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SULPHUR OXIDATION IN SAUDI ARABIAN AGRICULTURAL SOILS

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أكسدة الكبريت في الترب الزراعية بالمملكة العربية السعودية

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جمعت عينات التربة من ثمان مواقع في المملكة العربية السعودية وقد جرى تحليلها لتحديد التركيب الميكانيكي والكيميائي والميكروبي لكل تربة على حدة. ولدراسة أكسدة الكبريت فيهما، تم إضافة عنصر الكبريت بنسبة ١٪ لكل تربة وحضنت عند درجة ٢٥°م لمدة أربعة أسابيع. بعد كل أسبوع يتم تقدير محتوى كل تربة من الثيوسلفيت S_2O_3 و التتر اثيونيت S_4O_6 والسلفيت SO_4

أوضحت نتائج تحليل الترب أنها جميعها ترب رملية وأن الرقم الهيدروجيني لهذه الترب متعادل إلى قاعدي، كما وجد أن كل من تربة الخرج وتربة الرياض تحتويان على أعلى نسبة من الكبريتات، بمعدل ٤٩٣ و٤٠٠ ميكروجرام/ جرام تربة على التوالي. مع العلم بأن العدد الكلي للبكتريا والفطريات المؤكسدة للكبريت في هذان الموقعات كان أعلى بكثير من بقية المواقع التي تم دراستها .

بعد إضافة الكبريت إلى الترب الزراعية زادت نسبة كل من المنجنيز والحديد والأملاح الكلية الذائبة في التربة، بينما إنخفضت نسبة كربونات الكالسيوم ودرجة حموضة التربة في معظم الترب . وقد تمت مناقشة وعرض النتائج المتحصل عليها في الدراسة ومقارنتها مع الأبحاث الأخرى المنشورة في أماكن مختلفة من العالم .

Key words: S-oxidation, Sulphate production, Thiosulphate, Tetrathionate, S-oxidizing microorganisms.

ABSTRACT

Laboratory studies were conducted to determine the oxidation of elemental sulphur in eight localities of Saudi Arabian soils. The effect of sulphur application on related properties of calcareous soils in Saudi Arabia was also investigated. Elemental S (1%) was oxidized to sulphate in all soils tested. The maximum amount of sulphate was recorded in Alkharj (493 μg/g) and Riyadh (400 μg/g/) soils. These sites exhibited the largest numbers of S-oxidizing microorganisms. The addition of elemental sulphur to soils led to a marked increase in the concentrations of Mn and Fe, with a slight increase in T.S.S. in most soils. Also a large decline in soil pH and $CaCO_3$ was observed after amendment of soils with sulphur.

INTRODUCTION

Sulphur is an essential nutrient for plant growth. It is an abundant element in earth's crust but is often present in soil in limiting quantities or in forms unavailable to plants. Sulphur undergoes a number of transformations in soil which together form the sulphur cycle. These reactions are largely mediated by microorganisms (1). Also S-oxidation is a key transformation in the soil S cycle (2). The oxidation of elemental sulphur to sulphate in soils generally involves the formation of thiosulphate and tetrathionate (3), and is mediated by both chemolithotrophic and heterotrophic microorganisms (2).

Elemental sulphur and various sulphides are however, oxidized in the soil most actively by microorganisms. Various common heterotrophic soil bacteria, actinomycetes and fungi are capable of oxidizing elemental sulphur in soil (4). The bacteria using such molecules for energy are chiefly members of the genus *Thiobacillus*. It has been found that sulphur oxidation still occurs in some soils which lack *Thiobacilli* suggesting that in these soils other microorganisms are important sulphur oxidizers (2).

Species of *Arthrobacter, Bacillus, Flavobacterium,* and *Pseudomonas* oxidize elemental sulphur or thiosulphate to sulphate (1), and species of *Streptomyces* are able to generate thiosulphate from elemental sulphur (5). Filamentous fungi and yeasts oxidize powdered sulphur, and several heterotrophic bacteria convert thiosulphate to tetrathionate in the presence of organic nutrients (4). Many fungi can oxidize elemental sulphur and reduced forms of inorganic sulphur to sulphate (2). The ability of fungi to oxidize reduced sulphur in autoclaved soils has also been demonstrated (6).

