Eco. Env. & Cons. 29 (2) : 2023; pp. (571-575)
Copyright@ EM International
ISSN 0971–765X

DOI No.: http://doi.org/10.53550/EEC.2023.v29i02.006

Effect of Toluene, KNO_3 and Glucose on soil L-Glutaminase activity

M. Bhargava Narasimha Yadav[1], G. Padmaja[2] and M. Madhavi[3]

[1]Department of Soil Science and Agricultural Chemistry, PJTSAU, Rajendranagar, Hyderabad 500 030, India
Rajendranagar, PJTSAU, Hyderabad 500 030, India
Agricultural College, PJTSAU, Aswaraopeta 507 301, India

(Received 10 September, 2022; Accepted 5 November, 2022)

ABSTRACT

At the PJTSAU Department of Soil Science and Agricultural Chemistry, incubation tests were conducted to see how toluene, KNO_3 and glucose affect the activity of the soil enzyme L-glutaminase. Toluene has a considerable impact on the activity of L-glutaminase; it lowers enzyme activity in all soils. From 17.19 % to 36.06 %, the percentage drop in activity caused by the addition of toluene was more pronounced in black soils as compared to red soils. For six days, L-glutaminase activity increased in soils that had been incubated with either glucose or glucose-nitrate mixtures. Glucose and nitrate-treated soils were more active than soils that had only been nitrate-treated alone.

Key words: *L-glutaminase, Glucose, KNO_3, Toluene*

Introduction

Organically bound elements are converted into mineral form, which is easily absorbed by plants and essential for plant nourishment, by mineralization. Abiontic enzymes and soil microorganisms both aid in the mineralization of nutrients. Soil microorganisms depend on abiontic enzymes to catalyze a wide range of reactions necessary to maintain soil structure, organic waste decomposition, organic matter production and nutrient cycling (Dick, 1994). With the help of microorganisms, plants and soil animals, the biochemical processes of soil nutrient cycling can be carried out. Often, the soil N supply as a plant nutrient becomes deficient and additional sources are supplied to supplement the soil's natural fertility. The organic form of N is a major component of soil organic matter and may account for greater than 95% of the total N in most surface soils. About half of this organic N has not yet been identified. It has been estimated that about 20–40% of the total N in soils is present in the form of amino acids, but only a small portion of the amino acids are present in a "free" state and the major portion is bound to soil organic matter (Bremner, 1951). Sowden (1958) reported that a portion of the NH_4^+ released during acid hydrolysis was equivalent or nearly equivalent to the sum of aspartic acid-N and glutamic acid-N derived from asparagine and glutamine. The enzyme L-glutaminase (L-glutamine amido hydrolase E.C. 3.5.1.2) in soils hydrolyses L-glutamine to glutamic acid and ammonium, thus it is important in making the amide form of nitrogen available to plants.

The research of toluene affecting soil enzymes has great significance in soil enzymology and envi-

[3]Associate Dean,

ronmental science. Many researchers suggest that the function of toluene is to inhibit microbial uptake of the product, its impact is far wider, ranging from denaturation of proteins to permeabilization of cellular membranes, resulting in the inability to separate intra and extracellular enzyme activities, not to mention leakage of amino acids per se (Nannipieri *et al.*, 1996; Fuller and Scow, 1997; Burns and Dick, 2002). Weintraub and Schimel (2005) assumed that the toluene concentration was too low to effectively reduce immobilization; toluene is commonly included in the assay medium for determining the activity of soil enzymes. The problems associated with the assay of L-glutaminase were the elimination of enzymes produced by the growing population of soil microorganisms and the assimilation of reaction products during the assay of abiontic enzymes, as they don't include the enzymes accumulated by growing microorganisms. Hence, to overcome this problem, the present research work was carried out to determine the effect of toluene on L-glutaminase activity.

