International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)
Journal Home page: www.iscientific.org/Journal.html
© International Scientific Organization

Urease activity under salinity stress in calcareous soils of semi-arid regions of Iraq

Raghad S. Mouhamad[1], Ibrahim B. Razaq[1], Ali S. Fadhel[1], Shaimaa A. Yousir[1], Dhrgham I. Taha[1] and Munawar Iqbal[2,*]

[1]Soil and Water Resources Center, Ministry of Sciences & Technology, Baghdad –Iraq
[2]National Centre of Excellence in Physical Chemistry, University of Peshawar, Peshawar-25120, Pakistan

Abstract

The present study was conducted to appraise the urease activity under salinity stress from calcareous soil of lower Mesopotamian plain of Iraq. The soil selected was not under cultivation since last five year at the time of sampling. Soil samples of 0-15 cm depth were collected and soil characteristics were determined by standard methods. Urease activity was assayed by buffer method, which involved the estimation of ammonium produced from urea and toluene-treated tris (hydroxymethyl) amino-methane at 37ºC for 2 h. The correlation analysis was performed to appraise the relationship between urease activity and soil characteristics. The urease enzyme activity varied significantly among soil types and soil properties also affected it considerably. A high degree of correlation between urease activities and SAR was observed in clay loam and silty clay soils with R^2 values of 0.99 and 0.84, respectively. Furthermore, it was observed that the urease was considerably low where Na ion concentration was high versus Mg and Ca ions.

Key words: Soil type, soil characteristics, cations, enzyme activity and electrical conductivity

Full length article Received: 16-06-2014 Revised: 12-07-2014 Accepted: 19-07-2014 Available online: 31-07-2014
**Corresponding Author*, e-mail: bosalvee@yahoo.com

1. Introduction

Under dry conditions urea hydrolysis results in rapid N loss to the atmosphere through NH_3 volatilization process and urea fertilizer had been reported to less (20%) efficient due to this loss in the arid regions [1]. The urease enzyme activity is one of the reasons of this N loss to the atmosphere [2]. Kızılkaya and Hepflen [3] revealed the hydrolysis of urea-type substrates by urease enzymes and microbes are the major source of urease enzyme in soil. It is well known that urease enzyme in soil is responsible for the hydrolysis of urea fertilizer applied to soil into NH_3 and CO_2 with concomitant rise in soil pH. The stability of urease in soil is affected by several factors. Studies have shown that extracellular urease associated with soil organic mineral complexes is more stable than urease in the soil solution [4]. Humus-urease complexes extracted from soil are highly resistant to denaturing agents such as temperature; urease extracted from plants or microorganisms is rapidly degraded in soil [5]. Cropping history, organic matter content, soil amendments, heavy metals, moisture and temperature may affect urease activity [6]. Many studies have been conducted to evaluate the effect of incubating temperature on the activity of hydrolytic enzymes, although, most of these studies have involved finding out the optimum temperature for activity [7]. Furthermore, some studies also highlighted the effect of the incubation time on enzymes activities in soil of hydrolytic enzymes [8].

In view of the importance of enzyme activity in soil, it was hypothesized that soil properties are important in control enzyme activity. Therefore, this study was designed to evaluate the urease activity in different soil as a function of various soil physico-chemical properties from sami-arid region of Iraq.

2. Material and Methods

2.1. Study area

The study selected was flood plain 180 km south of Baghdad, at 32°30'0"–32.5° N and 45°20'0"–45.33° E. The area has semi-arid climate of low humidity and less than 120 mm annual precipitation rates. Soil types are typic torrifluvent hyper thermic soil and are undergone severe desertification since the mid-1980$_S$ due to climate changes in Iraq. The urea became the main N fertilizer source for agriculture after the development of agriculture industry since 1970 [9].