Little is known about the oxidation processes of elemental sulphur in Saudi Arabian soils and no studies appear to have reported a comparison of sulphur transformation between different regions of the Kingdom of Saudi Arabia.

The objective of this study is to investigate the microbial oxidation of elemental sulphur in selected Saudi soils. For this reason, an incubation study was conducted to determine the effect of sulphur application on availability of thiosulphate, tetrathionate, sulphate, some micronutrients and on other properties of calcareous soils in Saudi Arabia.

MATERIALS AND METHODS

Collection and analysis of soil samples

Soil samples were collected in sterile polyethylene bags from ten different localities at Saudi Arabia, five samples of each site from soil surfaces at a depth of 1-15 cm. 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 (7) were used for determination of the total soluble salts and calcium carbonates. Soil organic matter percentage was determined colorimetrically using the method described by Walinga *et al.* (8).

Determination of soil microbial numbers

Total viable bacteria, actinomycetes, S-oxidizing bacteria and S-oxidizing fungi were obtained using the dilution plate-count method. Soil samples (10 g, sieved to <2 mm) were shaken in 1/4 strength Ringer's solution (90 ml) and serially diluted. The final dilution (0.1ml) was then spread onto media, as described by Al-Gounaim *et al* (9) for the isolation of bacteria and actinomycetes. *Thiobacilli* and S-oxidizing fungi, were counted as described by Germida (10) and Wainwright (11) respectively.

Determination of S ions

Samples of soil were amended with elemental S (1% w/v flowers of sulphur), in order to determine the S-oxidation in different type of soils. The inoculated soils were then incubated in triplicate at 25°C for 4 weeks. Uninoculated controls were also included to account for any non-biological sulphur oxidation. Sulphate was extracted from soil using a 1:10 soil:LiCl (0.1M) slurry shaken for 15 minutes (100 throws min^{-1}) then filtered through Whatman No. 1 filter paper. After each 7 days sulphate was determined turbidimetrically using the method described by Hesse (12), while thiosulphate and tetrathionate were determined colorimetrically according to (13).

RESULTS

All soil samples tested, with exception of Hail soil, contained low percentages of organic matter (Table 1). The soils were slightly alkaline and sandy in texture in all cases, with exception of sandy loam and loamy sand soils in Qassium and Wadi dawaser respectively. The concentration of Cu, Fe, Mn and Zu were within the ranges reported earlier for some Saudi Arabian soils (14, 15). There was a high content of total soluble salts in Hail (0.51%) and Wadi dawaser (0.69%) soils. Riyadh, Aljouf and Wadi dawaswer soils exhibited the highest percentage of $CaCO_3$ which were 34.62, 24.3 and 24.92 respectively.

Changes in soil characteristics of different regions in Saudi Arabia after amendment of sulphur (1%) are shown in Table 2. It was found that after 4 weeks of incubation that the addition of elemental sulphur caused a marked decrease in soil pH and $CaCO_3$ with a slight increase in the total soluble salts (T.S.S.) of soil extract. Amendment of soil with sulphur increased also the amounts of available manganese and iron with less effect on zinc and copper. The influence of sulphur was obvious on soils with low amounts of carbonate and soluble salts.

Both of Alkharj and Riyadh soils were recorded as the highest counts of S-oxidizing bacteria and S-oxidizing fungi that were 81 and 70 x 10^3 bacteria and 77 and 68 x 10^3 fungi respectively (Table 3). Qassium soil exhibited the highest counts of actinomycetes with 97 x 10^3. While the maximum number of bacteria was observed in Alkharj soil, approximately three-fold higher than the other Saudi Arabian soils.

