl and  $\text{CaCl}_2$  were applied at a rate of 25 m.e./100 g. soil.

One-hundred grams of the soil were thoroughly mixed with 1, 2, 4, 6, 8 and 10 g. of clover straw in 200 ml conical flasks. To each flask, solution containing 25 m.e. of either NaCl or  $\text{CaCl}_2$  were added. The amount of salt solution added was 52.5 ml which was equivalent to 60% of the soil water holding capacity. The salt solution was added dropwise and the flasks were incubated for 28 days at a constant temperature room ( $29 \pm 1^\circ\text{C}$ ). Carbon dioxide evolved was measured at 4 days intervals. Each treatment was conducted in 6 replicates.

The closed technique with intermittent aeration and the absorption of the evolved  $\text{CO}_2$  by NaOH solution (Black *et al.*, 1965 and El-Shakweer, 1976) was adopted for the determination of  $\text{CO}_2$  evolution.

Statistical analysis of the data was carried out according to Steel and Torrie (1960).

## RESULTS AND DISCUSSION

The mean cumulative values of  $\text{CO}_2\text{-C}$  evolved after 28-days are presented in Table 1. It is clear that for each salt treatment, an initial rapid increase of  $\text{CO}_2$  evolution followed by a reduction in the rate of  $\text{CO}_2$  evolution occurred, by increasing the straw added to the soil. At 8-10% clover application, no more  $\text{CO}_2$  was evolved. However, the amount of  $\text{CO}_2$  evolved was 85.2% and 79% for  $\text{CaCl}_2$  respectively, of that of the control. Generally, the inhibition effects of NaCl and  $\text{CaCl}_2$  on decomposition of clover straw were significant.

Relationships between organic carbon contents of the clover straw or that of the clover straw + soil mixture as a substrate and the  $\text{CO}_2$  evolved are shown in Figures 1 and 2, respectively. Applying the Michaelis-Menten equation, the maximum velocities of  $\text{CO}_2$  evolution ( $V_{\text{max}}$ ) were 161.9, 145.0 and 126.3 mg.  $\text{CO}_2\text{-C}/100$  g. soil / 28 days for the control, NaCl and  $\text{CaCl}_2$  treatments respectively (Figures 1 and 2). The corresponding values of  $K_m$  (defined as the concentration required for  $\frac{1}{2} V_{\text{max}}$ ; Morris, 1978) were 300, 470 and 510 (Figure 1) and 837, 957 and 1040 (Figure 2). Differences in  $K_m$  values for the same treatment were apparently due to the nature of the substrate.

To test the applicability of Michaelis-Menten equation, the Lineweaver-Burk linear transformation:

Table 1- Salt effect on carbon dioxide evolved from a clay soil treated with increasing amounts of clover straw and incubated for 28 days.

Clover straw added, g./100 g. soil	Organic carbon, mg./100 g. soil		CO <sub>2</sub> -C evolved with treatments, mg./100 g. soil					L.S.D. (salts)
	Clover straw	Soil + clover straw	Control	NaCl	CaCl <sub>2</sub>	Mean		
0	0	527.2	9.5	12.7	11.9	11.4		
1	323.4	880.6	91.1	66.3	46.7	68.0		
2	706.8	1234.0	118.5	91.4	82.5	97.4		
4	1413.6	1940.8	140.1	120.3	111.8	124.1		
6	2120.4	2647.6	152.8	131.9	121.4	135.4		
8	2827.2	3354.4	161.9	144.6	126.8	144.4		
10	3534.0	4061.2	161.9	145.0	126.3	144.4		
Mean			119.4	101.7	89.6	—	1.2	
L.S.D. (additions)						1.3		

L.S.D. salt x additions = 2.3

$$\frac{1}{v} = \frac{1}{V_{\max}} + \frac{K_m}{V_{\max}(S)}$$

stated by Segel (1968) and Morris (1978) was adopted. Plotting values related to clover straw organic carbon or clover straw + soil mixture organic carbon as substrates (C) versus CO<sub>2</sub>-C evolution as product (v), are presented in Figures 3 and 4, respectively. The validity of application of the Lineweaver - Burk linear transformation considering clover straw carbon as a substrate was obtained in Figure 3.

