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Factors affecting L-asparaginase activity in soils

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Summary. Studies on the distribution of L-asparaginase in soil profile samples revealed that its activity generally decreases with sample depth and is accompanied by a decrease in organic C content. Statistical analyses indicated that L-asparaginase activity was significantly correlated (**$P<0.01$) with organic C ($r = 0.86$**) and total N ($r = 0.78$**) in the 26 surface soil samples examined. There was no significant relationship between L-asparaginase activity and the percentage of clay or sand. There was, however, a significant correlation between L-asparaginase activity and amidase ($r = 0.82$**) and urease ($r = 0.79$**) activities in the surface samples studied. The effects of 21 trace elements, 12 herbicides, 2 fungicides, and 2 insecticides on L-asparaginase activity in soils showed that most of the trace elements and pesticides, at the concentrations used, inhibited the reaction catalyzed by this enzyme. The degree of inhibition varied among soils. When the trace elements were compared, at the rate of 5 μmol g^{-1} soil, the average inhibition of L-asparaginase in three soils showed that Ag(I), Cd(II), Hg(II), Ni(II), Pb(II), and V(IV) were the most effective inhibitors (average inhibition ≥20%). The least effective inhibitors (average ≤10%) included Cu(I), Ba(II), Co(II), Sn(II), Zn(II), Al(III), Se(IV), As(V), and Mo(VI). Other trace elements that inhibited L-asparaginase activity in soils were Cu(II), Mn(II), As(III), B(III), Cr(III), Fe(III), Ti(IV), and W(VI). When the pesticides were compared, at the rate of 10 μg active ingredient g^{-1} soil, the average inhibition of L-asparaginase activity in three soils ranged from 4% with Merpan to 46% with Malaspray. Other pesticides that inhibited L-asparaginase activity in soils (average inhibition in parentheses) were Aatrex (17%), Alanap (21%), Amiben (18%), Banvel (12%), Bladex (24%), 2,4-D (17%), Dinitramine (19%), Eradicane (16%), Lasso (40%), Paraquat (33%), Sutan (39%), Treflan (7%), Menesan (18%), and Diazinon (33%).

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Recently, a simple method was developed for the assay of L-asparaginase activity (L-asparagine amidohydrolase, EC 3.5.1.1) in soil (Frankenberger and Tabatabai 1991). This enzyme, catalyzing the hydrolysis of L-asparagine with the production of ammonia and aspartic acid, plays an important role in N mineralization (Blagoveshchenskaya and Danchenko 1974). Koloskova and Murtazina (1978) found that L-asparaginase activity was significantly correlated with the humus content ($r = 0.97$), total N ($r = 0.96$), mineral N ($r = 0.64$), and readily hydrolyzed N ($r = 0.80$) in non-chernozemic soils.

L-Asparaginase has been characterized in terms of its molecular structure, mechanism of action, and synthesis during microbial growth. The molecular weight of L-asparaginase produced by *Escherichia coli* is approximately 130000, as determined by sedimentation equilibrium analysis (Wriston 1971). Its tetrameric structure is described as eight half-cystines per mol of *E. coli* B asparaginase, consistent with a four subunit model with one intrachain disulfide linkage per subunit (Greenquist and Wristin 1972). The NH_2-terminal amino acid is leucine and the COOH-terminal amino acid is tyrosine.

Ehrman et al. (1971) have reported two possible mechanisms of action for L-asparaginase catalysis. In mechanism I, the binding of L-asparagine with the enzyme precedes the release of ammonium, and the formation of the aspartyl enzyme. This intermediate reacts with water to form aspartic acid, or reacts with hydroxylamine to form hydroxamic acid. In mechanism II, aspartic acid and ammonium are released simultaneously without involving an acyl intermediate.

