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Nitrification in Some Soils from Saudi Arabia

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Abstract. Laboratory studies were conducted to determine the nitrification of ammonium sulphate, the hydrolysis of urea and oxidation of released ammonium, via nitrite, to nitrate in soil samples collected from eight localities of Saudi Arabian soils. The addition of ammonium sulphate and urea to soils led to a marked increase in the concentration of nitrate, with a slight increase in nitrite, in most soils. The maximum amount of nitrate was recorded in Alkharj (52 ppm) and Qassim (44 ppm) soils. These sites exhibited the largest number of nitrifying microorganisms. Hail soil (10 ppm) reached the lowest amount of nitrate production in the presence of added ammonium sulphate and urea.

Introduction

Nitrification is the biological formation of nitrite or nitrate from compounds containing reduced nitrogen [1]. The term generally refers to the oxidation of ammonium ion to nitrite and nitrate by specialized bacteria and fungi. A wide variety of heterotrophic fungi have been shown to be capable of oxidizing reduced forms of nitrogen [2]. A survey was made of the capacity of *Actinomycetes*, which are abundant in soil, to form nitrite and nitrate in an ammonium medium [3]. Although the ability of fungi to hydrolyze urea and nitrifying released ammonium were well established [4- 6]. Certain fungi and heterotrophic bacteria have a role to play in nitrification also, particularly in acid forest soils [7].

The nitrification in Saudi Arabian soils have been studied by a very few workers, though largely on the central part of Saudi Arabia [8-10]. No information is available that compares nitrification process of soils collected from different localities at Saudi Arabia.

Here we report the nitrification of ammonium sulphate, the hydrolysis of urea and oxidation of released ammonium, via nitrite, to nitrate in agricultural sandy soils collected from different localities at Saudi Arabia.. The soil characteristics and counts of soil microorganisms were also investigated in selected Saudi soils.

Materials and Methods

Collection and analysis of soil samples

Soil samples were collected in sterile polyethylene bags from eight different localities at Saudi Arabia, with five samples of each site from soil surfaces at a depth of 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 methods described by Jackson [11] was used for determination of the total soluble salts (T.S.S) and calcium carbonates content. Soil pH (a water-soil slurry 10:1) was determined with a glass electrode. Soil organic matter was determined colorimetrically using the method described by Walinga *et al.*, [12].

Nitrification and extraction of soil nitrogen ions

Soil samples (1.0 kg of each) were amended with either ammonium sulphate (5000 ppm) or urea (10000 ppm), in order to investigate the ammonium nitrification process, hydrolysis of urea and nitrification of released ammonium in soil.

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

Urea, nitrate and nitrite were extracted from soil with distilled water. Ammonium was extracted from soil using KCL (1.5N).. In all cases a 1:10 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 were determined. Ammonium was determined according to the indophenol blue method [13]; nitrate using an Orange I method [14] and nitrite colorimetrically as described by Hesse [15].

Numeration of soil microorganisms

For the quantitative estimation of fungi, actinomycetes and total bacteria the dilution plate method of Waksman as detailed by Johnson and Curl [16] was used. Soil (1 g) was shaken in sterile Ringer's solution (10 ml) for 15 min. Samples of the resulting suspension were then serially diluted in Ringer's solution. The final dilution was then spread on the surface of the medium. Incubation periods were 3 days at 30°C for total bacteria and 5-7 days at 30°C for total fungi and actinomycetes. Counts of nitrifying bacteria were determined according to the MPN method described by Nacos and Wolcott [17].

Results

The soil characteristics of different regions in Saudi Arabia are shown in Table 1. All soil samples tested contained low percentages of organic matter. The soils were slightly alkaline (pH 7.2-8.1). and sandy in texture in all cases, with the exception of sandy clay and loamy sand soils in Qassim and Wadi dawaser respectively. There was a high content of total soluble salts in Hail (0.51 %) and Wadi dawaser (0.69 %) soil samples. Both of Hail and Qassim soils had the highest concentrations of calcium carbonate (20.13 % and 15.22 %, respectively) and the lowest calcium carbonate concentration was recorded in Al-Kharj soil (1.87 %).

