The Libyan Journal of Agriculture: volume 8, 1979

Autotrophic Nitrification in Soils Irrigated with Diluted Seawater

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ABSTRACT

Application of seawater diluted to 10, 20 or 40% concentration in deionized water was found to exert no significant effect on soil pH. The electrical conductivity of these soils was found to increase in value to attain levels of 3.15, 4.87, and 8.00 mmhos/cm at 25°C after 6 hours of incubation respectively. Incorporation of neither concentration however exerted no deleterious effect on heterotrophic growth in soil. Counts of about 10 million cells/g were therefore obtained after 14 days of incubation.

Growth of *Nitrosomonas* was stimulated by seawater application at 10% concentration. Whence, counts of 1.6×10^5 cells/g were observed after 28 days of incubation. Conversely, a ten-fold growth suppression over the control treatment was noted for this bacterium in soil treated with 20% seawater. Incorporation of 40% seawater had virtually prevented *Nitrosomonas* propagation in soil.

Unlike *Nitrosomonas*, the 10% seawater treatment did not stimulate *Nitrobacter* growth in soil. Both 20 and 40% seawater applications had rendered *Nitrobacter* numbers undetectable by the procedure employed at 42 and 28 days of incubation respectively. These results suggest the possibility of nitrite accumulation in soil. Consequently, phytotoxicity is likely to occur under such conditions.

INTRODUCTION

The availability of limited supplies of fresh water in arid and semiarid regions has necessitated the implication of brackish water, diluted seawater and undesalinated seawater (5, 11) for irrigation purposes. Under certain experimental conditions, irrigation with water containing up to 6,000 ppm soluble salts has been claimed to have no deleterious effect on crop production (3, 10).

Application of such water is likely to suppress growth and activity of susceptable species of soil microorganisms. Although marine forms of autotrophic nitrifying bacteria have been occasionally isolated (12, 13), the terrestrial strains of these bacteria are more sensitive to salt stress than other soil microorganisms (7, 8, 15). Inhibition of these bacteria often results in an accumulation of the potentially phytotoxic nitrite anion (2). This occurs following the application of urea, anhydrous ammonia, or ammonium salts to soils with pH values above neutrality.

The purpose of this investigation was to evaluate the effect of seawater application at different dilution levels on the proliferation of indigenous *Nitrosomonas* and *Nitrobacter* in sandy soils. The soil samples were amended with 200 ppm ammonium sulfate-N, 100 ppm triple super-phosphate-P and 50 ppm potassium sulfate-K.

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MATERIALS AND METHODS

A representative 2 to 20 cm deep soil sample was collected from a recently reclaimed uncultivated strip at the experimental farm of the Faculty of Agriculture. The soil sample was air-dried and sieved through 2 mm mesh. Replicate 100.0 g soil portions were placed in 250 ml capacity cotton-plugged Erlenmeyer flasks. An aqueous solution of ammonium sulfate was pipetted on the surface of each soil portion to yield 200 ppm nitrogen. Similarly, 100 ppm triple superphosphate-P and 50 ppm potassium sulfate-K were added. All soil portions were brought to 60% water holding capacity with sterile deionized water or millipore filter-sterilized seawater diluted with sterile deionized water at the ratio of 1:10, 2:10, or 4:10 (v/v). All soil samples were incubated at 28°C at about 80% relative humidity for the required periods of time. Loss of moisture through evaporation was corrected with sterile deionized water periodically.

The soil pH and electrical conductivity were determined on a 1:1 soil-water extract.

Enumeration of heterotrophic soil bacteria was carried out using the plate-dilution frequency technique described by Harris and Sommers (6) on nutrient agar. The plates were incubated at 28°C for 4 days.

Nitrosomonas and *Nitrobacter* numbers were determined according to Alexander's most probable number technique (1). Five inoculated replicates were performed for each ten-fold serial dilution, and the 95% confidence interval was determined accordingly.

RESULTS AND DISCUSSION

Effect of incubation on soil pH

Incorporation of neither concentration of seawater into the soil had apparently affected the soil pH significantly (Table 1). However, a tendency of decline in pH values was observed after 14 days of incubation. Obviously, the acids produced through the microbial activity were subsequently neutralized due to the high carbonate contents of the soil which amounted to about 6.4%. The soil pH after 42 days of incubation was therefore maintained at 7.8 irrespective of the treatment performed. It seems that the prevailing soil pH had not adversley affected the performance of *Nitrosomonas* and *Nitrobacter* in soil. The activity of these bacteria is actually favoured by neutral and slightly alkaline soil conditions (4, 9, 14).