DeJong (1972) reported that L-asparaginase production approximately parallels the growth of *Streptomyces griseus* cells. Cells grown aerobically during the exponential phase yielded the highest levels of L-asparaginase. The amino acid, L-asparagine, can serve as the sole N

source for cell metabolism but not as the sole supply of C (Jones and Mortimer 1973). Several reports have shown that L-asparaginase is inducible not only by its substrate, L-asparagine, but also by its products, ammonium and L-aspartic acid (Foda et al. 1974; Eremenko et al. 1975; Albanese and Kafkewitz 1978). However, Jones and Mortimer (1973) reported that L-asparaginase from *Saccharomyces cerevisiae* is constitutive. It is now known that *S. cerevisiae* produces two L-asparaginases, one extracellular and inducible, and the other intracellular and constitutive (Dunlop and Roon 1975).

Studies of N cycling in soils have shown that trace elements inhibit nitrification and urease activity (Tabatabai 1977; Liang and Tabatabai 1978). The term "trace elements" is used here to refer to elements that are, when present in sufficient concentrations, toxic to living systems. Some of the trace elements that are known to inhibit L-asparaginase activity are Cu^{2+}, Zn^{2+}, and Hg^{2+}, each shown to be concentration-dependent (Cooney and Handschumacher 1970). Other inhibitors include *p*-chloromercuriphenyl-sulfonate, D-aspartic acid, methyl-DL-aspartic acid, DL-isoasparagine, and cysteine sulfonamide. Although, there are no reports on the effects of pesticides on L-asparaginase activity in soils, several investigators have shown that these agrochemicals can affect other enzyme-catalyzed reactions in soils such as amidase (Frankenberger and Tabatabai 1981 b), invertase, phosphatase, protease, urease, catalase, and dehydrogenase (Cervelli et al. 1978). Therefore, the objectives of the present work were: (1) to study the distribution of asparaginase activity in soil profiles; (2) to assess the effect of trace elements and pesticides on the activity of this enzyme in soils; and (3) to study the relationships between the activity of this enzyme and those of other enzymes involved in N cycling in soils.

Materials and methods

Twenty-six Iowa surface soil samples, selected to obtain a wide range in pH (4.6–8.3), organic C (0.43–5.32%), total N (0.041–0.426%) and texture (4–45% clay and 1–93% sand) were used properties of the three surface soils used in testing the effects of trace elements and pesticides have been reported in another paper (Frankenberger and Tabatabai 1991). The samples were sieved (2-mm screen) in the field-moist condition, air-dried by spreading the soils in a thin (< 2 cm) layer on clean paper, and left to dry at laboratory temperature (22 °C) for 2 days. These samples were stored in tightly sealed glass containers.

The profile samples were selected to give a range in organic C and L-asparaginase activity. Of the soil profiles studied, the Clarion, Nicollet, and Marshall soils were from cornfields, whereas the Webster and Marna soils were from soybean fields that had been cultivated before the samples were taken. Before use, each sample was air-dried and crushed to pass a 2-mm screen. A subsample of each soil was ground (< 100 mesh) for determination of organic C.

The soil analyses (organic C, total N, and percentage clay and sand) followed the methods described by Frankenberger and Tabatabai (1981 a). The soil enzymes assayed in this investigation were L-asparaginase (Frankenberger and Tabatabai 1991), aliphatic amidase (Frankenberger and Tabatabai 1980), and urease (Tabatabai and Bremner 1972).

The trace elements used were Fisher certified reagent-grade chemicals. Of these Ag(I), Cd(II), Co(II), Cu(II), Fe(II), Zn(II), and V(IV) were added as the sulfate; Cu(I), Ba(II), Hg(II), Mn(II), Ni(II), Sn(II), Al(III), Cr(III), and Fe(III) as the chloride; Pb as the 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.

The pesticides used included herbicides (Aatrex, Alanap, Amiben, Banvel, Bladex, 2,4-D, Dinitramine, Eradicane, Lasso, Paraquat, Sutan, and Treflan), fungicides (Menesan and Merpen), and insecticides (Diazinon and Malaspray). The active ingredients and trade and common names of the pesticides studied have been reported previously (Frankenberger and Tabatabai 1981 b).

