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Effect of Substrate Concentrations, Temperature and Cropping System on Hydrolysis of Urea in Soils

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ABSTRACT

The effect of substrate concentrations, temperature and cropping system on hydrolysis of urea in alkaline soils was studied under laboratory conditions. Soil samples were collected from berseem (*Trifolium alexandrinum*), chickpea (*Cicer arietinum*), cotton (*Gossypium hirsutum*) and wheat (*Triticum aestivum*) fields. Urea was applied at 0.28, 0.56, 0.84, 1.12, 1.40 and 1.68 mg N g^{-1} soil. Urea treated soil samples were incubated at 18°C and 32°C. At the end of the incubation period soil was extracted with 2 M KCl containing 5 mg phenyl mercuric acetate (PMA) L^{-1}. Unhydrolyzed urea was determined colorimetrically using Diacetyl monoxime method. The results showed that substrate concentrations, temperature and cropping system had significant effect on hydrolysis of urea in soil. At a concentration of 0.28 mg N g^{-1} soil urea was completely hydrolyzed in soil from berseem and cotton fields in one day at 32°C; whereas in chickpea and wheat soil, only 63 and 52% urea was hydrolyzed, respectively. Lowering the temperature of incubation retarded the hydrolysis of urea; 42.24, 64.02, 58.28 and 29.12% urea was hydrolyzed in soil under berseem, chickpea, cotton and wheat at 18°C. Urea concentration > 0.28 mg N g^{-1} soil reduced hydrolysis of urea in soil under berseem, chickpea, cotton and wheat both at 18 and 32°C. The results showed that urease activity was higher in soil under berseem and cotton as compared to that in chickpea and wheat soil.

Key Words: Hydrolysis; Urea; Urease activity; Berseem; Chickpea; Cotton; Wheat

INTRODUCTION

Urea added to soil as fertilizer or as animal urine is hydrolyzed enzymatically by soil urease ($NH_2CONH_2+H_2O \rightarrow 2NH_3+CO_2$) and resulting release of ammonia and rise in pH can lead to several problems including damage to germinating seedlings and young plants (Ouyang *et al*., 1998), nitrite toxicity and volatilization of urea N as ammonia, which may cause air and water pollution problems (Gasser, 1964; Hutchinson & Viets, 1969). Urea can be an inefficient N source due to rapid hydrolysis by soil urease leading to ammonia volatization, adverse effect of ammonia toxicity on seed germination and seedling growth (Watson, 2000).

The severity of problems related to the use of urea is dependent on the rate of hydrolysis of urea. The rate of hydrolysis of urea increases with an increase in urea concentrations until a maximum is reached, beyond which hydrolysis decreases (Singh & Nye, 1984; Lal *et al*., 1993). In order to overcome the problems associated with use of urea fertilizer it is imperative to study the hydrolysis of urea in agricultural soils.

Hydrolysis of urea is dependent on urease activity which is added to soil by a number of bacteria, fungi, actinomycetes, and fresh plant residues. Several workers have shown that urease activity is altered as a result of cultivation and difference in cropping system (Khan, 1970; Friedel *et al*., 1996; Barket & Dick, 1998; Klose & Tabatabai, 1999; Klose & Tabatabai, 2000). Speier *et al*. (1980) found urease activity in several soils to be initially similar under ryegrass and fallow conditions. With time, urease activity under ryegrass tended to remain constant or increase, but in follow soil urease activity consistently decrease.

Hamid *et al*. (1998) studied the effect of different concentrations of urea on pH changes in alkaline calcareous upland and low land soils. They noted a consistent increasing trend in soil pH as hydrolysis proceeded upto day 3 of incubation. Then pH of upland soil became normal in 6 days, whereas the pH of lowland soil remained elevated for extended period of time (14 days). Therefore a greater under standing of the influence of these variables on the rate of hydrolysis of urea would provide a guidance for modifying fertilizer management practices for higher efficiency of urea fertilizer.

The present study was conducted to determine the effect of urea concentrations, temperature and cropping system on hydrolysis of urea in alkaline soils under laboratory conditions.

MATERIALS AND METHODS

Bulk soil samples were collected from surface 0-15 cm from fields under berseem (*Trifolium alexandrium*) (S-1), chickpea (*Cicer arietinum*) (S-2), Cotton (*Gossypium hirsutum*) (S-3) and wheat (*Triticum aestivum*) (S-4), from NIAB farm, Faisalabad, situated at 31^0-25'N, 73^0-06'E. The soil samples were dried under shade at room temperature, crushed to pass through 2 mm sieve and analyzed for physico-chemical properties. On the average soil was 8.5% clay, 19.5% silt and 72.0% sand with pH 7.70, total N 0.07%, O.M. 1.40% and $CaCO_3$ 1.28%. The soil has been classified as Hafizabad series (Coarse loamy, mixed, Ustic hyperthermic Haplo Calcids).

