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The Interrelationship Between Microbial Numbers, Application Rate and Biodegradation Products of Two Organic Manures in a Sandy Soil

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ABSTRACT. The relationship between microbial numbers and rate of application and biodegradation products (mainly CO_2 and NH_3 evolution) of chicken manure (C.M.) and municipal refuse (M.R.) was determined using the data obtained during field incubation studies under centre-pivot sprinkler irrigation in Central Saudi Arabia.

Microbial numbers increased with increasing rate of manure application and with incubation time. Maximum microbial proliferation coincided with the optimum (feasible) rates of application of both manures with respect to CO_2 evolution previously reported by Abdel Magid *et al.* (1993). In microbial growth rate terms the effect of the optimum rate of application was more pronounced with C.M. than with M.R. at the 16th week of incubation.

After 16 weeks, highly significant correlations were obtained between microbial numbers and rates of treatment application, cumulative CO_2 and cumulative NH_3. The highest rates of CO_2 and NH_3 evolution coincided with the optimum rates of application of both manures.

A laboratory study of the effect of temperature on NH_3 evolution during the biodegradation of C.M. revealed that at high rates of application the magnitude of the temperature coefficient (Q_{10}) of NH_3 evolution is affected by the rate of application irrespective of

temperature. At application rates higher than the optimum biodegradation was insensitive to temperature.

The results obtained confirm our previous findings that the optimum economic rates of application of C.M. and M.R. occur between 8.25 to 16.5 and between 16.5 to 33.0 tonnes/ha, respectively.

The amount of organic material applied to marginal lands depends to a large extent on the amount of organic carbon it contains and on the availability of plant nutrients, especially nitrogen, to cultivated crops. This availability depends mainly on the capability of the soil to supply enough microbial numbers to degrade the organic materials added. Reinertsen *et al.* (1984) reported that readily available (Labile) C and N pools in organic materials determine the size of microbial biomass and rate of decomposition. However, microbial numbers are not always synonymous with microbial activity and therefore field measurements of microbial numbers require additional studies before their importance can be interpreted (Kowalenko *et al.* 1978).

A number of methods have been used to measure CO_2 and NH_3 evolution from soil (Tester *et al.* 1979, Orchard and Cook 1983, Smith and Hadley 1990, Abdel Magid *et al.* 1993). In our previous studies (Abdel Magid *et al.* 1993, 1994 and 1995) we have reported results pertaining to the biodegradability of chicken manure (C.M.) and municipal refuse (M.R) applied to field soils under the environmental conditions prevailing in Central Saudi Arabia. These studies did not involve assessment of the effect of adding the manures on microbial numbers in relation to rates of manure application and degradation. This paper reports the interrelationships between microbial numbers, rates of manure application, and cumulative CO_2 (CCO_2) and cumulative NH_3 (CNH_3) production during the biodegradation of C.M. and M.R. applied as biofertilizers in a winter wheat ecosystem in Central Saudi Arabia. In addition the temperature coefficient (Q_{10}) of NH_3 evolution was also measured.

Materials and Methods

The data used in this study were collected during the course of a previously reported study on the biodegradation of C.M. and M.R under field conditions (Abdel Magid *et al.* 1993). The salient properties of both manures, obtained from local sources, have also been previously reported (Abdel Magid *et al.* 1993). The characteristics of the sandy soil at the experimental site (Table 1) were determined as described by Page *et al.* (1982).

The rates and methods of application of both manures were described by Abdel

Magid *et al.* (1993): A month prior to winter wheat sowing each material was added and incorporated into field plots (4 x 2 m) at a depth of 10 cm at specified rates calculated on the basis of previous experience. The application rates were 0, 4.1, 8.25, 16.5 and 33.0, and 0, 16.5, 33.0, 49.5 and 66.0 tonnes/ha for C.M. and M.R, respectively.