In testing the effects of trace elements and pesticides on L-asparaginase activity, a 5-g soil sample in a 50-ml volumetric flask was treated with 1 ml of a solution containing 25 µmol of trace element or 50 µg of active ingredients of pesticide. This solution was added dropwise to moisten the whole soil sample. After 30 min of equilibration, L-asparaginase activity was assayed as described previously (Frankenberger and Tabatabai 1991). With this method, steam distillation is used to determine the NH_4^+ produced by L-asparaginase activity when the soil is incubated with 0.2 ml toluene, 9 ml 0.1 *M* THAM buffer (pH 10), and 1 ml 0.5 *M* buffered L-asparagine solution at 37 °C for 2 h. We used 8 ml of this buffer because the soil sample was treated with 1 ml of trace-element or pesticide solution. The levels of L-asparaginase activity obtained from the trace element- or pesticide-treated soils were compared with those obtained with 5 g soil treated with 1 ml water. The percentage inhibition of L-asparaginase activity by each trace element and pesticide was calculated from $[(A-B)/A]100$, where A is L-asparaginase activity of the untreated soil and β is L-asparaginase activity of the trace element- or pesticide-treated soil. All results reported are averages of duplicate determinations calculated on an oven-dry basis, moisture being determined from the loss in weight after drying the soil at 105 °C for 24 h.

Results and discussion

Distribution of L-asparaginase activity in soils

Table 1 shows the distribution of L-asparaginase activity and organic C content in five Iowa soil profiles. L-Asparaginase activity decreased markedly with depth and was associated with a decrease in organic matter content. Statistical analyses showed that L-asparaginase activity was significantly correlated (**$P<0.01$) with organic C in the Clarion ($r = 0.85$**), Nicollet ($r = 0.98$**), Marna ($r = 0.98$**), Marshall ($r = 0.72$*), and Webster ($r = 0.98$**) soils. The simple correlation coefficient for the pooled data (38 samples from five soil profiles) was 0.75**. Since most soil enzymes are believed to be of microbial origin, similar trends in distribution among microorganisms and soil enzymes are to be expected in profile samples. Khaziev and Burangolova (1965) reported

Table 1. Distribution of L-asparaginase activity and organic C in selected Iowa soil profile samples

Depth cm	Clarion		Nicollet		Marna		Marshall		Webster	
	OC[a]	AA[b]	OC[a]	AA[b]	OC[a]	AA[b]	OC[a]	AA[b]	OC[a]	AA[b]
0–15	1.93	16	3.07	33	4.19	64	2.19	110	3.14	69
15–30	1.51	14	1.36	18	2.24	26	2.11	24	1.44	24
30–45	1.11	4	0.93	9	1.10	9	1.40	11	0.90	15
45–60	0.62	7	0.54	7	0.76	6	1.04	10	0.61	12
60–75	0.40	6	0.32	9	0.43	5	0.68	8	0.37	15
75–90	0.30	7	0.18	4	0.34	7	0.41	6	0.20	7
90–105	0.16	4	0.16	2	0.30	5	0.36	6	0.15	5
105–115	0.16	4	ND[c]	ND[c]	0.30	3	0.31	4	ND[c]	ND[c]

[a] OC, organic C (%)
[b] AA, L-asparaginase activity (µg NH_4^+-N released g^{-1} soil $2\,h^{-1}$)
[c] ND, not determined

that activities of acid and alkaline phosphatase, nuclease, phytase, and glycerophosphatase in four chernozem profiles decreased with depth, and the distribution of these activities corresponded with the distribution of microorganisms.

