Source D 145 References 1/1/1977 0054

Effects of Trace Elements on Phosphatase Activity in Soils[1]

N. G. JUMA AND M. A. TABATABAI[2]

ABSTRACT

Studies to evaluate the effects on the activity of acid and alkaline phosphatases of 20 trace elements showed that all trace elements inhibit the activity of phosphatases in soils. Results showed that the relative effectiveness of the trace elements in inhibition of phosphatase activity depends on the soil. When the trace elements were compared by using 25 μmole/g soil, the average inhibition of acid phosphatase in three soils showed that Hg(II), As (V), W(VI), and Mo(VI) were the most effective (average inhibition >50%) and that Ba(II), Co(II), and Cr(III) were the least effective (average inhibition <10%) inhibitors. Other metals that inhibited acid phosphatase activity in soils were: Cr(II), Ag(I), Cd(II), Cu(II), Zn(II), Mn(II), Sn(II), Ni(II), Pb(II), Fe(III), B(III), Al(III), V(IV), and Se(IV); their degree of effectiveness varied in the three soils used.

The most effective inhibitors of alkaline phosphatase activity in soils were Ag(I), Cd(II), V(IV), and As(V). All the trace elements that inhibited acid phosphatase activity also inhibited alkaline phosphatase activity. The order of effectiveness of the trace elements in inhibition of alkaline phosphatase activity was different from that of inhibition of acid phosphatase activity. Generally, the inhibitory effect of trace elements was less in the presence of 2.5 μmole/g soil than in the presence of 25 μmole/g soil. In addition to the effect of trace elements, buffer ions inhibited acid and alkaline phosphatase activities in soils. Hydrated anions such as NO_3^-, NO_2^-, Cl^-, and SO_4^{2-} were not inhibitory in the presence and absence of buffer.

Additional Index Words: acid phosphatase, alkaline phosphatase, soil metals, soil enzymes, soil pollution, orthophosphate.

SOIL POLLUTION by heavy metals and other trace elements[3] is one of the major environmental problems associated with some industries. Use of As, Ba, B, Cd, Co, Cr, Cu, Mn, Hg, Mo, Ni, Se, Ag, Sn, V, and Zn and their compounds have been summarized recently by Page (12). Also, several of these elements are added to soils as fertilizers, as impurities in fertilizers, or as components of municipal and industrial wastes (1,2,9). In addition, some of these elements (e.g., Pb, V) are present in fuel oils and gasoline, emitted into the atmosphere upon burning and deposited on soils, especially near urban areas (9,18).

Research dealing with the chemistry of trace elements in soils and the toxicity of the various elements to biological systems, including accumulation in plants, is extensive (3,8,9), but little is known about the relative effects of trace elements on the biochemical processes in soils. Addition of trace elements to soils may affect microbial proliferation and enzymatic activities, possibly leading to a decrease in the rates of the biochemical processes in the soil environment. Phosphatases in soils and plant roots are largely responsible for mineralization of organic P in soils (4). It has been shown that several of these elements (e.g., Cu, Zn, Mo, W) inhibit potato (*Solanum tuberosum*) acid phosphatase activity (11) and that Mo inhibits acid phosphatase activity of tobacco leaves (*Nicotiana tabacum* L.) (13) and tomato roots (*Lycopersicon esculentum*) (15). Recently, Tyler (17) found a significant negative regression coefficient (−0.81***) between acid phosphatase activity and the sum of Cu and Zn concentrations in spruce (*Picea abies* L.) needle mor coniferous woodland around a brass foundry in Sweden. Also, while this manuscript was in preparation, a paper was published by Tyler (18) showing that V inhibits acid phosphatase activity in mor soils. Review of the literature revealed little information on the relative effectiveness of trace elements in inhibition of activity of soil acid and alkaline phosphatases. Therefore, this study was conducted to evaluate the effect of equimolar concentrations of trace elements on the activity of these enzymes in soils. Results obtained showed that all the 20 trace elements used inhibited the activity of acid and alkaline phosphatases in soils.