Table 1. Characteristics of the soil used

Variable	Value
pH (1 : 2.5 soil : water suspension)	8.0
ECe (dS m^{-1})	2.1
CEC (meq./100 g soil)	4.8
Organic carbon (%)	Traces
Total N (%)	Traces
Available P	7 ppm
$CaCO_3$ (%)	7.6
Sand (%)	91.2
Silt (%)	0.8
Clay (%)	8.0

Soil thermometers and tensiometers were inserted into the soils of treated and untreated plots to monitor the fluctuations in surface and subsurface temperature and moisture, respectively (Abdel Magid *et al.* 1993). Average soil temperature during the respective incubation intervals are reported in Table 2.

Table 2. Average surface and subsurface soil temperature at the incubation period specified

Soil layer	Incubation period (weeks)			
	4	8	12	16
	Soil temperature °C			
		C.M		
Surface	15.5	13.3	10.9	19.1
Subsurface	15.3	11.5	10.5	16.9
		M.R		
Surface	16.2	13.8	10.4	17.1
Subsurface	15.5	12.2	10.5	17.1
Average	15.6	12.7	10.6	17.6

Microbial respiration during 0, 4, 8, 12 and 16 weeks of field incubation was determined as described by Abdel Magid *et al.* (1993). The device used essentially consists of a plastic cylinder that is tightly closed at one end to confine the CO_2 evolving from the soil. To avoid localised heating of the traps used, the experimental site was sprinkler irrigated twice a day during the course of the experiment. Carbon dioxide evolution was monitored weekly by static absorption of the gas in NaOH standard solution and measured byback-titration with standard HCl.

Similarly, NH_3 evolution was monitored by static absorption of the gas in 0.5 M H_2SO_4 using the same device as for CO_2. Ammonia trapped in the H_2SO_4 was analysed by steam distillation after treatment with IM NaOH (Bremner and Edwards 1965). The amounts of CO_2 and NH_3 evolved during the various periods of incubation were reported as cumulative in kg/ha/day. Controls were included for background CO_2 and NH_3 corrections.

At 0, 4, 8, 12 and 16 weeks, samples from the amended and unamended soil were removed and rushed to the laboratory for microbial enumeration using the serial - dilution plate count technique as described by the FAO (1967).

In general, most of the metabolic reactions are affected by temperature. Thus NH_3 evolution was monitored at various temperatures, *viz.* 0, 10, 20, 30 and 40°C using a laboratory method similar to the one described by Abdel Magid and Tabatabai (1982). The data obtained were used to calculate the temperature coefficient (Q_{10}) of the NH_3 evolution process.

Results and Discussion

Addition of C.M. and M.R. to the sandy soil influenced microbial numbers and proliferation and consequently the biodegradation of both manures. Figures 1 (a and b) show the relationship between the rates of treatment application and microbial number over 16 weeks (cumulative 4-week intervals) of field incubation. The plots indicate that microbial numbers increase with increasing rate of manure application and time. A sigmoidal pattern is characteristic of bacterial growth (Alexander 1977), is more pronounced with C.M. than with M.R, which has a high C/N ratio and consequently a low mineralization rate. Maximum bacterial growth rate coincided with the application rate between 8.25 to 16.5 tonnes/ha for C.M. These results were in good agreement with our previous findings (Abdel Magid *et al.* 1993) that when C.M. and M.R were applied under field conditions the optimum (feasible) application rate of greatest economic return occurs at 8.25 and 33.0 tonnes/ha, respectively. Moreover, at application rates higher than 16.5 and 33.0 tonnes/ha biodegradation was insensitive to temperature (Abdel Magid *et al.* 1994).