Several soil properties affect the levels of soil enzyme activities. Figure 1 shows a direct relationship between L-asparaginase activity and organic C content in 26 surface soils studied. This relationship was significant ($r = 0.86^{**}$) and is similar to that of other soil enzymes, including amidase (Frankenberger and Tabatabai 1981 b), invertase (Ross 1975), phosphatase (Keilling et al. 1960), urease (Tabatabai 1977), pyrophosphatase (Tabatabai and Dick 1979), and rhodanese (Singh and Tabatabai 1978). The organic C content is often used as an index of biological activity of soils because it is significantly related to levels of soil enzyme activity. Total N was also significantly correlated with L-asparaginase activity ($r = 0.79^{**}$) in the surface soils tested (Fig. 2). A strong relationship was expected between the soil N content and the activity of this enzyme because of its association with organic matter and N transformations. There was no significant correlation between L-asparaginase activity and the percentage clay ($r = 0.25$) or sand ($r = 0.01$), or pH ($r = 0.26$). The lack of a significant relationship between L-asparaginase activity and the clay percentage was somewhat surprising because the persistence of soil enzymes has always been attributed to stabilization by binding to soil colloids. Apparently, L-asparaginase is strongly associated with humic complexes. There was, however, a significant relationship between L-asparaginase activity and amidase activity ($r = 0.82^{**}$) and urease activity ($r = 0.79^{**}$) in the 26 surface soils studied (Figs. 3 and 4). These amidohydrolases are specific but related, in that each act on C-N bonds other than peptide bonds and their substrates are linear amides.

Effect of trace elements on L-asparaginase activity in soils

Metal ions may inhibit enzyme reactions by complexing the substrate, by combining with the active sites of the enzyme, or by reacting with the enzyme-substrate complex. The mode of inhibition is dependent on the type of substrate used. Metal ions are generally assumed to inactivate enzymes by reacting with sulfhydryl groups, a reaction analogous to the formation of a metal sulfide. Sulfhydryl groups in enzymes may serve as integral parts of the catalytically active sites or as groups involved in maintaining the correct structural configuration of the enzyme protein.

Table 2 shows the effects of 21 trace elements on L-asparaginase activity in three soils. Inhibition by the trace elements varied considerably among the soils. When the average inhibition was compared by using 5 µmol trace element g^{-1} soil, L-asparaginase activity was inhibited by 5% with Ba(II) and As(V) to 47% with Ag(I). The most effective inhibitors were Ag(I), Cd(II), Hg(II), Ni(II), Pb(II), and V(IV), which showed an average inhibition $\geq 20\%$. The least effective inhibitors (average $\leq 10\%$) were Cu(I), Ba(II), Co(II), Fe(II), Sn(II), Zn(II), Al(III), Se(IV), As(V), and Mo(VI). Other trace elements that inhibited L-asparaginase activity in soils included Cu(II), Mn(II), As(III), B(III), Cr(III), Fe(III), Ti(IV), and W(VI).

The pH values of the trace-element solutions varied considerably. They ranged from 2.1 for Sn(II) solution to 9.6 for the As(III) and B(III) solutions. Tests indicated, however, that the deviation in pH values resulting from the addition of trace elements in the presence of THAM buffer (pH 10) did not exceed ±0.1 pH unit. Therefore, the inhibition of L-asparaginase activity in soils observed in the presence of the trace elements studied was not due to changes in pH of the incubation mixture, but was due