MATERIALS AND METHODS

The soils used (Table 1) were surface (0- to 15-cm) samples selected to obtain a range in pH, texture, organic matter content, and phosphatase activity. Before use, each sample was air dried and crushed to pass a 2-mm screen. In the analyses reported in Table 1, pH was determined by a glass electrode (soil/water ratio, 1:2.5), organic C by the method of Mebius (10), particle-size distribution by the pipette analysis of Kilmer and Alexander (7), and phosphatase activity by the method of Tabatabai and Bremner (16).

The trace elements used were Fisher certified reagent-grade chemicals. Of these, Ag(I), Cu(II), Cd(II), Zn(II), Fe(II), Co(II), and V(IV) were added as the sulfate; Cu(I), Hg(II), Sn(II), Mn(II), Ni(II), Ba(II), Fe(III), Cr(III), and Al(III) as the chloride; Pb(II) as the acetate or nitrate, and As(III), B(III), Se(IV), As(V), Mo(VI), and W(VI) as $NaAsO_2$, $Na_2B_4O_7$, H_2SeO_3, Na_2HAsO_4, H_2MoO_4, and Na_2WO_4, respectively.

In testing the effects of trace elements on activity of phosphatases, 1 g of soil in a 50-ml Erlenmeyer flask was treated with 1 ml of solution containing either 2.5 or 25 μmole of trace element. This solution was added dropwise to moisten the whole soil sample. After a 30-min equilibration, phosphatase activity was determined by the method of Tabatabai and Bremner (16). This method involves colorimetric determination of the *p*-nitrophenol released by phosphatase activity when soil is incubated with 3 ml of universal buffer, 1 ml of 5 m*M* buffered sodium *p*-nitrophenyl phosphate solution, and 0.25-ml toluene at 37°C for 1 hour. Universal buffer of pH 6.5 was used for assay of acid phosphatase activity and of pH 11 was used for assay of alkaline phosphatase activity as described by Eivazi and Tabatabai (5). The results of the phosphatase activity from trace element-treated soils were compared with those obtained with 1 g of soil treated with 1 ml of water. Percentage inhibition of phosphatase activity by each trace element was calculated from $(A - B/A)100$, where A is phosphatase activity of

Table 1—Properties of soils used.

Soil	pH	Organic C	Clay	Silt	Sand	Phosphatase activity† Acid	Alkaline
		%					
Harps	7.8	3.74	30	44	26	78	230
Okoboji	7.4	5.45	34	50	16	159	235
Webster	5.8	2.58	23	39	38	210	20

† *p*-Nitrophenol released per g soil/hour.

[1] Journal Paper J-8532 of the Iowa Agric. Home Econ. Exp. Stn. Ames, IA. Projects 1868, 2082, and 2112. Received 23 June 1976. Approved [illegible] 1976.

[2] Graduate Research Assistant and Associate Professor, respectively. Dept. of Agronomy, Iowa State Univ., Ames, IA 50011.

[3] The term *trace element* is used here to refer to elements that are, when present in sufficient concentrations, toxic to living systems.

9223

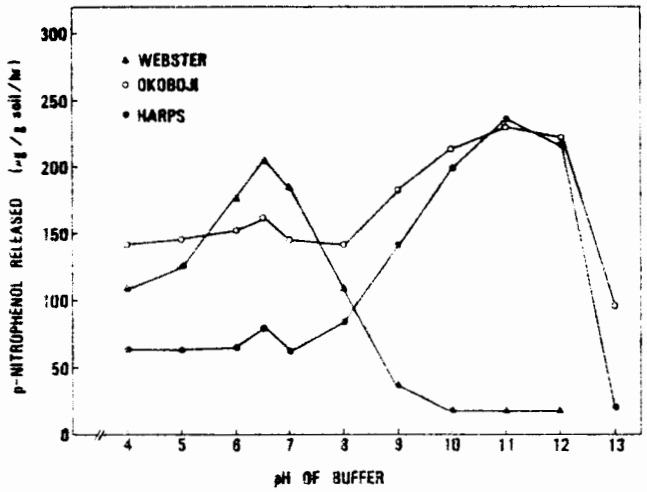

Fig. 1—Effect of pH of buffer on release of *p*-nitrophenol in assay of phosphatase activities in soils.

