NITRIFICATION OF NITROGEN FERTILIZERS UNDER SALINE CONDITION AND ORGANIC MATTER SUPPLEMENTATION.

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ABSTRACT

A laboratory experiment was conducted to study the effect of diluted Karoon lake water at Fayoum on nitrification of ammonium sulphate and urea fertilizers in the absence or presence of organic matter. Increased salinity delayed, suppressed or inhibited nitrification of both fertilizers. Ammonification of urea seemed to be independent of salinity. Nitrification, generally, was sensitive to salinity. In non-saline soil addition of narrow C/N ratio organic matter (Clover) enhanced ammonification and nitrification while wide C/N ratio ones (wheat straw) encouraged immobilization of mineral nitrogen. While salinity in presence of the two organic supplements, negatively affected ammonification, subsequent nitrification and even the immobilization process. Supplementing mineral fertilizer nitrogen with organic matter variably alleviated the adverse effect of salinity on biological nitrogen transformations in soil.

Key words: Salinity, organic matter, nitrification and nitrogen fertilizers.

INTRODUCTION

Microbial activity in soils is important in the cycling of nitrogen and other elements and also in the decomposition of organic matter. The use of high salinity water or addition of soluble salts to soil, result in decreased microbial activity (Johnson and Guenzi, 1963; Singh *et al.*, 1969; Agarwal *et al.*, 1971; Laura, 1974 and Paul and Clark, 1996). The increasing use of saline water in irrigation in order to overcome the lack of fresh water, specially in Fayoum Governorate, aroused concern about its effect on microbial transformation of mineral nitrogen fertilizers in soil. Nitrogen is a limiting factor for crop growth in saline condition, since availability of nitrogenous fertilizer in salt-affected soils depends upon the nature of the fertilizer, degree of salinity and absence or presence of organic matter in soil (Kuenen and Kobertson, 1988; Paul and Clark, 1996 and Subba-Rao, 1999).

Nitrification of mineral nitrogen fertilizers was found to increase with time and to decrease with the increase of salinity and was more from ammonium sulphate than urea (Ghandhi and Paliwal, 1976). This agrees with the findings of Singh *et al.* (1969); Broadbent and Nakashima (1971); Westerman and Tucker (1974) and Mc-Cormick and Wolf (1980). Ammonification seemed to be less sensitive to salts than nitrification process Ballmann and Conard (1998). As the concentration of salt increases ammonia accumulation increases in soil. Ammonification appeared to be, mostly, chemical in saline conditions (Singh *et al.* 1969; Laura 1974; and El-Shahawy and Mashhady 1984).

The present investigation based on a laboratory experiment, which extended to 12 wks, was undertaken to study the biochemical transformation of ammonium sulphate and urea nitrogen as influenced by different levels of saline Karoon lake water in absence or presence of organic matter.

MATERIALS AND METHODS

Unsaline loam soil containing 37.3% sand, 40.4% silt, 22.3% clay, 3.0% $CaCO_3$, 0.48% organic carbon, 0.054% total nitrogen, 15 ppm nitrate-nitrogen, 20 ppm ammonium-nitrogen, C/N ratio of 8.3, pH of 7.7 and T.S.S. 0.079% was used. Prior to the study, the soil was sieved through a 2 mm sieve, thoroughly mixed and distributed in 1000 cc capacity glass jars at the rate of 500 g/jar. Soils were fortified with appropriate amounts of ammonium sulphate or urea standard solutions to obtain a final concentration of 220 ppm ammonium nitrogen. Saline soils were prepared by adding different amounts of diluted Karoon lake water together with the nitrogen source to soil samples. Ground organic amendments (namely, clover and wheat straw) were also added at the rate of 2% w/w. Serial concentrations of salts (4000, 8000, 12000 and 16000 ppm representing 0.4, 0.8, 1.2 and 1.6%, respectively), were prepared by diluting saline water with fertilizer solution. The chemical composition of Karoon lake water used is illustrated in the following table:

Four levels of salinity (0.4, 0.8, 1.2 and 1.6%) were used and fifteen jars were prepared for each of the following treatments (4 salinity levels x 3 replicates in addition of treatment free of salts as a control)

1) ammonium sulphate
2) ammonium sulphate+clover
3) ammonium sulphate+wheat
4) urea
5) urea+clover
6) urea+wheat,
7) and 3 jars for plain soil.