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

Soil locality	Mechanical fraction %			Texture class	O.M %	pH	T.S.S %	$CaCO_3$ %
	Sand	Sand	Clay					
Al-Hassa	89.0	5.8	5.2	Sand	0.80	7.4	0.23	7.02
Al-Jouf	98.3	0.9	0.8	Sand	0.13	7.8	0.08	12.30
Al-Kharj	92.6	1.2	6.2	Sand	0.07	7.2	0.13	1.87
Hail	91.3	3.3	5.4	Sand	0.09	7.3	0.51	20.13
Qassim	56.4	6.1	37.5	Sandy clay	0.68	7.7	0.14	15.22
Riyadh	90.6	4.2	5.2	Sand	0.62	7.5	0.19	9.62
Tabouk	89.0	3.3	7.7	Sand	0.74	8.1	0.34	5.43
Wadi Dawaser	82.0	5.5	12.5	Loamy sand	0.02	8.0	0.69	11.92

Table 2 illustrates the counts of soil microorganisms at different regions in Saudi Arabia per gram soil. Both of Al-Kharj and Qassim soils recorded the highest counts of nitrifying bacteria and fungi being 94 and 73 x 10^3 MPN for nitrifying bacteria, 75 and 68 x 10^3 cfu/g for fungi, respectively (Table 2.). Riyadh soil exhibited the highest counts of actinomycetes 70 x 10^3 cfu/g. While the maximum number of total bacteria was observed in Al-Kharj soil, approximately 3-8-fold higher than the other Saudi Arabian soils.

Table 2. Counts of soil microorganisms (cfu/g) at different regions in Saudi Arabia per gram soil. (all values are means of six replicates ± S.D)

Soil locality	Total bacteria x 10^5	nitrifying bacteria x 10^3	Actinomycetes x 10^3	Total fungi x 10^3
Al-Hassa	22.7 ± 0.6	31 ± 1.9	50 ± 2.4	43 ± 1.1
Al-Jouf	13.5 + 0.4	43 + 3.1	33 ± 4.5	28 ± 3.0
Al-Kharj	89.7 ± 13.	94 ± 2.0	67 ± 1.7	75 ± 2.4
Hail	11.0 + 0.9	22 ± 4.1	28 ± 1.6	39 ± 1.0
Qassim	12.3 + 1.4	73 ± 2.7	45 ± 3.2	55 ± 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	60 ± 2.4	10 + 1.3	38 + 0.6

Addition of ammonium sulphate caused a slight decline of soil pH especially during the first two weeks of this study (Table 3), while amendment of soil with urea raised the soil pH by 2 to 3 units in most investigated soils.

Table 3. Changes in the pH of Saudi Arabian soils after addition of ammonium sulphate (5000 ppm) and urea-N (10000 ppm). All values are means of triplicates ± S.D.

Soil locality	Nitrogen forms added	Time in weeks			
		1	2	3	4
Al-Hassa	Ammonium	7.1 ± 0.1	7.2 ± 0.2	7.3 ± 0.0	7.3 ± 0.1
	Urea	7.6 ± 0.0	7.5 ± 0.1	7.4 + 0.2	7.4 ± 0.0
Al-Jouf	Ammonium	7.7 ± 0.2	7.8 ± 0.0	7.8 ± 0.1	7.8 + 0.0
	Urea	7.9 ± 0.0	7.8 + 0.1	7.8 ± 0.3	7.8 ± 0.1
Al-Kharj	Ammonium	6.9 ± 0.0	6.9 ± 0.2	7.0 ± 0.1	7.0 + 0.2
	Urea	7.5 ± 0.1	7.5 ± 0.1	7.4 ± 0.0	7.5 ± 0.1
Hail	Ammonium	7.3 ± 0.2	7.4 ± 0.1	7.3 ± 0.2	7.3 ± 0.0
	Urea	7.4 ± 0.1	7.3 + 0.0	7.3 ± 0.1	7.3 ± 0.1
Qassim	Ammonium	7.6 ± 0.1	7.6 ± 0.0	7.6 ± 0.0	7.6 ± 0.1
	Urea	7.9 ± 0.2	7.8 ± 0.3	7.8 ± 0.1	7.8 ± 0.0
Riyadh	Ammonium	7.4 + 0.1	7.4 ± 0.2	7.4 ± 0.3	7.5 ± 0.0
	Urea	7.7 ± 0.0	7.7 + 0.0	7.7 ± 0.0	7.6 ± 0.2
Tabouk	Ammonium	7.8 ± 0.0	7.8 ± 0.2	7.9 ± 0.3	7.9 ± 0.2
	Urea	8.3 + 0.0	8.4 + 0.1	8.3 + 0.0	8.2 + 0.2
Wadi Dawaser	Ammonium	7.9 + 0.2	7.9 ± 0.3	7.9 ± 0.1	7.9 ± 0.0
	Urea	8.2 ± 0.1	8.0 ± 0.2	8.2 ± 0.2	8.1 + 0.0

