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INFLUENCE OF *AZOTOBACTER* INOCULATION ON THE AVAILABILITY OF NITROGEN IN SOIL

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Abstract

The change of N in the availability pool was sigmoidal with control and P; sinusoidal with inocula and P as well as with organic matter and P. The response was quasi-sigmoidal with organic matter and inocula. Amongst the single and dual combinations, *Azotobacter chroococcum* in the presence of organic matter performed better. Interaction of inocula, P and organic matter showed flattened to sharp sigmoidal change in the availability of N. Both *A. chroococcum* and *A. macrocytogenes* in the presence of organic matter and higher dose of P exhibited better influence with well defined crest. Between the two inocula, *Azotobacter chroococcum* was more effective. Nitrogen availability was maximal at 20-day in all treatments under the experimental conditions.

Introduction

A few groups of soil inhabiting nitrogen fixing microorganisms convert molecular nitrogen into organic utilizable nitrogenous compounds. *Azotobacter*, a free-living nitrogen fixer is reported to influence yield of rice (Bhuiya and Mian 1977). Alongwith carbonaceous material the supply of phosphorus exert profound influence on microbial activity (Vancura and Macura 1960).

Inoculation of *Azotobacter* cells in the presence of organic matter, phosphatic and potassic fertilizers with respect to the availability of nitrogen in soil has not been reported in Bangladesh. With this end in view, an inoculation-incubation experiment was set up to evaluate the effects of two *Azotobacter* species for enhancing nitrogen mobilization. Addition of organic matter and

phosphorus as well as the combinations thereof on the availability of nitrogen in a nutrient deficient soil was investigated.

Materials and Methods

Soil sample was collected (0-15 cm) from cutlivated non-calcareous, Mirpur series, Dhaka. The chemical characteristics of the soil sample are presented in Table 1. According to USDA (1971) Soil Taxonomy. thc soil may be categorised as Fluvaquentic Haplaquept group.

Table 1. Chemical characteristics of the soil sample examined.

Soil series	pH	Organic matter (%)	CEC (meq/ 100g soil)	Available N	Available P (mg/100g soil)	Available K
Mirpur	5.8	0.72	5.5	9.0	0.6	130

Available NPK in the soil sample was extracted by Morgan's extractant. Determinations were made for pH with a Pye pH meter (soil : water ratio being 1:2.5), organic matter by wet oxidation method (Walkey and Black 1934), N by Kjeldahl method, P by chlorostanous reduced molybdophosphoric blue colour method by using Coleman Junior II Spectrophotometer, K by Flame Photometry using EEL Flame Photometer and CEC by normal ammonium acetate method.

The soil sample was processed and treated with three doses of phosphatic fertilizer (0, 85 and 170 kg P_2O_5/ha) and two doses of cowdung (0 and 1000 kg/ ha) as organic matter with a basal dose of potassic fertilizer (55 kg K_2O/ha). The treated soil samples (300g per container) were allowed to equilibrate at 30°C for two days at field moisture capacity prior to incubation. Thick suspension of *Azotobacter* cells (1.0 0.D at 600 nm) representing approximately 10^9 cells/ml was prepared from two species of *Azotobacter* (*A. chroococcum*, B_1 and *A. macrocytogenes*; B_2). The cultures were inoculated to the soil samples (1 ml/300g soil) and subjected to incubation for O, 10, 20 and 30 days. Identiflcation of *Azotobacter* species was done according to the method of Norris and Chapman (1968). At the and of each run, available nitrogen was determined. The experiment was arranged in a completely randomized block design with two replications.

Results and Discussion

The impact of *Azotobacter* inocula, phosphorus and organic matter as well as their combinations on the availability of nitrogen in the soil was studied. Results obtained are presented in Figs. 1-4.

Both the control and phosphorus treated samples showed a sigmoidal change in the availability pool of nitrogen (Fig. 1, curves II-IV). The increase in availability of nitrogen was maximum at 20-day run. Phosphorus curves deviated from the control depending upon the doses. Higher dose of phosphorus (170 kg P_2O_5/ha) was stimulatory or had some effect on the pool. This apparently caused a low availability possibly due to immobilization. In contrast, increase in nitrogen availability was evident with the lower dose of phosphorus(85 kg P_2O_5/ha). It may be presumed that addition of this low amount of phosphorus had modified the phosphorus deficiency in the soil and thus the changed situation probably favoured the mineralization. Alternatively, this perhaps could not