untreated soil and *B* is phosphatase activity of trace element-treated soil. All results reported are averages of duplicate analyses.

RESULTS AND DISCUSSION

Most soils show the greatest phosphatase activity near neutral pH and usually different optimum pH values are obtained by different buffer systems. The distinction between acid and alkaline phosphatases is based upon the marked difference in the pH ranges in which these enzymes are active. Therefore, an important requirement for the detection of acid and alkaline phosphatases is the use of the same buffer throughout the pH range desired. The universal buffer used has a buffering range from 3 to 12 and is suitable for this type of study. Using this buffer system, Eivazi and Tabatabai (5) recently showed that acid phosphatase [illegible] is predominant in acid soils and that alkaline phos[illegible] activity is predominant in alkaline soils, similar [illegible] shown in Fig. 1 and Table 1.

The effects of trace elements on acid phosphatase [illegible] in soils varied considerably among soils (Table 2). [illegible]ever, Hg(II), Cd(II), V(IV), As(V), W(VI), and [illegible] were the most effective inhibitors of the activity of [illegible]zyme in soils. The average inhibition observed [illegible] three soils by using 25 μmole trace element/g soil [illegible] from 48% with Cd(II) to 69% with W(VI). But only [illegible] As(V), W(VI), and Mo(VI) showed average in[illegible] >50%. Also, As(V) was a much stronger inhibitor than As(III). This is of importance because As(III) is readily oxidized to As(V) in soils under aerobic conditions. With few exceptions, reduction of the amount of trace elements added per g of soil by 10-fold (from 25 μmoles to 2.5 μmoles) decreased the degree of inhibition of acid phosphatase activity by these elements (Table 2). Other trace elements that inhibited acid phosphatase activity in soils [illegible] Cu(I), Ag(I), Cu(II), Zn(II), Mn(II), Sn(II), Ni(II), [illegible] Fe(II), Ba(II), Co(II), Cr(III), Fe(III), B(III), Al(III), [illegible] Se(IV). Of these Ba(II), Co(II), and As(III) were the [illegible] effective inhibitors (average inhibition <10%). Lead [illegible]tate inhibited acid phosphatases activity in the three [illegible] the same extent as lead nitrate.

Table 3 shows the effects of trace elements on [illegible] phosphatase activity in Okoboji and Harps soils. Among the 20 elements studied, Ag(I), Cd(II), Hg(II), V(IV), [illegible] As(V) were the most effective inhibitors. With 25 [illegible] trace element/g soil, the average inhibition observed [illegible] the two soils ranged from 47% with Hg(II) to 80% [illegible] Ag(I). But only Ag(I), Cd(II), V(IV), and As(V) showed average inhibition >50%. Like the acid phosphatase activity, reduction of the amount of trace elements added [illegible]