Figures 1-4 show that the Saudi Arabian soils oxidized ammonium sulphate and ammonium released from hydrolysis of urea forming nitrite and nitrate. The oxidation of ammonium, as measured by both loss of ammonium and increases in nitrite and nitrate, varied from one soil to another. Since the significant difference of nitrifying microorganisms counts in Saudi Arabian soils, which are capable of nitrification, we were interested in determining their ability in this respect compared with each other.

All of Saudi Arabian soils hydrolysed urea leading to the formation of similar large amounts of ammonium (Figs. 1 & 2). This decline in urea was associated with a concomitant increased in net ammonium, nitrite and nitrate production. The concentration of added urea decreased more rapidly in Al-Kharj soil compared to the other Saudi Arabian soils tested. Al-Kharj soil followed by Qassim soil recorded the highest hydrolysis of urea being 2723 ppm and 2456 ppm (Fig. 2) of released ammonium respectively. While the lowest hydrolysis of urea was observed in Hail soil with 78 ppm ammonium released at the end of the incubation period. Therefore, Al-Kharj and Qassim soils were particularly active in released ammonium process forming the maximum amount of ammonium at the end of the incubation period.

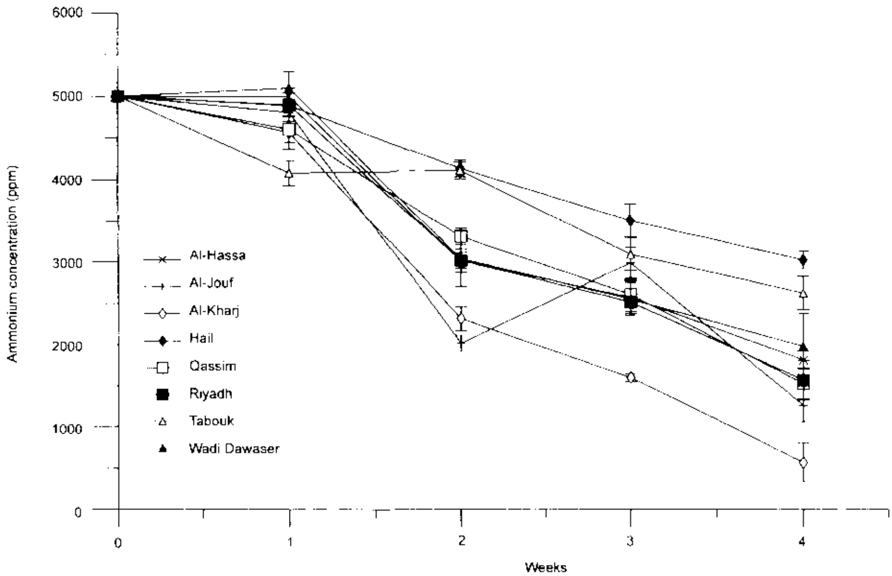

Fig. 1. Changes in ammonium concentration in some soils from Saudi Arabia following the addition of ammonium sulphate (5000 ppm).