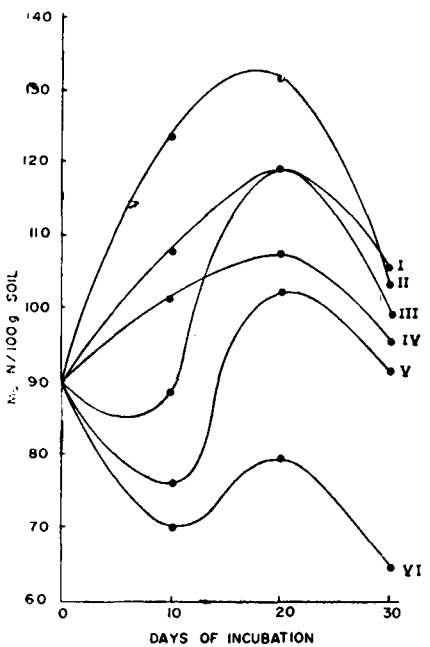

Fig. 1. Availability of nitrogen as influenced by inocula, phoshorus and organic matter.
Legend : I=C_1, II=P_1, III=Control. IV=P_2, V=B_2, VI=B_1.
B_1, B_2=*Azotobacter chroococcum* and *A. macrocytogenes* respectively;
P_1, P_2=85 and 170 kg P_2O_5/ha respectively.
C_1=10000 kg organic matter/ha.

create conditions congenial for vigorous microbial activity and/or the rate of mineralization exceeded the increased demand of microbes. Consequent upon the phenomena a discrease in nitrogen availability had resulted.

The application of organic matter and inocula individually (Fig. 1, curves I, V-VI) and the combinations of inocula and phosphorus (Fig. 2, curves II-V), and the combinations of inocula and phosphorus (Fig. 3, curves III, V) influenced the availability of nitrogen more or less in the same fashion. The availability curve for those are almost sinusoidal (horizontally oriented S-type) and are indicative of continual ups and downs in the availability of the nutrient. After an initial depression upto about 10 days, the curves showed a rise in the availability pool and attained a maximal value at about 20-day followed by a

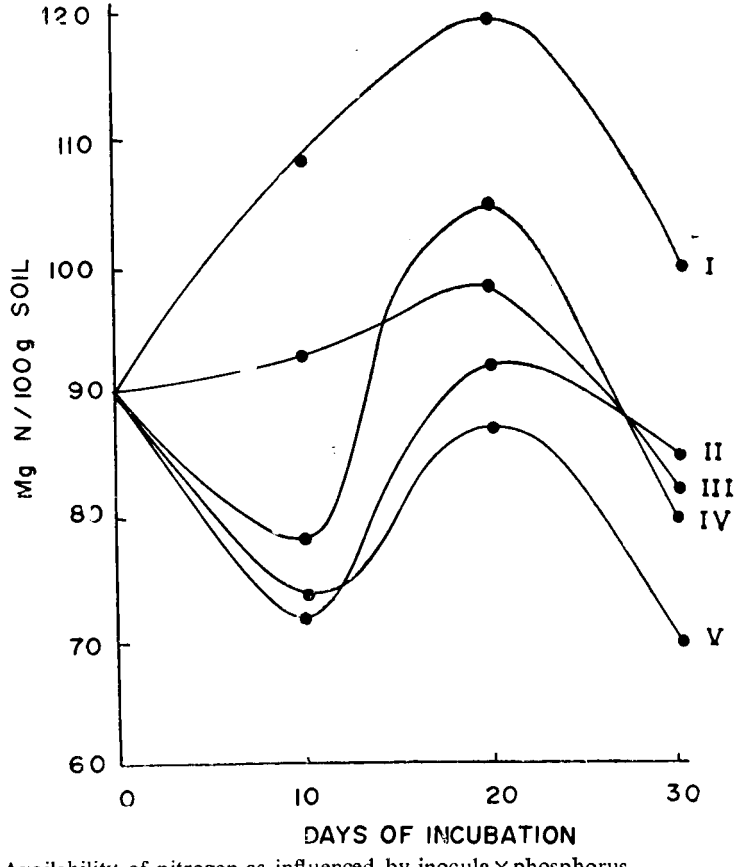

Fig. 2. Availability of nitrogen as influenced by inocula × phosphorus.
I=Control, II=B_2P_1, III=B_2P_2, IV=B_1P_2, V=B_1P_1
For Legend : see Fig. 1.

