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UREA HYDROLYSIS AND UREASE ACTIVITY IN SAUDI ARABIAN SOILS

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تحولات اليوريا ونشاط إنزيم اليورييز في الترب الزراعية بالمملكة العربية السعودية

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جُمعت عينات التربة من ثمان مواقع مختلفة في المملكة العربية السعودية وقد جرى تحليلها لتحديد تركيبها الميكانيكي ، كما تم تقدير تحولات اليوريا ونشاط إنزيم اليورييز في جميع هذه الترب .
أوضحت نتائج التحليل أن معظم الكمية المضافة من اليوريا (١٠ملجم يوريا/جم تربة) تحولت إلى أمونيا في جميع الترب ، وذلك خلال أربعة أسابيع . إضافة اليوريا إلى التربة أدت إلى زيادة واضحة في تركيز أيون الأمونيوم في أغلب الترب . ولقد ظهر أعلى معدل لأيون الأمونيوم في تربة منطقة الخرج (٤ ملجم/جم تربة) والتي سجلت أفضل مستوى لنشاط إنزيم اليورييز (٨٥ ميكروجرام يوريا/جم تربة/ساعة) مقارنة بجميع المواقع المدروسة .

Key Words: Urea hydrolysis, urease enzyme, soil, ammonia.

ABSTRACT

Soil samples were collected from eight different localities in Saudi Arabia, and analysed for mechanical properties as well as for urea hydrolysis. The urease enzyme activity of these calcareous soils was also investigated. Urea applied (10 mg urea - N/g soil) was almost totally hydrolysed to ammonia in all soils tested within four weeks. The addition of urea to soils led to a marked increase in the concentration of NH_4^+ in most soils. The maximum amount of NH_4^+ was recorded in Alkharj soil (4.0 mg/g soil), a site which exhibited the highest activity of urease enzyme (85 µg Urea/g/h).

INTRODUCTION

The use of urea as a nitrogen fertlizer in world agriculture has increased dramatically in recent years. Each year, approximately 500,000 tons of urea are applied to farms in Saudi Arabia (1). it is predicted that the importance of urea will continue to increase due to the advantages it has over other nitrogen fertilizers. Urea is hydrolyzed by the soil enzyme urease. The hydrolysis of urea proceeds according to the following equation (2):

$$NH_2{\cdot}CO{\cdot}NH_2 \rightarrow NH_2{\cdot}COOH{+}NH_3 \rightarrow CO_2 + 2NH_3 \rightarrow H_2{\cdot}CO_3{+}2NH_3$$

The presence of urease enzyme in the soil is of paramount importance to the utilization of urea in agricultural applications. Urea has been considered as a slow-release fertilizer since it must undergo two transformations in the soil before it becomes available to most crops. The first transformation of urea is urease enzyme catalyzed hydrolysis to carbonate ion and ammonium ion. The second transformation is nitrification in which ammonium ion is oxidized in the soil via microbilogical processes initially to nitrite and subsequently to nitrate (3).

Urease activity tends to be high in non-calcareous soils under dense vegetation and low in saline and gleyed soils or soils lacking organic matter (4). The rate of urea hydrolysis increases with increase in urea concentration until the urease enzyme becomes saturated with substrate (5, 6).

The aim of the present study is to investigate the urea hydrolysis and released ammonia in the soils of various regions of Saudi Arabia. Concurrently, urease enzyme activity was studied because it is catalysing the transformation of urea into ammonia.

MATERIALS AND METHODS

Collection and analysis of soil samples

Soil samples were collected in sterile polyethylene bags from eight different localities in Saudi Arabia, five samples from each site collected at different depths from 0-15 cm. mechanical analysis of the soil was made by the sieve method and soil texture was determined using the soil texture triangle. The method described by Jackson (7) was used for determination of the total soluble salts (T.S.S.). Soil pH was determined with a glass electrode in a water-soil slurry (10:1). Soil organic matter percentage was determined colorimetrically using the method described by Walinga *et al* (8).

Nitrification and extraction of soil nitrogen ions

Soil samples were amended with urea (110 mg urea-N g-1 soil), in order to determine the urea transformation into NH_4^+ in soil. The soil samples were incubated in closed polythene bags with a small hole to allow for gas exchange. The bags were set up in triplicate. The soils were moistened to a water potential of -0.9 MPa and incubated at 25°C for 5 weeks.

Urea was extracted from soil with distilled water and NH_4^+ was extracted from soil using KCL (1.5 N). In all cases a 1:10 soil extractant ration was used and the slurry was shaken for 15 min (100 throws min-1). After being shaken, the soil slurries were filtered through Whatman No.1 filter paper and the concentration of nitrogen ions determined. Urea was determined according to Douglas and Bremner (9) and NH_4^+ by the indophenol blue method (10).

Determination of urease enzyme

For estimation of urease activity the method of Douglas and Bremner (9) was used. Triplicate samples of soil (5 g) were placed in universal bottles, and 4 ml of 0.5 M sodium acetate buffer (pH 6.5), 1 ml of toluene and 10 ml of the substrate (5 mM urea-N) solution were added. The reaction mixtures were incubated for 8 h at 25°C on an orbital shaker (150 throws min^{-1}). After this time the reaction was stopped by the addition of 2 M potassium chloride-phenyl mercuric acetate reagent (20 ml). The flasks were shaken for a further 30 minutes, then filtered through Whatman No.1 filter papers and the urea concentration of the extract analysed at 525 nm in a Pye Unicam SP 500 spectrophotometer.

Urea concentration was calculated from a urea-N standard curve (0-10 μg ml^{-1}) prepared on the day of analysis.