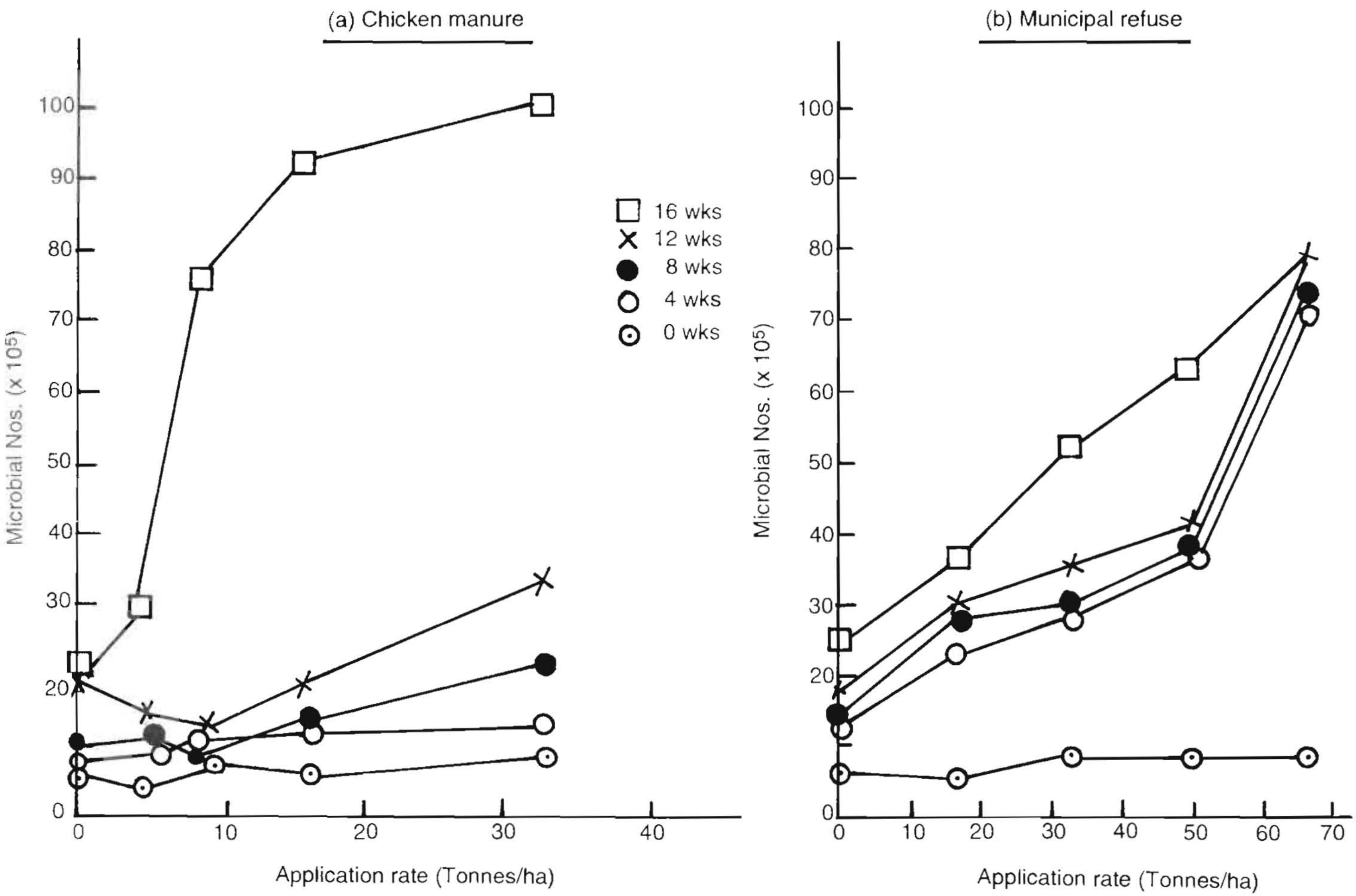


Fig. 1 (a and b). The relationship between rate of application of manure and microbial numbers at different incubation periods under field conditions:
a) Chicken manure b) Municipal refuse.

The correlation matrix for the relationship between microbial number and rate of manure application, cumulative CO_2 and cumulative NH_3 as shown in Table 3, revealed highly significant correlations at 16 weeks. Earlier, significant and nonsignificant correlations were obtained. The inconsistent statistics could be attributed to the variation in soil temperature (Table 2) between the incubation intervals, (the 8 week and 12 week intervals being the coldest). Edwards (1975) reported that variation in winter temperature results in differences in organic material biodegradation and in the rates of evolution of the biodegradation products.

