Journal of the Rubber Research Institute of Sri Lanka, (1997) 79, 1-10

UREA HYDROLYSING POTENTIAL OF THE RUBBER GROWING SOILS OF SRI LANKA

R S Dharmakeerthi, Lalani Samarappuli, S N Silva and A Yakandawala

(Accepted 11 September 1996)

ABSTRACT

The ability of the rubber growing soils to hydrolyse urea under natural conditions was evaluated by measuring the urease activity using a non buffer method. The temporal variability of urease activity was also investigated during the period February 1994 to February 1995. The urease activity has been ranged from 14.5 to 292.4 µg NH_4^+ - N released/g/h with a mean of 83.84 in all soils. The Matale series soils had a significantly high level of urease activity (120.22) compared to that of the Parambe series (47.88). The level of urease activity in these soils was found to be much higher than the values reported earlier for the same soils. During the first several months after wintering, i.e. *from April to September, urease activity was high where as during the period of November, December and January the activity was low in all the series studied. In Parambe and Homagama series soils the urease activity was as low as zero in the months of November and December. The significance of high and low levels of urease activity with regard to urea fertilization in the rubber growing areas was discussed.*

Key words: Non-buffer method, rubber soils, temporal variability, urease activity

INTRODUCTION

Although small amount of soil applied urea can be absorbed by plants directly (Webster, 1954), most of it has to be converted to inorganic form (ammonia) before absorption. Soil applied urea is converted to plant available forms enzymatically, and urease (urea amidohydrolase, EC 3.5.1.5) is responsible for the hydrolysis of urea to carbondioxide and ammonia (Conrad, 1940a; Conrad, 1940b). Urease first converts urea to ammonia and carbomic acid (Fig.1) which is hydrolysed chemically to ammonia and CO_2 later (Gould *et al.*, 1986). Therefore, the effectiveness of urea as a nitrogen fertilizer depends on the level of urease activity in the soil where it is applied.

Although considerable information is available on urease activity in tropical and temperate soils, only a little is known of the level of activity in Sri Lankan soils (Bhavanandan & Fernando, 1970; Silva & Perera, 1971). The investigation of these authors were based on a buffer method (Hoffman, 1963). However, according to Zantua and Bremner (1975), the buffer method does not represent the true ability of soils to hydrolyse urea. They suggested that to obtain

$$CO(NH_2)_2 + \text{Urease} \rightleftharpoons \text{Urease}[CO(NH_2)_2]$$
$$\Updownarrow +H_2O$$
$$NH_3 + NH_2COOH + \text{Urease}$$
$$\downarrow +H_2O$$
$$NH_3 + CO_2$$

Fig.1 Mechanism of urease-catalized hydrolysis of urea.

an index of the ability of the urease in soil to hydrolyse urea under natural conditions, a non buffer method is obviously superior to a buffer method. Since there is a paucity of information on the true urea hydrolysing potential in rubber growing soils, an attempt was made to investigate the level of urease activity in these soils using a non buffer method (Zantua & Bremner, 1975).

Further, several workers have observed a seasonal variation in urease activity (Palma & Conti, 1990; Ross *et al.,* 1984; Stott & Hogedorn, 1980). Understanding this variation is most important in adjusting the fertilizer programme during a year. Therefore, this study was also extended to monitor the differences in urease activity during a year in the rubber growing soils of Sri Lanka.

MATERIALS AND METHODS

Experiment 1: Level of urease activity

Six locations from each soil series in the rubber growing areas (Silva, 1969) were selected and at each location surface sample was collected from the top 0-15cm. Sampling was done during the months, June to October, when the degree of decomposition of the leaf litter would have reached a similar stage. All soil samples were removed from existing rubber fields, in between four rubber trees avoiding the manuring circle. Samples were air dried immediately, passed through a 2mm screen and stored at room temperature ($27 \pm 2°C$) until being analyzed.