RESULTS

The soil characteristics of the different regions in Saudi Arabia are shown in Table 1. All soil samples tested contained low percentages of organic matter (Table 1). The soils were slightly alkaline (pH 7.2-8.1). and sandy in texture in all cases, with exception of sandy loam and loamy sand soils in Qassim and Wadi Dawaser respectively. There was a high content of total slouble salts in Hail (0.51%) and Wadi Dawaser (0.69%) soils.

All of Saudi Arabian soils hydrolysed urea leading to the formation of comparable large amounts of ammonia (Fig 1-8). This decline in urea was associated with a concomitant increase in net NH_4^+ production. The hydrolysis of urea, as measured by both loss of urea and increase in NH_4^+ varied from one soil to another.

The concentration of added urea decreaed more rapidly in Alkharj soil compared to the other Saudi Arabian soils tested. Alkharj soil followed by Qassim soil induced the highest hydrolysis of urea (0.8 and 2.5 mg g^{-1} soil respectively, Fig. 3 and 5), while the lowest hydrolysis of urea was observed in Hail soil (5.4 mg g^{-1} soil) at the end of the incubation period (Fig. 4).

Alkharj soil was particularly active in releasing ammonia, forming over 4.0 mg of NH_4^+ soil at the end of the incubation period (Fig.3) followed by Wadi Dawaser soil with 3.2 mg of NH_4^+ soil (Fig.8). The relatively largest amount of NH_4^+ was formed by the soils of Alkharj and Wadi Dawaser; the concentration of this ion exceeding 2.0 mg g^{-1} soil from the 1st week sample onwards (Fig.3 and 8). On the other hand the Hail soils (Fig.4) exhibited the lowest amount of NH_4^+ 1.5 mg of NH_4^+ g^{-1} soil at the end of the incubation period.

The urease activity of the different Saudi Arabian soils are given in Fig. 9. The soil samples tested show wide variabilities in their urease activities which were found to vary between 8.3 -85 μg ureahydrolysed per gram of soil per hour. Alkharj and Qassium soils exhibited the highest urease activity (85 and 70 μg urea / g soil respectively). Moderate urease activity was exhibited by the Riyadh, Alhassa, Aljouf and Wadi Dawaser soils (40, 30, 27 and 33 μg Urea/g soil respectively). Slight urease activity was shown by the Hail soil.

DISCUSSION

Following diffusion of urea into the soil samples, and exposure of urea to urease enzyme throughout the soil, the urea concentration remaining in the samples decreases reapidly as the urea is hydrolysed by urease enzyme. the results demonstrate that urea applied to the soil surface in aqueous solution is almost totally hydrolysed in all Saudi Arabian soils by urease enzyme in approximately four weeks.

The organic matter content of the soil samples shown in Table 1 varies between 0.02-0.80%. Sandy soils such as Saudi Arabian soils typically exhibit orgnaic matter values in the range of 0.10 -0.75% (1). The presence of orgnaic matter in soil that promote microbial activity will stimulate production of urease by soil microorganisms. Thus soil organic matter content normally correlates with urease activity, the soil organic matter stimulaes microbial activity and subsequent urease activity (11, 12). In addition, urease enzyme occurs in the soil in forms in which it is protected against degradation, i.e., in the form of stabilized urease-humus organic complexes or through an assocaition with clay particles (13).

The pH values of the Saudi Arabian soils presented in Table 1 are in the range 7.2-8.3. This range coincides with one of the pH optima of urease, i.e., pH range 7-8 (14). The pH range of the soils studied is conducive to attainment of the maximal rate of efficiency of the urease-catalyzed hydrolysis of urea. The urease activity of the soils studied is dependent on several factors including soil pH and organic matter.

The total soluble salts has been reported to have no effect on urea hydrolysis but enhances ammonia volatization and inhibits nitrification with simultaneous accumulation of nitrite in the soil (15, 16).

The ammonium concetration occurs abundantly in soils receiving applications of urea as a fertilizer. Optimum NH_4^+ production occurred in Alkharj soil which appears to exhibit the highest activity of urease anzyme. The trend of urea hydrolysis in case of the other soils seems to be consistent with the findings of previous studies [1 6, 12, 15]. Minimum NH_4^+ production recorded in soil of Hail appears to be correlated with the lowest activity of urease enzyme occurring in this site.

In conclusion, the addition of urea to soils led to a marked increase in the concentration of ammonium in most soils. The maximum amount of ammonium was recorded in Alkharj soil which exhibited the highest activity of urease enzyme.

REFERENCES

[1] **Peters, E., M. Rafi, M. bakalla, A. Shammas, M. Baroudi and M.Dawood, 1997.** Urease *anzyme* activity in some Saudi soils. Abstracts of the First Saudi Symposium on Agricultural Science. p, 29.

[2] **Blakeley, R.L., E. C. Webb and B. Zerner, 1969.** Jack bean urease (EC 3515). A new purification and reliable rate assay. Biochem, 8: 1984-1990.

[3] **Killham, K., 1994.** Soil Ecology, Cambridge University Press.

[4] **Bremner, J.M. and R. L. Mulvaney, 1978.** Urease activity in soils. In: *soil Enzymes* (Ed, Burns, R.G). Academic Press, London, pp 149-196.

[5] **Tabatabai, M.A. and J.M. Bremner, 1972.** Assay of urease activity in soils. Soil Biol. Biochem. 4, 479-487.

[6] **Overrein, L.N. and P.G. Moe, 1967.** Factors affecting urea hydrolysis and ammonia volatization in soil. Soil Sci. Soc. Am. Proc., 31: 57-61.