Materials and Methods

Thirty soil samples (10 red soils and 20 black soils) of varying physico-chemical properties representing, various cropping systems were collected from the Rajendranagar campus of Hyderabad by the quartering method. Thirty soil samples were collected and analysed for various physical and physico-chemical and chemical properties. From above 30 soil samples, three black soils and three red soils were selected to study the effect of toluene on the activity of L-glutaminase and physico-chemical properties of selected soil were presented in Table 1.

Effect of Toluene on L-glutaminase activity

Soil sample (10 g) was taken in a 150 ml conical flask and adds 0.4 ml of toluene, to which 12 ml of 0.1 M THAM buffer of pH 8 was added. The flasks were gently swirl to mix the contents followed by addition of 8 ml of 0.125 M L-glutamine were added, so that concentration substrate was 50 mM. The flasks were gently shaken for few seconds and covered with polythene paper. Then the contents were incubated at 37±0.5 °C for 4 hours in BOD incubator. After incubation, reaction was terminated by addition of 30 ml of KCl-Ag_2SO_4 solution. The contents were agitated on mechanical shaker for 30 min to release all NH_4^+ formed and the suspension was allowed to settle and filtered. In the controls the same procedure described above was followed but the toluene was not added to soil. The rate of NH_4^+ released was estimated by modified indophenol blue method as described Dorich and Nelson (1983) as modified by Yadav *et al.* (2022).

L-glutaminase activity in the presence of glucose and potassium nitrate

10 g of soil (red soil 1 and black soil 1) was taken in 150 ml conical flask. Nitrogen was added as KNO_3@ 0.2 mg N/g soil in one treatment. In another treatment, KNO_3 was added at 0.2 mg N/g soil and glucose was added at 3 mg/g soil. The soil was thoroughly mixed with 10 ml of water. In the controls the same procedure described above was followed but the glucose and potassium nitrate was not added to soil. These were incubated for 0, 3, 6, 10 days and the L-glutaminase activity was quantified by modified indophenol blue method as described Dorich and Nelson (1983) as modified by Yadav *et al.* (2022) at the end of these periods. All these were carried out in triplicates.

Result and Discussion

Toluene's effect on soil L-glutaminase activity is illustrated in Table 2, which is shown in Figures 1 and 2. The L-glutaminase activity in toluene-treated soils

Table 1. Physico-chemical characteristics of the six representative soil samples

S. No .	pH	EC (dS/m)	OC (%)	Available N (Kg/ha)	Available P_2O_5 (Kg /ha)	Available K_2O (Kg/ha)	Total N (%)	Clay (%)
Black soil1	7.98	0.152	1.25	200.70	81.24	734.72	0.170	16.00
Black soil 2	7.81	0.154	1.16	280.70	68.23	675.36	0.238	18.00
Black soil 3	8.23	0.479	0.84	150.50	92.30	659.68	0.128	12.00
Red soil1	6.98	0.080	0.90	190.53	56.40	514.08	0.161	24.00
Red soil 2	7.56	0.153	0.51	113.00	41.03	285.60	0.096	16.00
Red soil 3	7.58	0.163	0.82	113.00	41.03	294.50	0.146	12.00

was generally lower than in untreated soils, as shown in the Table 2. L-glutaminase activity measured in the presence of toluene is derived from extracellular L-glutaminase Adsorbed on soil colloids or free enzymes, where as the activity measured in the absence of toluene includes activity derived from metabolizing microorganisms, i.e. includes activity produced by microorganisms during the assay. Increased amidase activity in the absence of toluene may encourage the manufacture of this enzyme by soil microbes, according to Frankenberger and Tabatabai (1980). Furthermore, toluene additions reduced soil enzyme hydrolysing activities, potentially due to the destruction of cell-bound L-glutaminase or inhibition of extracellular enzymes. The results reported on the use of toluene were contradictory, with several authors showing increased activity of enzymes by the addition of toluene and a decrease in activity, especially for soil urease and phosphatase (Tabatabai and Bremner, 1972; Skujins, 1978). However, in the present study, toluene was used as a microbial biostatic agent because of its incubation period of 4 hours. The percentage decrease in activity owing to the addition of toluene ranged from 17.19% to 36.06%, and the activity was considerably more pronounced in black soils than red soils. Tabatabai (1982) reported similar results for Amidase, but Frankenberger and Tabatabai (1991 a, b) discovered that the activity of L-glutaminase and L-asparaginase were higher in buffered toluene soils than in untreated soils. They speculated that enhanced activity in the presence of toluene could be related to a change in microbial cell membrane permeability to substrate and enzyme reaction products. So, it can be utilized in enzyme assays as a biostatic agent (Jackson and DeMoss, 1965; Levinthal *et al.*, 1962; Rao, 1989; Srinivas, 1993). In the absence of toluene, phosphatase activity was exactly related to the specific surface area of the soil particles in a study of the influence of toluene on phosphatase activity in soils of diverse structures. The enzymatic activity was reduced in the presence of toluene (Burns, 1978).