Table 3. Correlation matrix for the relationship between microbial numbers and rate of application, cumulative CO_2 and cumulative NH_3 evolution at the incubation period indicated

Parameter	Incubation period (weeks)				
	0	**4**	**8**	**12**	**16**
	Correlation matrix[a]				
Rate of application:					
CM	0.433	0.986***	0.946**	0.880*	0.863*
MR	0.674	0.937**	0.913**	0.909*	0.998***
Cumulative CO_2 :					
CM	0.572	0.966**	0.737	0.620	0.985***
MR	0.837*	0.809	0.774	0.781	0.954***
Cumulative NH_3 :					
CM	0.270	0.962***	0.921***	0.762	0.998***
MR	0.394	0.871**	0.803*	0.741	0.936***

[a]:*, **, *** Indicate significance at the 5, 1 and 0.1% levels.

Decomposition of C.M and M.R over time as a function of microbial activity is best described in terms of evolution of CO_2 and NH_3 under field conditions at the respective incubation intervals. It is evident from Tables 4 and 5 that muchless NH_3 than CO_2 is evolved from the ammended soils. The high rate of CO_2 evolution, according to Wessen and Berg (1986), could be attributed to the content of highly soluble carbon compounds which degraded first in soil, followed by the structural polysaccharides (cellulose and hemicellulose). In percent carbon terms the effect is more pronounced with C.M compared with M.R (Table 4). Moreover, the plots in Fig. 2 (a,b,c and d) are similar in pattern to those shown for microbial number in Fig. 1a and Fig. 1b. This supports the hypothesis that these biodegradation products were produced as a result of microbial activity.

Table 4. Percentage of organic carbon evolved as carbon dioxide during each period of field incubation at the specified rate of application of chicken manure and municipal refuse*

Application rate (Tonnes/ha)	Incubation period (Weeks)										
	Chicken manure					Application rate (Tonnes/ha)	Municipal refuse				
	0	4	8	12	16		0	4	8	12	16
	%						%				
4.1	3.7 (6.2)	13.9 (23.6)	20.5 (34.7)	25.2 (42.5)	31.9 (53.3)	16.5	1.5 (18)	3.0 (36.9)	4.1 (49.5)	4.9 (60.4)	3.4 (–)
8.25	4.2 (14.0)	17.8 (60.5)	30.9 (105.2)	38.9 (132.4)	43.9 (149.7)	33.0	0.9 (22.4)	3.1 (76.6)	6.3 (154.2)	8.1 (198.2)	9.3 (227.5)
16.5	2.5 (17.2)	10.9 (94.1)	18.5 (125.9)	23.6 (160.6)	26.9 (183.6)	49.5	0.6 (22.2)	2.2 (80.4)	4.7 (172.6)	6.1 (224.1)	7.0 (257.6)
33.0	1.3 (17.4)	6.0 (81.8)	12.1 (165.3)	15.2 (207.2)	17.4 (236.8)	66.0	0.5 (22)	1.8 (88.3)	4.0 (195.9)	5.4 (263)	5.8 (284.8)

* Parentheses indicate rate of CO_2 evolution kg/ha/day.

Table 5. Percentage of total nitrogen evolved as NH_3 during each period of field incubation at the specified rate of application of chicken manure and municipal refuse

Application rate (Tonnes/ha)	Incubation period (Weeks)										
	Chicken manure					Application rate (Tonnes/ha)	Municipal refuse				
	0	4	8	12	16		0	4	8	12	16
	%						%				
4.1	0.01	0.04	0.08	0.15	0.19	16.5	0	0.04	0.11	0.15	0.19
8.25	0.01	0.04	0.10	0.17	0.21	33.0	0	0.03	0.05	0.10	0.12
16.5	0.01	0.08	0.13	0.20	0.21	49.5	0	0.02	0.06	0.09	0.10
33.0	0.01	0.09	0.10	0.13	0.14	66.0	0	0.02	0.05	0.07	0.09