[7] **Jackson, M.L., 1962.** Soil and plant analysis.Constable & Co. Ltd. London.

[8] **Walinga, I., M. Kithome, L. Novozamsky, V. J.K. Houba and J.J. van der Lee, 1992.** Spectrophotmetric determination of organic carbon in soil. Commun. Soil Sci. Plant Anal., 33 (15 &16): 1935-1944.

[9] **Douglas, L.A. and J. M. Bremner, 1970.** Colorimetric determination of microgram quantities of urea. Anal. Letters, 3: 79-87.

[10] **Wainwright, M. and G.J.F. Pugh, 1973.** The effect of three fungicides on nitrification and ammonification in soil. Soil Biol. Biochem, 5:577-584.

[11] **May, P.B. and L.A. Douglas, 1976.** Assay for soil urease activity. Plant Soil 45, 301-305.

[12] **Mulvaney, R.L. and J. M. Bremner, 1981.** Control of urea transformations in soils. In: Soil Bichemistry (Eds, Paul, E.A. and Ladd, J.N). Marcel Dekker, New York. Vol.5, pp 153-196.

[13] **Pettit, N. M., A.R.J. Smith, R.B. Freedman and R.G. Burns. 1976.** Soil urease activity, stability and kinetic properties. Soil Biol. Biochem. 8: 479-484.

[14] **Rachhpal-Singh and P.H. Nye, 1984.** The effect of soil pH and high urea concentration on urease activity in soil. J. Soil Sci. 35: 519-527.

[15] **Gould, W.D., F.D. Cook and G.R. Webster, 1973.** Factors affecting urea hydrolysis in several Alberta soils. plant Soil 38: 394-401.

[17] **Wickramasinghe, K.N., S. Sivasubramaniam and P. Nalliah, 1982.** Urea hydrolysis in some tea soils. Plant Soil 62: 473-477.

Table 1.

Soil characteristics of different regions in Saudi Arabia (n=3)

Soil Locality	Mechanical Fraction % Sand	Silt	Class Clay	Texture %	O.M. %	pH	T.S.S %
Alhassa	89.0	5.8	5.2	Sand	0.80	7.4	0.23
Aljouf	98.3	0.9	0.8	Sand	0.13	7.8	0.08
Alkharj	92.6	1.2	6.2	Sand	0.07	7.2	0.13
Hail	91.3	3.3	5.4	Sand	0.09	7.3	0.51
Qassim	56.4	6.1	37.5	Sandy loam	0.68	7.7	0.14
Riyadh	90.6	4.2	5.2	Sand	0.62	7.5	0.19
Tabouk	89.0	3.3	7.7	Sand	0.74	8.1	0.34
Wadi dawaser	82.0	5.5	12.5	Loamy sand	0.02	8.0	0.69

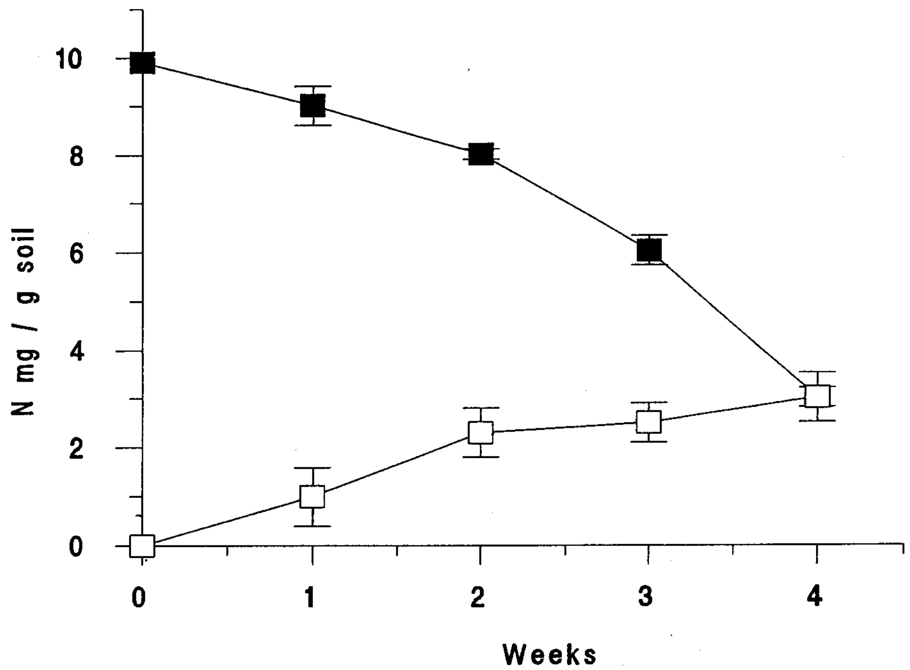

Fig. 1: Urea hydrolysis (10 mg urea -N/g soil) in Alhassa soil, all values are means of triplicates ± S. D. (■,Urea; , Ammonium).