fall. The trough attained during the initial period of incubation was probably due to the utilization of the mineralized nitrogen by the inocula and native microbes for their growth and activity. The trough shifted towards the left in the organic matter treated sample (Fig. 1, curve I). The presence of energy source possibly enhanced the depletion of available nitrogen due to assimilation by the growing microbes in the initial stage of incubation as compared to other treatments. At 20-day run, the availability afflux may possibly be due to the mineralization of native organic nitrogen which might have exceeded the bacterial utilization. The declining phase common in all the Figures after 20-day was probably due to exhaustion of mineralizable materials in the samples. The inocula might have used sufficient nitrogen for their growth and multiplication

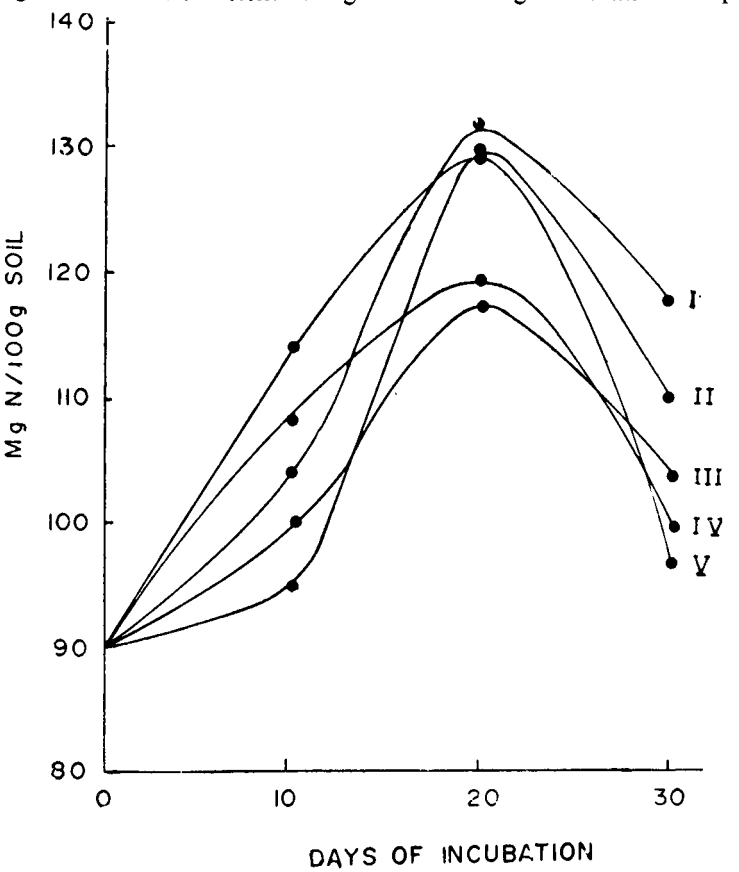

Fig. 3. Availability of nitrogen as influenced by inocula × organic matter, I=B_1B_2, II=C_1B_1, III=C_1P_1, IV=Control, V=C_1P_2.
For Legend : see Fig. 1.

causing a temporary shortfall in availability pool. Although phosphorus alone (Fig. 1, curves II, IV) gave sigmoidal change in available nitrogen but inoculation resulted the curve to a sinusoidal one (Fig. 2, curves II-V) with pronounced reduction in availability at 20-day. However, in the presence of organic matter, nitrogen availability was not appreciable (Fig. 3, curve I-III, V).

The combined effect of organic matter and inocula gave quasi sinusoidal curves (Fig. 3, curves I-II) of available nitrogen and resulted better impact than phosphorus (Fig. 1, curves II, IV), inocula (Fig. 1, curves V-VI), and phosphorus and inocula (Fig. 2, curves II.V) treatments. Both organic matter (Fig. 1, curve I) and inocula alone (Fig. 1, curves V-VI) produced deeper trough. As expected their combinations (Fig. 3, curves I-II) did not