Table 2—Effects of trace elements on acid phosphatase activity in soils.†

Trace element		Percentage inhibition of acid phosphatase activity in soil specified			
Element	Oxidation state	Harps	Okoboji	Webster	Average
Cu	I	28	43	51(18)	40.7
Ag		38	15	9(9)	20.7
Hg	II	52	53	63(12)	56.0
Cd		51	48	44(14)	47.7
Cu		26	36	44(11)	35.3
Zn		32	33	30(5)	31.7
Mn		16	12	62(11)	30.0
Sn		15	21	41(12)	25.7
Ni		19	21	23(11)	21.0
Pb acetate		19	17	24(3)	20.0
Pb nitrate		18	15	26(4)	19.7
Fe		11	18	27(3)	18.7
Ba		3	8	3(6)	6.3
Co		8	5	1(0)	4.7
Cr	III	25	27	30(5)	27.3
Fe		5	26	40(7)	23.7
B		1	22	33(2)	18.7
Al		8	14	34(10)	18.7
As		1	16	9(4)	8.7
V	IV	45	55	49(30)	49.7
Se		39	34	24(16)	32.3
As	V	39	62	80(33)	60.3
W	VI	45	69	94(75)	69.3
Mo		41	68	93(69)	67.3

† 25-μmole trace element/g of soil. Figures in parentheses indicate percentage inhibition of acid phosphatase activity using 2.5-μmole trace element/g of soil.

Table 3—Effects of trace elements on alkaline phosphatase activity in soils.†

Trace element		Percentage inhibition of alkaline phosphatase activity in soil specified		
Element	Oxidation state	Harps	Okoboji	Average
Ag	I	93	67(28)	[illegible]
Cu		30	32(8)	[illegible]
Cd	II	78	61(27)	[illegible]
Hg		53	41(3)	[illegible]
Zn		59	28(3)	[illegible]
Sn		25	38(4)	[illegible]
Pb acetate		33	21(9)	[illegible]
Pb nitrate		38	15(4)	[illegible]
Cu		20	23(9)	[illegible]
Mn		25	18(9)	[illegible]
Fe		22	20(5)	[illegible]
Ni		22	12(8)	[illegible]
Co		16	6(5)	[illegible]
Ba		17	5(3)	[illegible]
Cr	III	27	39(14)	[illegible]
As		35	19(9)	[illegible]
Fe		19	32(4)	[illegible]
Al		20	25(4)	[illegible]
B		14	12(11)	[illegible]
V	IV	61	60(21)	[illegible]
Se		30	35(15)	[illegible]
As	V	75	57(32)	[illegible]
W	VI	29	32(5)	[illegible]
Mo		25	22(12)	[illegible]

† 25-μmole trace element/g of soil. Figures in parentheses indicate percentage inhibition of alkaline phosphatase activity using 2.5-μmole trace element/g of soil.

fold (from 25 μmoles to 2.5 μmoles) generally re- in a decrease in inhibition of alkaline phosphatase ac- in soils. Also, As(V) was more effective inhibitor of e phosphatase than was As(III). All other trace ele- that inhibited acid phosphatase activity also inhibited e phosphatase activity in soils. The orders of effec- ss of the trace elements as inhibitors of acid phospha- ctivity were different from those as inhibitors of alka- hosphatase activity (Table 1 and 2).

tal ions may inhibit enzyme reactions by complexing bstrate, by combining with the protein active group of nzymes, or by reacting with the enzyme-substrate lex. The mode of inhibition is dependent on the type bstrate used. Little information is available, however, nibition of phosphatases by various metal ions. Studies e effect of metal ions on the action of acid phosphatase ed from tomato leaves have shown that, with *p*-ni- enyl phosphate as a substrate, Cu(II), Zn(II), and I) are strong inhibitors of this enzyme (13). The inhibi- seems of a noncompetitive nature. Metal ions generally ssumed to inactivate enzymes by reaction with sulfhy- groups, a reaction analogous to the formation of a l sulfide(14). Sulfhydryl groups in enzymes may serve tegral parts of the catalytically active sites or as groups ived in maintaining the correct structural relationship of enzyme protein.