Soil pH (1:2.5,0.01N $CaCl_2$), organic carbon (OC) (Walkley, 1947), total nitrogen (TN) (Bremner & Mulvaney, 1982), calcium carbonate equivalent (CCE) (Bundy & Bremner, 1972), and texture (Day, 1965) was determined (Table 1). Soil urease activity was assayed by a non-buffer method (Zantua & Bremner, 1975) with modifications as described below.

10g of soil was placed in a 100ml Erlynmeyor flask, and 10ml 1.5M urea solution was added after adjusting the water holding capacity of the soil to 60%. The contents were mixed and incubated at 37°C for 5 hours. Following this 30 ml of the inhibitor KCl-$HgCl_2$ solution (100ml 2M KCl + 0.5g $HgCl_2$) was added and shaken for 30 min. and contents filtered through Whatman No.542 filter paper. For each sample a control was run in the same manner, but the addition of the inhibitor solution preceeded the addition of the substrate. The ammonia contents in the filtrates were determined colorimetrically using a Technicon auto analyzer. Each sample was run in duplicate with urease activity expressed as µg NH_4^+-N released per gramme of oven dried soil per hour at 37°C.

Table 1. *Properties of rubber growing soils of Sri Lanka*

Series	pH	OC%	TN%	CCE%	Sand%	Silt%	Clay%
Matale	4.08	1.85	0.38	0.22	53.9	10.7	35.4
Ratnapura	3.57	2.16	0.37	0.04	61.6	9.6	28.8
Agalawatta	3.53	1.31	0.44	0.08	74.2	6.9	18.9
Homagama	3.50	1.93	0.44	0.08	69.5	7.9	22.6
Boralu	3.54	1.29	0.39	0.02	72.1	6.5	21.4
Parambe	3.81	1.18	0.44	0.06	58.3	12.7	29.0

Experiment 2: Temporal variability of urease activity

A location from each soil series was selected and a plot of 6 x 6m was marked in each location. Locations selected were from existing mature rubber fields of the same age, with the same clone and situated on comparable terrain. A composite sample of 10 auger holes from the surface (0-15cm) was drawn avoiding the manuring circle at every month from February 1994 to February 1995. The samples were air dried immediately, passed through 2mm sieve and stored under refrigerator conditions until the time of analysis. Urease activity of each sample was measured according to the method described in experiment 1.

RESULTS

Urease activity in rubber growing soils ranged from 14.5 to 292.4 with mean urease activity of 83.84 in all soils (Table 2). The Matale series soils exhibitted the highest mean urease activity (120.22) where the Parambe series had the lowest level of activity (47.88) and they were significantly different. In all other series there were no significant difference in the level of urease activity.

Urease activity in these soils showed correlations with some soil physical and chemical properties, with organic carbon and total nitrogen showing a significant possitive correlation with urease activity in all soil studied (Table 3). A weak negative relationship was observed between pH and urease activity in all soils, yet it was not significant.

Table 2. *Urease activity in rubber growing soils of Sri Lanka*

Series	UA (μg NH_4^+-N released /g/h)	
	Range	Mean*
Matale	50.03-292.4	120.22[a]
Ratnapura	47.3-189.8	88.08[ab]
Agalawatta	48.5-116.7	82.31[ab]
Boralu	41.6-170.10	83.47[ab]
Homagama	43.0-129.2	81.11[ab]
Parambe	14.5-71.20	47.88[b]

* - Values with the same letter are not significantly different

Table 3. *Correlation coefficients of urease activity with different soil properties*

Property	correlation coefficients(r)
pH	-0.304
OC	0.463**
TN	0.441**
CCE	-0.073
Sand	-0.201
Silt	0.050
Clay	0.228

**- Significant at 1% probability level

The variation observed in urease activity during the year is more or less simmilar in all soil series. A high enzyme activity was recorded from April to October with a slight reduction during the months of July and Augest. A very low activity was observed during the months of November, December and January (Fig. 2). Urease activity is almost zero in Homagama and Parambe series during these months.