	Salts	E.C.	Cations meq/L				Anions meq/e			
pH	%	(dSm^{-1})	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	CO_3^{-}	HCO_3^{-}	Cl^{-}	$SO_4^{=}$
7.5	3.75	58.58	20.9	88.9	467.1	46.6	-	3.5	548.5	74.2

Total jars were thus 93. After thorough mixing, soil moisture was raised to 50% of WHC using distilled water, and the jars were incubated at 30^{0}C. Soil moisture was kept constant by daily compensating evaporative loss, during the experimental period, with distilled water. Chemical analyses included the determination of ammonium and nitrate nitrogen, according to Bremner (1965) were conducted at 0, 14, 28, 56 and 84 days intervals. Mineral nitrogen was extracted using 2 *N* KCl and ammonium was determined by alkaline distillation in the presence of MgO. Combined nitrite and nitrate were then determined in a second distillation using Devardas alloy. While microbiological analysis was limited to the determination of nitrifier counts (Alexander and Clark, 1965) using MPN technique. MPN figures were obtained from Cochran (1950) tables. Sampling was discontinued when (nitrite + nitrate) nitrogen formed represented 95% of the total inorganic nitrogen (NH^+_4 + NO^-_2 + NO^-_3) of the soil or after 84 days.

RESULTS AND DISCUSSION

Effect of Salinity on Nitrification of Ammonium Sulphate and Urea

The periodical changes in the levels of ammonium and nitrate nitrogen of nitrified ammonium sulphate and urea fertilizers in soil as affected by salinity are shown in Figs. (1 and 2). The data showed that nitrification of ammonium sulphate was greatly decreased by increased salinity and was completely inhibited at 0.8, 1.2 and 1.6% salinity (Laura, 1974; Sindhu and Cornfield, 1967 a, b and Paul and Clark, 1996). Ammonium sulphate in control soil (no salinity) nitrified completely within 56 days and maximum nitrification rate was obtained after 14 days. While 0.4% salinity suppressed nitrification for 28 days and then slightly increased till the end of the experiment. The increase in nitrate-N was proportional to the decrease in ammonium with slight loss not more than 7% in total soluble nitrogen which does not support the finding of Ghandhi and Paliwal (1976) and was closely similar to that of Laura (1974). Nitrification of urea was found to be in slower rate than ammonium sulphate in unsaline soil (control) and this may be due to that ammonium ions are readily available for conversion to nitrate but urea has to undergo hydrolysis, converting it first into ammonium carbonate (Ghandhi and Paliwal 1976) or to the increase in soil pH following the hydrolysis of urea and ammonia formation which is unfavorable condition to nitrifying bacteria. Increasing levels of salts causes slightly decreases ammonification of urea, especially in high levels of salinity, which ascertained the suggestion of Singh *et al.* (1969) and Laura (1974) that in saline condition, ammonification of urea is mostly chemical and may be partially biological.

Also Mc-Cormick and Wolf (1980) stated that ammonification is less sensitive to salinity than nitrification that led to accumulation of ammonium-N in salt affected soils. The nitrification of ammonia formed from urea followed the same trend as in ammonium sulphate but in somewhat slower rates.

Following up the periodical changes in nitrifier counts are shown in Table 1. It seemed that ammonium sulphate, irrespective of salinity, was more preferable than urea for nitrifiers. In no salt treated soil, nitrifier counts reached maximum after two weeks and were 380 and $300x10^2g^{-1}$ soil in ammonium sulphate and urea fertilizer, respectively. At different levels of salinity, there was a relatively slight difference between ammonium sulphate and urea in nitrifier counts all over the experimental period. But in general the count decreases as levels of salinity increases.