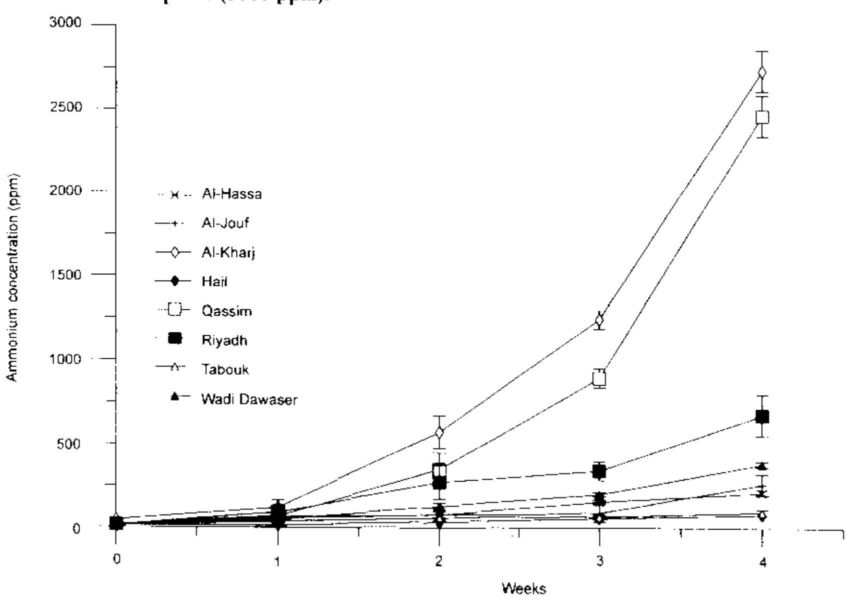

Fig. 2. Changes in ammonium concentration in some soils from Saudi Arabia following the addition of urea-N (10000 ppm).

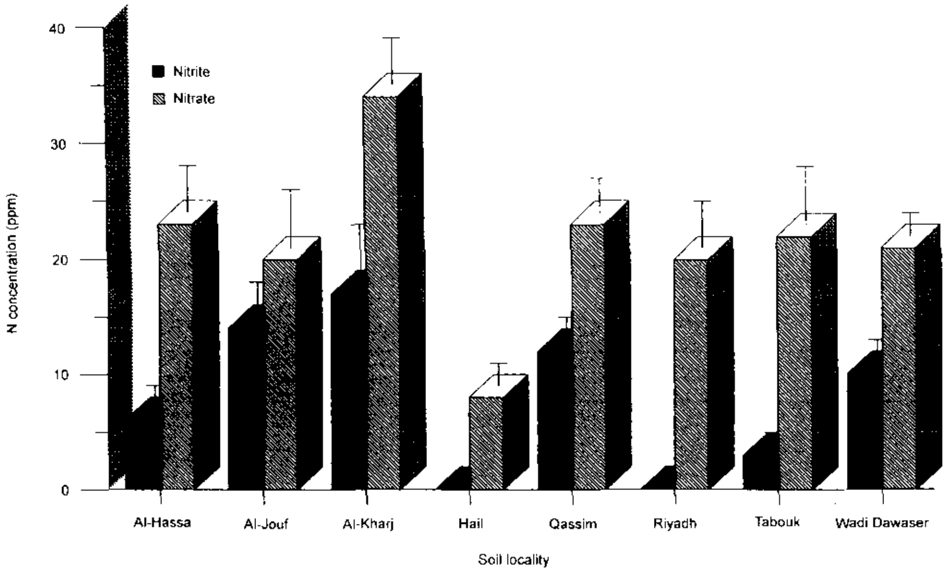

Fig. 3. Changes in nitrite and nitrate concentration in some soils from Saudi Arabia following the addition of ammonium sulphate (5000 ppm).