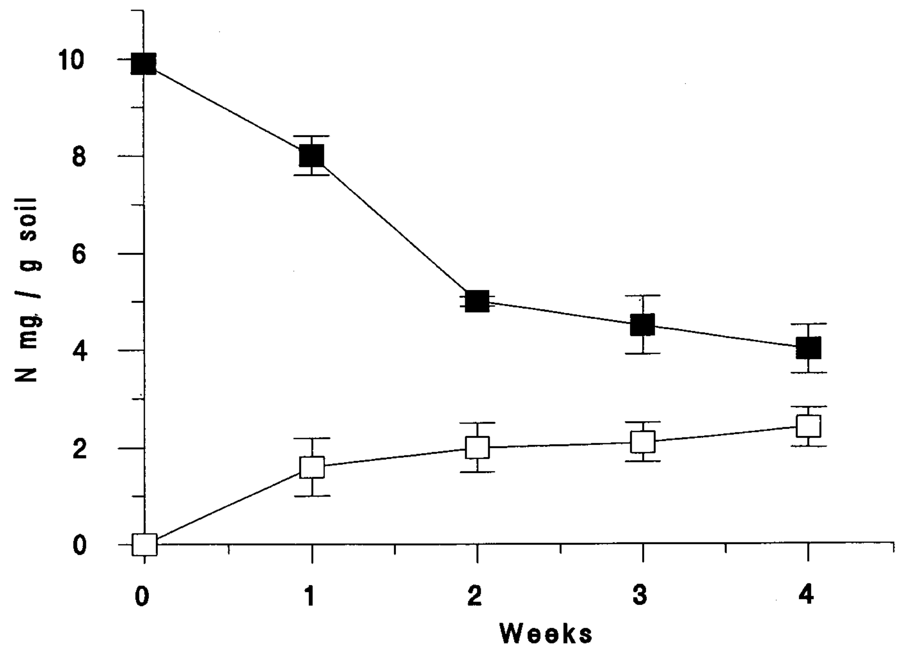

Fig.2: Urea hydrolysis (10 mg urea-N/g soil) in Aljouf soil, all values are means of triplicates ± S. D. (■,Urea; , Ammonium).

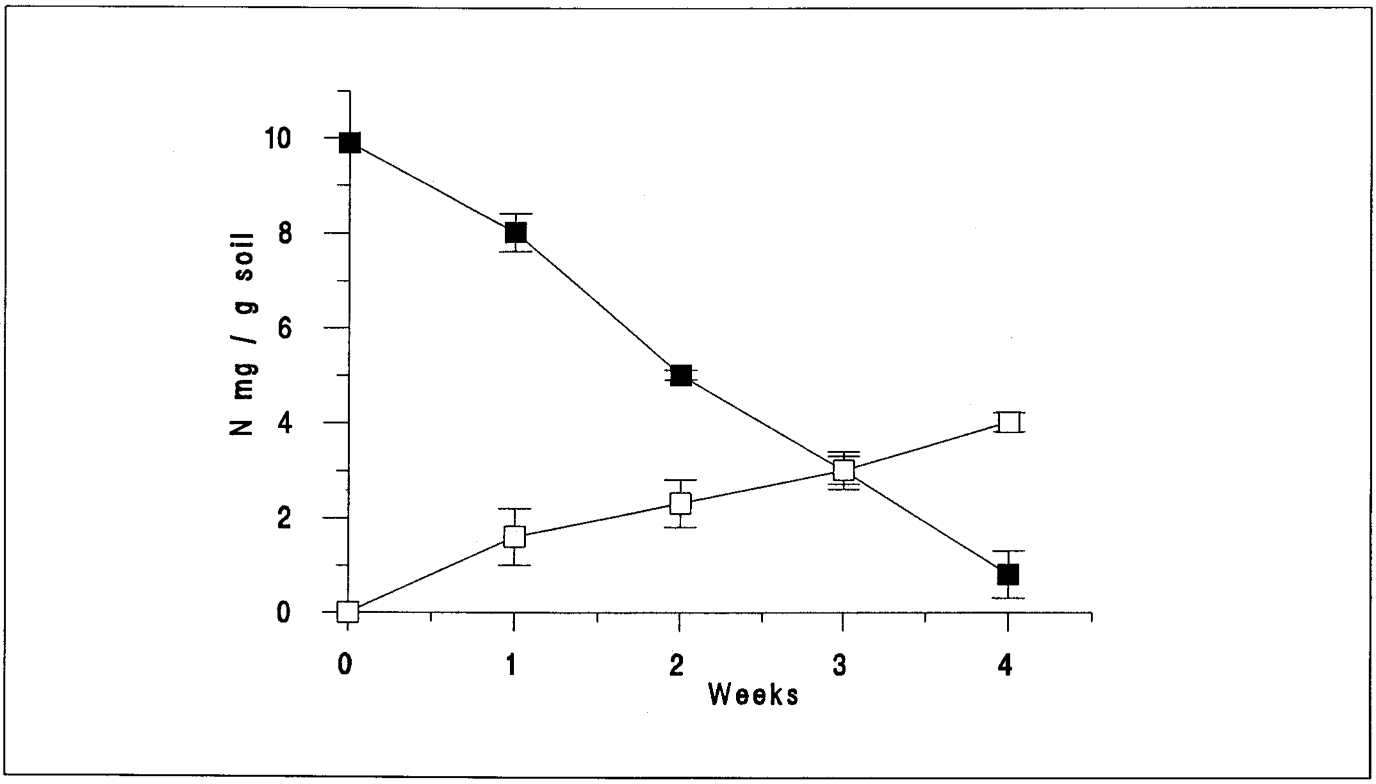

Fig. 3: Urea hydrolysis (10 mg urea -N/g soil) in Alkharj soil, all values are means of triplicates ± S. D. (■,Urea; , Ammonium).