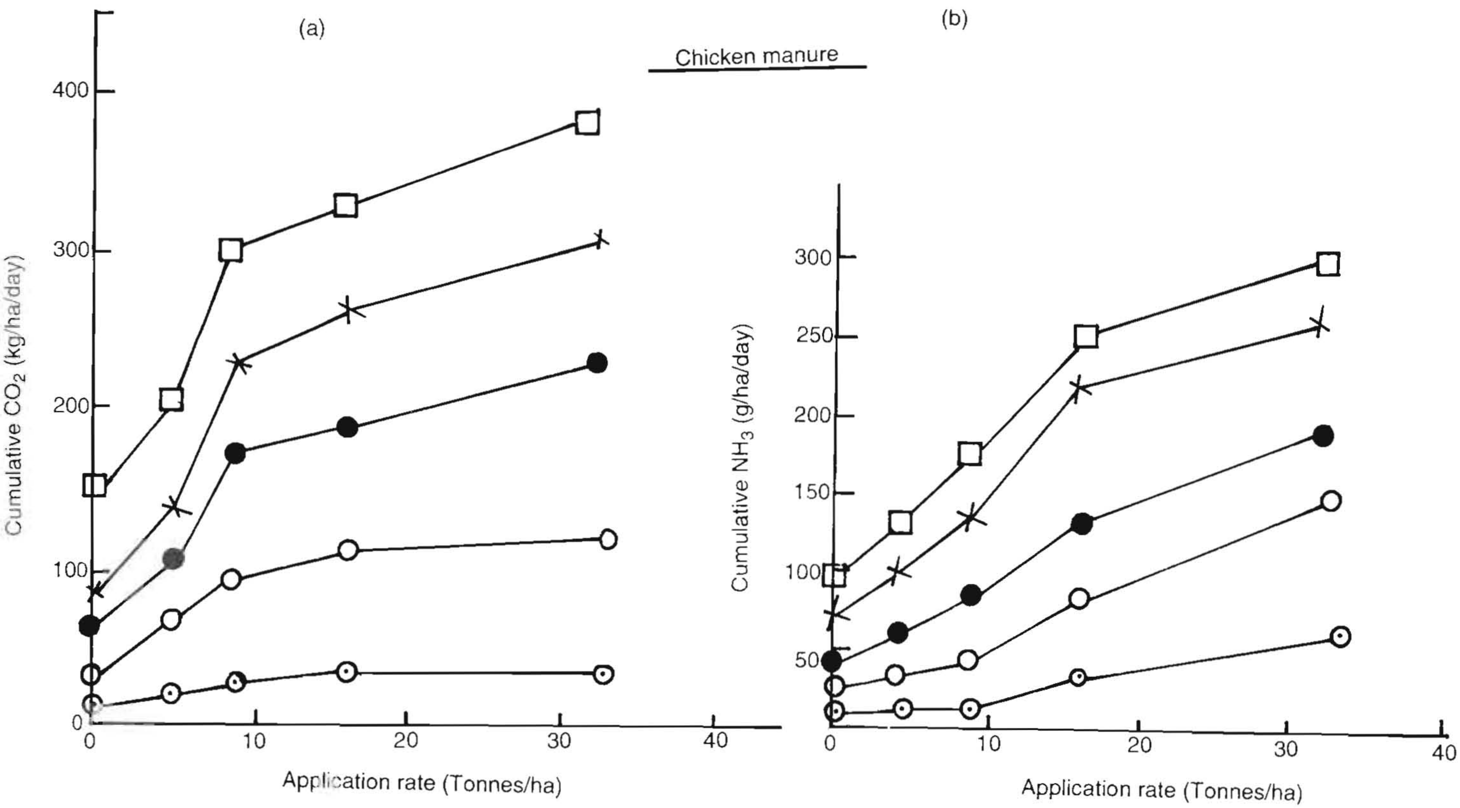