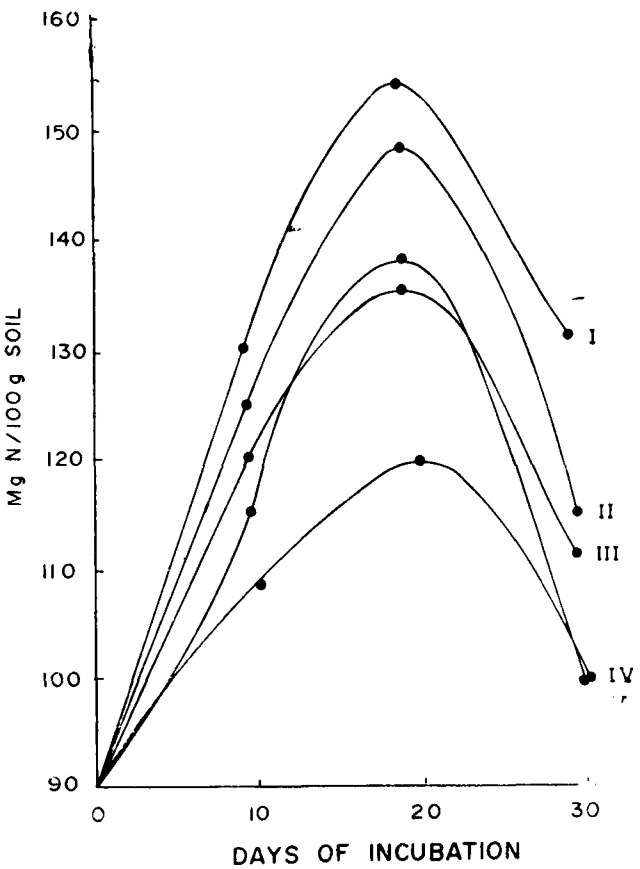

Fig. 4. Availability of nitrogen as influenced by inocula × phosphorus × organic matter. I=$C_1B_2P_2$, II=$C_1B_1P_2$, III=$C_1B_1P_1$, IV=Control, V=$C_1B_2P_1$.
For Legend ; see Fig.1.

cause marked depression in the trough showing a negative interaction. Alexander (1976) also reported the favourable response of bacterial inocula in the presence of organic matter with respect to the availability of nitrogen.

The availabilitv of nitrogen was greatly enhanced due to the applications of inocula, phosphorus and organic matter in the combinations. The availability curves showed flattened sigmoidal to sharp sigmoidal change (Fig. 4, curves I-III, V) and same as lone phosphorus (Fig. 1, curves II, IV). In fact all the combined effect of inocula, phosphorus and organic matter was better than those of lone treatment of inocula, phosphorus, organic matter, and combined effect of inocula and phosphorus, inocula and organic matter, and organic matter and phosphorus. However, the combination of $C_1B_2P_2$ (Fig. 4, curve I) appeared to be the best with well defined crest. This implies that the activty of inocula in the presence of added organic matter coupled with higher dose of phosphorus (170 kg P_2O_5/ha) produced the best results (Fig. 4, curves I-II). It is apparent from the curves that *Azotobacter chroococcum* (B_1) appeared to be superior to *A. macrocytogenes* (B_2) in respect of nitrogen availability.

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Short Communication

RELEASE OF SULPHATE SULPHUR FROM GYPSUM AND ELEMENTAL SULPHUR IN SOIL UNDER SUBMERGED CONDITION

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Submergence of a soil creates unique chemical and biological environment which markedly affects sulphur transformations (Freney and Boonjawat, 1983). Few workers (Freney and Boonjawat 1983, Kamprath and Till 1983) have studied the sulphur transformations occurring in flooded soils as a whole and even fewer (Chaudhury and Cornfield 1967) have examined the reactions occurring in the aerobic or anaerobic zones. A measure of relative effectiveness of sulphate and elemental sulphur as plant nutrients on lowland and upland soils is needed. In this study release of sulphate sulphur from gypsum and elemental S in soil under submerged condition was observed in an incubation experiment.

Soil samples (0-15 cm depth) collected from Naraibag and Kalma series were silty loam and silty clay, pH being 6.7 and 7.7, organic matter, 2.05 and 0.94 per cent; total and available S, 32.9, 350 and 9.5, 210 ppm respectively. 10g (2 mm) soil sample was taken in each test tube (2.5 cm x 15 cm size) and mixed thoroughly with gypsum and elemental S each @ 0, 10, 20, 30 and 40 kg S/ha. A separate treatment containing calcium oxide (70.5 kg/ha) was also included in the experiment to compare the additional effect, if any, of calcium coming fromgypsum. The test tubes were kept submerged (3 cm water above soil surface) and covered with polythene film. The treated samples were incubated at 30°C ± 1°C. The treatments with three replications were randomized in block.

Soil samples were collected periodically at 0, 7, 15, 30 and 60 days of incubation to determine calcium dihydrogen phosphate (500 ppm P) extractable sulphate turbidimetrically following the method of Chesin and Yien (1950) modified by Sakai (1978).