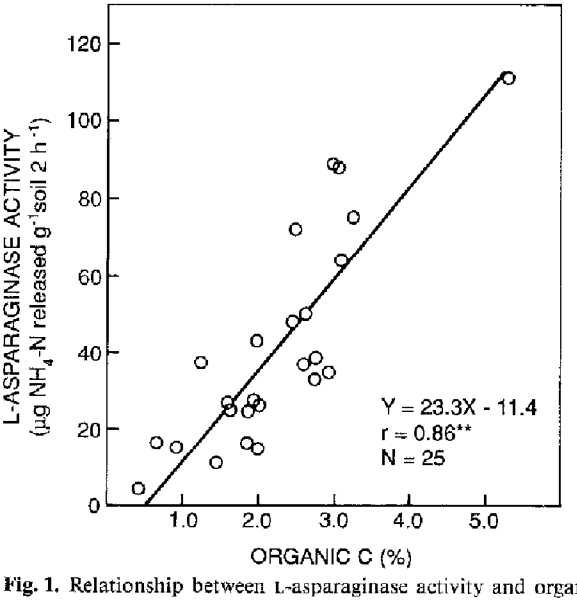

Fig. 1. Relationship between L-asparaginase activity and organic C in soils

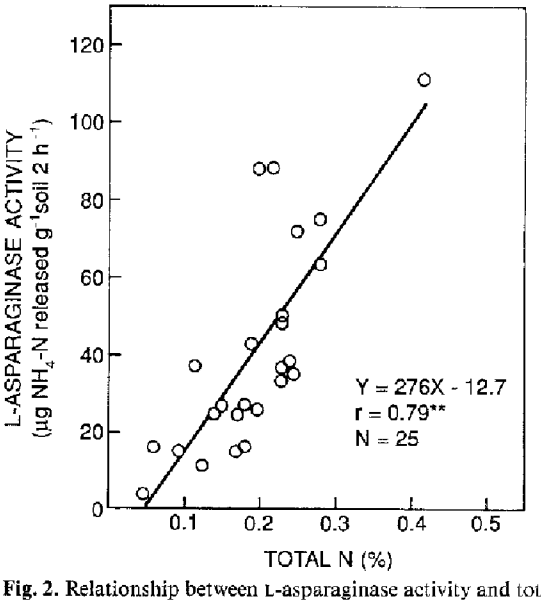

Fig. 2. Relationship between L-asparaginase activity and total N in soils

Table 2. Effects of trace elements on L-asparaginase activity in soils

Trace elements (5 μmol g^{-1} soil)		Inhibition of L-asparaginase activity (%)			
Element	Oxidation state	Harps soil	Muscatine soil	Okoboji soil	Average
Ag	I	48	44	50	47
Cu		8	3	8	6
Ba	II	4	6	4	5
Cd		20	19	24	21
Co		15	5	6	9
Cu		20	19	19	19
Fe		7	9	5	7
Hg		22	38	28	29
Mn		20	14	13	16
Ni		19	23	18	20
Pb		21	38	18	26
Sn		7	12	4	8
Zn		10	7	8	8
Al	III	2	14	6	7
As		22	8	12	14
B		11	7	20	13
Cr		13	21	12	15
Fe		12	13	9	11
Se	IV	9	14	7	10
Ti		13	17	19	16
V		16	19	28	21
As	V	5	3	8	5
Mo	VI	3	4	6	4
W		13	16	24	18
LSD[a] ($P<0.05$)		0.6	0.8	0.8	

[a] LSD, least significant difference

Table 3. Effects of pesticides on L-asparaginase activity in soils

Pesticide[a]	Inhibition of L-asparaginase activity (%)			
	Harps soil	Muscatine soil	Okoboji soil	Average
Herbicide				
Aatrax	12	21	18	17
Alanap	22	20	20	21
Amiben	12	18	25	18
Banvel	10	17	8	12
Bladex	17	23	33	24
2,4-D	13	14	24	17
Dinitramine	11	16	31	19
Eradicane	11	19	17	16
Lasso	34	44	41	40
Paraquat	27	33	39	33
Sutan	31	46	40	39
Treflan	11	4	6	7
Fungicide				
Menesan	24	18	11	18
Merpan	3	5	5	4
Insecticide				
Diazinon	27	40	31	33
Malaspray	40	44	54	46
LSD[b] ($P<0.05$)	1.7	1.7	1.3	

[a] 10 μg active ingredient of pesticide g^{-1} soil
[b] LSD, least significant difference

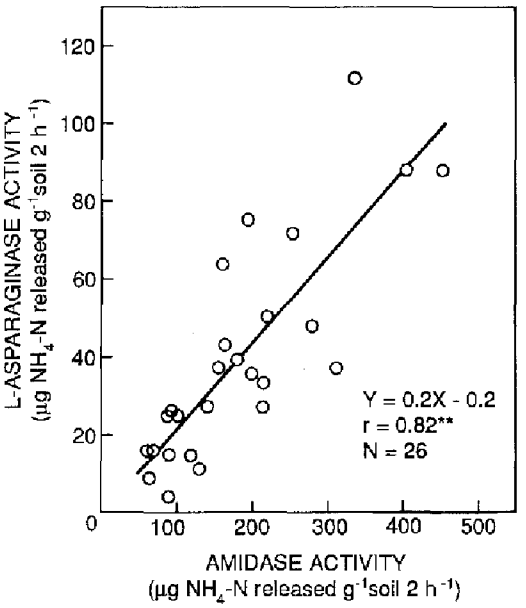

Fig. 3. Relationship between L-asparaginase activity and amidase activity in soils