Table 1 Effect of incubation on soil pH.

Incubation time, days	Soil pH			
	Seawater concentration in soil moisture, %			
	0	10	20	40
¼	7.8	7.9	7.8	7.8
7	7.9	7.9	7.8	7.8
14	7.4	7.4	7.5	7.6
21	7.5	7.6	7.6	7.5
28	7.5	7.6	7.6	7.6
35	7.7	7.6	7.6	7.6
42	7.8	7.8	7.8	7.8

Soil electrical conductivity

Although the initial salt content of the soil was 0.42 mmhos/cm at 25°C, the electrical conductivity of the moist soil was increased to 2.20 mmhos/cm within 6 hours of incubation merely because of fertilizer applications and hydration of indigenous salts. Within the same period of incubation time, values of 3.15, 4.87 and 8.00 mmhos/cm were recorded for soil samples treated with seawater diluted to 10, 20 or 40% concentration respectively (Table 2). The electrical conductivity of each treatment tended to increase during the initial three weeks of incubation with seawater. Thus, values of 4.56, 5.67 and 8.51 mmhos/cm were obtained respectively. The gradual dissociation of the soil salt components had been shown thereafter to approximate equilibration. Whence, all succeeding values fluctuated around their corresponding maxima at 3 weeks. The relative increase in soil electrical conductivity observed within each treatment did not appear to impose a substantial effect on *Nitrosomonas* and *Nitrobacter* growth in soil (Tables 4 and 5).

Heterotrophic soil bacteria

Table 3 clearly demonstrates the degree of tolerance of these bacteria to the salinity levels applied in this experiment. Growth appreciation of these bacteria in soils treated with diluted seawater continued to increase approaching counts of 6.9×10^6, 1.07×10^7 and 1.01×10^7clls/g after 14 days of incubation respectively. A considerable decline in numbers was observed afterwards, probably because of substrate depletion. The rapid response of these bacteia and the extend at which they propagated had undoubtedly contributed to growth repression of *Nitrosomonas* and *Nitrobacter* in soils treated with 20 and 40% seawater (Tables 4 and 5).

Growth of *Nitrosomonas* in soil

Numbers of *Nitrosomonas* were shown to increase from 1.2×10^2 to 9.2×10^4 cells/g soil within 28 days of incubation with deionized water (Table 4).

A significant increase in numbers over the control treatment was observed in soil treated with 10% seawater after 14 and 21 days of incubation. The bacterium continued to grow however in this soil to attain 1.6×10^5 cells/g after 28 days of incubation. Growth stimulation in this case could be attributed, at least partially, to the added trace metals contained in seawater. Analyses of the original soil sample for available iron, zinc, copper and manganese indicated a marked defficiency of zinc which amounted to about 0.15 ppm only.

Though a tendency of growth appreciation of *Nitrosomonas* was noted in soil incubated with 20% seawater for 21 days, further incubation resulted in a ten-fold decline in numbers of the bacterium over the control treatment at 28 days. However, incorporation of 40% seawater had virtually prevented *Nitrosomonas* propagation in soil. Counts lower than those originally present in soil were therefore recorded after 42 days of incubation.

Growth of *Nitrobacter* in soil

Nitrobacter growth in soil treated with deionized water was shown to reach its maximum of 1.3×10^4 cells/g at 21 days of incubation (Table 5). The maximum growth observed in this case, though at a week earlier, represented only one-seventh of the corresponding count of *Nitrosomonas* (Table 4).

Table 2 Effect of incubation conditions on soil electrical conductivity.

Incubation time, days	Electrical conductivity, mmhos/cm@			
	Seawater concentration in soil moisture, %			
	0	10	20	40
¼	2.20	3.15	4.87	8.00
7	2.37	3.92	5.52	8.08
14	2.97	4.51	5.46	7.49
21	3.10	4.56	5.67	8.51
28	2.84	4.09	5.42	8.21
35	2.96	4.31	5.30	8.40
42	2.72	4.14	5.45	8.12
@Regression coefficient:	+0.0932	+0.1196	+0.450	+0.0614
t (calculated):	1.688	1.444	0.9375	0.9192

Table 3 Numbers of heterotrophic bacteria in soil incubated at 60% water holding capacity with deionized water or diluted seawater.

Incubation time, days	Bacteria, thousands/g@			
	Seawater concentration in soil moisture, %			
	0	10	20	40
¼	410	760	410	390
7	4,700	6,600	5,900	8,000
14	9,600	6,900	10,700	10,100
21	390	290	1,170	2,900
28	690	560	660	1,170
35	610	400	240	280
42	660	420	350	410

@95% confidence interval was 2.47 d to d/2.47, where d is the number of bacteria.