Table 2. Effect of Toluene on soil L-Glutaminase activity

Different soils	With Toluene	Without Toluene
Red soil1	13.80	16.20
Red soil 2	6.62	8.75
Red soil 3	6.38	8.18
Black soil 1	9.50	14.86
Black soil 2	14.30	19.13
Black Soil 3	7.80	9.42
	C.D. (5 %)	SE(d) ±
Toluene application (A)	0.228	0.11
Soil (B)	0.394	0.19
A X B	0.558	0.269

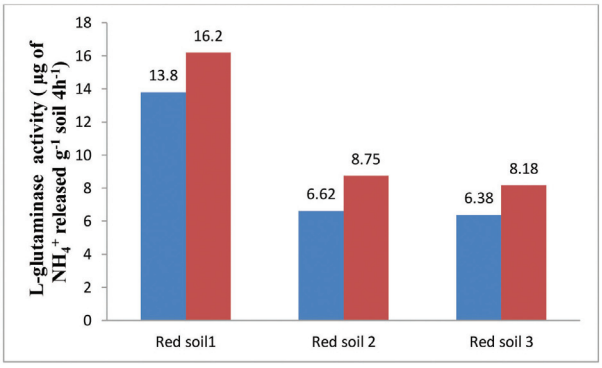

Fig. 1. Effect of toluene on soil L-glutaminase activity in Red soil

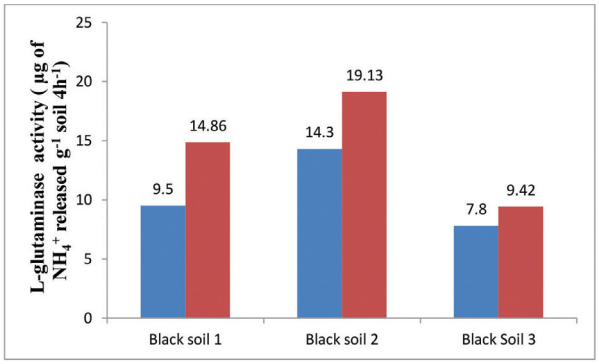

Fig. 2. Effect of toluene on soil L-glutaminase activity in Black soil

Table 3 and 4 show the effects of pre-incubating 10 grams of red and black soil with glucose and potassium nitrate, as seen in Figure 3 and 4. In soils incubated with nitrate alone or with glucose and

