

Nitrogen Cycling In Soil Cultivated With Sugar Beet

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Abstract. Soil cultivated with sugar beet in Saudi Arabia, analysed mechanically and chemically. Four varieties of sugar beet used in the first attempt of sugar beet cultivation in Saudi Arabia. The four varieties of cultivated sugar beet are vision, universe, roberta and sonja. The characteristics of a sandy soil tested supported the best growth of sugar beet plant. Results indicated that the soil samples have low cation exchange capacities. Growth of sugar beet varieties recorded a sufficient level with the exception of sonja variety in shoots measurements and vision variety in roots measurements. The highest content of sucrose found in universe variety that was 18 %. Hydrolysed urea to ammonium and oxidised ammonium in soil cultivated with sugar beet led to a marked increase in nitrate concentration, but trace amounts of nitrite have been detected. However this increase in amounts of nitrate associated with activity of heterotrophic nitrifying fungi. Seven isolates species belonging to three nitrifying fungal genera isolated from soil samples. *Penicillium* was predominant and represented by three species followed by *Aspergillus* and *Fusarium* with two species each.

Key words: Nitrifying fungi, Nitrogen cycling, *Beta vulgaris*, *Penicillium*, *Aspergillus*, *Fusarium*.

Introduction

Sugar beet is a biennial herb grown as annual for its fleshy roots containing large amounts of sugar used for the manufacture of sugar. Sugar beet is a native to northern Europe and developed as a sugar source primarily in Germany (Van Royen, 1954), but now grown in several countries in Asia, Africa and America. Optimum temperature of sugar beet is about 21° C. While moisture requirements of sugar beet are moderate, with the largest amounts needed during the middle of the period of vegetative growth. When the sugar beet is grown under irrigation in areas of warmer climate, the beets can be seeded in the fall or winter months, and harvested in the summer. Mahendrappa et al., 1966 reported that a careful regulation of water supply aids in attaining high sugar content (16-20 % w/w sucrose). Since sugar beet is one of

the very important economic crops, so it is worth to try to cultivate it in Saudi Arabia as other country do.

Four varieties of the common sugar beet, *Beta vulgaris* Linn, var, *ropa* Dum, Chenopodiaceae, were used in the first attempt of sugar beet cultivation in Saudi Arabia. The four varieties of cultivated sugar beet are vision, universe, roberta and sonja.

Sugar beet growth and the percentage of sugar content in this crop are affected by soil microbial processes such as nitrification, S-oxidation and P-solubilization (Al-Falih, 1995). Nitrification is the biological formation of nitrate or nitrite from compounds containing reduced nitrogen (Alexander, 1977). The term generally refers to the oxidation of ammonium to nitrite and nitrate by specialised fungi. A wide variety of heterotrophic fungi have been shown to be capable of oxidizing reduced forms of

nitrogen (Eylar and Schmidt, 1959). Although the ability of fungi to hydrolyse urea and nitrify is well established (Killham, 1986). Certain fungi and heterotrophic bacteria have a role to play in nitrification also, particularly in acid forest soils (Killham, 1994).

This study aims to evaluate the possibility of growing sugar beet in Saudi Arabia. Here I report the activity of nitrifying fungi in agricultural sand soil cultivated with sugar beet, particularly nitrification of ammonium sulphate and urea in soil. This led me to isolate some species of nitrifying fungi responsible.

Materials and Methods

Collection and Analysis of Soil Samples

Field experiment was carried out in Saudi Arabia, during the successive growing season October 2002, to attempt growing sugar beet. The soil type is sandy, mechanical and

Sugar beet Shoots and Roots Measurements and Sugar Extraction

Four varieties of the common sugar beet, *Beta vulgaris*, were grown in Saudi Arabia. These were vision, universe, roberta and sonja. Each variety was growing in a complete block design measuring 75 m square.

Shoots and roots of each plant were weighted separately for fresh weight, while dry weight of total leaves was determined using an oven at 108° C for 48h. Leaf area for each plant of sugar beet varieties was estimated by photocopying and weighting. Number of roots per m square for each variety were counted. In all previous measurements five replicates of each variety of sugar beet plant were used. Water irrigation was given on a weekly basis.