Effect of salinity on ammonium sulphate and urea nitrogen transformations in soil supplemented with organic amendments:

Figures 3 and 4 illustrate the effect of different levels of salinity in the absence or presence of two organic supplements with different nitrogen content, on mineral nitrogen transformations of ammonium sulphate in the soil. Data obtained show that the addition of narrow C/N ratio amendment (Clover) at the rate of 2% (w/w), to soil fortified soil with additional amount of nitrogen amounting to 500 ppm. In no salt treated soil, transformation of nitrogen took place in clover, as well as in ammonium sulphate in the same time, followed by active nitrification which led to formation of 410 ppm total soluble nitrogen (18 ppm-ammonium and 392 ppm nitrate-N)

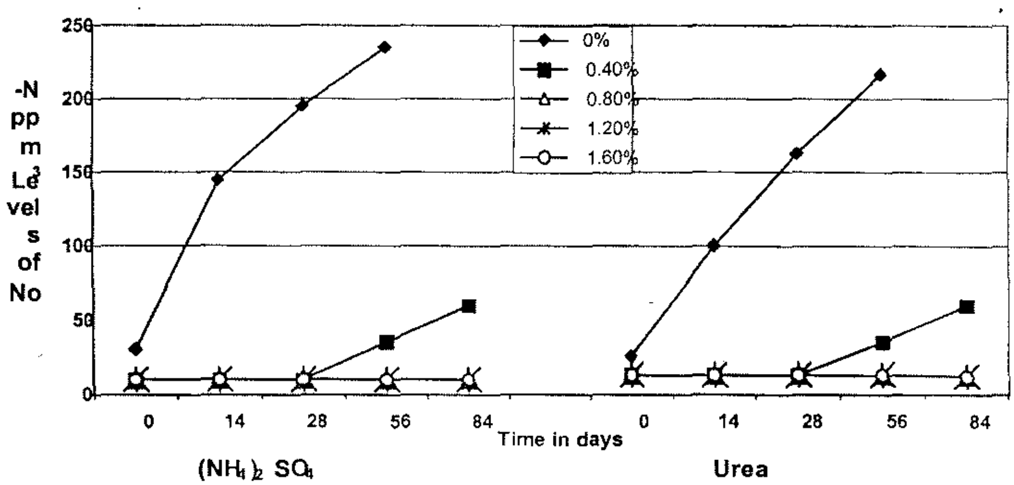

Fig. (1) Changes in NO_3-N of $(NH_4)_2SO_4$ and Urea as affected by salinity

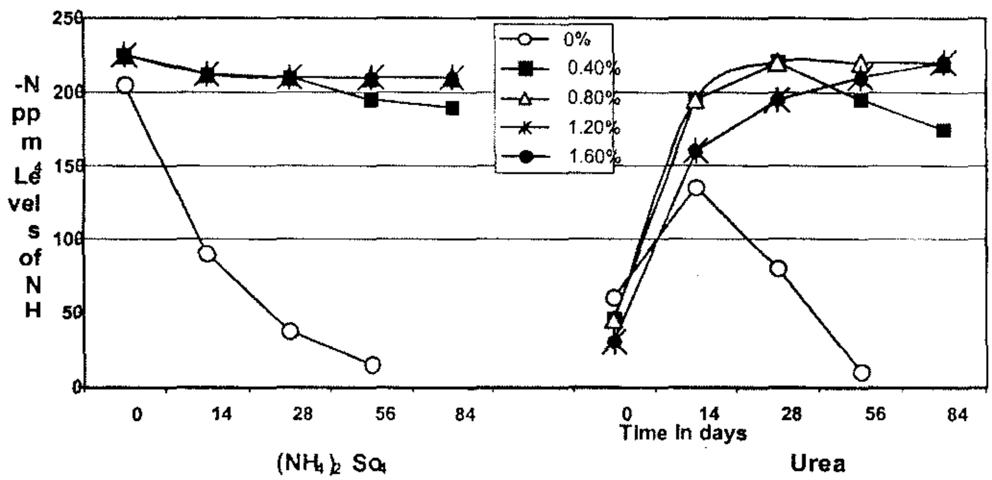

Fig (2) Changes in NH_4-N of $(NH_4)_2SO_4$ and Urea as affected by salinity

Table (1) Changes in nitrifier counts (x 10^2/g) as affected by salinity, mineral nitrogen fertilizers and organic matter supplementation.