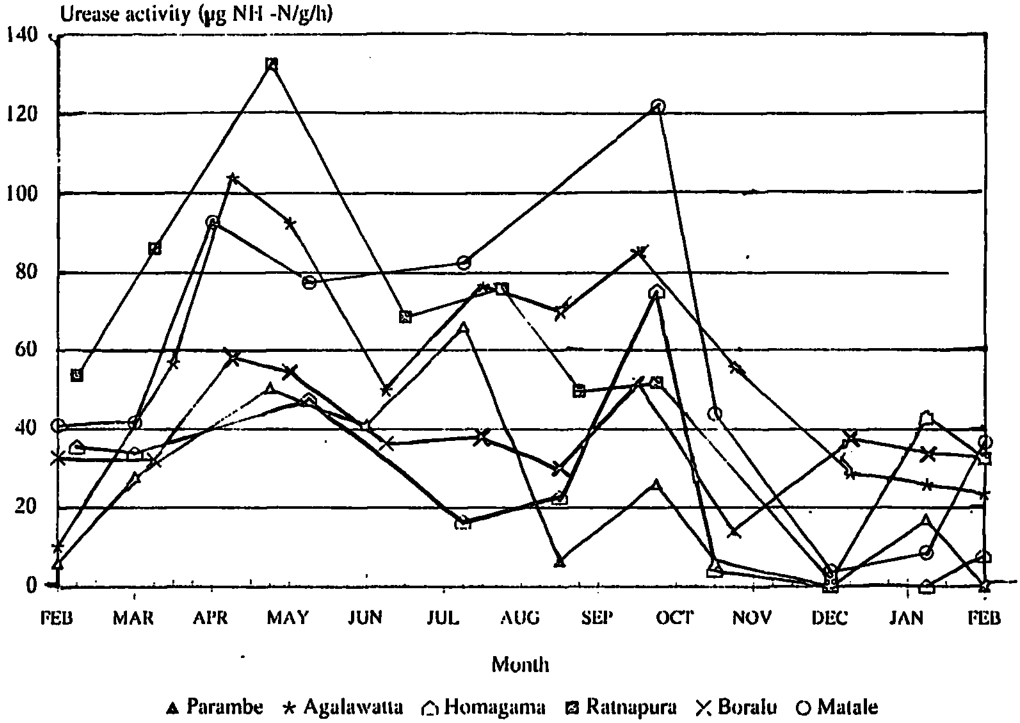

Fig.2. Temporal variability of urease activity in rubber growing soils of Sri Lanka

DISCUSSION

The mean urease activity of 83.84 in all the soils shows that these soils exhibit a considerably high enzyme activity compared to most of the other soils in the world (Table 4). Silva and Perera (1971), using a buffer method, estimated a mean urease activity of 52.25 μg NH_4^+-N released/g/h for the same soils series in 1971 and this value was lower than the value recorded in this study (83.84) by using a non-buffer method. But, according to Zantua and Bremner (1975) a non-buffer method should have recorded much less urease activity than a buffer method. One reason for such an increase in the level of urease activity could be the continuous use of urea as N fertilizer. Yogaratnam and Perera (1981) have observed a high urease activity when urea was applied for 9 years (77.6 μg NH_4^+-N released/g/h) than when no N fertilizers were applied (37.55 μg NH_4^+-N released/g/h) in Boralu series soils. Similar observation was made by Bhavanandan and Sundaralingam (1971) also in some tea soils of Sri Lanka. This increase may be attributed to the proliferation of micro-organisms due to the continuous use of urea as the N source which is essential for the growth and proliferation of micro organisms.