Sucrose was extracted from roots of sugar beet (10 g) by shaking for 15 min. in boiling

Table 1. Certain characteristics of the soil used in the experiment (All values are means of triplicates).

Mechanical fraction			Texture class	Organic matter %	CaCO ₃ %	PH	E.C. mmhos/cm	T.S.S. %	HCO ₃ %	SO ₄ %
Sand	Silt	Clay								
91.3	3.3	5.4	Sand	0.13	24.30	7.7	0.12	0.83	0.036	0.038

chemical properties of the soil are shown in (Table 1).

Mechanical analysis of the soil was made by the sieve method. Soil pH was determined with a glass electrode with a water soil slurry (10:1). The methods described by Jackson (1962) were used for soil chemical analysis included the determination of the conductivity as well as the estimation of the percentage content of total soluble salts, bicarbonates, sulphates, and calcium carbonates. Soil organic matter percentage was determined colorimetrically using the method described by Walinga *et al.* (1992).

water (100 ml). Sucrose concentration in sugar beet extract was determined by the dinitrosalicylic acid method (Bernfeld, 1955).

Determination of soil nitrifying fungi

Soil nitrifying fungi were isolated by the dilution plate technique as adopted earlier by Bokhary *et al.*, 1984. Soil (1 g) was shaken in sterile Ringers solution (10 ml) for 15 min. Samples of the resulting suspension was then serially diluted in Ringers solution. Six replicates of the final dilution was spread on the surface of Czapek-Dox Agar (Oxoid). Rose bengal (0.03 g/l) was also added in the medium to reduce the spread of fast growing fungi,

while streptomycin sulphate (0.033 g/l) was added to eliminate bacterial growth. Inoculated plates were incubated at 25° C for 7 days. The fungal genera and species were identified according to Raper and Fennell (1965), Gilman (1971), Pitt (1979), Ramirez (1982) and Nelson *et al.*, (1983).

Nitrification and Extraction of soil Nitrogen ions

Soil samples were amended with either ammonium sulphate (5 mg N g⁻¹ soil) or urea (10 mg urea-N g⁻¹ soil), in order to respectively determine the effect of soil nitrifying fungi on the process of nitrification of ammonium, hydrolysis of urea and nitrification of released ammonium in soil.

The soil samples were incubated in polythene bags, closed with a small hole to allow for gas exchange. The bags were set up in triplicate. The soils were moistured to a water potential of -0.9 MPa and incubated at 25°C for 4 weeks.

Ammonium was extracted from soil using KCL (1.5N), nitrate and nitrite were extracted from soil with distilled water. In all cases a 1:10

(Wainwright and Pugh, 1973); nitrate using an Orange 1 method (Middleton, 1959) and nitrite colorimetrically as described by Hesse (1971).

Results and Discussion

Soil and Plant analysis

The results of mechanical and chemical analysis of 1:5 soil extract (Jackson, 1962) for samples taken from soil cultivated with sugar beet in Saudi Arabia are given in Table 1. The particle size distribution of the soil samples show that the soil texture class is sandy. It is clear from the results presented in Table 1 that the content of total soluble salts is very low, not exceeding 0.083 %, and is mainly represented by sulphates. The soil reaction was slightly alkaline, pH value is 7.7. Alkalinity may be referred to the high content of CaCO₃, which amounted to about one-fourth of the weight of the oven-dry soil. The percentage of organic matter and bicarbonates are low. However the effect of organic matter, sulphates and bicarbonates content of soil looks negligible since soil samples contained low percentage. The result, consistent with the findings of earlier studies on some Saudi Arabian soil (Ali

Table 2. Shoots measurements of different varieties of sugar beet plant (Values are means of 5 replicates, standard deviation).