The release of sulphate from gypsum and elemental S in Naraibag and Kalma soils during every sampling was statistically significant at 5 to 0.1 per

cent level (Figs. 1 - 2). The data showed that amount of sulphate estimated varied with rates and sources of added S. However, the trend of sulphate release

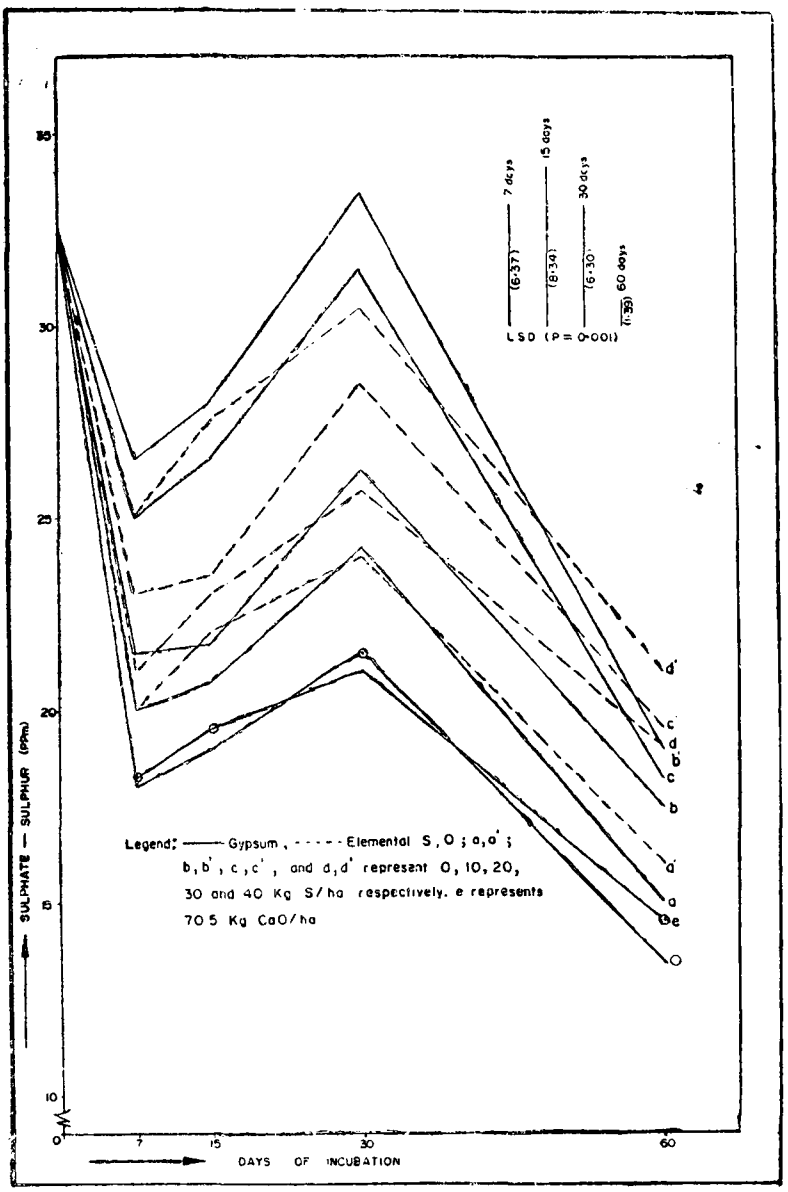
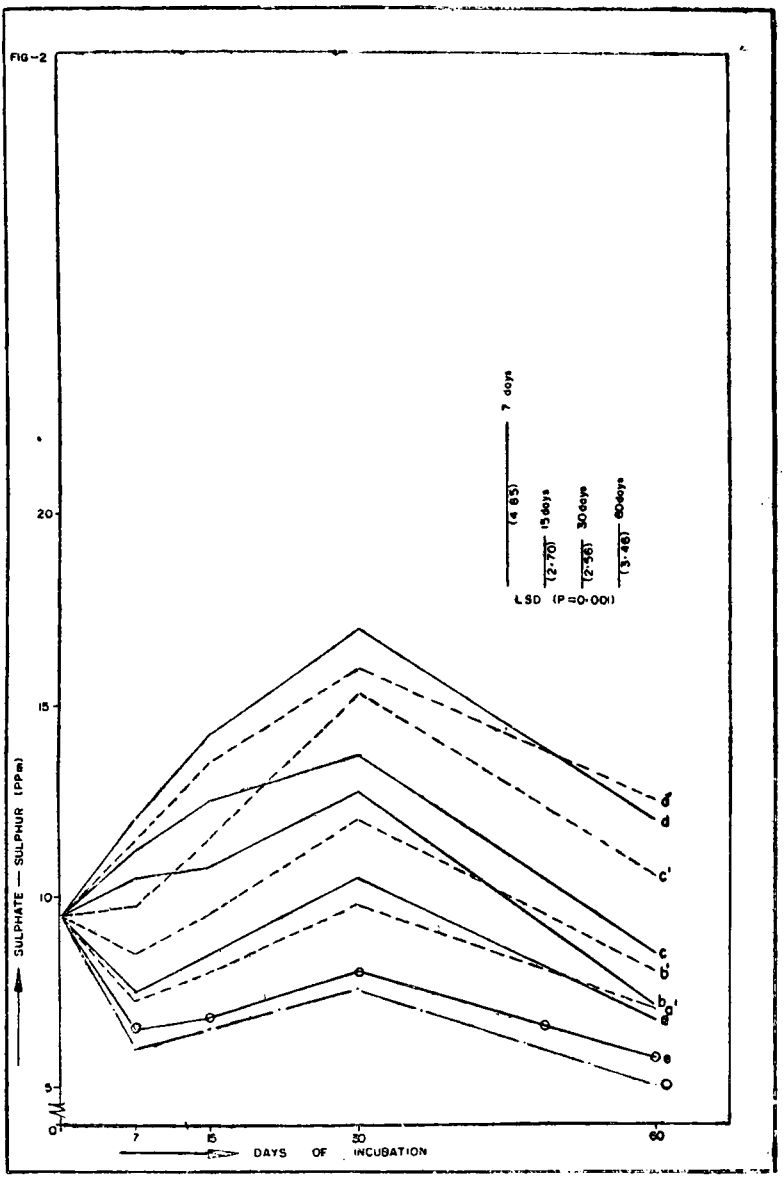


