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Nitrogen Immobilization and Mineralization as Affected by Fertilizer Application in Soils

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MINERALIZATION and immobilization processes were studied in soil samples treated with ammonium sulphate, calcium ammonium nitrate, urea, urea ammonium phosphate and/or nitrophosphate along with unhumified dung. The conversion of fertilizer nitrogen into non-KCL extractable factions occurred within sixteen days and was relatively rapid in the samples treated with urea and urea ammonium phosphate. The mineral nitrogen content during incubation studies was highest in calcium ammonium nitrate treated soils. Amino acid nitrogen appeared to be the main fraction involved in the immobilization and mineralization of nitrogen in soil. Dung behaved as an efficient nitrification inhibitor and slowed the release of nitrogen from fertilizers.

Keywords: Mineralization, Immobilization, Dung, Nitrification inhibitor, Assimilation, Microorganisms.

The conversion of inorganic nitrogen to organic form through assimilation by microorganisms during the decomposition of organic residues is an important aspect of the nitrogen cycle in soil (Negm *et al.*, 1998; El-Douby *et al.*, 2001 and El-Amry *et al.*, 2001). The quantity of nitrogen required to meet the needs of the microbial population depends on the nature of the organic amendments, kinds of nitrogen supplied to the microorganisms, moisture, temperature, pH and aeration (Broadbent, 1968; Broadbent Nakashima, 1970; Kai *et al.*,1969 and Overrein, 1970). The present investigation deals with immobilization and release of nitrogen in soil samples treated with nitrogenous fertilizers such as ammonium sulphate, calcium ammonium nitrate, urea, urea ammonium phosphate and nitrophosphate and unhumified dung. The rates and amounts of change were estimated from chemical characteristics of newly immobilized and subsequently released nitrogen in the various nitrogen fractions in acid hydrolysate of soil samples at different stages of incubation. Also, various workers (Huber *et al.*, 1969 and Locascio & Fiskell, 1971) have tried to increase the efficiency of utilization of nitrogenous fertilizers through devices such as the coating of fertilizers and use of nitrification inhibitors (Hamouda *et al.*, 1999; Badran *et al.*, 2000; Habib *et al.*, 2001 and Farghly, 2001). Incorporation of partially decomposed dung, a cheaper source of nitrogen, along with fertilizers in soil may also have good prospects (Arafat ,1998; Hassouna & Hassanein, 2000; Shalan *et al.*, 2001; Allam *et al.*, 2001; El-Sayed 1995; El-Sayed 1997 a&b and El-Sayed, 1998).

Material and Methods

The study was made on a sandy loam soil (0-15 cm) of the Faculty of Agriculture Farm in Assiut, El-Azhar University, which fits the order of Aridisols (irrigated), the suborder of Orthids and the great group of Calciorthids, according to the 7^{th} Approximation system of soil classification (Soil Survey Staff, 1994). The soil contained 0.08 and 0.50% of nitrogen and carbon, respectively and had a pH of 8.6. Electrical conductivity of the saturation extract was 1.5 dS m^{-1}. The soil sample was passed through a 2-mm sieve and subsamples used for the study.

Incubation and determination

Air-dried samples (50 g) were weighed into 100 ml Erlenmeyer flasks and treated with 2.4 g of unhumified dry dung (containing 0.833% N and 30.4% C to give a C/N ratio 36.9) and 200 ppm of nitrogen in the form of ammonium sulphate, urea, calcium ammonium nitrate, urea ammonium phosphate (28% N and 28% P_2O_5) and nitrophosphate, (20% N and 20% P_2O_5). There were two more treatments, namely, (soil + dung) and (soil + fertilizer). The flasks were loosely covered with polyethylene film and placed in an incubator maintained at 30°C for periods of 16, 32, 50, 70, 80, and 110 days. Moisture content in all treatments was maintained at 60% of maximum water holding capacity (Chapman and Pratt, 1961). Ammonium and nitrate nitrogen produced at the different incubation intervals were extracted with 2 N KCl and determined using MgO and Devardas alloy in a specially designed micro-distillation apparatus (Srivastava, 1969; FAO, 1980 and Cottenie, 1980). The procedure adopted for microbially immobilized nitrogen was a modification of the Van Slyke method of protein analysis as followed by Stewart *et al.* (1963a); Page *et al.* (1982) and

Evenhuis & DeWaard (1978). After the mineralized nitrogen was extracted with 2 N KCl, a sample was hydrolyzed with 6 N HCl. Distillable acid-soluble nitrogen (designated as amino sugar $+NH_4$-N)was determined by distilling the filtrate with excess NaOH. Total filtrate (hydrolyzable) nitrogen was determined by the conventional Kjeldahl procedure. The difference between these two was designated as an amino acid nitrogen (non-distillable acid-soluble N). Data were statistically analysed according to Steel & Torrie (1982) and SAS (1988).