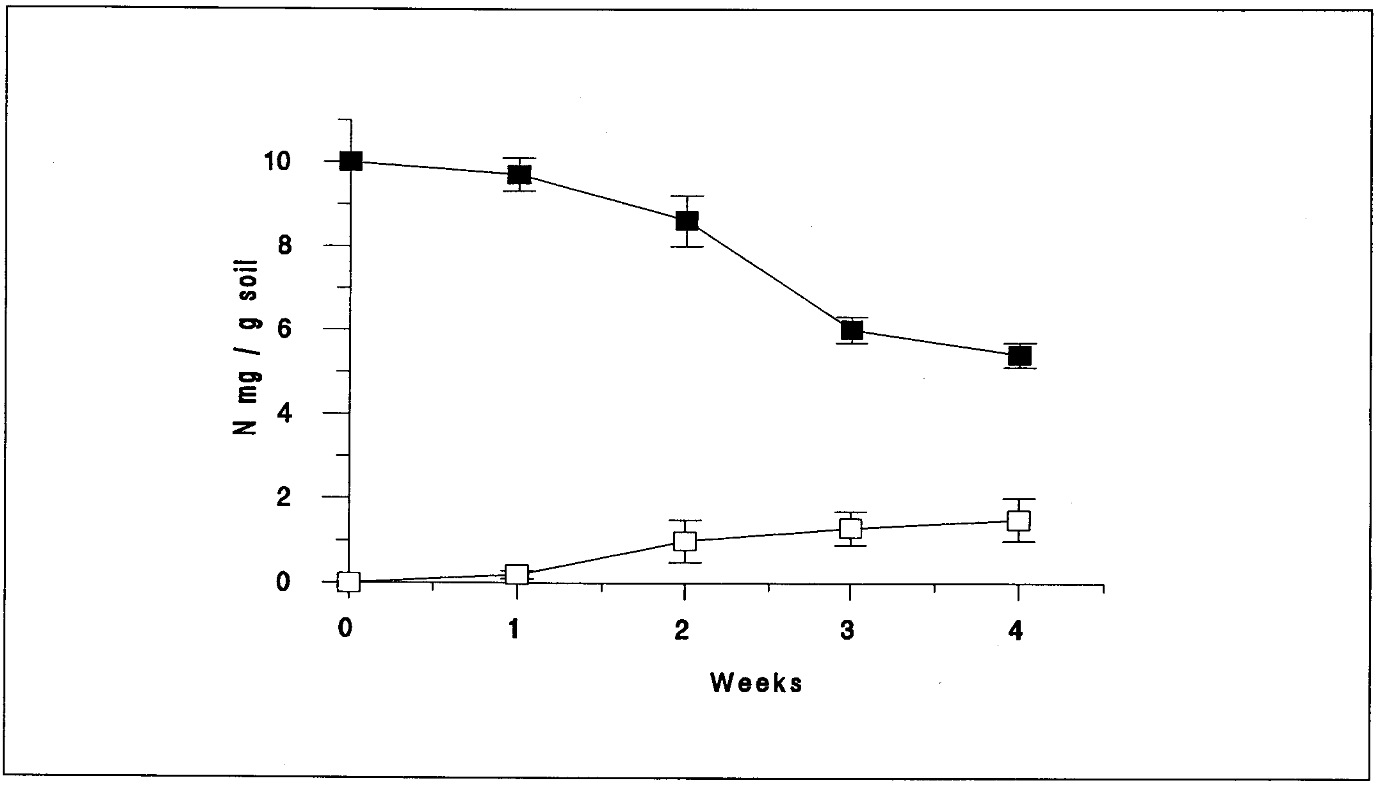

Fig. 4: Urea hydrolysis (10 mg urea-N/g soil) in Hail soil, all values are means of triplicates ± S. D. (■,Urea; , Ammonium).