Salinity %	Time in days									
	1	14	28	56	84	1	14	28	56	84
	$(NH_4)_2SO_4$					Urea				
0.0	19	380	260	23	12	19	300	290	70	18
0.4	18	98	73	22	12	16	102	93	55	28
0.8	16	43	18	12	12	15	47	19	18	18
1.2	16	23	18	12	12	16	22	18	12	12
1.6	16	20	18	12	12	16	19	18	12	11
	Clover + $(NH_4)_2SO_4$					Clover + Urea				
0.0	21	570	343	220	96	16	522	353	230	101
0.4	17	115	83	42	26	16	111	99	57	33
0.8	16	92	55	26	18	14	86	60	30	19
1.2	15	32	22	18	18	13	31	26	19	18
1.6	15	21	16	16	15	13	20	16	16	15
	Wheat + $(NH_4)_2SO_4$					Wheat + Urea				
0.0	14	100	43	22	7	14	86	44	28	7
0.4	15	98	90	43	11	16	96	93	48	12
0.8	13	15	21	18	11	13	12	22	18	11
1.2	13	12	11	10	10	13	12	11	11	11
1.6	13	12	11	10	10	13	12	11	12	10
Plain	14	12	11	7	7	14	12	11	7	7

at the end of the experimental period. While the addition of different levels of salinity caused deleterious effect on both processes, the effect was more pronounced on nitrification, than ammonification.

Addition of 0.4 and 0.8% salts delayed ammonification and nitrification but the later was more affected thus leading to accumulation of ammoniacal nitrogen, *i.e.* 363 ppm (171 NH_4-N and 192 NO_3-N) and 341 ppm (173 NH_4-N and 168 NO_3-N) total soluble nitrogen, respectively. Similar results were obtained by Singh *et al.* (1969), Broadbent and Nakashima (1971), Westerman and Tucker (1974); Mc-Cormick and Wolf (1980) and Saad *et al* (1996). Westerman and Tucker (1974), stated that dilute concentrations of salts plus ammoniacal fertilizer stimulated mineralization of soil nitrogen. This so-called "priming effect" was not detected in this experiment in non-amended and amended soils. At 1.2% and 1.6% salinity, ammonification was greatly retarded and nitrification was completely inhibited leading to accumulation of most of the ammonia formed *i.e.* 290 ppm (259 NH_4-N and 31 NO_3-N) and 243 ppm (221 NH_3-N and 22 NO_3-N) total soluble nitrogen, respectively.

It was very clear that addition of clover at the rate of 2% alleviated to, great extent, the adverse effect of salinity on salinity treatments could have been the addition of nitrifiers naturally present in the organic amendments. Rankov (1965) and