Results and Discussion

The results of nitrogen mineralization (ammoniacal and nitrate nitrogen forms) and immobilization (hydrolysable ammonium, amino-sugar and amino-acid forms of nitrogen) as affected by fertilizer application at different incubation intervals, with and without unhumified dung, are presented in Tables 1-4 inclusive.

TABLE 1. Mineralization of nitrogen as affected by different feitilizer application (ppm).

Soil Treatments		Incubation time (days)							Average
		16	32	50	70	80	100	110	
Control	NH_4-N	23.5	19.5	20.1	31.3	28.5	21.8	13.0	22.5
	NO_3-N	22.6	26.5	26.1	26.2	44.5	63.4	84.4	42.0
	total	46.1	46.0	46.2	57.5	73.0	85.2	97.4	
Ammonium Sulphate	NH_4-N	9.3	5.8	9.9	14.1	18.4	20.1	14.4	13.1
	NO_3-N	52.1	73.1	94.0	108.1	125.0	160.5	197.1	115.7
	total	61.4	78.9	103.9	122.2	143.4	180.6	211.5	
Urea	NH_4-N	0.8	6.0	13.3	13.6	13.9	10.6	6.5	9.2
	NO_3-N	36.5	58.2	100.1	143.6	176.0	197.1	218.5	132.9
	total	37.3	64.2	113.4	157.2	189.9	207.7	225.0	
Calcium Ammonium Nitrate	NH_4-N	9.8	16.0	15.1	16.7	16.9	14.6	12.6	14.5
	NO_3-N	37.1	71.6	84.2	137.3	172.7	200.1	226.0	132.7
	total	46.9	87.6	99.3	154.0	189.6	214.7	238.6	
Urea Ammonium Phosphate	NH_4-N	1.8	7.8	4.0	16.6	7.0	8.1	10.0	7.9
	NO_3-N	38.9	59.3	105.5	127.6	158.1	180.1	205.6	125.0
	total	40.7	67.1	109.5	144.2	165.1	188.2	215.6	
Nitrophosphate	NH_4-N	17.6	7.9	35.0	9.2	43.9	35.1	2.5	21.6
	NO_3-N	42.4	56.2	80.9	151.0	131.0	152.6	198.8	116.1
	total	60.0	64.1	115.9	160.2	174.9	187.7	201.3	
Average	NH_4-N	10.5	10.5	16.2	16.9	21.4	18.4	9.8	
	NO_3-N	38.3	57.5	81.8	115.6	134.6	159.0	188.4	
	total	48.8	68.0	98.0	132.5	156.0	177.4	198.2	

$LSD_{0.05}$ for NH_4-N = 7 8 NO_3-N 23.0

TABLE 2. Effect of dung on the mineralization of nitrogen (ppm).

Soil Treatments		Incubation time (days) 16	32	50	70	80	100	110	Average
Dung	NH_4-N	22.8	16.6	16.8	25.1	33.6	50.1	13.6	25.5
	NO_3-N	7.5	10.0	11.7	20.5	23.6	27.1	83.4	26.3
	total	30.3	26.6	28.5	45.6	57.2	77.2	97.0	
Dung + Ammonium	NH_4-N	13.3	22.9	26.3	25.1	24.9	24.4	18.2	22.2
Sulphate	NO_3-N	27.5	21.0	22.9	25.6	27.3	71.5	115.7	44.5
	total	40.8	43.9	49.2	50.7	52.2	95.9	133.9	
Dung + Urea	NH_4-N	3.2	8.6	8.6	23.7	26.2	24.6	14.1	15.6
	NO_3-N	20.0	31.4	44.0	41.1	50.4	109.6	115.1	58.8
	total	23.2	40.0	52.6	64.8	76.6	134.2	129.2	
Dung + Calcium	NH_4-N	7.6	20.1	20.6	23.0	21.1	20.7	7.5	17.2
Ammonium	NO_3-N	20.1	29.2	50.5	61.8	69.1	104.0	150.7	69.3
Nitrate	total	27.7	49.3	71.1	84.8	90.2	124.7	158.2	
Durg + Urea	NH_4-N	9.0	7.5	12.7	8.8	4.8	11.1	12.1	9.4
Ammonium	NO_3-N	17.3	30.0	30.8	45.1	57.6	101.9	137.0	60.0
Phosphate	total	26.3	37.5	43.5	53.9	62.4	113.0	149.1	
Dung+Nitrophosphate	NH_4-N	1.5	5.1	9.0	7.3	11.4	10.1	9.0	7.6
	NO_3-N	26.1	18.4	30.8	46.2	86.1	113.1	137.7	65.5
	total	27.6	23.5	39.8	53.5	97.5	123.2	146.7	
Average	NH_4-N	9.6	13.5	15.7	18.8	20.3	23.5	12.4	
	NO_3-N	19.8	23.3	31.8	40.1	52.4	87.9	123.3	
	total	29.4	36.8	47.5	58.9	72.7	111.4	135.7	

