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Microbiological Transformations of Two Ammoniacal Fertilizers under Saline And/ or Organic Matter Fortification

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Abstract: In unsaline loam soil, a laboratory experiment was undertaken studying the effect of using diluted saline water from Karoon lake at Fayoum Governorate, on transformations and subsequent nitrification of two ammoniacal fertilizers, namely $(NH4)_2SO_4$ and urea, in the presence or absence of natural organic amendments, namely clover and wheat straw. Ammonification and nitrification of both fertilizers delayed, suppressed or inhibited by increasing salinity levels. Ammonification of urea appeared to be independent of salinity, but generally, nitrification was sensitive to it. Addition of narrow C/N organic supplements (clover) in non-saline soil enhanced ammonification and subsequent nitrification while wide C/N one (wheat straw) encouraged immobilization of mineral nitrogen. In presence of the organic supplements, salinity showed negative effect on ammonification and subsequent nitrification and even the mineralization process. Generally, the adverse effect of salinity on biological transformations of the two mineral N-fertilizers used was variably alleviated in presence of organic supplements.

Key words: salinity, ammonification, nitrification, N-fertilizer, organic amendments

INTRODUCTION

Soil microorganisms play an important role in the cycling of most elements especially nitrogen and also in the decomposition of organic debris. Soil irrigation with high saline water may result in decreasing microbial activity (Laura, 1974; Paul and Clark, 1996; Rysgaard *et al*, 1999; Subba-Rao, 1999; Wong *et al*., 2005; Azam *et al.*, 2005; Jin *et al*., 2007; and Santoro and Enrich 2009). 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 (Rysgaard *et al*, 1999; Azam and Farooq, 2003; Wong *et al*, 2005; Jin *et al*, 2007 and Santoro and Enrich 2009). 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). 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; Subba-Rao, 1999, Strauss, *et al* 2002; Azam and Farooq, 2003; Wong *et al*, 2005; Gill *et al*, 2006; and Singh and Kashyap, 2007). The need to use saline water in irrigation compensating the shortage in fresh water, especially in Fayoum Governorate, aroused the concern of its effect on microbial transformation of mineral nitrogen fertilizers in soil. Therefore, the present investigation which based on a laboratory experiment extended to 12 weeks, was undertaken to study the biological 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% Ca CO3, 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

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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 in this study was as follow: pH, 7.3; EC, 58.58 (dSm^{-1}); Salts, 3.75%. Ca^{++}; Mg^{++}, Na^{+}, K^{+} were 20.9, 88.9, 467.1 and 46.6 meq/L, respectively, and $CO3^{=}$, $HCO3^{-}$, Cl^{-}, and $SO4^{=}$ were 0.0, 3.5, 548.5 and 74.2, respectively. 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: (1) Ammonium sulphate, (2) Ammonium sulphate + clover, (3) Ammonium sulphate + wheat , (4) Urea, (5) Urea + clover, (6) Urea + wheat, (7) three jars for plain soil (a total 93 jars). After thorough mixing, soil moisture was raised to 50% of WHC using distilled water, and jars were incubated at 30°C. Soil moisture was kept constant by daily compensating evaporative loses, during the experimental period, with distilled water. Chemical analyses included the determination of ammonium and nitrate nitrogen, according to Bremner (1965) was conducted at 1, 14, 28, 56 and 84 day's 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 ($NH4^{+}$ +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 (Sindhu and Cornfield, 1967a,b; Laura, 1974; Paul and Clark, 1996; Wong *et al*, 2005 and Santoro and Enrich 2009). 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 8% 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; Azam and Farooq 2003; and Jin *et al*, 2007), 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 slightly decreases ammonification of urea, especially in high levels of salinity, which ascertained the suggestion of Singh *et al*., 1969; Laura, 1974; Paul and Clark, 1996; Rysgaard *et al*., 1999; Azam *et al*., 2005; that in saline condition, ammonification of urea is mostly chemical and may be partially biological. Also Mc-Cormick and Wolf (1980) found that ammonification is less sensitive to salinity than nitrification which 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 control soil, nitrifier counts reached maximum after two weeks and were 380 and 300 $x10^{2}$ g^{-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 illustrated 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 soil. Data obtained show that the addition of narrow C/N ratio amendment (clover) at the rate of