Parameters	Sugar beet varieties			
	Vision	Universe	Roberta	Sonja
Total leaves fresh wt. (g)	175.4 ± 10	215.6 ± 30	188.5 ± 17	241.3 ± 24
Total leaves dry wt. (g)	35.1 ± 2	45.2 ± 5	39.2 ± 4	50.6 ± 9
Single leaf area (cm)	150.9 ± 33	176.3 ± 14	200.4 ± 30	250.7 ± 27

soil extractant ratio was used and the slurry was shaken for 15 min (100 throws min⁻¹). After being shaken, the soil slurries were filtered through Whatman No1 filter paper and the concentration of nitrogen ions determined. Ammonium by the indophenol blue method

and Abou-Heila 1984, Hashem 1993, Abdel-Hafez 1981, Youssef and El-Sheikh 1981).

Table 2 records shoots measurements of different varieties of sugar beet plant. The results show that the highest leaves fresh and dry weight was given by the varieties of sonja

Table 3. Roots measurements of different varieties of sugar beet plant (Values are means of 5 replicates, standard deviation).

Parameters	Sugar beet varieties			
	Vision	Universe	Roberta	Sonja
Total roots No./ m ²	7.53 ± 1	8.1 ± 0.5	7.77 ± 2.1	7.16 ± 0.9
Single root fresh wt. (g)	450.6 ± 21	810.3 ± 56	616.2 ± 34	345.7 ± 13
Single root sucrose %	10.1 ± 0.8	18.0 ± 1.2	15.3 ± 1.4	12.3 ± 0.7

Table 4. Nitrifying Fungi isolated from soil cultivated with sugar beet in Saudi Arabia.

Species	No. of colonies per gram of soil
<i>Aspergillus flavus</i> Link ex Fries	33 ± 10
<i>Aspergillus niger</i> Van Tieghem	51 ± 12
<i>Penicillium chrysogenum</i> Thom	20 ± 5
<i>Penicillium notatum</i> Westling	13 ± 2
<i>Penicillium expansum</i> Link ex S.F. Gray	7 ± 3
<i>Fusarium oxysporum</i> Scheldon	19 ± 11
<i>Fusarium solani</i> (Martius) Saccardo	4 ± 2

and universe. Also the leaf area of sonja was larger than the other varieties. Vision recorded the lowest measurement of leaves fresh weight, leaves dry weight and leaf area.

However, in the case of roots measurements (Table 3) the variety of universe looks the most successful plant for cultivation in soil tested. Sugar beet roots represented the most important part as a source of sucrose, therefore their numbers and fresh weight were reflecting a suitable comparison between sugar beet varieties. Universe have grown more than eight roots per meter square, but the other varieties were about seven roots per meter square. Root fresh weight was found to be 810.3, 616.2, 450.6 and 345.7 gram in the variety of univers, roberta, vision and sonja respectively. The highest sucrose content showed in a variety of universe with 18.0%; followed by roberta with 15.3%, however in Britain the sucrose percentage of sugar beet is reaching 16% (Al-Falih and Wainwright, 1996). Vision variety was forming 10.1 % of sucrose only. The

sucrose content of sugar beet is varied from 12 to 20 % according to many factors including soil nutrients, soil texture, soil pH, temperature, irrigation and variety selection and breeding of sugar beet (Al-Falih, 1995). The results suggest that univers variety of sugar beet plant might be used to cultivate in Saudi Arabian soils.

Nitrifying fungi

Seven fungal species belonging to three genera nitrifying fungi have been isolated from soil cultivated with sugar beet in Saudi Arabia (Table 4). The genus *Penicillium* was predominant with three species followed by *Aspergillus* and *Fusarium* with two species each. *Penicillium*, *Aspergillus* and *Fusarium* are common soil mycoflora which were isolated earlier from different types of soil in Saudi Arabia (Bokhary *et al*, 1984, Hashem 1991, Hashem 1994, Bokhary and Parvez 1992a, b). The highest number of colonies of soil nitrifying fungi isolated was *Aspergillus niger*, which was 51 colonies per gram of soil, while the lowest were *Penicillium expansum* and

Fusarium solani which were 7 and 4 colonies per gram of soil respectively. Nitrification is generally considered to be mediated by chemoautotrophic bacteria and heterotrophic fungi (Killham, 1986).