$LSD_{0.05}$ for: NH_4 - N =8.9 NO_3-N = N.S.

Immobilization of nitrogen occurred when fertilizer was applied to the soil. It became more pronounced in the presence of unhumified organic matter. Broadbent & Nakashima (1970); Stewart *et al.* (1963b); Negm *et al.* (1998) and Badran *et al.* (2000) also found increased amounts of tagged organic nitrogen when they incorporated tagged mineral nitrogen in soils. The accumulation of mineral nitrogen in a soil is a net effect of two processes which occur

TABLE 3. Effect of dung and nitrogen fertilizers on hydrolysable NH_4^+ amino-sugar-N fraction (ppm) of soil.

Soil + Treatment	Incubation period (days)							Average
	16	32	50	70	80	100	110	
Soil*	213.7	233.7	234.1	252.9	267.7	279.4	300.2	254.5
Ammonium sulphate*	246.9	260.4	274.3	274.1	278.8	296.1	325.8	279.5
Urea*	253.4	287.2	292.9	295.6	297.3	308.6	322.7	294
Calcium ammonium nitrate*	244.4	264.2	292.1	293.5	311.3	318.6	368.6	299
Urea-ammonium phosphate*	241.4	268.4	278.2	282.6	302.2	306.1	312.2	284.4
Nitrophosphate*	249.6	272.3	249.2	278.0	258.3	378.1	312.7	285.5
Average*	241.6	264.4	270.1	279.5	285.9	314.5	323.7	
Dung**	257.4	286.6	289.4	288.1	310.6	328.1	375.6	305.1
Dung + ammonium sulphate**	305.9	317.3	316.9	325.1	347.2	351.8	363.0	332.5
Dung + urea**	299.0	296.6	339.6	330.5	366.0	381.8	417.1	347.2
Dung + calcium ammonium nitrate**	319.6	319.1	344.6	325.2	352.1	354.5	388.7	343.4
Dung + urea ammonium phosphate**	313.2	321.7	328.5	343.4	360.4	358.1	366.1	341.6
Dung + Nitrophosphate**	317.7	324.1	341.2	345.9	355.8	368.1	390.2	349
Average**	302.1	310.9	326.7	326.4	348.7	357.1	383.5	
Average of all treatment	271.9	287.7	298.4	303.0	317.3	335.8	353.6	

*$LSD_{0.05}$ = 18.8 **$LSD_{0.05}$ = 18.6

TABLE 4. Effect of dung and nitrogen fertilizers on amino-acid-nitrogen (ppm) fraction of soil.

Soil + Treatment	Incubation period (days)							Average
	16	32	50	70	80	100	110	
Soil*	355.0	323.0	279.0	231.0	187.0	80.0	49.0	214.9
Ammonium sulphate*	386.0	356.0	328.0	282.0	271.0	80.0	37.0	248.6
Urea*	382.0	317.0	278.0	258.0	162.0	80.0	38.0	216.4
Calcium ammonium nitrate*	470.0	353.0	309.0	283.0	239.0	99.0	36.0	255.6
Urea-ammonium phosphate*	610.0	361.0	342.0	260.0	182.0	77.0	45.0	268.1
Nitrophosphate*	393.0	354.0	332.0	292.0	257.0	80.0	52.0	251.4
Average*	432.7	344.0	311.3	267.7	216.3	82.7	42.8	
Dung**	612.0	594.0	569.0	557.0	247.0	102.0	29.0	387.1
Dung + ammonium sulphate**	715.0	676.0	669.0	625.0	510.0	150.0	129.0	496.3
Dung + urea**	744.0	688.0	562.0	513.0	361.0	118.0	60.0	435.1
Dung + calcium ammonium nitrate**	724.0	657.0	628.0	570.0	354.0	108.0	61.0	443.1
Dung + urea ammonium phosphate**	751.0	656.0	640.0	618.0	587.0	124.0	84.0	494.3
Dung + Nitrophosphate**	735.0	708.0	608.0	595.0	524.0	130.0	71.0	481.6
Average**	713.5	663.2	612.7	579.7	430.5	122.0	72.3	
Average of all treatment	573.1	503.6	462.0	423.7	323.4	102.4	57.6	