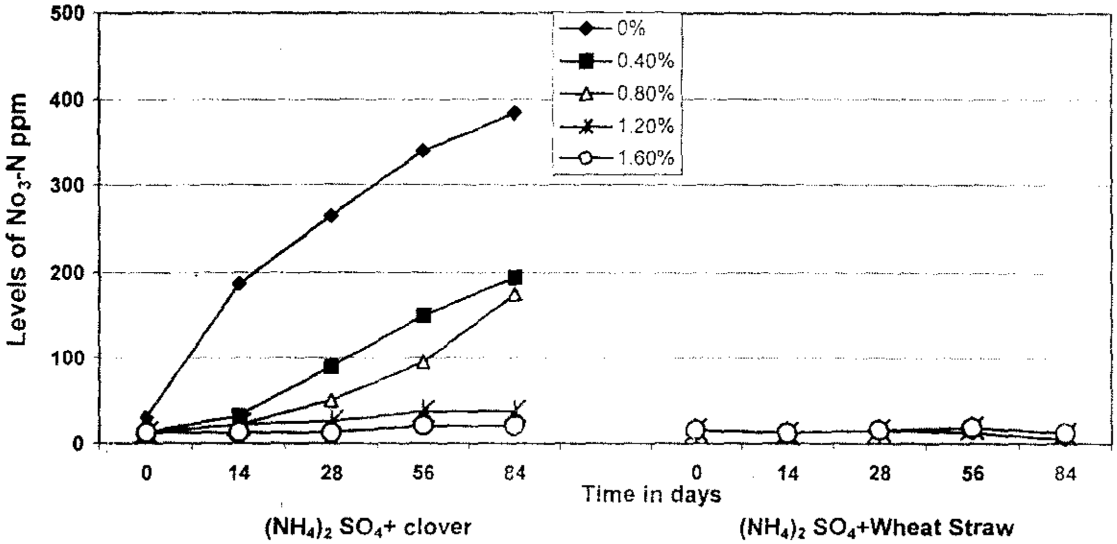

Fig. (3) Changes in NO_3-N of $(NH_4)_2SO_4$ as affected by salinity and type of organic matter

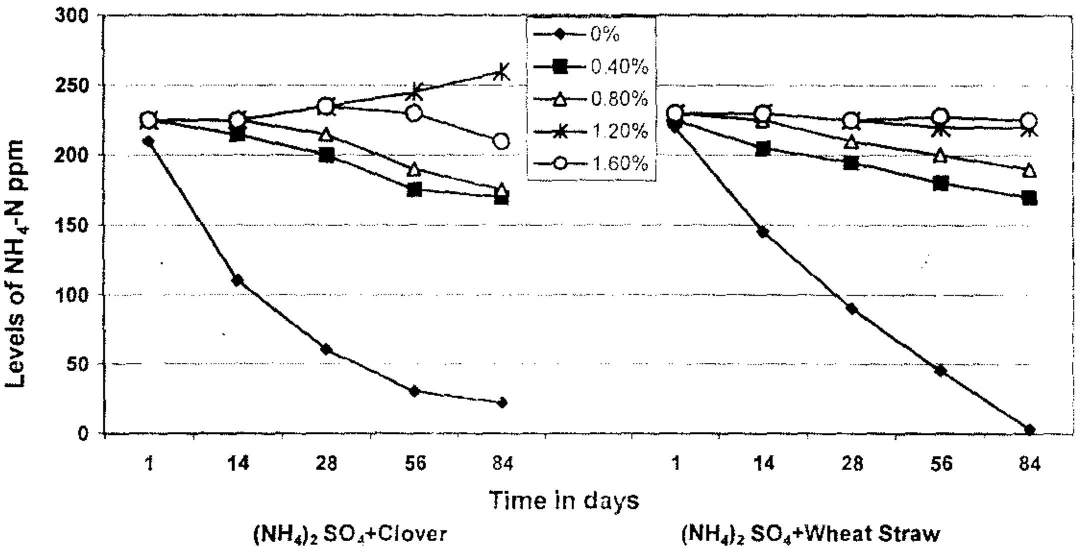

Fig. (4) Changes in NH_4 of $(NH_4)_2$ SO_4 as affected by salinity and type of organic matter

ammonification and nitrification of ammonium sulphate and clover-N. Mc-Cormick and Wolf (1980) found comparable trends attributed alleviation of the adverse effect of salts by organic amendments to the dilution caused by adding organic amendment. Another reason for the persistence of nitrification in relatively high Wes...man and Tucker (1974) stated that increased salt concentration might solubilize soil organic matter making it more easily mineralized at relatively low and medium salt concentrations, while a decrease occurred at higher salt concentrations which inhibited autotrophic nitrifiers.