Table 4. *Comparison of urease activity of some selected soils*

Soil	Value*	Method			Reference
		Substrate conc.**	Buffer	Toluene	
1. Rubber soils Sri Lanka	14.0-292.4	42,000	-	-	This study
2. Rubber Soils Sri Lanka	6.95-90.42	46,600	Na citrate citric acid (pH - 6.7)	+	Silva & Perera, 1971
3. Rubber Soils Malaysia	1.96-14.10	1000	-	-	Tan, 1982
4. Tea soils Sri Lanka	13.63-64.5	46,000	Citrate buffer (pH-6.7)	+	Bavanandan & Fernando, 1970
5. Indian soils	2.00-26.2	466.7	-	-	Singh *et al.*, 1991
6. Phillipine soils	6.22-24.89	1000	-	-	Sahrawat, 1980
7. Australian soils	10.17-194.33	23,300	K citrate-citric acid (pH 6.7)	+	McGarity & Myers, 1967
8. Iowa soils	0-52.87	1000	-	-	Zantua *et al.*, 1977
9. Iowa soils	13.5-180.00	1120	THAM (PH-9.0)	+	Tabatabai & Bremner, 1972

* - All values were converted to µg NH_4^+ - N released/g of soil/h

** - µg of urea -N/g of soil

The rapid hydrolysis of urea due to high urease activity can result in high soil pH values and high ammonium ion concentrations which are conducive to accumulation of ammonia. The major problems observed in such a situation are the loss of volatile ammonia gass (Yogaratnam & Perera, 1981; Mengal *et al.*, 1982) and ammonia toxicity to germinating seedlings (Kiss *et al.*, 1975). Since the level of urease activity in these soils is very high, the risk of loss of nitrogen with surface application of urea could be more now than when it was first introduced to the rubber plantations. Therefore, utmost care should be taken to make urea an effective fertilizer in rubber growing areas. One effective way of controlling ammonia volatilization under Sri

Lankan conditions may be subsurface application of urea in rubber plantations. However, urea may not be effective in the Matale series soils where the mean urease activity is as high as 120.22 µg NH_4^+-N released/g/h in addition to high pH levels.

The enzyme activity in soils was affected by some soil physical and chemical properties. However, Zantua *et al.* (1977) showed that the most of the variations in urease activity observed in soils could be accounted for organic matter content. It has been reported that urease activity is correlated positively with OC and TN of the soil (Speir *et al.*,1980; Dash *et al.*, 1981; Frankenberger & Dick, 1983; Reynolds *et al.*, 1985). Our findings are in agreement with the above observations where urease activity is significantly positively related to OC and TN. Chemical analysis of these soils indicates that OC and pH are high in Matale series soils where as the lowest organic carbon content is in Parambe series soils (Table 1). Silva (1971) has also reported similar observations in evaluating the nutritional status of these soils. The observed differences in the urease activity *i.e.* the highest activity in Matale series soils and the lowest in the Parambe series soils, could be attributed to the differences in OC in these soils. However, it was observed that at higher levels of OC there was a dicrease in the level of urase activty in the Ratnapura series soils. This could be due to the increase in the level of urease inhibitory substances present in the organic materials in these soils. Sivepalan *et al.* (1983) reported that polyphenols present in organic materials inhibit the soil urease activity.

Rubber plants shed their leaves in January and February and this provides organic materials to the soil. Incorporation of organic materials increases the urease activity in soils and then decreases to the original level with time (Nannipieri *et al.*, 1982; Nannipieri *et al.*, 1983; Al-Rashidi & Al-Jabri, 1990). The high urease activity observed from April to October may be due to the increased organic matter content in the soil as a result of wintering. Further, moisture content also has a direct impact on soil urease activity and inhibition of urea hydrolysis at low soil water potentials have been reported (Bouwmeester *et al.*, 1985; Reynolds & Walf, 1987). Rubber growing regions experience two dry periods during a year one in January to March and then the other in July to August. Urease activity of these soils was low during these two periods, the lowest being in November to March.