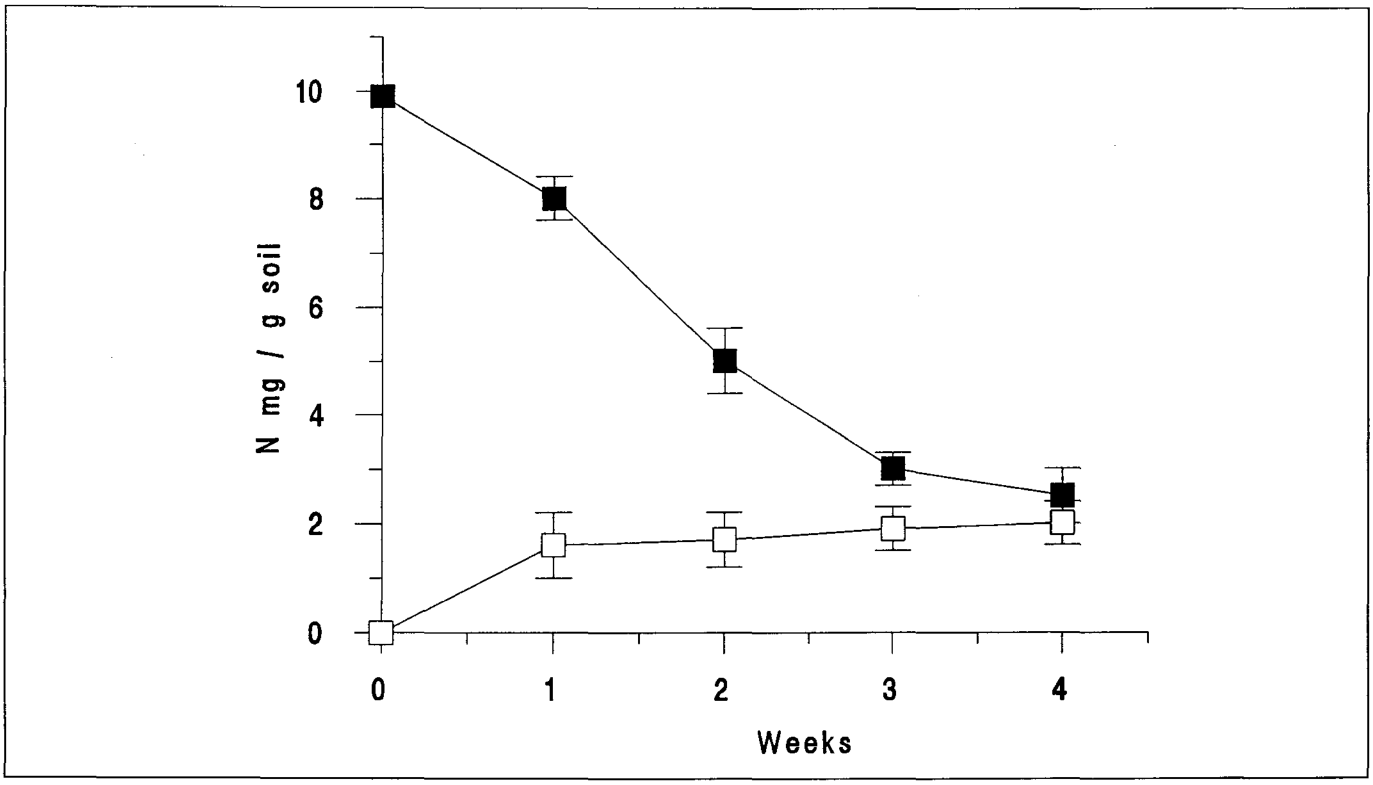

Fig. 5: Urea hydrolysis (10 mg urea-N/g soil) in Qassim soil, all values are means of triplicates ± S. D. (■,Urea; , Ammonium).

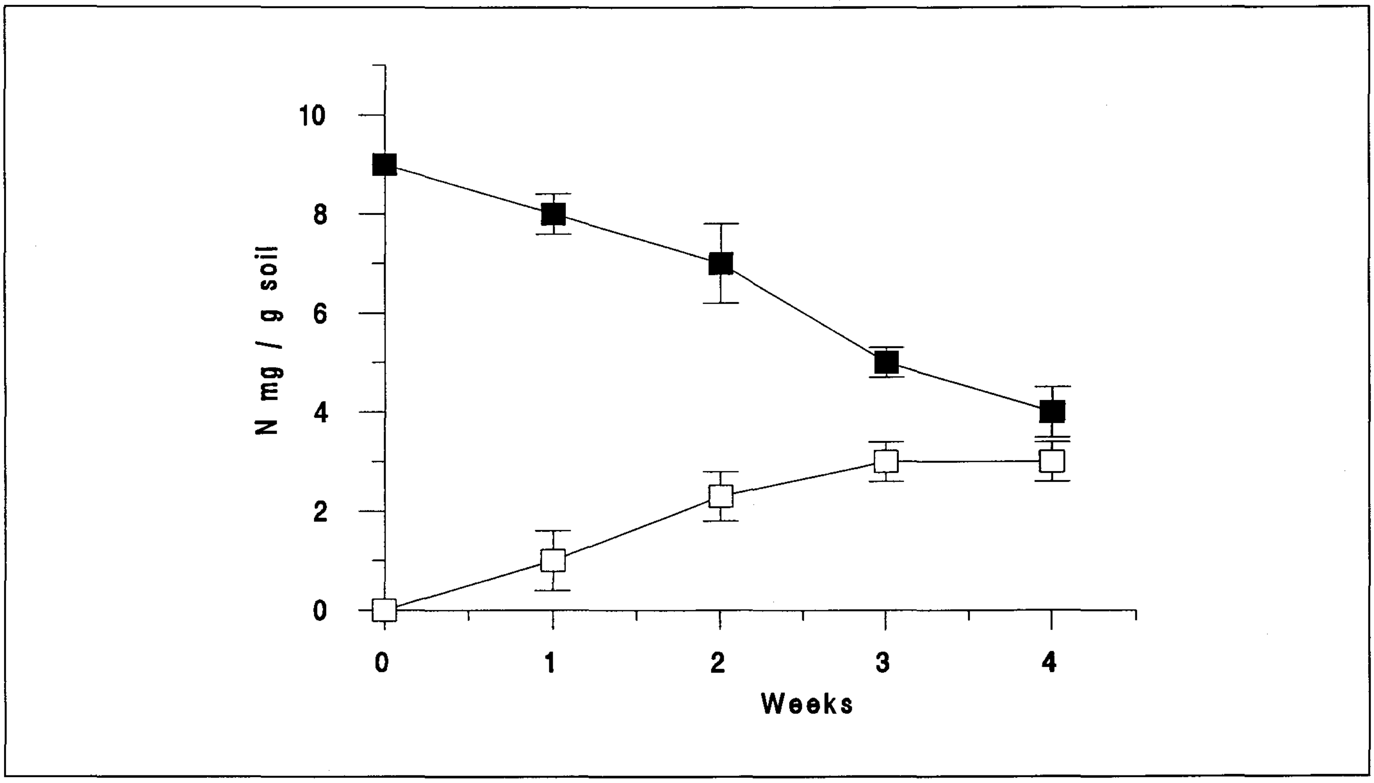

Fig. 6: Urea hydrolysis (10 mg urea-N/g soil) in Riyadh soil, all values are means of triplicates ± S. D. (■,Urea; , Ammonium).