Particle size distribution was determined by

Bouyoucos hydrometer method (Moodie *et al*., 1959). Soil pH was measured using glass electrode on pH meter (Hinna HI-840-Japan). Total N was determined by Kjeladhl digestion and steam distillation method (Jackson, 1982). Organic matter was determined by wet oxidation method of Walkley-Black as described by Jackson (1982). Calcium carbonate was determined by Puris method (Puri, 1931).

For studying hydrolysis of urea, 5 g soil was weighed in 100 mL conical flasks. Urea solutions were applied to soil samples to get 0.28, 0.56, 0.84, 1.12, 1.40 and 1.68 mg N g^{-1} soil concentrations. Water in the soil was maintained at 20% during incubation period. Urea treated Soil samples were incubated at 18°C and 32°C for 1, 2, 3, 7, 10 and 14 days.

After incubation soil was extracted with 50 mL 2M KCl solution containing 5 mg phenyl mecuric acctate (PMA) L^{-1}. Soil suspension was shaken for 1h and then filtered through Whatman No. 42 filter paper.

In soil extracts unhydrolysed urea was estimated by using Diacetyl monoxime colorimetric method as described by Douglas and Bremner (1970). The intensity of red colour developed was measured at 527 nm wavelength using spectrophotometer (Bausch & Lomb Spectronic 21).

Urea N content of the extracts was calculated from urea N standard curve. The hydrolysed urea was calculated by substraction of unhydrolysed urea N from the total urea N added. The percentage of urea hydrolysed was calculated. Data were subjected to analysis of variance.

RESULTS AND DISCUSSION

The soil samples differed significantly (Table I) in their capacity to hydrolyse urea; S-3 (soil from cotton field) had the maximum urease activity and S-4 (soil from wheat field) had the least urease activity. Urea hydrolysis was rapid at low concentration. At 0.28 mg N g^{-1} soil concentration complete hydrolysis occurred in S-3; whereas 94.50% in S-1, (soil from berseem field), 63.06% in S-2 (soil from chickpea field) and 52.38% in S-4 (soil from wheat field) urea was hydrolysed in 1 day. At the highest concentration (1.68 mg N g^{-1} soil) 79.96% urea was hydrolysed in S-3. In S-1, S-2 and S-4 only 33.53, 25.56 and 24.02% urea was hydrolysed. Higher concentrations appeared to inhibit the hydrolysis of urea.

Maximum percentage of the urea hydrolysed at lower rates is in close conformity to the results of Beri and Brar (1978) who reported 100% hydrolysis of urea at 0.2 mg N g^{-1} soil within 20-56 h in alkaline subtropical soils. The lower hydrolysis of urea at higher rates of urea are in agreement with the results reported by Broadbent *et al*. (1958) and Thormahlen and Preez (1992) that the time taken to complete the hydrolysis in soils increased almost linearly with increasing urea concentrations. With lowering the incubation temperature to 18°C urea hydrolysis was reduced to 1/3 to 1/2 of that observed at 32°C in various soil samples (Table II). At the lowest level of urea (0.28 mg N g^{-1} soil), 29.12 to 64.02% urea was hydrolysed under different crops; whereas at the highest concentration (1.68 mg N g^{-1} soil)

Table I. Effect of substrate concentrations, temperature and cropping systems on hydrolysis of urea in soil at 32°C in 1 day

Treatments	Urea (% hydrolysed)				
(mg N g^{-1} soil)	S-1	S-2	S-3	S-4	Means
0.28	94.52^{a}	63.06^{d}	100.00^{a}	52.38^{ef}	77.49^{A}
0.56	55.71^{de}	32.88^{ghi}	85.12^{b}	25.99^{hij}	49.92^{B}
0.84	46.17^{f}	32.13^{ghi}	73.01^{c}	26.72^{j}	43.01^{C}
1.12	46.58^{f}	26.30^{hij}	78.79^{bc}	28.64^{ghij}	45.06^{C}
1.40	39.90^{g}	26.66^{hij}	79.04^{bc}	23.88^{ij}	41.62^{C}
1.68	33.53^{gh}	25.56^{hij}	79.96^{bc}	24.02^{ij}	40.77^{C}
Means	52.22^{B}	34.43^{C}	82.65^{A}	29.27^{D}	

Table II. Effect of substrate concentrations, temperature and cropping systems on hydrolysis of urea in soil at 18°C in 1 day