All of Saudi Arabian soils oxidized elemental sulphur forming thiosulphate, tetrathionate and the terminal oxidation product sulphate (Fig 1-8). Alkharj soil was particularly active in this process forming over 490 $\mu g\ g^{-1}$ of sulphate at the end of the incubation period (Fig. 3) followed by Riyadh soil with 400 $\mu g\ g^{-1}$ of sulphate (Fig. 6). The relatively largest amount of tetrathionate (189 $\mu g\ g^{-1}$) was formed by the soil of Qassium; the concentration of this ion exceeding the sulphate from 2nd week sample onwards (Fig. 5). On the other hand the Qassium, Tabouk (Fig. 7) and Wadi dawaser (Fig. 8) soils reached the lowest amount of sulphate with 89, 80 and 78 $\mu g\ g^{-1}$ of sulphate respectively at the end of the incubation period.

Table 1
Soil characteristics of different regions in Saudi Arabia (n = 3)

Soil locality	Texture class	O.M %	pH	T.S.S %	$CaCO_3$ %	heavy metal content (μg/g) Cu	Fe	Mn	Zn
Alhassa	Sand	0.80	7.4	0.23	15.00	29.0	30.6	2.4	10.7
Aljouf	Sand	.13	7.8	0.08	24.30	10.1	23.1	4.5	6.7
Alkharj	Sand	0.07	7.2	0.13	1.87	5.8	15.2	7.0	18.0
Hail	Sand	1.03	7.3	0.51	1.22	24.6	19.1	2.1	6.3
Qassium	Sandy loam	0.68	7.7	0.14	12.44	11.7	31.1	8.9	9.2
Riyadh	Sand	0.62	7.5	0.19	34.62	7.1	28.1	3.4	4.2
Tabouk	Sand	0.74	8.1	0.34	7.43	11.1	30.1	9.2	5.8
Wadi dawaser	Loamy sand	0.02	8.0	0.69	24.92	12.3	28.2	12.4	6.3