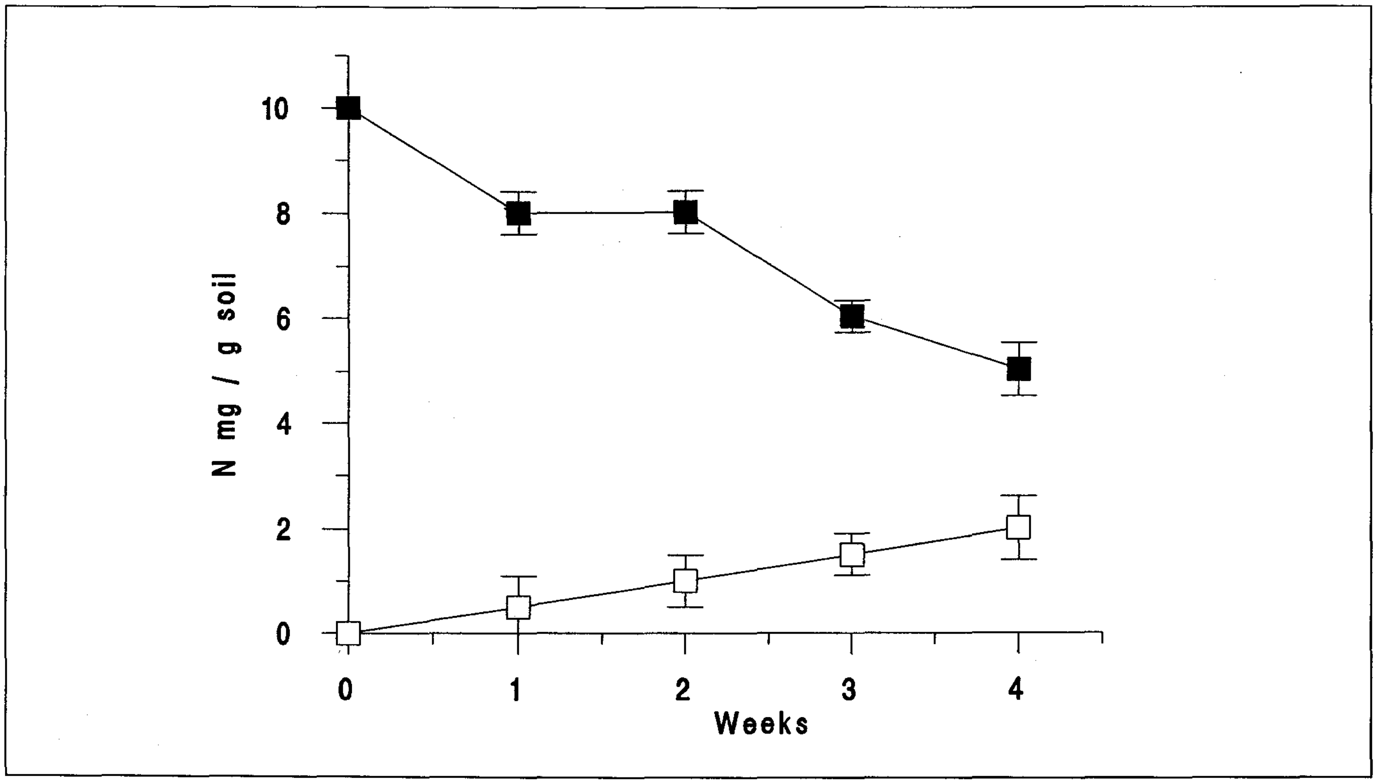

Fig. 7: Urea hydrolysis (10 mg urea-N/g soil) in Tabouk soil, all values are means of triplicates ± S. D. (■,Urea; , Ammonium).