Treatments	Urea (% hydrolysed)				
(mg N g^{-1} soil)	S-1	S-2	S-3	S-4	Means
0.28	42.24^{c}	64.02^{a}	52.28^{b}	29.12^{f}	46.19^{A}
0.56	37.04^{e}	41.16^{cd}	40.53^{d}	26.95^{g}	36.42^{B}
0.84	35.42^{e}	21.47^{j}	36.37^{e}	24.64^{hi}	26.60^{C}
1.12	23.38^{i}	17.89^{l}	25.87^{gh}	19.47^{k}	21.63^{D}
1.40	16.74^{l}	14.66^{m}	23.94^{i}	9.90^{o}	16.31^{E}
1.68	16.42^{l}	12.69^{n}	25.76^{gh}	8.54^{o}	15.85^{E}
Means	26.62^{B}	28.65^{B}	34.11^{A}	19.77^{C}	

Means followed by the same letter do not differ significantly at 5% level of probability according to Duncan's Multiple Range Test; S-1=Soil collected from berseem field ; S-2=Soil collected from chickpea field; S-3=Soil collected from cotton field; S-4=Soil collected from wheat field

only 8.54 to 25.76% urea was hydrolysed at 18°C as compared to 24.02 to 79.96% observed at 32°C in various soil samples. Invariably cold temperature slowed down the hydrolysis of urea. The soil samples from fields of four crops under study differed significantly in their capacity to hydrolyse urea at 18°C as well.

The effect of temperature on urea transformation has been reported by several workers. Kumar and Wagenet (1984) observed that urease activity increased linearly with temperature from 5 to 35°C in all soils, though from 35 to 45°C the increase was less and not according to the same linear relationship. The percentage increase from 5 to 35°C was 23, 32 and 23 in Kilburn, Dagor and Nibley soils, respectively. Gould *et al*. (1973) found urease activity continued to increase above 37°C. Roberge and Knowles (1968), however, found that urea hydrolysis was approximately proportional to temperature in the 10 to 40°C range and decreased at higher temperature. Kumar *et al*. (2000) observed with increasing temperature from 15 to 45°C a linear increase in urease activity in two soils. Dowling (1998) found soil temperatures of 13 to 25°C adequate for rapid hydrolysis of urea.

The significant differences among the soil samples in hydrolysing urea observed in the present study could be attributed to cropping system, the physico-chemical properties being similar. The soil from wheat field had the lowest urease activity and that from cotton field had the highest urease activity.

The alteration of soil enzymatic activities resulting from cultivation or differences in cropping system has been demonstrated by several researchers (Khan, 1970; Pancholy

& Rice, 1973; Voets *et al*., 1974; Klein & Koths, 1980; Speir *et al*., 1980; Reynolds *et al*., 1985; Bandick & Dick, 1999; Klose & Tobatabai, 2000; Nosheen, 2002). Khan (1970) noted significantly higher soil enzymatic activities in plots under a 5- year rotation of grains and legumes than in wheat fallow system. Voets *et al*. (1974) found a decrease in urease activity in soil after plant cover removal and Klein and Koths (1980) found greater urease activity in no tillage corn plots than in conventional tillage plots. Reynolds *et al*. (1985) noted that urea hydrolysis was greater in soil samples from pasture than in samples from cultivated fields. It appeared that the pasture vegetation exhibited a major impact on urea hydrolysis rates and the variability of urea hydrolysis rates. Bandick and Dick (1999) while assaying enzyme activities in soil samples collected from vegetable crop rotation plots found that enzyme activities were higher in continuous grass field than in cultivated fields. Klose and Tabatabai (2000) working with soil samples taken from corn, soybean and oats fields found that urease activity in soil was significantly affected by crop rotation. Nosheen (2002) reported that urease activity was more prominent in soil samples form cultivated fields than the uncultivated soils.

Activity of urease tends to increase with the size of the microbial population and organic matter contents (Reynold *et al*., 1985; Klose & Tabatabai, 2000). The presence of relatively fresh plant residues often result in abundant supplies of urease (Zantva & Bremner, 1976). Greatest activity of urease occurs in the rhizosphere where microbial activity is high and where it can be excreted from plant roots. The variation in hydrolysis of urea in the present study in different soil samples could be attributed to the plant residues addition and rhizosphere urease which varies with plant species.

CONCLUSIONS

It is concluded from the results of this study that urea hydrolysis rates were affected by concentrations of substrate, temperature and the cropping system. Higher substrate concentration (>0.28 mg N g^{-1} soil) and lower temperature inhibited hydrolysis of urea. Hydrolysis of urea was highest in soil from cotton field and lowest in soil from wheat field at 32^{o}C.

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