Nitrification of ammonium sulphate and urea

The hydrolysis of urea, as measured by increases in ammonium (Fig 1a), was stimulated throughout the 4 weeks incubation period, as was the subsequent nitrification of released ammonium to nitrate and nitrite (Fig 1b). Urea was hydrolysed leading to the formation of large amounts of ammonium over 3 mg per gram of soil at the end of the incubation period. Ammonium produced by

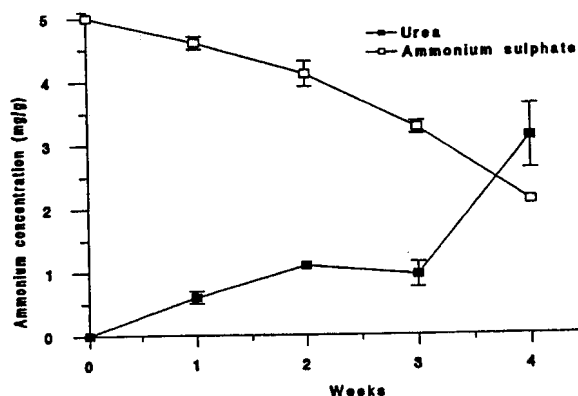


Fig 1a. Changes in ammonium concentration in soil.

urea hydrolysis was oxidized to nitrate, while nitrite was only formed transiently in trace amounts towards the end of the incubation period (Fig 1b). Nitrification led to a reduction in the pH of the soil. The pH of the soil was markedly decreased, to around pH 6 as a result of rapid nitrification, and therefore nitric acid production (Fig 2a).

Ammonium sulphate added was nitrified forming nitrate and again transient and small amounts of nitrite (Fig 2b). The concentration of added ammonium decreased rapidly

throughout the 4 weeks incubation period (Fig 1a). This decline in ammonium was associated with a decrease in soil pH (Fig 2a) and a concomitant stimulation in net nitrate production. Here the pH of the soil did not fall as was the case when urea was hydrolysed and then nitrified.

Nitrate concentration in the soil after addition of urea was 2 times higher than that after addition of ammonium sulphate, which may be due to the fast leaching of nitrate from the soil through irrigation water. In general the nitrification process in soil tested is looks average effective at nitrifying (Agarwal *et al*, 1971; El-Shahawy and Ghazi 1983 and El-

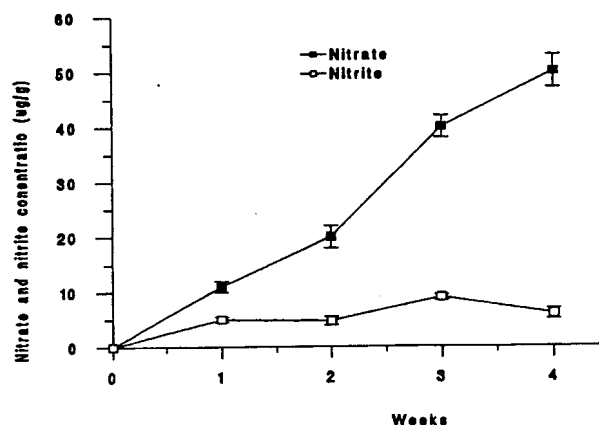


Fig 1b. Changes in nitrate and nitrite concentration in soil after addition of urea-N.

Shahawy and Amer 1984, Al-Falih and Wainwright, 1995a, Al-Falih and Wainwright, 1995b, Al-Falih and Wainwright, 1996).

In recent years, our understanding of the ecology of soil nitrification has changed. Two new aspects have become particularly evident. The first has been the realisation that nitrification is not as restricted by soil pH as was traditionally thought. The second aspect of this change in our understanding about nitrifier ecology is that two types of nitrification are now recognised; chemoautotrophic and hetero-

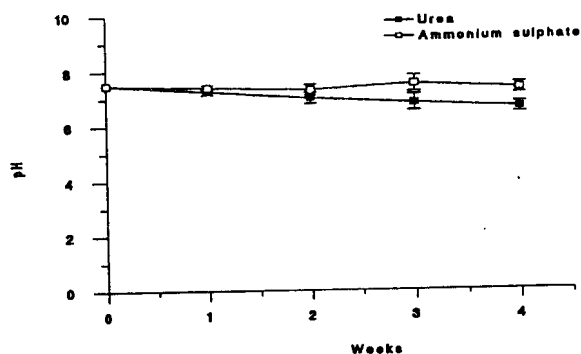


Fig 2a. Changes in soil pH.

trophic nitrification (Killham, 1994).