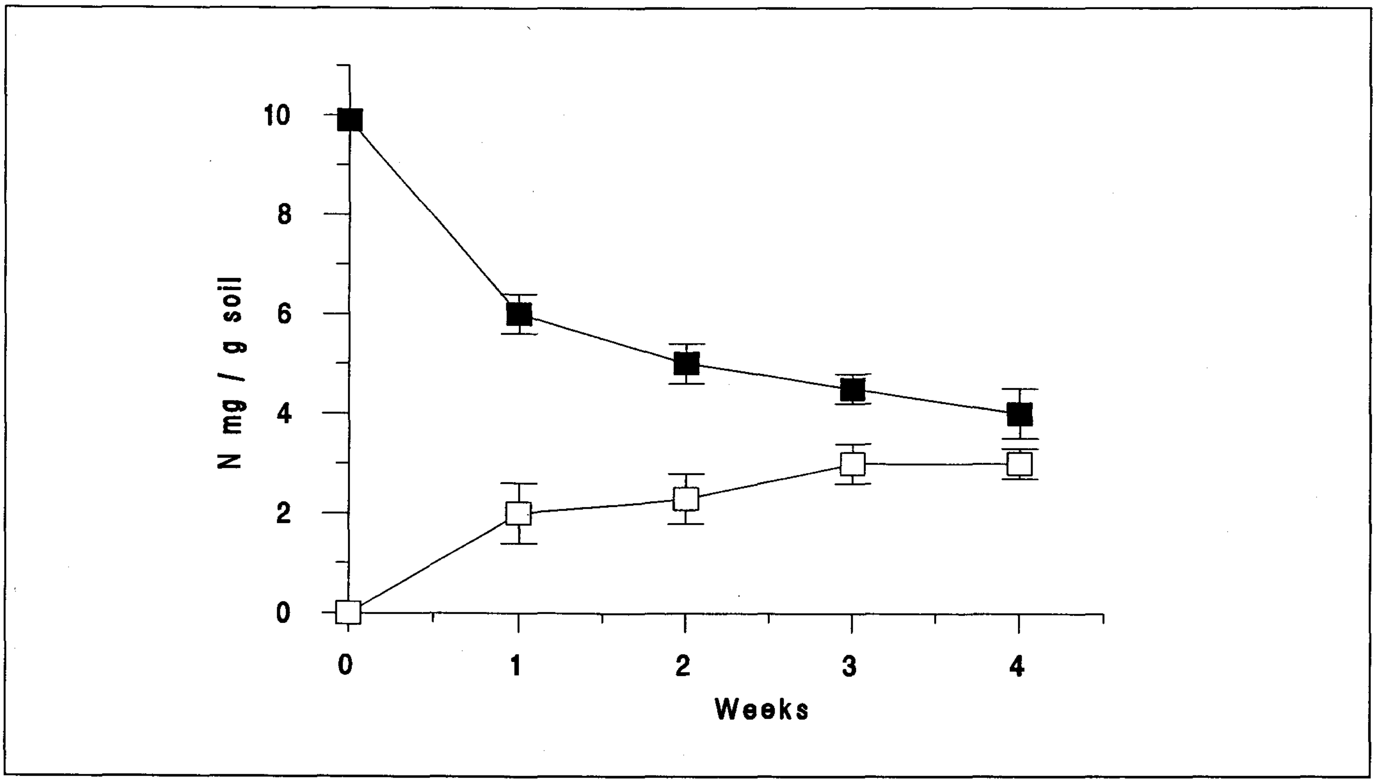

Fig. 8: Urea hydrolysis (10 mg urea-N/g soil) in Wadi Dawaser soil, all values are means of triplicates ± S. D. (■,Urea; , Ammonium).

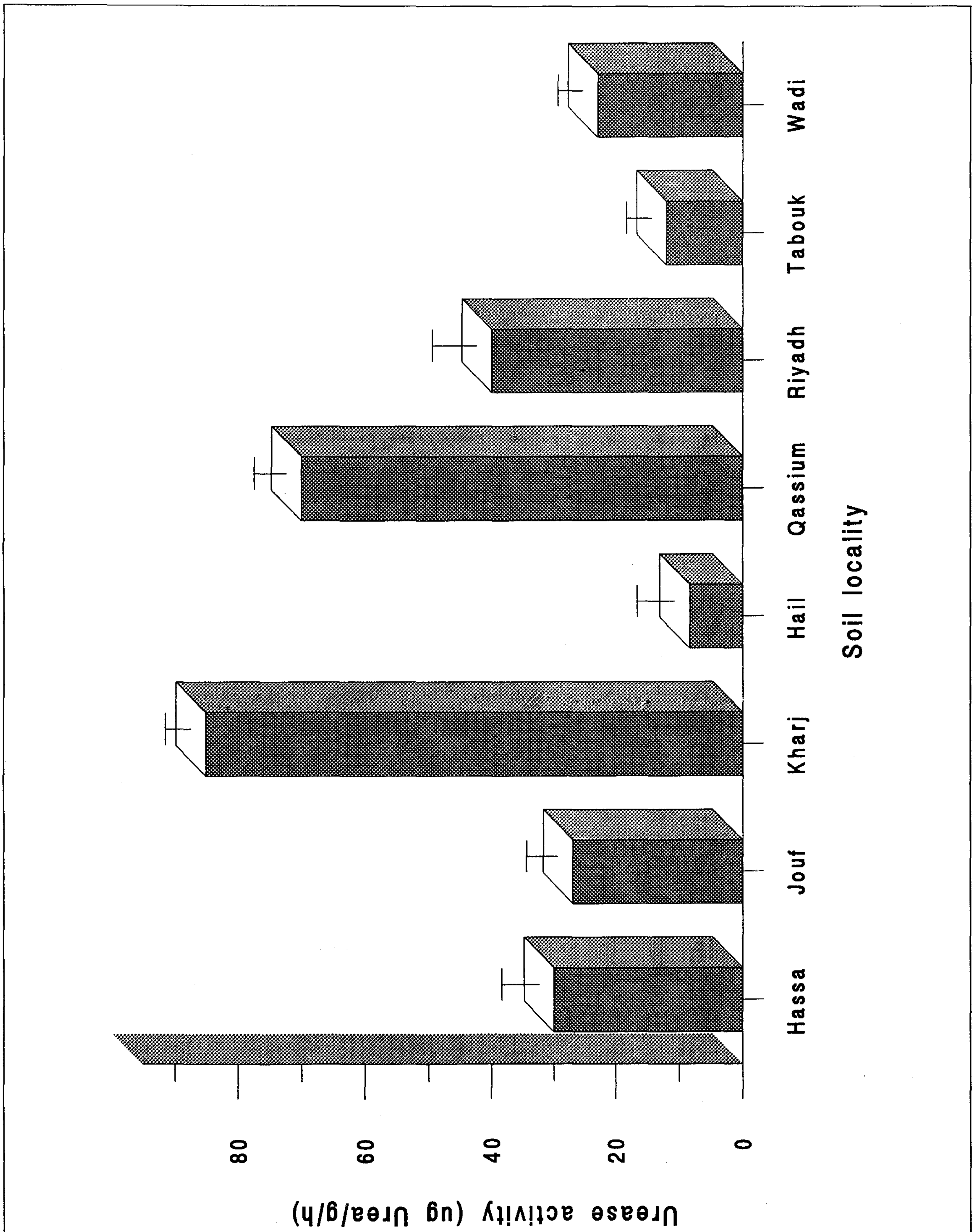

Fig. 9: Urea activities (10 μg Urea/g/1h) in Saudi Arabian soil, collected from different regions, all values are means of triplicates ± S.D.