To prove such validity, the regression lines were found to be:

$$\begin{aligned} \text{For control} &: \frac{1}{v} = 5.66 + 0.1900 \frac{1}{C} \\ \text{For NaCl} &: \frac{1}{v} = 5.99 + 0.3265 \frac{1}{C} \\ \text{For CaCl}_2 &: \frac{1}{v} = 5.68 + 0.5356 \frac{1}{C} \end{aligned}$$

Statistical analysis showed that there were non-significant difference between the three intercept values. This indicates identical  $V_{\max}$  value for NaCl, CaCl<sub>2</sub> and the control. The differences between the slope values of the regression lines, however, are found to be significant. The different slope values reflex different  $K_m$  values. On this basis, the calculated  $K_m$  values of the control, NaCl and CaCl<sub>2</sub> treatments were 336, 545, and 943 mg./100 g. soil/28 days, respectively. Such higher  $K_m$  value than that of the control indicates an inhibition effect.

It could be concluded, from the obtained results that the decomposability of clover straw organic carbon obeyed the Michaelis-Menten equation and that NaCl and CaCl<sub>2</sub> salts inhibited the decomposition process. However, the native organic carbon of the soil confused the application of this equation. The decomposability of plant materials seemed to differ from that of the native soil organic matter (Allison, 1973), thus, their kinetic considerations are different.

According to Morris (1978) considerations and the results obtained for NaCl and CaCl<sub>2</sub> treatments (Fig. 3), the two salts behaved as competitive inhibitors. The competitive inhibitor combines with the enzymes catalyzing the decomposition process at its substrate-binding sites and decreases the overall velocity of the decomposition process of clover straw. The magnitude of inhibition would depend on the nature and concentration of the inhibitor in the substrate (Morris, 1978).

A modified Michaelis-Menten equation in the presence of a competitive inhibitors had been proposed by Segel (1968) and Morris (1978) as follows:

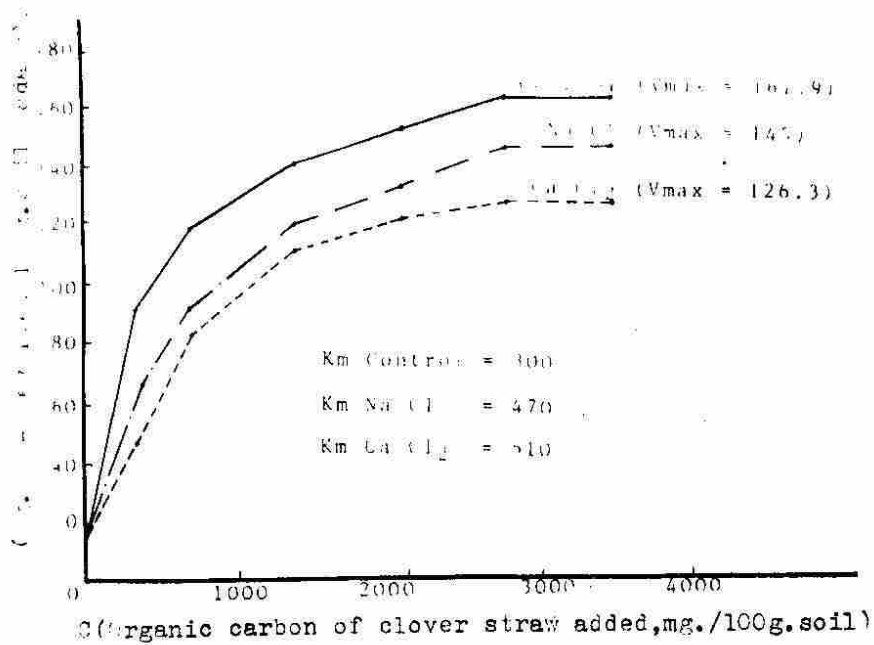


Figure 1 : Plotting of V versus C.