he pH values of the trace element solutions varied con- rably. They ranged from 2.1 for the Sn(II) solution to for the As(III) and B(III) solutions. Because of this ation in pH, the percentages inhibition of phosphatase vity of the Harps, Okoboji, and Webster soils by trace ments in the absence of buffer were, in most cases, ter than those obtained for acid phosphatase activity in presence of buffer. In some instances (e.g., B(III) in ps and Okoboji soils), results showed activation of sphatase activity in the absence of buffer. Measurement H of the trace element-treated soils indicated that the pH es of the soil-trace element-water systems varied con- ly and deviated markedly from the pH of the system absence of trace element, indicating that the increase nhibition and the activation observed in the absence of fer were due to changes in the pH of the incubation mix- s. However, tests showed that the acid and alkaline sphatase activities of the soils used were not affected n the 1 g soil was treated with 1 ml of water adjusted to 2 with dilute H_2SO_4 or to pH 10 with dilute NaOH solu- and allowed to equilibrate at room temperature for 30 before assay of phosphatase activity in the presence of fer. The results suggested that the pH of the trace ele- t solutions did not affect phosphatase activity and the erved inhibitions by the trace elements in the presence of fer were totally due to the effect of these ions on acid and line phosphatase activities of soils.

ts showed that with use of buffer pH 6.5 for assay of hosphatase activity, the pH of the soil-buffer mixture from 6.5 with Webster soil to 7.0 with Harps and oji soils. With buffer pH of 11 for assay of alkaline atase activity, the pH of the soil-buffer mixtures was for Harps and Okoboji soils. The deviation in pH ulting from addition of trace elements in the pres- niversal buffer did not exceed ±0.3 pH unit.

Experiments with 25 μmole of NaCl or K_2SO_4/g soil indicated that the Na^+, Cl^-, and SO_4^{2-} associated with the trace elements studied did not have any effect on the activity of acid and alkaline phosphatases in soils.

Because of the similarity in the ionic structure of WO_4^{2-}, MoO_4^{2-}, $HAsO_4^{2-}$, and PO_4^{3-} and because phosphate is added to soils as a fertilizer, we studied the effect of PO_4^{3-} (as KH_2PO_4) on the activity of acid and alkaline phosphatases in the three soils used. Results indicated that 25 μmole phosphate/g soil caused a marked inhibition of acid phosphatase activity, 32% in Okoboji soil, 35% in Webster soil, and 62% in Harps soil. The corresponding percentage inhibition at 2.5 μmole/g soil were 5%, 6%, and 26%, respectively. Weimberg and Orton (19) showed that, like borate, molybdate, and arsenate, phosphate ion inhibits the *S. mellis* (yeast) acid phosphatase activity, although higher concentrations of phosphate were required for the same degree of inhibition as was obtained with either arsenate or molybdate. Inhibition of acid phosphatase activity by these anions seems of a competitive nature because studies by Spencer (15) have shown the competitive behavior of molybdate for the active sites of tomato acid phosphatase. Our work indicated, however, that the phosphate ion was a less effective inhibitor of alkaline phosphatase than of acid phosphatase activity in Harps and Okoboji soils. The percentage inhibition of alkaline phosphatase activity was 28% in Okoboji soil and 40% in Harps soil at 25 μmole phosphate/g soil. The results at 2.5 μmole phosphate/g soil were 2% and 6%, respectively.

Studies of phosphatase activity in the absence of buffer showed that, at 25 μmole/g soil, phosphate ion led to inhibition values ranging from 35% in Webster soil to 69% in Harps soil. The corresponding percentage inhibition at 2.5 μmole/g soil were 14% and 30%, respectively. Related anions such as NO_2^- and NO_3^- were not inhibitory in the presence or absence of buffer.

The inhibition of acid and alkaline phosphatase activities by orthophosphate ion is of particular importance because these enzymes are responsible for hydrolysis of soil organic P and because, besides its addition to soils as a fertilizer, orthophosphate is the end product of P mineralization in soils. Halstead (6) found a positive relationship between the decrease in phosphatase activity of several Canadian soils incubated at room temperature for 9 months and the amount of organic P mineralized over the same period. He also reported, however, that addition of phosphate (concentration not specified) before incubation had no appreciable effect on phosphatase activity in soils. Our results clearly indicate that phosphate ion inhibits both acid and alkaline phosphatase activities in soils. The degree of this inhibition is dependent on the amount of phosphate added.