*$LSD_{0.05}$ = N.S. **$LSD_{0.05}$ = N.S.

simultaneously but oppose each other, *i.e.*, mineralization and immobilization. The content of mineral nitrogen after an incubation period of 110 days in case of the control was 97.4 ppm. It was increased to 201.3, 211.5, 215.6, 225.0 and 238.6 ppm by the application of 200 ppm of fertilizer nitrogen as nitrophosphate, ammonium sulphate, urea ainmonium phosphate, urea and calcium ammonium nitrate, respectively. The highest concentration of mineral nitrogen in samples treated with calcium ammonium nitrate may be partly due to the calcium content of the fertilizer which might have maintained favourable conditions for nitrifying bacteria by controlling the decline in soil pH (Millbank, 1959; El-Sayed, 1997 a&b and El-Sayed, 1998) and partly due to a lesser amount of NH_4-N present in this fertilizer. There is already considerable infoimation showing that NO_3-N is as a good source of nitrogen as ammonium for the heterotrophic microflora of the soil but that in the presence of NH_4 and NO_3 the microorganisms use ammoniurn preferentially (Jansson *et al.*, 1955; Broadbent & Nakashima, 1970; Metwally & Khamis, 1998 and Hassouna & Hassanein, 2000). The mineral nitrogen decreased rapidly at first and changed slowly thereafter. Broadbent (1968); Stewart *et al.* (1963 a&b); Khamis & Metwally (1998) and El-Zaher nd El-Kafoury (1999) noted that under conditions favouring biological immobilization, there is an initial period of very rapid decrease in the level of inorganic nitrogen which reaches a minimum within two weeks or less, followed by a period of net mineralization of nitrogen after the needs of the soil population are met. The data in Table 1 indicate that the incorporation of fertilizer nitrogen into non KCl extractable fractions occurred relatively quickly in the urea and urea ammonium phosphate treatments, more than in treatments with ammoniacal and nitrate forms of fertilizers. The results are consistent with the findings of Overrein (1970); Beheiry *et al.* (1998) and Hassanein & El-Shebiny (2000).

A small amount of nitrogen could be accounted for as exchangeable ammonium, but most of the mineral nitrogen was in nitrate form. Nitrate nitrogen increased further with increased incubation tune. Most of the fertilizer nitrogen was transformed into a form resistant to acid hydrolysis and some of it was converted into hydrolysable ammonium + amino sugar and amino acid forms. Nommik (1957); Aziz *et al.* (1998) and Hassanein and Hassouna (2000) have described as a function of organic matter the situation in which well decomposed organic matter (humic substances) and living or fresh organic residues exist as two interacting organic pools in which applied nitrogen repeatedly cycles and gradually reverts to increasingly more stable forms (El-Sayed, 1995 and Kristensen *et al.*, 2000).