The differences in urease activity during the year has to be well understood in adopting a proper fertilizer programme in rubber plantations in order to obtain the maximum out put from the costly N fertilizers. In Parambe and Homagama series soils, urease activity was almost zero from November to January and even in other soils it is considerably low during this period. Urea applied in these months may remain unhydrolyzed for a certain period of time. The unhydrolysed urea can be leached down to lower depth of soil profile (Broadbent *et al.*, 1958) or washed away, especially if heavy torrential rain falls soon after its application. These possibilities can result in lower efficiency in urea application.

ACKNOWLEDGEMENT

The authors are thankful to Mr. A M A Perera, Miss. Dayvi Senaratna and Miss. Sagari Kudaligama for the technical assistance given during the study.

REFERENCES

Al-Rashidi, K and Al-Jabri, M M (1990). Nitrification and urea hydrolysis in amended calcareous saline soils. *Arid Soil Research and Rehabilitation* 4(4), 243-252.

Bhavanandan, V P and Fernando, V (1970). Studies on the use of urea as a fertilizer for tea in Ceylon. II. Urease activity in tea soils. *Tea Quarterly* 41(2,3 & 4), 94-106.

Bhavanandan, V P and Sundaralingam, S (1971). The effect of the nitrogen fertilizer on urease activity, soil acidity and leaf nutrient content on yield of tea. *Tea Quarterly* 42(1 & 2), 40-47.

Bouwmeester, R J B, Velk, P L G and Stampe, J M (1985). Effect of environmental factors on ammonia volatilization from a urea-fertilized soil. *Soil Science Society of America Journal* 49(2), 376-381.

Bremner J M and Mulvaney C S (1982) Nitrogen Total. In: *Methods of Soil analysis - part 2,* pp.621-622 (Eds. A L Page, R H Millar and D R Keeney), American Society of Agronomy, Inc., Midson, Wisconsin, USA.

Broadbent, F E, Hill, G N and Tyler, K B (1958). Transformations and movement of urea in soils. *Soil Science Society of America Proceedings* 22(4), 303-307.

Bundy, L G and Bremner J M (1972). A simple titrimetric method for determination of inorganic carbon in soils. *Soil Science Society America Proceedings* 36, 273-275.

Conrad, J P (1940a). Hydrolysis of urea in soils by thermolabile catalysis. *Soil Science* 49(3), 253-263.

Conrad, J P (1940b). The nature of the catalyst causing the hydrolysis of urea in sols. *Soil Science* 50(2), 119-134.

Dash, M C, Mishra, P C, Mohanty, R K and Bhatt, N (1981). Effects of specific conductance and temperature on urease activity in some Indian soils. *Soil Biology & Biochemistry* 13(1), 73-74.

Day, R P (1965). Particle fractionation and particle-size analysis. In: *Methods of Soil Analysis-* Part 1, pp.562-566 (Eds. C A Black, D D Evans, J L White, L E Ensminger, and F E Clark), American Society of Agronomy, Inc., Madison, Wisconsin, USA.

Frankenberger, W T Jr. and Dick, W A (1983). Relationships between enzyme activities and microbial growth and activity indices in soil. *Soil Science Society of America Journal* 47(5), 945-951.

Gould, W D, Hagedorn, C and McCready, R G L (1986). Urea transformations and fertilizer efficiency in soil. *Advances in Agronomy* 40, 209-238.

Hoffman, E (1963). Urease. In: *Method of Enzymatic Analysis*, pp.913-1916 (Ed H U Bergmeyer), Acadamic Press, N.Y.

Kiss, S, Dargan-Bularda, M and Radulescu, D (1975). Biological significance of enzymes accumulated in soil. *Advances in Agronomy* 27, 25-87.

McGarity, J W and Myers, G M (1967). A survey of urease activity in soils of Nothern New South Wales. *Plant and Soil* 27(2), 217-238.

Mengel, D B, Nelson, D W and Huber, D M (1982). Placement of nitrogen fertilizers for no-till and conventional till corn. *Agronomy Journal* 74(3), 515-518.