Table 4 Growth of *Nitrosomonas* in soil incubated at 60% water holding capacity with deionized water or diluted seawater.

Incubation time, days	*Nitrosomonas* cells/g@			
	Seawater concentration in soil moisture, %			
	0	10	20	40
¼	120	100	180	130
7	1,100	790	17	15
14	6,400	35,000	2,100	240
21	7,900	51,000	16,000	220
28	92,000	160,000	9,200	350
35	35,000	54,000	430	54
42	5,400	16,000	410	24

@95% confidence interval was 3.30 d to d/3.30 where d is the number of bacteria.

Table 5 Growth of *Nitrobacter* in soil incubated at 60% water holding capacity with deionized water or diluted seawater.

Incubation time, days	*Nitrobacter*, cells/g@			
	Seawater concentration in soil moisture, %			
	0	10	20	40
¼	75	93	120	61
7	6,200	1,400	2,300	950
14	4,900	1,700	1,700	450
21	13,000	2,300	1,300	40
28	1,700	12,000	1,100	None
35	1,700	1,300	780	None
42	1,400	600	None	None

@95% confidence interval was 3.30 d to d/3.30, where d is the number of bacteria.

Unlike *Nitrosomonas*, the 10% seawater treatment did not stimulate growth of *Nitrobacter*. Conversly, growth of *Nitrobacter* in this case proceeded at a significantly slower rate than that noted in the control treatment.

The relatively normal growth of *Nitrobacter* initiated during the first week of incubation with 20% seawater was gradually suppressed by the treatment conditions. Accordingly, cells of this bacterium were rendered undetectable after 42 days of incubation. Incorporation of 40% seawater, however, resulted in an even higher rate of decline in numbers of *Nitrobacter* in soil. Following the 21 days observation, values below the detection limits of the procedure were continuously recorded for this bacterium.

In view of these results and the knowledge available about the optimum requirements of *Nitrosomonas* and *Nitrobacter* (2, 4), it is concluded that accumulation of the potentially phytotoxic nitrite is likely to occur under such conditions in soil irrigated with diluted seawater. Further research is needed, however, to confirm this point. Eventually, the nitrogen fertilizer employed, both kind and rate of application, remains the factor of primary importance if toxicity should be avoided.

ACKNOWLEDGEMENTS

The author is indebted to M. H. Saleem for his technical assistance and to B. A. Al-Rawi for helpful advice on analysis of experimental data.

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النترجه في الترب المروية بمياه البحر المخففة

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المستخلص

أجريت هذه التجارب لمعرفة تأثير استعمال مياه البحر المخففة بالمياه العذبة في الزراعة على نشاط باكتريا التربة المؤكسدة للامونيا (نيتروسوموناس) والمؤكسدة للنيترايت (نيتروباكتر) . وقد دلت النتائج على ما يلي :—

١ — ازدياد ملحوظ في نمو النيتروسوموناس نتيجة لمعاملة التربة بمياه البحر بتركيز ١٠٪ بحيث أصبح عددها ١٦٠ ٠٠٠ خلية في الغرام الواحد من التربة بعد ٢٨ يوما من المعاملة . وعلى العكس من ذلك فإن معاملة التربة بمياه البحر بتركيز ٢٠٪ أدت إلى انخفاض في سرعة نمو هذه الباكتريا بحيث أصبح عددها مساويا إلى ١ / ١٠ من مقدارها في تربة المقارنة بعد ٢٨ يوما من المعاملة كذلك . ولم تتمكن هذه الباكتريا من التكاثر في الترب المعاملة بمياه البحر بتركيز ٤٠٪ .

٢ — لم تستجب النيتروباكتر لمعاملة التربة بمياه البحر بتركيز ١٠٪ ولكن عددها في الترب المعاملة بمياه البحر بتركيز ٢٠ و٤٠٪ قد اقترن بانخفاض ملحوظ بحيث أصبح مجرد الكشف عنها في التربة بعد ٤٢ يوما من المعاملة بالحالة الأولى و٢٨ يوما من المعاملة بالحالة الثانية أمراعسيرًا .

٣ — وعلى ذلك فإن النتائج المذكورة تدل على إمكانية تراكم أيون النايترايت في التربة مما قد يؤدى إلى تسمم النباتات المزروعة تحت تلك الظروف ما لم يراع في ذلك نوع وكمية الأسمده النيتروجينية المستعملة .