LITERATURE CITED

1. Allaway, W. H. 1971. Feed and food quality in relation to fertilizer use. p. 533–556. *In* R. A. Olson (ed.). Fertilizer technology and use. Soil Sci. Soc. Am., Madison, Wis.
2. Berrow, M. C., and J. Webber. 1972. Trace elements in sewage sludges. J. Sci. Food Agric. 23:93–100.
3. Chaney, F. C. 1973. Crop and food chain effects of toxic elements in sludges and effluents. p. 129–141. *In* Recycling municipal sludges and effluents on land. Nat. Assoc. State Univ. Land Grant Coll., Washington, D.C.

4. Cosgrove, D. J. 1967. Metabolism of organic phosphates in soil. p. 216–228. *In* A. D. McLaren and G. H. Peterson (eds.). Soil biochemistry. Vol. 1. Marcel Dekker, New York.
5. Eivazi, F., and M. A. Tabatabai. 1977. Effects of trace elements on urease activity in soils. Soil Biol. Biochem. 9(1): 9–13.
6. Halstead, R. L. 1964. Phosphatase activity of soils as influenced by lime and other treatments. Can. J. Soil Sci. 44:137–144.
7. Kilmer, V. J., and L. T. Alexander. 1949. Methods of making mechanical analyses of soils.. Soil Sci. 68:15–24.
8. Kubota, J., and W. H. Allaway. 1972. Geographic distribution of trace element problems. p. 525–554. *In* J. J. Mortvedt, P. M. Giordano, and W. L. Lindsay (eds.). Micronutrients in agriculture. Soil Sci. Soc. Am., Madison, Wis.
9. Lagerwerff, J. V. 1972. Lead, mercury, and cadmium as environmental contaminants. p. 593–636. *In* J. J. Mortvedt, P.M. Giordano, and W. L. Lindsay (eds.). Micronutrients in agriculture. Soil Sci. Soc. Am., Madison, Wis.
10. Mebius, L. J. 1960. A rapid method for the determination of organic carbon in soils. Anal. Chim. Acta 22:120–124.
11. Naganna, B., A. Raman, B. Venugopal, and C. E. Sripathi. 1955. Potato pyrophosphatase. Biochem. J. 60:215–223.
12. Page, A. L. 1974. Fate and effects of trace elements in [illegible] applied to agricultural land: A literature review. Environ[illegible] tection Agency, Cincinnati, Ohio.
13. Shaw, J. 1966. Acid phosphatases from tobacco leaves. [illegible] chem. Biophys. 117:1–9.
14. Shaw, W. H. R., and D. N. Raval. 1961. The inhibition of [illegible] metal ions at pH 8.9. J. Am. Chem. Soc. 83:3184–3187.
15. Spencer, D. 1954. The effect of molybdenum on the [illegible] tomato acid phosphatases. Aust. J. Biol. Sci. 7:151–160.
16. Tabatabai, M. A., and J. M. Bremner. 1969. Use of *p*-[illegible] phosphate for assay of soil phosphatase activity. Soil Biol. [illegible] 1:301–307.
17. Tyler, G. 1974. Heavy metal pollution and soil enzyme activity. [illegible] Soil 41:303–311.
18. Tyler, G. 1976. Influence of vanadium on soil phosphatase activity. J. Environ. Qual. 5:216–217.
19. Weimberg, R., and W. L. Orton. 1963. Repressible acid [illegible] monoesterase and constitutive pyrophosphatase of *Saccharomyces mellis*. J. Bacteriol. 86:805–813.