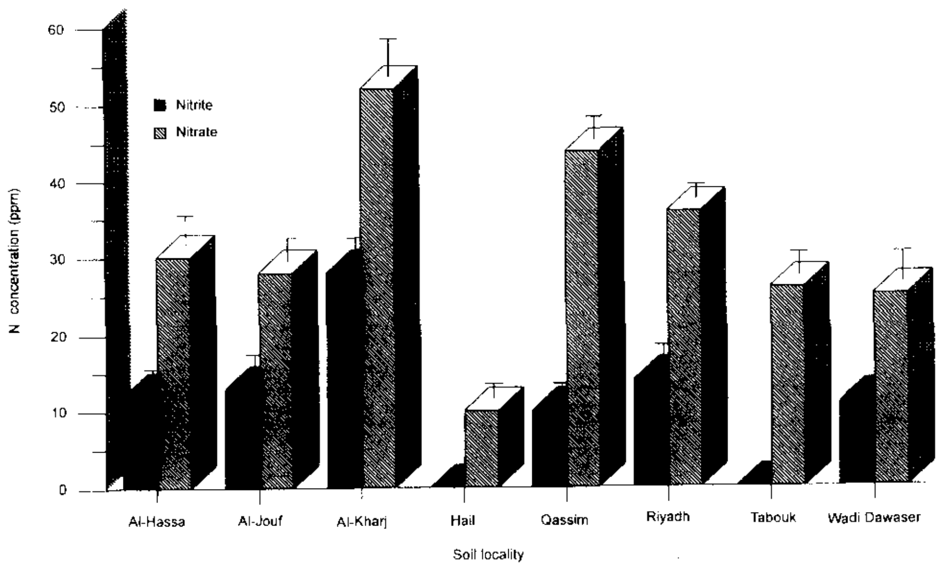

Fig. 4. Changes in nitrite and nitrate concentration in some soils from Saudi Arabia following the addition of urea-N (10000 ppm).

As can be seen urea hydrolysis was largely correlated with increased of ammonium concentration in soils tested, especially in the case of Al-Kharj and Qassim soils (Fig. 2). Therefore the urea hydrolysis increased soil pH and accumulated ammonium ions.

Overall Al-Kharj soil appeared to be the most active in the nitrification of ammonium sulphate, the hydrolysis of urea and oxidation of released ammonium, via nitrite, forming nitrate (Figs. 3 and 4). In the nitrification of ammonium sulphate most of the soils studied produced betwen 20 - 23 ppm nitrate over the 4 weeks incubation period while Al-Kharj soil produced 34 ppm nitrate (Fig. 3). Also during the oxidation of released ammonium the relatively largest amount of nitrate (52 ppm) was formed by the soil of Al-Kharj (Fig. 4). On the other hand the Hail, Tabouk and Wadi Dawaser soils reached the lowest amount of nitrate with 10, 26 and 25 ppm respectively at the end of the incubation period.

However, in all soils tested the concentration of nitrite ions never exceeded the concentration of nitrate formed. The results show a small amount of nitrite in all sites with some exception, such that there was no amount of NO_2^- detected in the Hail and Tabouk soils (Fig 4). Nitrite ion is usually considered to be intermediates and rarely exceed the concentration of nitrate. As a result, NO_2^- ion was only formed transiently in trace amounts towards the end of the incubation period.

Discussion

In the past, nitrification process was thought to be restricted to species of *Nitrosomonas* and *Nitrobacter* only. But in recent years, our understanding of the ecology of soil nitrification has changed. The new aspect about nitrifier ecology has become particularly evident that two types of nitrification are now recognised; chemoautotrophic and heterotrophic nitrification [7]. The ability of heterotrophs to oxidize reduced forms of nitrogen has long been recognised. A wide variety of heterotrophic microorganisms have been shown to be capable of oxidizing reduced forms of nitrogen, including gram negative and gram positive bacteria, spore and non-spore formers, an obligate anaerobes, fungi and species of actinomycetes [2-7].

All of the soil samples oxidised ammonium to nitrate and nitrified the ammonium produced by urea hydrolysis to form nitrate. Net nitrate production varied among the soils depending on whether the ammonium was provided directly or as a hydrolysis product of urea. Generally however, higher concentrations of nitrate were detected when urea rather than ammonium sulphate was provided as the N source. Similar results were reported earlier [6;7;9;10].

Saudi Arabian soils nitrified the ammonium produced by urea hydrolysis to form nitrate, while nitrite was only formed transiently in trace amounts towards the end of the incubation period. In addition they were also able to nitrify ammonium sulphate forming nitrate and again transient and small amounts of nitrite were produced. The

NO_2^- is usually formed as transitory intermediates during soil oxidation of ammonium [4;6].