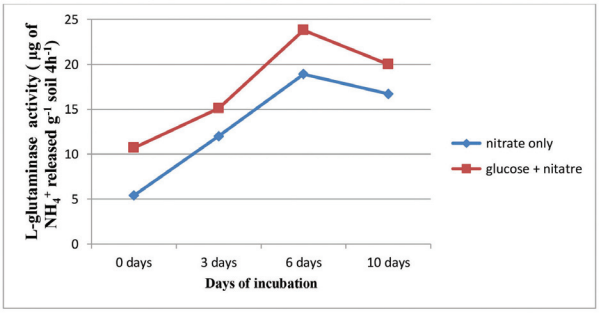

Fig. 3. Effect of KNO_3 and Glucose on soil L-glutaminase activity in Red soil

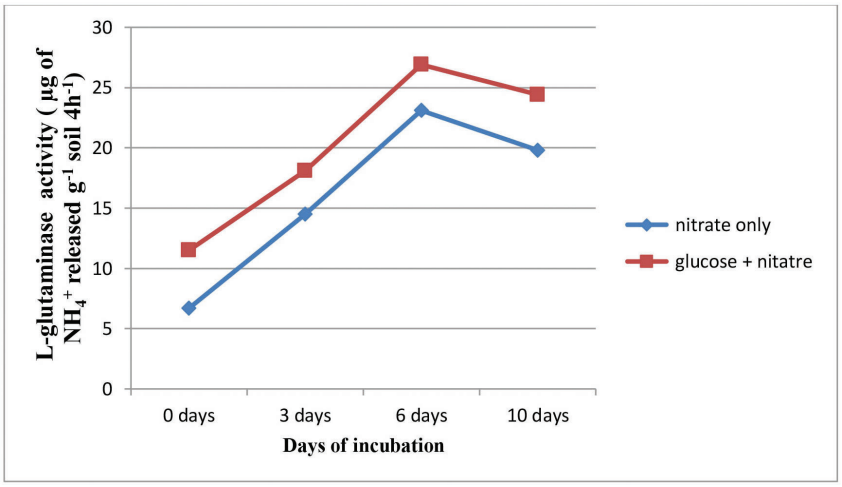

Fig. 4. Effect of KNO_3 and Glucose on soil L-glutaminase activity in Black soil

nitrate, L-glutaminase activity increased steadily from 0 to 6 days. The activity of the soils treated with glucose and nitrate was consistently higher than that of the soils treated alone with nitrate. In glucose-treated soils, L-glutaminase activity was most likely increased because glucose acted as a carbon source for microorganisms, which in turn increased L-glutaminase activity. Similar results for soil urease activity were reported by Sahrawat, 1983; Pal and Chhonkar, 1981.

Conclusion

For assay of L-glutaminase activity toluene was used as a microbial biostatic agent, in all the experiment soils activity of L-glutaminase was lowered by

Table 3. Effect of KNO_3 and Glucose on soil L-glutaminase activity in Red soil 1

Days of incubation	L- glutaminase activity (µg of NH_4^+ released g^{-1} soil $4h^{-1}$) with only nitrate	L- glutaminase activity (µg of NH_4^+ released g^{-1} soil $4h^{-1}$) with glucose + nitrate
0 days	5.40	10.70
3 days	12.00	15.10
6 days	18.90	23.80
10 days	16.70	20.00
	C.D. (5 %)	SE(d) ±
Incubation with (A)	0.627	0.293
Days of incubation (B)	0.887	0.415
Factor (A X B)	1.254	0.587

Table 4. Effect of KNO_3 and Glucose on soil L-glutaminase activity in Black soil 1

Days of Incubation	L-glutaminase activity (µg of NH_4^+ released g^{-1} soil $4h^{-1}$) with only nitrate	L-glutaminase activity (µg of NH_4^+ released g^{-1} soil $4h^{-1}$) with glucose + nitrate
0 days	6.7	11.5
3 days	14.5	18.1
6 days	23.1	26.9
10 days	19.8	24.4
	C.D. (5 %)	SE(d) ±
Incubation with (A)	0.693	0.324
Days of incubation (B)	0.98	0.459
Factor (A X B)	1.386	0.648

addition of toluene. Glucose affects microbe proliferation by providing as a source of organic material and L-glutaminase enzyme activity is triggered by glucose.

Conflict of Interest

Authors declare that they have no conflict of interest.

Acknowledgements

The authors express sincere thanks to the PJTSAU and ICAR for funding research.

References

Bremner, J.M. 1951. A review of recent work on soil organic matter. *International Journal of Soil Science.* 2: 67-82.

Burns, R. G. and Dick, R. P. 2002. *Enzymes in the Environment: Activity, Ecology, and Applications.* Marcel Dekker, New York.