O.M = Organic Matter
T.S.S = Total Soluble Salts

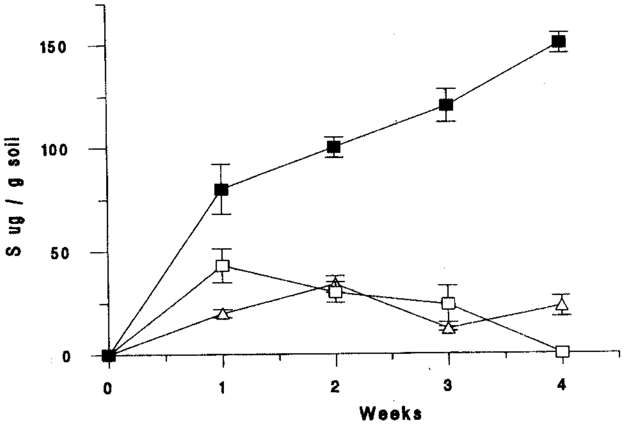

Fig. 1: Alhassa soil

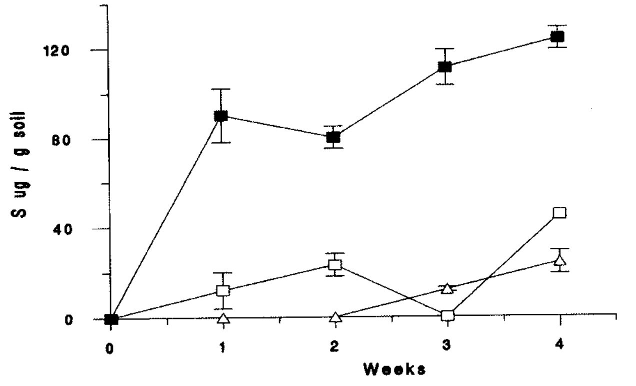

Fig. 2: Aljouf soil

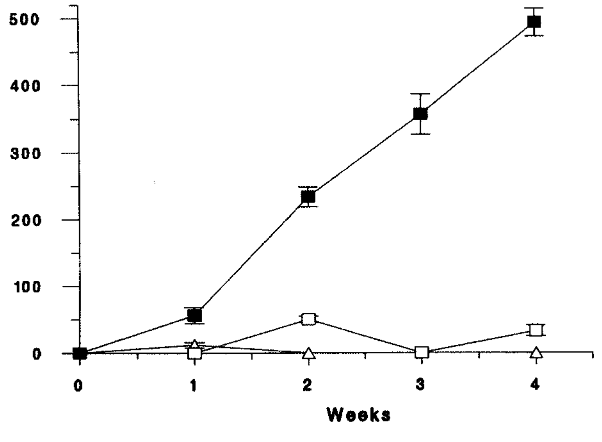

Fig. 3: Alkharj soil

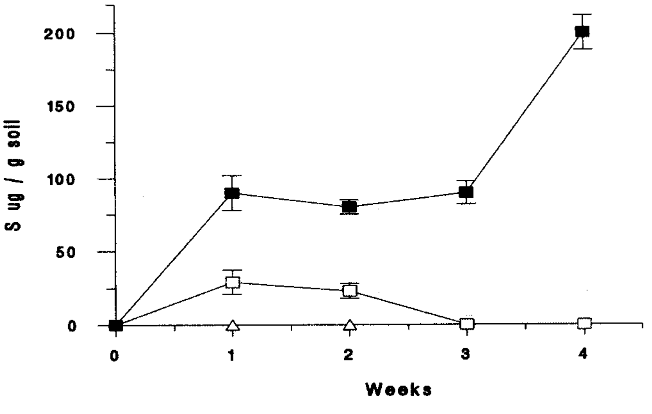

Fig. 4: Hail soil

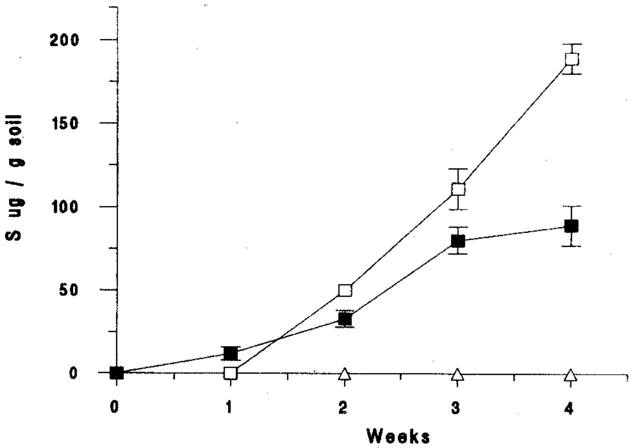

Fig. 5: Qassium soil

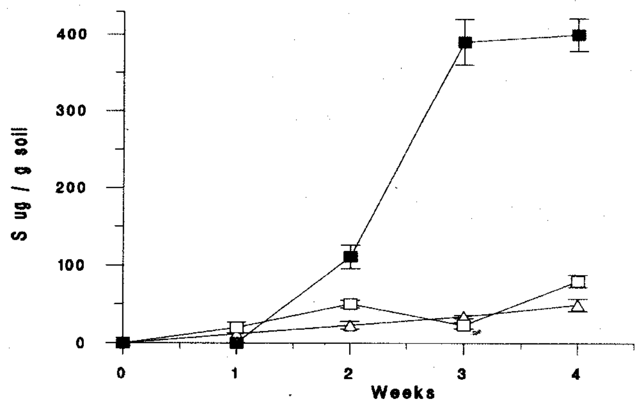

Fig. 6: Riyadh soil

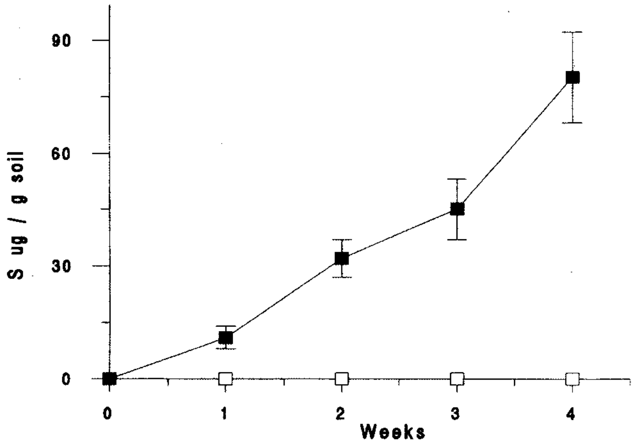

Fig. 7: Tabouk soil

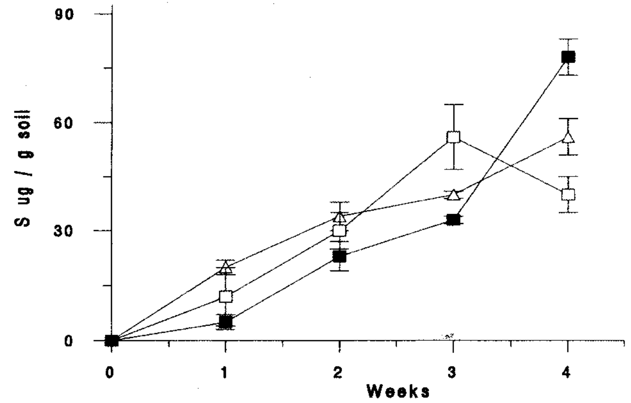

Fig. 8: Wadi dawaser soil

Legends:

Fig. 1: Oxidation of elemental sulphur (1%) in Alhassa soil, all values are means of triplicates ±S.D. (Δ, Thiosulphute; □, Tetrathionate; ■, Sulphate).

Fig. 2: Oxidation of elemental sulphur (1%) in Aljouf soil, all values are means of triplicates ±S.D. (Δ, Thiosulphute; □, Tetrathionate; ■, Sulphate).

Fig. 3: Oxidation of elemental sulphur (1%) in Alkharj soil, all values are means of triplicates ±S.D. (Δ, Thiosulphute; □, Tetrathionate; ■, Sulphate).

Fig. 4: Oxidation of elemental sulphur (1%) in Hail soil, all values are means of triplicates ±S.D. (Δ, Thiosulphute; □, Tetrathionate; ■, Sulphate).