Fig. 1. Changes in extractable sulphate with time in Naraibag soil.
Legend : O; A, A'; B, B'; C, C'; and D, D' represent 0, 10, 20, 30 and 40 kg S/ha respectively. E represents 70.5 kg CaO/ha.



Days of incubation

Fig. 2. Changes in extractable sulphate with time in Kalma soil. Legend same as Fig. 1.

was similar in both the soils. The amount released from two sources did not differ very much. The release of sulphate increased gradually from 7 days of incubation with an initial immobilization upto 7 days in most of the treatments On reaching maximum the value decreased slowly with time. Sakai (1978) also

reported from an incubation experiment the highest concentration of sulphate after 7 days. This was followed by gradual and sharp decline with time. Anaerobic incubation of soil caused an increased mineralization of sulphur as reported by a number of workers (Freney 1958; Barrow 1961; Williams 1967; Tabatabae and Bremner 1972).

It can be seen that in either of the soils maximum amount of sulphate recorded was at 30 days run. With time the concentration of sulphate decreased. This declining phase may be associated with microbial immobilization of S. Similar views were also expressed by Alexander (1961) who reported that the inorganic product of S is utilized by the microflora for cell synthesis.

On comparison of the sources, it may be seen that the amount of sulphate released from gypsum during 60 days run was significantly lower than from elemental S irrespective of the treatments in both the soils. However, the amount of sulphate measured during 30 days run was slightly higher from gypsum than from elemental S though not significantly. This suggested that assimilation of sulphate by soil population is favoured by gypsum.

It is noted that about 30 to 74 per cent and 23 to 66 pe cent of S was mineralized during 60 days incubation from Naraibag and Kalma soil respectively. Attoe and Olson (1965) reported that at the end of a 4-week incubation period, nearly 50 per cent of added sulphur had been oxidized. Though low, Zhu *et al.* (1982) also found that 3.8 to 15.6 per cent of the organic sulphur was mineralized during a 10-week incubation of soil under saturated conditions. Mineralization of only about 2 per cent of organic sulphur during two week incubation period was reported by Williams (1967). This variable rate of mineralization might be associated with soil properties and environmental conditions. The release of sulphate was generally higher in Naraibag soil as compared to Kalma soil irrespective of the treatments. This was probably due to higher organic matter content of the former soil.

Soil samples treated with calcium oxide showed no significant change in extractable sulphate in both the soils over control. This indicated that calcium in gypsum had either limited or no contribution on sulphate release from gypsum.

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