Burns, R.G. 1978. Enzyme activity in soil: Some theoretical and practical considerations. In R.G. Burns (eds.) - *Soil Enzymes*, Academic Press, London, 295- 340.

Dick, R.P. 1994. Soil enzyme activities as indicators of soil quality. In J.V. Doran, D. C., Coleman, D.F., Bezdicek and V.A. Stewart (eds.) - *Defining soil quality for Sustainable Environment*. Soil Science Society of America, American Society of Agriculture, Madison. 107-124.

Dorich, R.A. and Nelson, D.W. 1983. Direct colorimetric measurement of ammonium in potassium chloride extracts of soil. *Soil Science Society of American Journal.* 47(4): 833-836.

Frankenberger, W.T. Jr. and Tabatabai, M.A. 1991b. L-Glutaminase activity of soils. *Soil Biology & Biochemistry.* 23(9): 869-874.

Frankenberger, W.T.Jr. and Tabatabai, M.A. 1980. Amidase activity in soils: I. Method of assay. *Soil Science Society of America Journal.* 44: 282-287.

Frankenberger, W.T.Jr. and Tabatabai, M.A. 1991a. L-Asparaginase activity of soils. *Biology and Fertility of Soils.* 11: 6-12.

Fuller, M.E. and Scow, K. M. 1997. Impact of trichloroethylene and toluene on nitrogen cycling in soil. *Applied and Environmental Microbiology.* 63: 4015-4019.

Jackson, R.W. and DeMoss, J.A. 1965. Effects of toluene on *Escherichia coli*. *Journal of Bacteriology.* 90: 14-20.

Levinthal, C., Signer, E.R. and Fetherhof, K. 1962. Reactivation and hybridization of reduced alkaline phosphatase. *Proceedings of the National Academic of Science U.S.A*. 48: 193-198.

Nannipieri, P., Sastre, I., Landi, L., Lobo, M. C., Pietramellara, G. 1996. Determination of extracellular neutral phosphomonoesterase activity in soil. *Soil Biology & Biochemistry.* 28: 107-112.

Pal, S. and Chhonkar, P.K. 1981. Urease activity in relation to soil characteristics. *Pedobiologia.* 23: 152-158.

Rao, S.V. 1989. *Distribution, Kinetics and some interactions of urease and phosphatases in soils. Ph D Thesis.* Acharya N G Ranga Agricultural University, Hyderabad.

Sahrawat, K.L. 1983. Relationship between soil urease activity and other properties of some tropical wetland rice soil. *Fertilizer Research.* 4: 145-150.

Skujins, J.J. 1978. History of abiontic soil enzyme research. In Burns R.G (eds.) *Soil Enzymes*. Academic Press, New York, 1-49.

Sowden, F.J. 1958. The forms of nitrogen in the organic matter of different horizons of soil profiles. *Canadian Journal of Soil Science.* 38: 147-154.

Srinivas, D. 1993. *Phosphomonoesterase activity in some soils of Andhra Pradesh. Distribution, kinetics and variation due to plant cover, pesticide application and submergence. Ph D Thesis.* Acharya N G Ranga Agricultural University, Hyderabad.

Tabatabai, M.A. 1982. In A.L. Page (eds.) - Methods of Soil Analysis, Part-2, *Soil Enzymes*, American Society. Agron, Madison, 903-948.

Tabatabai, M.A. and Bremner, J. M. 1972. Assay of urease activity in soils. *Soil Biology & Biochemistry.* 4: 479-487.

Weintraub, M. N. and Schimel, J. P. 2005. Seasonal protein dynamics in Alaskan arctic tundra soils. *Soil Biology & Biochemistry.* 37: 1469-1475.

Yadav, M. B. N., Padmaja, G., Anjaiah, T., Kumari, J. A. and Madhavi, M. 2022. Standardisation of assay of L-glutaminase activity in arid and semi arid tropical soils of south India. *Indian Journal of Ecology.* 49 (4): 1357-1362.