2.2. Soils and Analysis

Soil samples were collected early spring (April 2012) from the upper 30 cm of two different regions. Soil sample was stored at 4°C in a field moist condition. Microbiological properties were studied for the field-moist soil. The soil moisture content was measured after drying at 105°C for 24 h. All results of microbiological properties were expressed on the basis of moisture-free weight**.** The pH and electrical conductivity were measured in an aqueous extract (1/5 s/w) [10]. Available P was extracted from sodium bicarbonate and determined by the method reported elsewhere [11]. Exchangeable K, Mg and Na were extracted with ammonium acetate and were measured by flame photometry. Ammonium and Nitrate were determined by the Kjeldahl method [12] and texture was determined by the hydrometer method [13].

2.3. Urease Assay

Urease activity was assayed by the method of Hoffmann and Teicher [14]. Toluene (0.25 mL), 0.75 mL citrate buffer (pH, 6.7) and 1 mL of 10% urea substrate solution were added to 1 g soil and incubated for 3 h at 37°C. The formation of ammonium was determined as reported by Bremner and Mulvaney [12]. Results were expressed as μg NH_4-N g^{-1} $2h^{-1}$ of dry soil.

2.4. Statistical Analysis

The data of urease assays were examined statistically. All experiments were performed in triplicate and data thus obtained was averaged. Analysis of variance (2-way ANOVA) was carried out using the 3 factors arranged in a randomized complete block design. Means were compared using correlation tests, with a significance level of $P<0.01$. All statistical calculations were performed using MSTAT and SPSS (version 11.0).

3. Results and Discussion

Characteristics of soils used in this study are shown in Table 1. The soils texture was clay, clay laom and silty clay texture and calcareous in nature. There was a significant difference in soil properties among soils. The EC, OC and total nitrogen content varied significant and there was slight variation in pH values. As it can be seen in Table 1, The, EC, pH, OC and N (%) were in the range of 20.1-51.4 dS/m, 7.1-8.33, 0.04-0.55 mgg^{-1} and 0.23-0.48%, respectively.

3.1. Effect of soil physico-chemical characteristics on urease activity

Urease activities detected in soils under salinity stress are shown in Fig. 1. The urease activity recorded to be in the range of 30-160 μg NH_4-N g^{-1} $2hr^{-1}$ dry soil. It was observed that the EC affected the urease activity significantly and the enzyme activity was also observed different among soil textures. In clay loam soil, the urease activity was ranged from 80-160 μg NH_4-N g^{-1} $2h^{-1}$ dry soil, whereas it was 40-100 μg NH_4-N g^{-1} $2h^{-1}$ dry soil in silty clay and 140-160 μg NH_4-N g^{-1} $2h^{-1}$dry soil in clay. The EC value greatly affect the urease activity and urease activity was higher at 20 dS/m, then decreased as the EC values increased to 30-35 dS/m and above this value the urease activity again increased (Fig. 1). It is well known that the EC is directly related to salt concentration and type of salt in soil and the salt concentration may vary among soil types that might be responsible in EC variation and urease activities. Statistical analysis shows that EC in the range of 20-30 ds/m correlated negatively with urease activity that may be explained by the fact that increase in salinity decrease the microflora in soil and resultantly the enzyme activity is likely to decrease [15]. While EC range of 30-≤40 dS/m in the same soil texture was positively correlated with activity of urease. In this case the positive correlation between EC with enzyme activity may be due to salt movement (concentration) that is controlled by adsorption on clay particles or encapsulated in humic complexes [16]. Previously, similar trend has been reported regarding enzymatic activity and EC [17]. Author’s shown both types of relationships between EC and enzyme activity in certain ranges. A significant and positive correlation between clay content and urease activity has also been reported form Iowa and Trinidad soils [18]. Similar to this study, Frankenberger and Tabatabai [19] also revealed a correlation of enzymes activity with clay contents and a negative relationship between urease activities and sand contents in ten surface non-cultivated California soils. The silty clay and clay loam soil showed exponentially negative correlations between urease activity and EC in 20-30 ds/m range. At 20 ds/m urease activity was 100 and 160 μg NH_4-N g^{-1} $2h^{-1}$ dry soil for silty clay and clay loam, respectively, whereas it reached to 70 and 130 μg NH_4-N g^{-1} $2h^{-1}$ dry soil for silty clay and clay loam, respectively for EC values greater than 40 ds/m. Furthermore, it was observed that soil texture revealed positive liner correlation with urease activity where salt concentration was high which may explain that urease activities depended on microbial biomass and greater stabilization of extracellular urease by soil properties. This means inhibition of enzyme activity reduced when soluble slats were increased from antagonism and homogenates participated in the active site of the urease enzyme [20].