The addition of wide C/N ratio organic matter (wheat straw), in the absence of salts, led to disappearance of most of native soluble nitrogen as well as ammonium sulphate nitrogen within eight weeks. Total soluble nitrogen was 17 ppm at the end of the experiment, which was less than plain soil (35 ppm). The effect of increasing levels of salts on immobilization of ammonium sulphate nitrogen is presented in Fig. 3 and 4. Generally, data proved that immobilization was active than nitrification process which led to decreases in ammonia nitrogen levels in the soil without corresponding increases in nitrate nitrogen. The addition of 0.4, 0.8, 1.2 and 1.6% salts led to accumulation of 192 ppm (171 NH_4-N and 21 NO_3-N), 195 ppm (177 NH_4-N and 18 NO_3-N), 217ppm (201 NH_4-N and 16 NO_3-N), and 233 ppm (217 NH_4-N and 16 NO_3-N) total soluble nitrogen, respectively, at the end of the incubation period. It is worth mentioning that there were no significant differences between levels of salinity and formation of nitrate throughout the experimental period. Immobilization of soluble nitrogen was significantly affected by $\geq$ 1.2% salts. Similar trends were observed by Jansson *et al.* (1955) who added that micro-organisms prefer NH_4-N to NO_3-N whereas Broadbent and Tyler (1962) observed that nitrates were immobilized by soil micro-organisms when it was the only form of available nitrogen.

Figures 5 and 6 illustrates the effect of different levels of salinity on the transformations of urea nitrogen in the presence or absence of narrow and wide C/N ratio organic amendments. It is clear from figures that nearly the same trends of changes in ammonium sulphate existed also in urea fertilizer. The only difference was that urea needs slightly more time, at the beginning, for hydrolysis to ammonium nitrogen, then followed nearly the same trends.

Changes in nitrifier counts, in narrow C/N ratio organic amendment soils indicated that, the combined addition of ammonium sulphate or urea with 2% clover maximized nitrifier counts irrespective of salinity.

Nitrifier counts irrespective of salinity. The addition of different levels of salinity decreased nitrifier counts but the magnitude of the effect was less than that when any of the mineral fertilizer studied was added alone. Presence of 1.6% salts greatly decreased nitrifier counts to levels similar to plain soil. The addition of wide C/N ratio organic amendment (wheat straw) in combination with ammonium sulphate or urea slightly increased nitrifier counts in non-saline soil. This was expected since soluble nitrogen needed for nitrifier rapidly disappeared from soil. The addition of different levels of salts affected nitrifier counts in two ways. First directly by decreasing the activity of nitrifier because nitrifiction, as well known, is more sensitive to salinity than ammonification. Secondly, which was indirect, immobilization was less sensitive to salinity and proceeded faster than nitrification thus leading to disappearance of available nitrogen in the soil so that nitrifiers lacked their sole source of energy. This effect was more pronounced at $\geq$ 0.8% salinity.

It may, therefore, be concluded that under saline conditions, mineralization and subsequent nitrification processes were delayed, suppressed or inhibited increasingly as salinity increased. The addition of organic amendments alleviated, in salt affected soils, the adverse effect of salinity on microbiological transformations of mineral nitrogen fertilizers in soil.

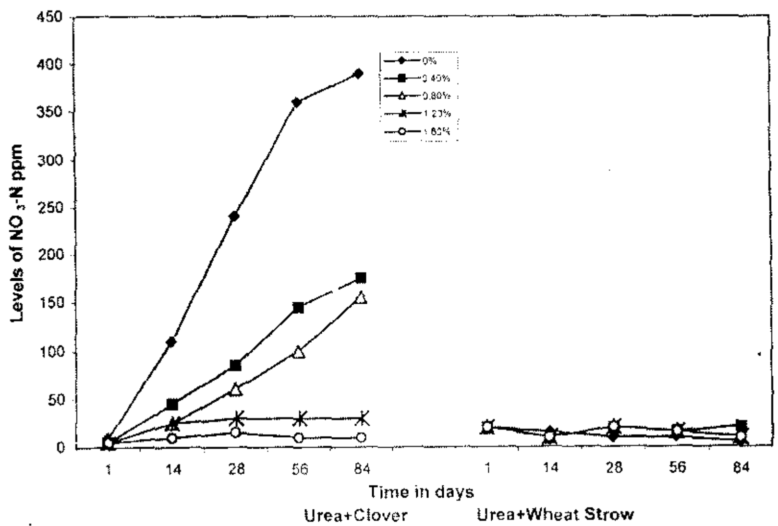

Fig. (5) Changes in NO_3-N of Urea as affected by salinity and type of organic matter.