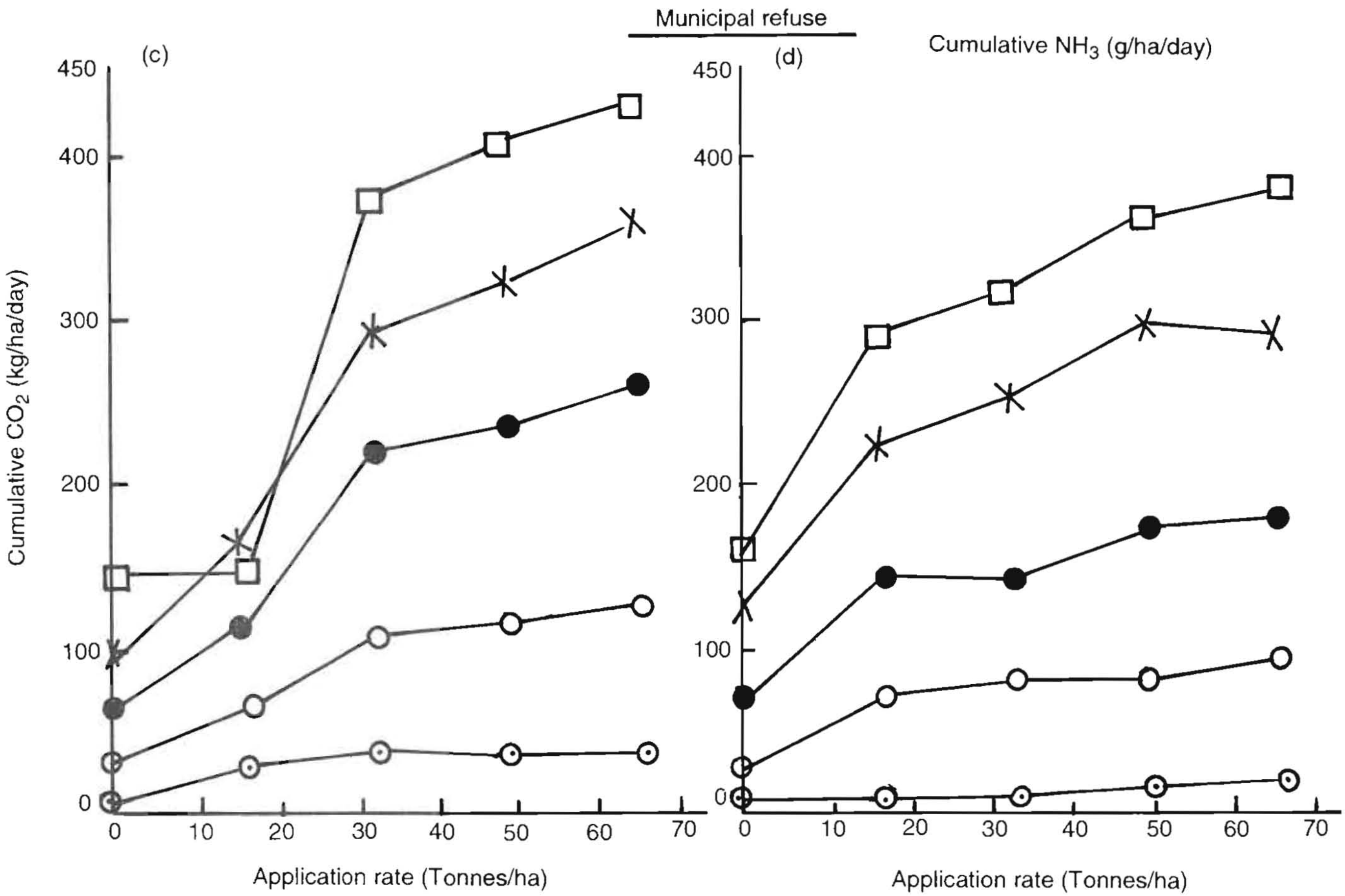