The obvious explanation for this observed stimulation in nitrification of ammonium and hydrolysis of urea and nitrification of released ammonium is that it was due to the activity of the populations of heterotrophic nitrifying fungi including *Aspergillus flavus*, *Aspergillus niger*, *Penicillium chrysogenum*, *Penicillium notatum*, *Penicillium expansum*, *Fusarium oxysporum* and *Fusarium solani*.

Conclusions

In general conclusion the characteristics of a sandy soil in Saudi Arabia supported the best growth of sugar beet plant. Results indicated that the soil tested have low cation exchange capacities. Although the activity of soil nitrifying fungi, some of which have been isolated such as genera of *Aspergillus*, *Penicillium* and *Fusarium*, will contribute in the beneficial of soil fertility to be rich in nitrogen ions. Transformation of ammonium or urea in soil tested led to a marked increase in available nitrate that was associated with activity of heterotrophic nitrifying fungi. As a result, the observed growth of sugar beet varieties recorded a sufficient level with the exception of sonja variety in shoots measurements and

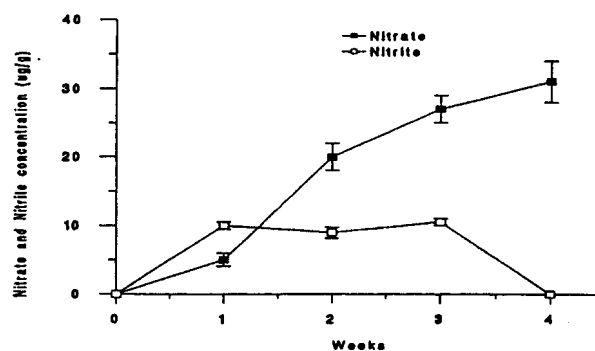


Fig 2b. Changes in nitrate and nitrite concentration in soil after addition of ammonium sulphate.

vision variety in roots measurements.

Finally this work shows the possibility of cultivating sugar beet in Saudi Arabia. The results recommend cultivation of universe variety in Saudi Arabian soils because of the high content of sucrose that was found to be 18%.

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تحولات النيتروجين في التربة التي زرع فيها بنجر السكر

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الملخص: تم جمع عينات مختلفة من التربة التي زرع فيها بنجر السكر في المملكة العربية السعودية وقد جرى تحليلها ميكانيكياً وكيميائياً. جربت أربعة أصناف من نبات بنجر السكر لمحاولة زراعتها لأول مرة في المملكة العربية السعودية، وهي فيشن وينيفيرس وروبيرتا وسونجا. أوضحت نتائج تحليل التربة أنها رملية وقاعدية وفقيرة في محتواها المعدني والعضوي. وأعطت الأصناف المختلفة من نبات بنجر السكر نمواً جيداً، باستثناء المجموع الخضري لصنف سونجا والمجموع الجذري لصنف فيشن. وجد من خلال التحليل الكيميائي للنباتات أن أعلى مستوى للسكر كان في صنف ينيفيرس والذي قدر بنسبة وصلت إلى ١٨ %.

أدت تحولات اليوريا إلى أمونيوم وأكسدة الأمونيوم في التربة التي زرع فيها بنجر السكر إلى زيادة كبيرة في تركيز النيترات NO₃ وزيادة قليلة في تركيز النيتريت NO₂، حيث ترتبط الزيادة في تركيز النيترات NO₃ بنشاط فطريات التآزت في التربة.

تم في هذا البحث عزل سبعة أنواع من فطريات التآزت تابعة لثلاثة أجناس، ووجد أن الفطر *Penicillium* من أكثر الأجناس السائدة في التربة التي زرع فيها بنجر السكر وكان ممثلاً بثلاثة أنواع، ثم يليه الفطر *Aspergillus* والفطر *Fusarium* والممثلين بنوعين لكل منهما.