Immobilization of Fertilizer Nitrate Applied to a Swelling Clay Soil in the Field[1]

D. E. Kissel, S. J. Smith, W. L. Hargrove, and D. W. Dillow[2]

ABSTRACT

Low recoveries of N fertilizer by crops on Houston Black clay prompted this investigation. Our objectives were to determine how much fertilizer nitrate can be biologically immobilized under field conditions when fertilization exceeds crop requirements and to determine when immobilization occurs during the growing season. Calcium nitrate tagged with 9.6 atom % ^{15}N was applied to field microplots at 328 kg N/ha and grain sorghum (*Sorghum bicolor* L.) was planted in rows across each microplot. The various components of the N balance were measured by sampling microplots at different times during the season.

Only 2.1 kg N/ha was immobilized during the first 47 days after fertilization and planting. The rate of immobilization increased rapidly during the next 60 days and an additional 60 kg N/ha was immobilized. This increase was apparently in response to an increase in soil temperature above 22°C.

About 91% of the fertilizer N was recovered, indicating that no more than 9% was denitrified, in spite of ample water that was received as rain or irrigation water during the study. A net immobilization (an increase in soil organic N relative to an unfertilized plot) of 53 to 73 kg N/ha was measured on the fertilized plot. This net immobilization suggests that the organic N content of swelling clay soils that have historically been subjected to exhaustive cropping may increase slowly and gradually reach a higher equilibrium level due to N fertilizer use.

Additional Index Words: **N fertilizer efficiency, N balance, N cycling, ^{15}N.**

Immobilization of inorganic N has received considerable attention in recent years, and the principles of immobilization and mineralization of N in soils have been fairly well established.

Net immobilization of N during the process of plant residue decomposition has been shown to reach a maximum very quickly after the addition of a C source to soil (Allison and Klein, 1962; Winsor and Pollard, 1956). Allison and Klein (1962) found immobilization by wheat straw was maximum about 20 days after the start of decomposition. At that time the C/N ratio in the partially decomposed [illegible] was about 25. After the initial rapid decomposition, [illegible] of mineralization of immobilized N had been fa[illegible] rather slow (Broadbent and Tyler, 1962; Bart[illegible] 1965). However, the rate of mineralization of reco[illegible] mobilized N has been from 10 to 15 times greater [illegible] of indigenous organic N (Broadbent and Nakashima, [illegible] Legg et al., 1971). The relative availability of im[illegible] N declined with time in these studies.

Allison and Klein (1962) concluded that the N [illegible] ment in the decomposition process depended on "[illegible] tion of the materials; on environmental conditions [illegible] fect the nature of the flora and rate of decay; and on [illegible] of incubation." In this regard, Hiltbold et al. (1950) [illegible] that two to four times more N was immobilized in c[illegible] than in fallowed soils. Nômmick (1968) found that [illegible] an originally acid raw humus substantially inc[illegible] mobilization of mineral N.

Although many studies have been reported on the process of N immobilization, only a few have studied its [illegible] and importance under field conditions (Westerman [illegible] 1972). In a recent N balance field study we obs[illegible] considerable applied N was immobilized during the [illegible] ing season (Kissel et al., 1976). The purpose of the pr[illegible] study was to measure the rate of immobilization of applied NO_3^--N during the growing season. A relatively high rate of N was applied to insure that sufficient NO_3^--N was [illegible] able for immobilization throughout the cropping season.

[1]Contribution from Texas Agric. Exp. Stn., Texas A&M Univ. [illegible] Station, Texas, in cooperation with the ARS-USDA, Durant, OK. Received 30 Sept. 1976. Approved 19 Nov. 1976.

[2]Associate Professor, Texas Agric. Exp. Stn., Temple, Tx 7650[illegible]; [illegible] Scientist, USDA Water Quality Management Laboratory, Durant, OK 74701; Technician I, Texas Agric. Exp. Stn., Temple, Tx 7650[illegible]; and Biological Laboratory Technician, USDA Water Quality Management Laboratory, Durant, OK 74701, respectively.