Fig. 2 (a,b,c and d). The relationship between rate of application of manure and cumulative CO_2 (Fig. 2a) and cumulative NH_3 evolution (Fig. 2b) at different incubation periods under field conditions:
a and b = Chicken manure c and d = Municipal refuse.


Municipal refuse
(c)
Cumulative CO$_2$ (kg/ha/day)
450
400
300
200
100
0
0
10
20
30
40
50
60
70
Application rate (Tonnes/ha)
(d)
Cumulative NH$_3$ (g/ha/day)
450
400
300
200
100
0
0
10
20
30
40
50
60
70
Application rate (Tonnes/ha)

The amount of N mineralized in the form of NH_3 as a percentage of total N added is small, comprising between 0.14 to 0.21% and between 0.09 to 0.19% at the 16th week of incubation for C.M and M.R., respectively (Table 5). However, with advancement of incubation time, the amount of added N mineralized in the form of NH_3 increases as the application rate increases up to the optimum application rates mentioned before (8.25 and 33.0 tonnes/ha, for C.M. and M.R, respectively). Premi and Cornfield (1971) reported that N mineralization increased with time at low levels of organic material addition but that there were wide differences in mineralization rates. This is expected, since at excessively high rates of application the substrate concentration inhibits (limits) the activity of microbial enzymes involved in the biodegradation process.

The small magnitude of NH_3 evolution could be attributed to low soil temperature (Table 2), and consequently low N mineralization rate. Moreover, incorporation and placement of the biofertilizers at a 10 cm depth could have restricted NH_3 evolution through its absorption in the soil solution at the subsoil sites. Absorption of NH_3 on soil particle surfaces is unlikely since this soil has low cation exchange capacity (Table 1). Tester *et al.* (1977) reported low NH_3 evolution from garbage compost ammended soil. The greatest quantity of NH_3 evolved in their experiment was as low as 0.04% of the N added. They attributed these results to low pH and low mineralization rate of the compost. Under the conditions of this study, the inhibitory effect of pH on N mineralization is unlikely since the soil of the experimental site contains an adequate amount of $CaCO_3$ (Table 1). Therefore, low rate of N mineralization or immobilization could be the most plausible explanation. Terman *et al.* (1973) reported that the application of municipal compost to N-deficient soil resulted in N immobilization.

Since temperature is one of the primary environmental variables controlling the N mineralization process in soil we determined the temperature coefficient (Q_{10}) of NH_3 evolution under the conditions of this study using the classical Vant Hoff's equation:

$Q_{10} = K_{t+10} / K_t$ where:

Q_{10} = temperature coefficient

K = NH_3 evolution rate at a temperature t and K_{t+10} is the NH_3 evolution rate at a temperature 10°C higher.

Similar to the results obtained with the soil respiration rate under the same experimental conditions (Abdel Magid *et al.* 1994) the Q_{10} values obtained at the temperatures of incubation used in this laboratory study (Table 6) showed no general trend. The Q_{10} values were generally higher at low to medium temperatures than at high temperatures. At the highest rate of manure application, Q_{10} was insensitive to temperature. The data confirm that high Q_{10} values (low rates of application) are indicative of impassive mineralization rates (impassive biodegradation) while low Q_{10} values (high rates of application) are indicative of passive mineralization rates. Moreover, in similar previous studies other workers (Stanford *et al.* 1973, Abdel Magid, 1980. Buyanovsky *et al.* 1986) reported Q_{10} values between 1.6 - 4.4 for soil N mineralization and soil respiration. In all these studies the lowest Q_{10} values occur at the highest temperature of incubation. Nobel (1983) indicated that low Q_{10} values *i.e.* low energies of activation, in many biochemical reactions, are associated with insensitivity to temperature, thus confirming the invalidity of very high applications of organic manures to increase crop production.