Urea, whether added to soils as fertilizer or as animal urine, is hydrolyzed by the soil enzyme urease. The urea hydrolysis increases soil pH and accumulate ammonium ions [1,7]. Hydrolysis of urea associated with a concomitant increased in net ammonium, nitrite and nitrate production, consistent with the results of other workers [4-6;10]. So the marked increased of ammonium concentration in soils was attributed to the urea hydrolysis and oxidation process of nitrogen ions that caused by the activity of soil microorganisms. Fungi, Actinomycetes and heterotrophic bacteria, which are abundant in soil, undoubledly play an important, and in some cases dominant, role in the process of nitrification to form nitrite and nitrate [5-7].

Optimum nitrate production occurred in Al-Kharj and Qassim soils, such that they exhibited the highest counts of nitrifying bacteria and fungi.. As a result of these inputs the microbial population of Al-Kharj and Qassim soils is likely to have been adapted to ammonium oxidation and urea hydrolysis, so that when ammonium sulphate or urea was added, it was nitrified faster than that in the other soils studied. The trend of ammonium oxidation and urea hydrolysis in case of the other soils are therefore more typical, consistent with the findings of previous studies [8- 10; 18- 20].

This investigation shows that the addition of ammonium sulphate caused a slight decrease of soil pH, but urea hydrolysis associated with an increase of pH values. Oxidation of ammonium to nitrate via nitrite is acidifying the soil. This process, however, is generally not strongly acidifying under natural conditions and it is only associated with a marked shift in soil pH after the input of an ammonium-based fertilizer or a manure, or in a weakly buffered sandy soil [7]. The abundance of calcium carbonates in Saudi Arabian soils is what makes the soil strongly buffered. The soil pH reflects the carbonate contents and is controlled by calcium carbonate. Tisdale *et al.*, [21, pp. 84-123] reported that soil pH can rise by 2 or 3 units in the immediate vicinity of a urea fertilizer granule.

The Al-Kharj and Qassim were the most active soils in the process of oxidized ammonium to nitrate and nitrified the ammonium produced by urea hydrolysis to form nitrate in the ammonium and urea amended soils over the 4 weeks incubation period. Al-Hassa, Riyadh and Al-Jouf soils were average in this respect. On the other hand, the Hail soil reached the lowest amount of nitrate production in the presence of added ammonium sulphate and urea which may referred to the lowest viable counts of nitrifying microorganisms occurring in this site.

The nitrifying microorganisms occur abundantly in soils receiving applications of nitrogen as a fertilizer, either in organic (sewage, etc.) or inorganic forms. It was shown, for example, that soils having large number of nitrifying bacteria and nitrifying fungi were capable of oxidizing ammonium more rapidly than the soils less in numbers of nitrifying microorganisms [1; 4; 7].

In conclusion, the addition of ammonium sulphate and urea to soils led to a marked increase in the concentration of nitrate, with a slight increase in nitrite, in most soils. The maximum amount of nitrate was recorded in Al-Kharj and Qassim soils, sites which exhibited the largest number of nitrifying microorganisms. Hail soil reached the lowest amount of nitrate production in the presence of added ammonium sulphate and urea.

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عملية التأزت في عدد من الترب الزراعية بالمملكة العربية السعودية

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ملخص البحث. أجريت تجربة معملية لدراسة تحولات كبريتات الأمونيوم واليوريا وأكسدة الأمونيوم المتحررة إلى نيتريت NO_3 مرورًا بالنيترات NO_2في عيّنات التربة التي جمعت من ثمان مواقع في المملكة العربية السعودية. إضافة كبريتات الأمونيوم واليوريا أدت إلى زيادة كبيرة في تركيز النيتريت NO_3 وزيادة قليلة في تركيز النيرات NO_2.

وجد في هذه الدراسة أن أعلى تركيز للنيتريت NO_3 سجل في كل من تربة الخرج (٥٢ جزء من المليون) وتربة القصيم (٤٤ جزء من المليون) وهذين الموقعين يحتويان على أعلى معدّل لميكروبات التأزت. بينما ظهر أقل تركيز للنيتريت NO_3 في تربة منطقة حائل بمعدّل ١٠ جزء من المليون بعد إضافة كبريتات الأمونيوم واليوريا.