3.2. Correlation between urease activities and soil physico-chemical characteristics

The correlation matrix demonstrated the relationships between soil characteristics and urease activity that discounted the use of multiple linear regressions due to co-alignment, including the relationships between SAR (Sodium adsorption ratio, mmol/L), pH, NH^{4+}, NO^3, Na^+, Mg^{2+} and Ca^{2+} on urease activity. The results regarding correlation between urease activity and soil physico-chemical characteristics are given in Table 2. Results showed that soil pH had significant but native impact on urease activity and the clay loam showed the highest correlation of -0.92, while it was -0.80 and -0.88 for clay and silty clay soil, respectively. Regarding pH effect on urease activity on urease activities Fidaled and Lavecchia (2003) revealed that the molecular dissociation constants for the free enzyme are not affected by substrate binding and pH negative impact might be this reason. High pH soil affects availability of nutrients and controls the composition and diversity of microbial community and also less prone to denaturation and biological degradation by humic polymers which protect enzymes against microbiological degradation [8] (Nelson *et al.,* 2008). The SAR showed positive and a high degree of correlation with urease activity, while it was negative with clay soil. The relationship between urease activities and ammonium was high and insignificant among soil types, whereas nitrate showed high positive correlation with urease activity in clay soil and negative correlation was recorded for clay loam and silty clay soil.