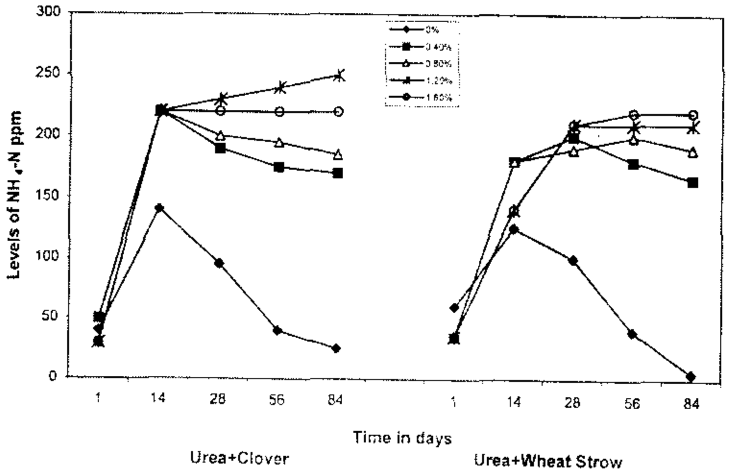

Fig. (6) Changes in NH_4-N of Urea as affected by salinity and type of organic matter.

REFERENCES

Agarwal, A. S.; Singh, B. R. and Kanekiro, Y. (1971). Ionic effect of salts on mineral nitrogen release in an allophonic soil. Soil Sci. Soc. Amer. Proceed., **35**: 454-457.

Alexander, M. and Clark, F. F. (1965). Nitrifying bacteria. Methods of Soil Analysis Part 2. Chemical and Microbiological Properties. No. 9 in the series of Agronomy, American Society of Agronomy Incorporation, Madison, Wisconsin, U.S.A. 1477-1483.

Ballmann, A. and Conrad, R. (1998). Influnce of O_2 availability on NO and N_2O release by nitrification and denitrification in soils. Global Change Biol., **4**: 387-396.

Bremner, J. M. (1965). Inorganic forms of nitrogen. Methods of Soil Analysis Part 2: Chemical and Microbiological Properties. No. 9 in the series of agronomy. American Society of Agronomy Incorporation, Madison, Wisconsin, U.S.A. 1179-1237

Broadbent, F. E. and Nakashima, T. (1971). Effect of added salts on nitrogen mineralization in three California soils. Soil Sci. Soc. Amer. Proceed., **35**: 457-460.

Broadbent, F. E. and Tyler, K. B. (1962). Laboratory and greenhouse investigations of nitrogen immobilization. Soil Sci. Soc. Amer. Proceed., **26**: 459-462.

Cochran, W. G. (1950). Estimation of bacterial densities by means of the "most probable number". Biometrics, **6**: 105-116.

El-Shahawy, R. M. and Mashhady, A. S. (1984). Nitrification of ammonium sulphate and urea fertilizers under saline condition. ZentralBlatt Microbiology, **139**: 343-347.

Ghandhi, A. P. and Paliwal, K.V. (1976). Mineralization and gaseous losses of nitrogen from urea and ammonium sulphate in salt-affected soils. Plant and Soil, **45**: 247-255.

Jansson, S. L.; Hallam, M. J. and Bartholomew, W. V. (1955). Preferential utilization of ammonium over nitrate by microorganisms in the decomposition of oat straw. Plant and Soil, **6**: 382-390.

Johnson, D. D. and Guenzi, W. G. (1963). Influence of salts on ammonium oxidation and carbon dioxide evolution from soil Soil Sci. Soc. Amer. Proceed., **27**: 663-666.