Table 6. Temperature coefficient, Q_{10}, of NH_3 evolution from C.M. at the temperature and application rate indicated

Application rate (Tonnes/ha)	Temperature of incubation (°C)			
	10	20	30	40
	Q_{10}			
0.0	1.2	1.2	1.1	1.1
4.1	1.4	1.3	1.1	1.1
8.25	1.8	2.0	1.1	1.4
16.5	1.9	3.0	2.8	–
33.0	1.2	1.1	1.0	1.1

Conclusion

The biodegradation of chicken manure and municipal refuse, applied at different rates, was studied under field conditions using a 4-week incubation interval (cumulative 16 weeks).

Microbial numbers increased as the rate of application increased and over time. Maximum bacterial growth coincided with the application rates ranging between 8.25 and 16.5 tonnes/ha for chicken manure. This result agrees with our previous findings, (Abdel Magid *et al.* 1993, 1995) that the optimum rates of greatest economic returns occur at these rates.

At the 16th week of incubation, highly significant correlations were obtained between microbial numbers and rate of application, cumulative CO_2 and cumulative NH_3 evolved. Little NH_3, as compared to CO_2, was evolved from the amended soil during incubation. The low rate of NH_3 evolution was attributed to some of the factors known to affect NH_3 evolution from soil. The most plausible of these factors is the low rate of N mineralization as a result of low temperature.

Laboratory studies of the temperature effect revealed that the temperature coefficient, Q_{10}, of NH_3 evolution is similar in magnitude to the Q_{10} obtained by other workers for similar temperature dependent proceses.

These results demonstrate the following:

a) Seasonal variations with respect to the prevailing temperature must be considered when applying organic materials to soil as biofertilizers. This is important in view of the fact that biodegradation and consequently nutrient cycling are temperature - dependent processes.

b) Application of liberal rates (over use) of organic manures is of no subtantial benefits in terms of economic returns in crop production.

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العلاقة بين أعداد الميكروبات ومعدل الإضافة ونواتج التحلل الميكروبي لبعض الأسمدة العضوية في التربة الرملية

هجو محمد عبد الماجد و رأفت خلف الله ربيع
و رجاء عليوه أحمد صبره و شفيق ابراهيم عبد العال

قسم التربة والمياه - كلية الزراعة والطب البيطري - فرع جامعة الملك سعود بالقصيم
ص .ب ١٤٨٢ - بريدة - المملكة العربية السعودية

تم تحديد العلاقة بين أعداد الميكروبات ومعدل الإضافة ونواتج التحلل الميكروبي لسمادي الدواجن ونفايات المدن (بصورة رئيسية تطاير ثاني أوكسيد الكربون والنشادر) وذلك في دراسة تحضين حقلية تحت ظروف الري بالرش المحوري في المنطقة الوسطى بالمملكة العربية السعودية ، ويمكن تلخيص النتائج في الآتي :

إزدادت أعداد الميكروبات مع زيادة معدل إضافة السمادين وتقدم فترة التحضين -هذا الأثر- أي زيادة أعداد الميكروبات - ظهر بصورة واضحة في تحلل سماد الدواجن في الأسبوع السادس عشر .

عموما فإن النتائج قد أظهرت وجود إرتباط معنوي بين أعداد الميكروبات ومعدل إضافة الأسمدة ومعدل التطاير التراكمي لثاني أوكسيد الكربون والنشادر في

الأسبوع السادس عشر على التوالي ، كما أن أعلى معدل للتطاير يتزامن مع معدل الإضافة الأمثل للسمادين .

الدراسات المعملية لأثر درجة الحرارة أظهرت أن المعامل الحراري (Q_{10}) لتطاير النشادر في سماد الدواجن يتأثر بمعدل الإضافة بصرف النظر عن درجة الحرارة . كما أن تحلل السماد لا يستجيب لدرجة الحرارة في معدلات الإضافة الأعلى من المعدل الأمثل .

هذه النتائج أكدت ما وصلنا إليه في الدراسات السابقة وهو أن المعدل الاقتصادي الأمثل لإضافة سمادي الدواجن ونفايات المدن يقع ما بين ٢٥ ,٨ إلى ٥ ,١٦ وبين ٥ ,١٦ إلى ٣٣ طن للهكتار على التوالي .