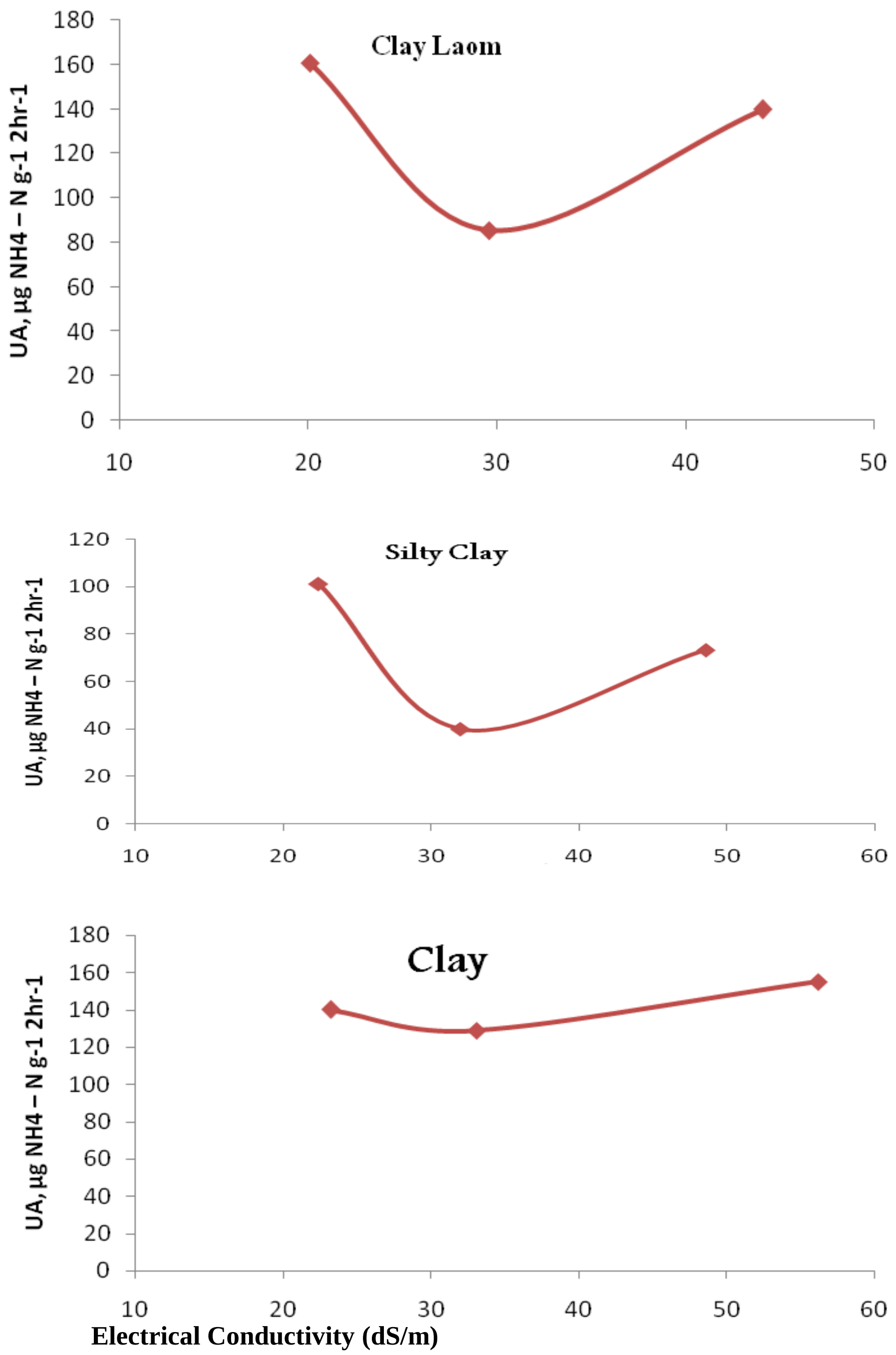

Fig. 1. The urease activity (UA) in different soil texture at different EC values

Table 1. Descriptive statistics for selected properties of soils

Soil Texture	Ece	pH	O.C	N%
Clay Loam	20.1	7.1	0.04	0.23
	29.6	8	0.05	0.3
	44.1	7.67	0.04	0.25
Silty Clay	20.1	8.24	0.37	0.36
	32	8	0.25	0.43
	48.6	8	0.3	0.32
Clay	23.2	8	0.54	0.48
	33.1	7.75	0.55	0.45
	51.4	8.33	0.51	0.31

Where: Ece (1:5 soil: solution), O.C (organic Carbon), N%: Total Nitrogen

Table 2. Correlation between urease activities (UA) and physico-chemical characteristics of soils, ** significant at $P \leq 0.01$

Characterises	Clay	Clay loam	Silty Clay
pH	-0.80581**	-0.92007**	-0.88601**
SAR	-0.51439	0.999512**	0.840254**
Ammonium	-0.38623 NA	-0.29829 NA	-0.28346 NA
Nitrate	0.995401**	-0.89989**	-0.80382**
Sodium	-0.01057 NA	0.038974 NA	-0.27439 NA
Magnesium	0.995475**	-0.64795	-0.40087
Calcium	0.860238**	-0.26278 NA	-0.20233 NA

UA, urease activity; **, significant at P≤0.01; NA, not significant.

The metallic ions such as sodium, magnesium and calcium showed negative and non-significant correlation, while sodium for clay loam (non-significant) and magnesium and calcium (significant) for clay soil revealed positive correlation with urease-activity. Insignificant and native relationship were observed between sodium and urease activity in two soil texture reached (-0.01 and -0.27) in clay and silty clay respectively. But an insignificant and positive relation between urease activity and clay loam soil. It is clear from results that sodium ion is more effective in decreasing urease activity, while Mg and Ca least which indicates that sodium ion competed urease on exchange sites and enhanced the chalet enzyme and inhibition.

4. Conclusion

The enzymatic activity is an important factor in soil and in present investigation urease activity was evaluated in different soil types as a function of soil physico-chemical properties such as soil texture, EC, pH, SAR and ions. The correlation between soil characteristics and urease activity was performed by statistical analysis. It was found that soil physico-chemical properties have significant effect on urease activity in soil. Furthermore, the urease activity was also found to be different among soil textures. Form results of present study, it is concluded that the soil physico-chemical properties must be taken into account while evaluating the enzymatic activities of soil.

References

[1] C.O. Da Ros, C. Aita and S.J. Giacomini. (2005). Ammonium volatilization in the application of urea on the soil surface in the no-tillage system. Ci. Rural. 35: 799-805.