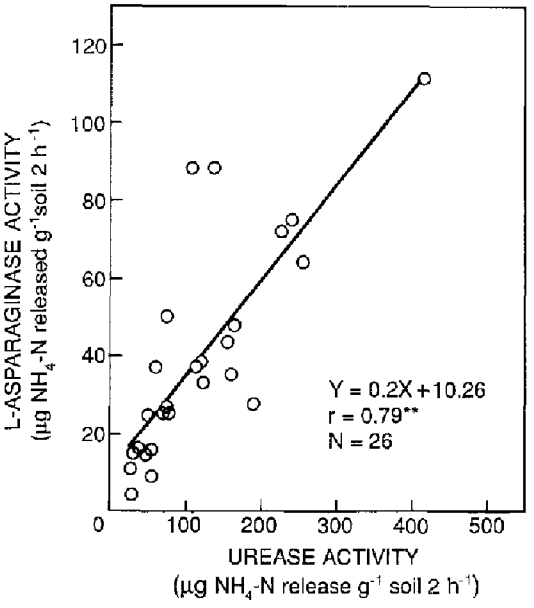

Fig. 4. Relationship between L-asparaginase activity and urease activity in soils

to a reaction between the trace element and L-asparaginase functional groups. Tests indicated that at 5 μmol NaCl and K_2SO_4 g^{-1} soil, K^+, Na^+, Cl^-, and SO_4^{2-} associated with the trace elements studied did not have any effect on L-asparaginase activity in soils. Nor was NO_3^- inhibitory at this concentration.

Effects of pesticides on L-asparaginase activity in soils

Table 3 shows the effects of 12 herbicides, 2 fungicides, and 2 insecticides on L-asparaginase activity in soils. As with the trace elements, the relative effectiveness of the pesticides as inhibitors varied among the soils. When the pesticides were compared at the rate of 10 μg active ingre-

dient g^{-1} soil, the average inhibition of L-asparaginase activity in three soils ranged from 4% with Merpan to 46% with Malaspray. Of all the pesticides tested, the most effective inhibitors (>20%) were Alanap, Bladex, Lasso, Paraquat, Sutan, Diazinon, and Malaspray. The least effective inhibitors (<10%) were Treflan and Merpan. Other pesticides that inhibited L-asparaginase activity included Aatrex, Amiben, Banvel, 2,4-D, Dinitramine, Eradicane, and Menesan.

The pH values of the pesticide solutions varied from 3.2 with Merpan to 9.9 with Bladex. However, when the pesticide solutions were added to the reaction mixture in the presence of THAM buffer (pH 10) in the assay of soil L-asparaginase activity, there were no significant changes in pH (±0.2 pH unit).

The effects of pesticides on soil enzymes are concentration-dependent but often show very little inhibition at relatively high levels of application. For example, Juma (1976) showed that by using 50 µg active ingredients of pesticide g^{-1} soil, inhibition of acid phosphatase activity ranged from 0% with Diazinon and Malaspray to 11% with Alanap. Other studies showed that these pesticides inhibited amidase in three soils, with average inhibition values ranging from 2% with Dinitroamine, EPTC plus R-25788, and Captan to 10% with Butylate (Frankenberger and Tabatabai 1981b). But the inhibition of L-asparaginase activity by pesticides showed a much greater range, suggesting that addition of some of these pesticides to soils could lead to a reduction in the amount of N derived from soil organic matter. The effects of agrochemicals on other soil enzyme activities deserve further studies, because several of these enzymes are involved in nutrient cycling and plant nutrition.

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