Kuenen, J. G. and Kobertson, L. A. (1988). Ecology of nitrification and denitrification., The Nitrogen and Sulpher Cycles eds. Cole, J. A. and Ferguson, S. PP. 162-218. Cambridge University Press, ISBN Cambridge.

Laura, R. D. (1974). Effects of neutral salts on carbon and nitrogen mineralization of organic matter in soil. Plant and Soil, **41**: 113-127.

Mc-Cormick, W. R. and Wolf, C. D. (1980). Effect of sodium chloride on carbon dioxide evolution, ammonification and nitrification in a Sassafras sandy loam. Soil Biol. Biochem., **12**: 153-157.

Paul, E. A. and Clark, F. E. (1996). Ammonification and Nitrification part-8., Soil Microbiology and Biochemistry PP. 181-197. Academic press. Incorporation. 525 B Street, San Diego, ISBN, California.

Rankov, V. (1965). Effect of soil salinity on relationship of Azotobacter and other soil microorganisms. Soils and Fertilizers, **28**: 3882.

Saad, O. A. O., Lehmann, S. and Conrad, R. (1996). Influence of thiosulfate on nitrification, denitrification and production of nitric oxide and nitrous oxide in soil. Biology and Fertility of Soils, **21**: 152-159.

Sindhu, M. A. and Cornfield, A. H. (1967a). Effect of sodium chloride and moisture content on ammonification and nitrification in incubated soil. J. Sci Food Agric., **18**: 505-506.

Sindhu, M. H. and Cornfield, A. H. (1967b) comparative effect of varying levels of chlorides and sulphates of sodium, potassium calcium and magnesium on ammonification and nitrification during incubation of soil. Plant and Soil, **27**: 468-472.

Singh, B. R.; Agarwal, A. S. and Kanehiro, V. (1969). Effect of chloride salts on ammonium nitrogen release in two Hawaiian soils. Soil Sci. Soc. Amer. Proceed., **33**: 557-560.

Subba-Rao, N. S. (1999). Nitrification and Denitrification. Soil Microbiology pp 270-273., Fourth Edition, Science Publishers, Inc. U.S.A., P.B. 699. Enfield, ISBN New Hampshire 03748

Westerman, R. L. and Tucker, T. C. (1974). Effect of salts and salts plus nitrogen-15-labeled ammonium chloride on mineralization of soil nitrogen, nitrification and immobilization. Soil Sci. Soc. Amer. Proceed., **38**:602-605.

الملخص العربي

التأزت البيولوجي للأسمدة النيتروجينية المعدنية في الظروف الملحية وإضافات مواد عضوية

ربيـــع محمد الشهاوي و خالـــــد محمد عطالله

قسم الميكروبيولوجيا الزراعية – كلية الزراعة بالفيوم–جامعة القاهرة

أجريـــت تجـــربة معملـــية لدراسة تأثير مياه بحيرة قارون بعد تخفيفها علي عملية التأزت لبعض الأسمدة المعدنية الأزوتية (سلفات أمونيوم – يوريا) في وجود وعدم وجود مادة عضوية (برسيم أو قش القمح).

وجـــد أنـــه بـــزيادة الملوحة بالتدريج من صفر إلي ١٦٠٠ جزء في المليون أخرت أو ثبطت لفترة أو أوقفت عملية التأزت تماماً. كما وجد أيضاً أن عملية النشدرة لليوريا لم تتأثر بالملوحة مطلقاً بينما عملية التأزت كانت بوجه عام حساسة للملوحة. إضافة المـــواد العضـــوية ذات نسبة الكربون للأزوت الضيقة (برسيم) تشجع من عملية النشدرة ثم التأزت بينما المواد ذات النسبة الواسعة (قـــش القمح) تشجع عملية تحول النيتروجين المعدني إلى عضوي. بينما إضافة المادة العضوية إلى السماد المعدني الأمونيومي يقلل بدرجة كبيرة من تأثير الملوحة الضار علي عملية النشدرة والتأزت وكذلك عملية تحول النيتروجين المعدني إلى نيتروجين عضوي.