[2] D.E. Kissel, M.L. Cabrera and S. Paramasivam. (2008). Ammonium, ammonia and urea reactions in soils. Pp. 101-156. In Nitrogen in Agricultural Systems. Agronomy Monograph No. 49. J.S. Schepers and W.R. Raun, eds. ASA-CSSA-SSSA, Madision, WI.

[3] R. Kızılkaya and F. Hepflen. (2007). Microbiological properties in earthworm Lumbricus terrestris L. cast and surrounding soil amended with various organic wastes. Commun. Soil Sci. Plant Anal. 38: 2861-2876.

[4] B.H. Byrnes and A. Amberger. (1989). Fate of broadcast urea in a flooded soil when treated with N-(n-butyl)thiophospheric triamide, a urease inhibitor. Fertil. Res. 18: 221-231.

[5] M.I. Zantua and J.M. Bremner. (1977). Stability of urease in soils. Soil Biol. Biochem. 9: 135-140.

[6] Z. Yang, S. Liu, D. Zheng and S. Feng. (2006). Effects of cadium, zinc and lead on soil enzyme activities. J. Environ. Sci. 18: 1135-1141.

[7] M.A. Tabatabai. (1994). Enzymes, In: methods of soil analysis. microbiological and biochemical properties, Part 2 (Eds: R.W. Weaver, S. Augle, P.J. Bottomly, D. Bezdicek, S. Smith, M.A. Tabatabai and A. Wollum) Soil Science Society of America- Madison, Pp. 775-783.

[8] K.A. Nelson, P.C. Scharf, L.G. Bundy and P. Tracy. (2008). Agricultural management of enhanced-efficiency fertilizers in the north-central United States. Online. Crop Management doi:10.1094/CM-2008-0730-03-RV. Plant Management Network.

[9] R. S. Mohammed, I.B. Razaq and S.A. Al-Saedi. (2012). Role of organic agriculture in reducing the level of nitrates in soil and water. Basra Agriculture Scientific Journals. 25: 639-646.

[10] A.L. Page. (1982). Methods of Soil Analysis. Part 2. Chemical & microbiological properties Amer. Soc. of Agron. Madison, Wisconsin, U.S.A.

[11] J. Ryan, G. Estefan and A. Rashid. (2001). Soil and plant analysis laboratory manual. International Center for Agricultural Research in the Dry Areas (ICARDA). Syria.

[12] J.M. Bremner and R.L. Mulvaney. (1978). Urease activity in soils. In: Soil enzymes (Ed: R.G. Burns). Academic Press- New York, Pp. 149-196.

[13] P. Cookson and G.L. Lepiece. (1996). Urease enzyme activity of soils of Batinah region of Sultanate of Oman. J Arid Environ. 32: 225–238

[14] Hoffmann, G.G. und K. Teicher. 1961. Ein Kolorimetrisches Verfahren zur Bestimmung der Urease Aktivitat in Boden. Z. Pflanzenernahr. Bodenk. 91: 55-63.

[15] J.M. Norton. (2008). Biological denitrification. p. 173-200. In Nitrogen in Agricultural Systems. Agronomy Monograph No. 49. J.S. Schepers and W.R. Raun, eds. ASA-CSSASSSA, Madision, WI.

[16] K. Takishima, T. Suga and G. Mamiya. (1988). The structure of jack bean urease. The complete amino acid sequence, limited proteolysis and reactive cysteine residues. Eur J Biochem. 175: 151–165.

[17] K. Susanne and M.A. Tabatabai. (2008). Urease activity of microbial biomass in soils. Soil Biology and Biochemistry. 31: 205-211.

[18] M.I. Zantua and J.M. Bremner. (1977). Stability of urease in soils. Soil Biol. Biochem. 9: 135-140.

[19] W.T.J. Frankenberger and M.A. Tabatabai. (1991). Factors affecting Lglutaminase activity in soils. Soil Biol Biochem. 23: 875–879.

[20] Shi R, Stein K, Schwedt G: 1997. Determination of mercury(II) traces in drinking water by inhibition of an urease reactor in a flow injection analysis (FIA) system. Fresenius J Anal Chem, 357:752–755.