Fig. 5: Oxidation of elemental sulphur (1%) in Qassium soil, all values are means of triplicates ±S.D. (Δ, Thiosulphute; □, Tetrathionate; ■, Sulphate).

Fig. 6: Oxidation of elemental sulphur (1%) in Riyadh soil, all values are means of triplicates ±S.D. (Δ, Thiosulphute; □, Tetrathionate; ■, Sulphate).

Fig. 7: Oxidation of elemental sulphur (1%) in Tabouk soil, all values are means of triplicates ±S.D. (Δ, Thiosulphute; □, Tetrathionate; ■ Sulphate).

Fig. 8: Oxidation of elemental sulphur (1%) in Wadi dawaser soil, all values are means of triplicates ±S.D. (Δ, Thiosulphute; □, Tetrathionate; ■, Sulphate).

Table 2

Changes in soil characteristics of different regions in Saudi Arabia after amendment of sulphur (n = 3)

Soil locality	pH	T.S.S %	$CaCO_3$ %	heavy metal content (μg/g) Cu	Fe	Mn	Zn
Alhassa	6.2	0.23	13.00	29.0	38.7	5.1	10.8
Aljouf	7.4	0.11	20.30	10.2	33.4	9.9	6.6
Alkharj	5.3	0.15	1.77	5.7	20.2	25.1	18.1
Hail	5.4	0.54	1.10	24.6	29.6	13.0	6.2
Qassium	6.2	0.18	10.64	11.6	48.1	8.9	8.4
Riyadh	7.4	0.22	30.41	7.2	50.3	9.4	4.2
Tabouk	6.5	0.39	7.03	11.4	44.1	18.2	5.9
Wadi dawaser	7.7	0.75	20.79	12.0	41.2	28.7	6.2

Table 3

Counts of soil microorganisms at different regions in Saudi Arabia per gram soil (n = 3)