Nannipieri, P, Ceccanti, B, Cervelli, S and Conti, C (1982). Hydrolases extracted from soil: kinetic parameters of sevaral enzymes catalyzing the same reaction. *Soil Biology & Biochemistry* 14(5), 429-432.

Nannipieri, P, Muccini, L and Ciardi, C (1983). Microbial biomass and enzyme activities: production and persistence. *Soil Biology & Biochemistry* 15(6), 679-685.

Palma, R M and Conti, M A (1990). Urease activity in Argentine soils: field studies and influence of sample treatment. *Soil Biology & Biochemistry* 22(1):105-108.

Reynolds, C M and Wolf, D C (1987). Effect of soil moisture and air relative humidity on ammonia volatilization from surface applied urea. *Soil Science* 143(2), 144-152.

Reynolds, C M, Wolf, D C and Armbruster, J A (1985). Factors related to urea hydrolysis in soil. *Soil Science Society of America Journal* 49(1), 104-108.

Ross D J, Speir, T W, Janine C, Cowling C and Whale, K N (1984) Temporal fluctuations in biochemical properties of soil under pasture. II. Nitrogen mineralization and enzyme activities. *Australian Journal of Soil Research* 22, 303-317.

Sahrawat, K L (1980). Control of urea hydrolysis and nitrification in soil by chemicals - prospects and problems. *Plant and Soil* 57(2-3), 335-352.

Silva, C G (1969). Provisional classification of Rubber Soils of Ceylon. *Journal of the Rubber Research Institute of Malaysia* 21(2),

Silva, C G (1971). An evaluation of the nutritional status of the rubber soils of Ceylon. *Quarterly Journal of the Rubber Research Institute of Ceylon* 48, 147-159.

Silva, C G and Perera, A M A (1971). A study of the urease activity in rubber soils of Ceylon. *Quarterly Journal of the Rubber Research Institute of Ceylon* 47(1&2), 30-36.

Singh, J P, Vinod Kumar and Dahiya, D J (1991). Urease activity in some benchmark soils of Haryana and its relationship with various soil properties. *Journal of Indian Society of Soil Science* 39, 281-285.

Sivapalan, K, Fernando, V and Thenabadu, M W (1983). Humified phenol-rich plant residues and soil urease activity. *Plant & Soil* 70, 143-146.

Speir, T W, Lee, R, Pansier, E A and Carnis, A (1980). A comparison of sulfates, urease and protease activities in planted and fallow soils. *Soil Biology & Biochemistry* 12(3), 281-291.

Stott, D E and Hagedorn, C (1980). Interrelations between selected soil characteristics and arysulfatase and urease activities. *Soil Science and Plant Analysis* 11, 935-955.

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

Tan, K A (1982). Studies on Nitrogen in Malaysian soils. II Urea hydrolysis and transformations. *Journal of Rubber Research Institute of Malaysia* 30(1), 19-30.

Walkely, A (1947). A critical examination of a rapid method for determining organic carbon in soils. Effect of variations in digestion conditions and of inorganic soil constituents. *Soil Science* 63, 251-263.

Webster, G C (1959). *Nitrogen Metabalism in Plants.* Row Peterson and Co., White Plains, New York, 152pp.

Yogaratnam, N and Perera, A M A (1981) Urea as a nitrogen fertilizer for rubber plantations in Sri Lanka. II Agronomic investigations. *Journal of the Rubber Research Institute of Sri Lanka* 59, 20-30.

Zantua, M I and Bremner, J M (1975). Comparison of methods of assaying urease activity in soils. *Soil Biology & Biochemistry* 7, 291-295.

Zantua, M I, Dumenil, L C and Bremner, J M (1977). Realationship between soil urease activity and other soil properties. *Soil Science Society of America Journal* 41(2), 350-352.

(Received 12 August 1996)