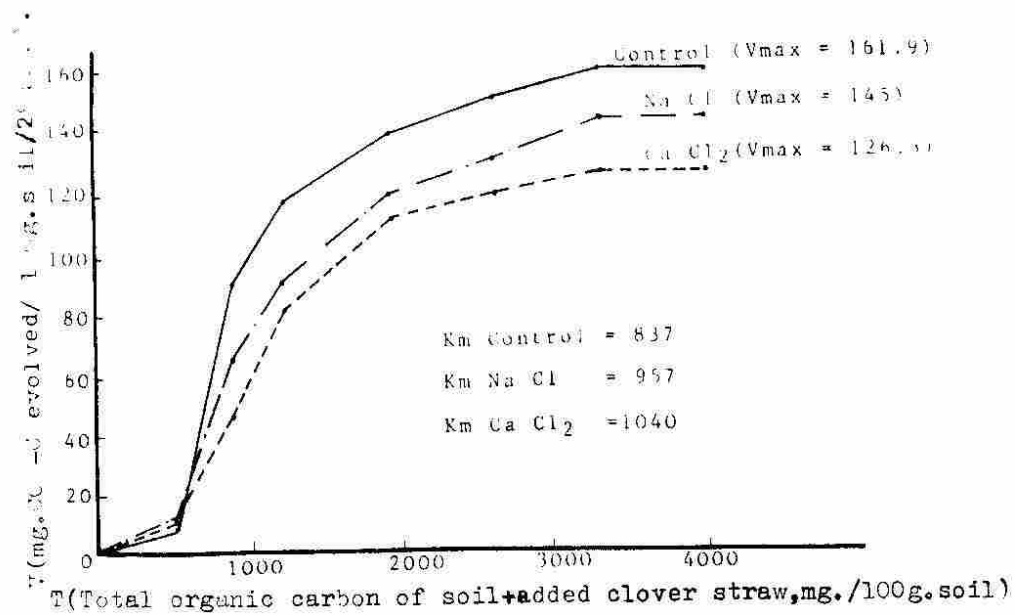


Figure 2: Plotting of V versus T.

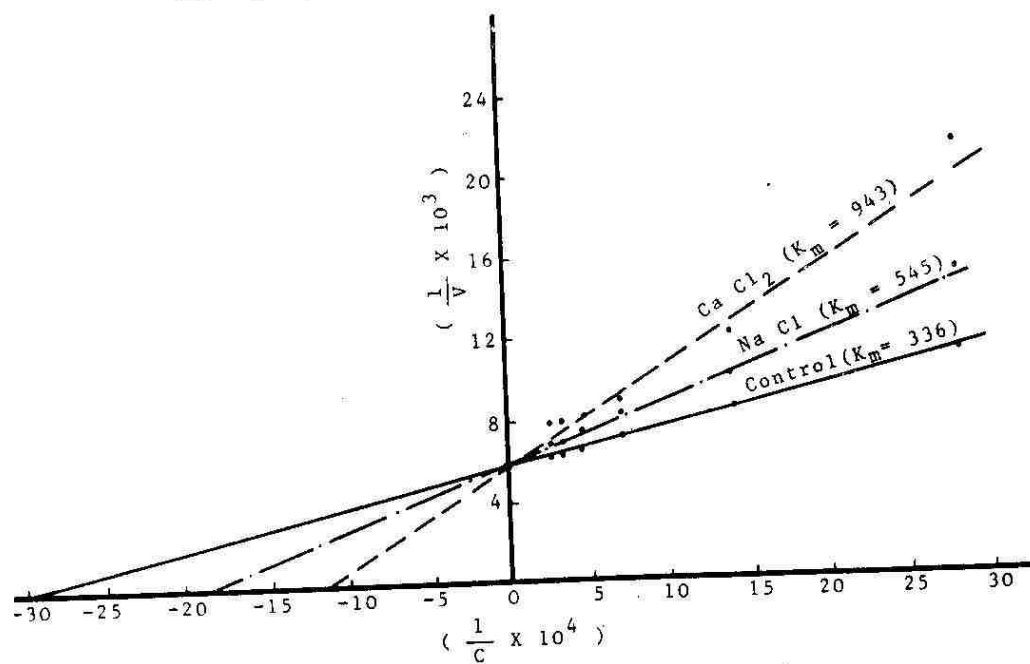


Figure 3 : Plotting of  $\frac{1}{v}$  versus  $\frac{1}{C}$ .