When dung was applied to the samples, the accumulation of mineral nitrogen was reduced and was found to be even lower than in the controls. One interesting feature is that the amounts of mineral nitrogen in dung and (dung + fertilizer) treated samples were almost similar up to 80 days of incubation, though in later treatments, 200 ppm fertilizer nitrogen were added. These results indicate that microorganisms derived some nitrogen from the native organic source and that the conversion of the NH_4-N to the NO_3-N form under these conditions was as rapid as was the conversion of organic nitrogen to NH_4. The results correspond to the findings of Walker *et al.* (1956); Arafat (1998) and El-Douby *et al.* (2001) who reported an accelerated mineralization of humus in soil after addition of flesh organic substances. When dung was mixed in the samples along with fertilizer, the amino acid content in the initial stages was found to be higher than in samples treated with dung alone. This may be due to more favourable conditions for microbial growth. Microorganisms decomposing the organic matter multiple rapidly, resultin, in vigorous synthesis of microbial protoplasm. The protoplasm has a rather high N content (3-12%), in most cases higher than the decomposing subutrate. The mineral nitrogen will, therefore, be used for the formation of that protoplasm and, if the C/N ratio of the substrate is wide, all the mineralized nitrogen will be reabsorbed by microorganisms and converted into amino acids as body building material (Stewart *el al.*, 1963 a; Cheng & Kurtz, 1963; Chu & Knowles, 1966; Hamouda *et al.*, 1999; Allam *et al.*, 2001 and Farghly, 2001). The results are in conformity with the observations of Broadbent (1968) who found less ammonium and non hydrolyzable nitrogen and more amino acid nitrogen during initial periods of fertilizer application followed by their transformation into organic substances of increasing molecular weight and low availability (Malpassi *et al.*, 2000).

After 100 days of incubation, the mineral nitrogen content in samples treated with dung plus fertilizer increased over control. This may be due to narrowing of the C/N ratio of the dung as decay proceeds and the energy supply diminishes. The microbial population decreases because of the reduced food supply and ultimately a new equilibrium is reached. Therefore, if a crop is to receive a continual supply of nitrogen during the period of its growth, it is necessarv that the mineralization rate exceeds that of immobilization, the usual situation in nature. The average mineral nitrogen which was 29.4 ppm after 16 days of incubation increased to 135.7 ppm (Table 2) after 110 days. The effect of this increase in mineral nitrogen was observed on the amino acid fraction of nitrogen which decreased sharply. After 16 days of incubation, the average figures for amino acid content were 432.7 ppm in fertilizer and 713.5 ppm in fertilizer + dung treatments, and these decreased to 42.8 and 72.3 ppm, respectively (Table 4) after 110 days. Hydrolyzable (ammonium + the amino sugar nitrogen fraction) Table 3 showed a slight gain. Thus it seems that the amino acid fraction has been the most important in contributing to the release of nitrogen in mineral form. Stewart *et al.* (1963 a&b); Habib *et al.* (2001) and Shalan *et al.* (2001) found that changes in the nondistillable acid soluble nitrogen fractions of soil hydrolysates (largely amino acid N) were closely related to inverse changes in inorganic nitrogen of the soil and underwent fairly rapid turnover in their experiment. The results further correspond to the findings of Porter *et al.* (1964); Moore &Russell (1968) and El-Amry *et al.*, 2001) who observed a marked decline in amino acid content of a soil after crop harvesting. They believed that amino acid nitrogen in soils might be more susceptible to mineralization than other forms of organic soil nitrogen. Cornfield (1957) also noted earlier that amino acid content of soils is affected by cropping, cultivation and fertilizer application.

Conclusions

The accumulation of mineral nitrogen in a soil is a net effect of two processes which occur simultaneously but oppose each other, *i.e.*, mineralization and immobilization.

Immobilization of nitrogen occurred when fertilizer was applied to the soil. It became more pronounced in the presence of unhumified organic matter.

Dung behaved as an efficient nitrification inhibitor and slowed the release of nitrogen from fertilizers.

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تمثيل ومعدنة النيتروجين المتأثر بإضافة الأسمدة فى التربة

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تم دراسة عمليات المعدنة وتمثيل العناصر فى التربة المضاف إليها كل من كبريتات الأمونيوم، ونترات الكالسيوم الأمونيومى، واليوريا، وفوسفات اليوريا الأمونيومى، والنيتروفوسفات إلى جانب سماد الروث الجاف.

تتحول الأسمدة النيتروجينية إلى عناصرها الأولية بدون إستخلاصها بكلوريد البوتاسيوم ويحدث فى مدى ١٦ يوما، ويكون سريعا نسبيا فى العينات المعاملة مثل اليوريا، وفوسفات اليوريا الأمونيومى.

تم دراسة عنصر النيتروجين خلال فترة التحضين ووجد أن نسبته مرتفعة فى التربة المعاملة بنترات الكالسيوم الأمونيومى.

توجد الأحماض الأمينية النيتروجينية كمكون أساسى كأجزاء رئيسية معقدة خلال عمليتى المعدنة وتمثيل العناصر فى نيتروجين التربة.

يتصرف سماد الروث سلوكا فعالا بواسطة مثبط النترتة (التأزت)، حيث يتحرر النيتروجين ببطء من خلال الأسمدة.