Soil locality	Total bacteria x 10^5	Actinomycetes x 10^3	S-oxidizing bacteria x 10^3	S-oxidizing fungi x 10^3
Alhassa	22.7 ± 0.6	31 ± 1.9	50 ± 2.4	53 ± 1.1
Aljouf	13.5 ± 0.4	43 ± 3.1	33 ± 4.5	28 ± 3.0
Alkharj	89.7 ± 13.0	31 ± 2.0	81 ± 1.7	77 ± 2.4
Hail	11.0 ± 0.9	52 ± 4.1	28 ± 1.6	39 ± 1.0
Qassium	12.3 ± 1.4	97 ± 2.7	45 ± 3.2	25 ± 0.4
Riyadh	32.1 ± 0.6	31 ± 5.0	70 ± 1.4	68 ± 1.9
Tabouk	22.7 ± 4.0	51 ± 3.1	17 ± 2.5	22 ± 1.5
Wadi dawaser	14.5 ± 0.8	64 ± 2.4	10 ± 1.3	18 ± 0.6

However, in all soils tested the concentration of polythionate ions never exceeded the concentration of sulphate formed. The results show a small amount of thiosulphate and tetrathionate in all sites with some exception, such that there was no amount of S_2O_3 detected in the Hail (Fig. 4), Qassium (Fig. 5) and Tabouk soils (Fig. 7). S_4O_6 was totally absent from Tabouk soil. Thiosulphate and tetrathionate ions are usually considered to be intermediates and rarely exceed the concentration of sulphate. As a result, S_2O_3 and S_4O_6 ions were only formed transiently in trace amounts towards the end of the incubation period. Oxidation of elemental sulphur led to a marked reduction in the pH of the soil due to the formation of sulphuric acid (Table 2).

DISCUSSION

Optimum sulphate production occurred in Alkharj and Riyadh soils, such that they exhibited the highest counts of S-oxidizing bacteria and S-oxidizing fungi. As a result of these inputs the microbial population of Alkharj and Riyadh soils is likely to have been adapted to S oxidation, so that when elemental S was added, it was oxidized faster than that in the other soils studied. Amendment of soils with elemental sulphur caused a marked decline in pH values and total soluble salts in all sites. Nevell and Wainwright (16) reported that the soil pH fell to a lower value following elemental sulphur amendment. However no significant reduction in pH of Aljouf, Riyadh and Wadi dawaser soils occurred as there was a large amount of $CaCO_3$ in these soils, that working as a buffer in soil solution. The significant increase in amounts of Fe and Mn elements as well as the T.S.S in most soils may be caused by increased soil acidity followed by addition of sulphur. Similar results were reported earlier in Egyptian soils (17), in Iraqi soils (18) and in Jordanian soils (19).

Qassium and Wadi dawaser soils appear unusual, because they form large amounts of tetrathionate and thiosulphate when oxidizing elemental S; ions which are usually considered to be intermediate and rarely exceed the concentration of sulphate. These S oxyanions are usually formed as transitory intermediates during soil oxidation of elemental S (3, 20). The trend of S-oxidation in case of the other soils is therefore more typical of heterotrophic S-oxidizers, consistent with the findings of previous studies (2, 16). Heterotrophs undoubtedly play an important, and in some cases dominant, role in the oxidation of sulphur in soils, particularly where populations of chemoautotrophic S-oxidizing bacteria are low (21). Minimum sulphate production recorded in soils of Tabouk and Wadi dawaser which may be referred to the lowest viable counts of S-oxidizing microorganisms occurring in these sites.

The S-oxidizing microorganisms occur abundantly in soils receiving applications of sulphur as a fertilizer, either in organic (sewage, etc.) or inorganic forms. It was shown, for example, that soils having large numbers of S-oxidizing bacteria and S-oxidizing fungi were capable of oxidizing sulphur more rapidly than the soils less in numbers of S-oxidizing microorganisms (1, 4).

In conclusion, the addition of elemental sulphur to soils led to a marked increase in the concentration of manganese and iron, with a slight increase in total soluble salts, in most soils. Also a large decline in soil pH and $CaCO_3$ were observed after amendment of soils with 1% sulphur. The maximum amount of sulphate was recorded in Alkharj and Riyadh soils, sites which exhibited the largest numbers of S-oxidizing microorganisms.

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