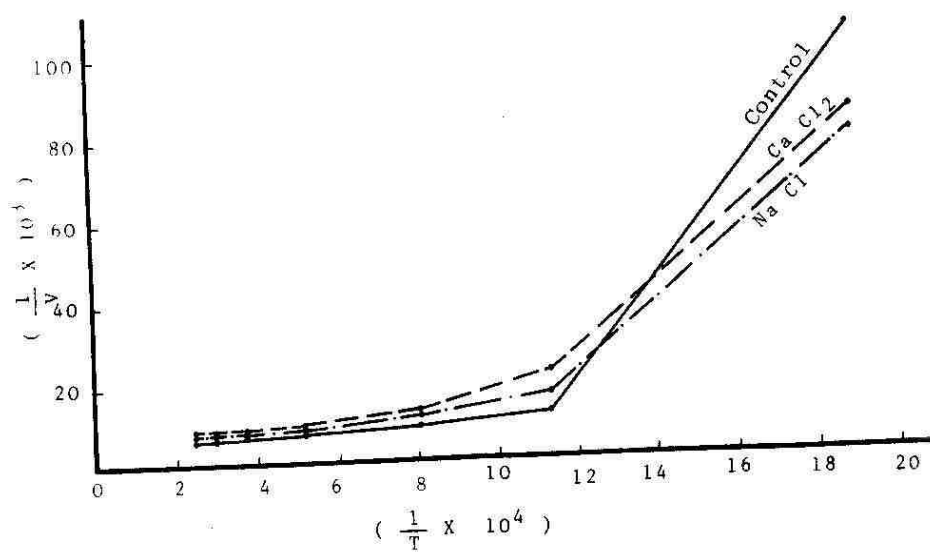


Figure 4 : Plotting of  $\frac{1}{v}$  versus  $\frac{1}{T}$

$$\frac{v}{v_{\max}} = \frac{(S)}{K_m \left(1 + \frac{I}{K_i}\right) + (S)}$$

Where  $K_i$  is the dissociation constant of the enzyme-inhibitor complex and  $I$  is the inhibitor concentration. Inversion of this equation, then dividing by  $(v_{\max})$ , the following is obtained:

$$\frac{1}{v} = \frac{1}{v_{\max}} + \frac{K_m \left(1 + \frac{I}{K_i}\right)}{v_{\max}(S)}$$

A comparison between the above equation and that of Lineweaver-Burk linear transformation shows that the competitive inhibitor alters the value of the demonstrator constant ( $K_m$ ), so that, it has the new value  $K_m \left(1 + \frac{I}{K_i}\right)$  which is referred as "the applied Michaelis - Menten constant ( $K_{m,app}$ )". Thus, on the basis of the  $K_m$  values calculated from the regression lines, the value obtained with the control is actually a  $K_m$  value while that of NaCl and  $CaCl_2$  treatments resembled  $K_{m,app}$  values. Therefore, the dissociation constant ( $K_i$ ) of the enzyme-salt complex were found to be 41.2 m. mole/100 g. soil for NaCl treatment and 15.8 m. mole/100 g. soil for  $CaCl_2$  treatment. The relatively lower dissociation constant of the enzymes -  $CaCl_2$  complex proves that lower velocity of the decomposition process of clover straw is expected with  $CaCl_2$  than with NaCl. This indicates that calcium ions have higher potential to form complexes with such enzymes and substrate molecules.

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