2% (w/w), fortified soil with additional amount of nitrogen amounting to 500 ppm. In control soil, transformations of nitrogen take 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) 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 NH4-N and 168 NO_3-N) total soluble nitrogen, respectively. This result is in agreement with those obtained by Singh *et al.,* 1969; Broadbent and Nakashima, 1971; Mc-Cormick and Wolf, 1980; and Saad *et al.*1996. However, it was reported that dilute concentrations of salts plus ammoniacal fertilizer stimulated mineralization of soil nitrogen (Westerman and Tucker, 1974; Gill *et al*, 2006; and Singh and Kashyap, 2007). 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 ammonification and nitrification of ammonium sulphate and clover-N. McCormick 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 salinity treatments could have been the addition of nitrifiers naturally present in the organic amendments. Rankov (1965) and Westerman and Tucker (1974) stated that the salt 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 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 Figs. 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), 217 ppm (201 NH_4-N and 16 NO_3-N) and 233 ppm (217 $NH4\text{-}_N$ and 16 NO_3-N) total soluble nitrogen, respectively, at the end of the incubation period. It is worth mentioning that there were no great differences between levels of salinity and formation of nitrate throughout the experimental period. Immobilization of soluble nitrogen was greatly affected by > 1.2% salts. Similar trends were observed by Jansson *et al.* (1955) who added that microorganisms prefer NH_4-N to NO_3-N whereas Broadbent and Tyler (1962) observed that nitrates were immobilized by soil microorganisms when it was the only form of available nitrogen.

Figures 5 and 6 showed 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 more time at the beginning to undergo hydrolysis to ammonium nitrogen, and 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.
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 nitrification, 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 > 0.8% salinity.

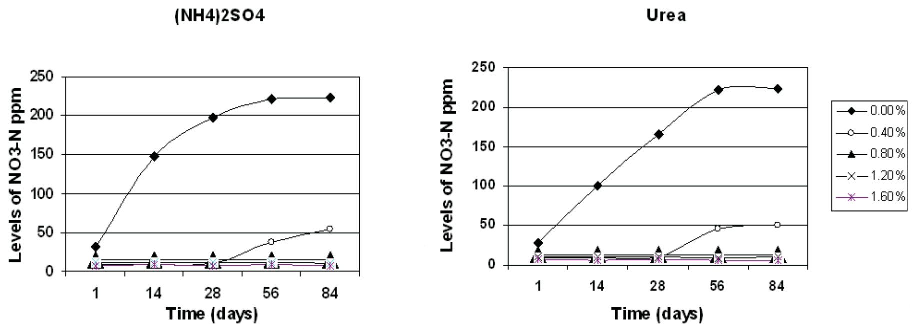

Fig. 1: Levels of No_3-N of $(NH_4)_2$ SO_4 and urea as affected by salinity (ppm-N)

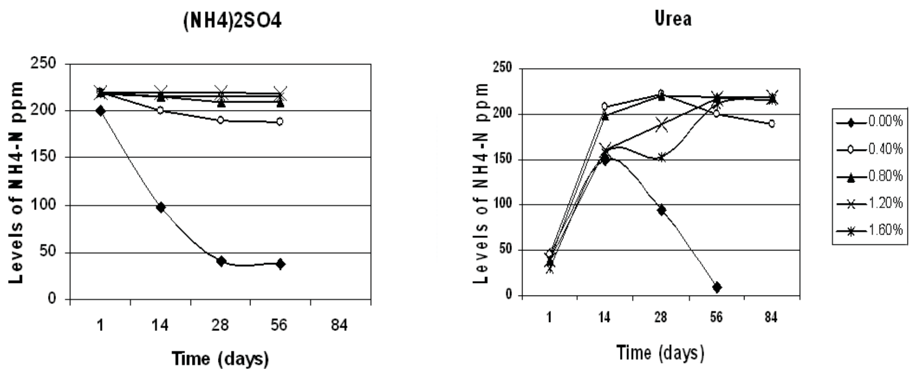

Fig. 2: Levels of NH_4-N of $(NH_4)_2$ So_4 and urea as affected by salinity (ppm-N)

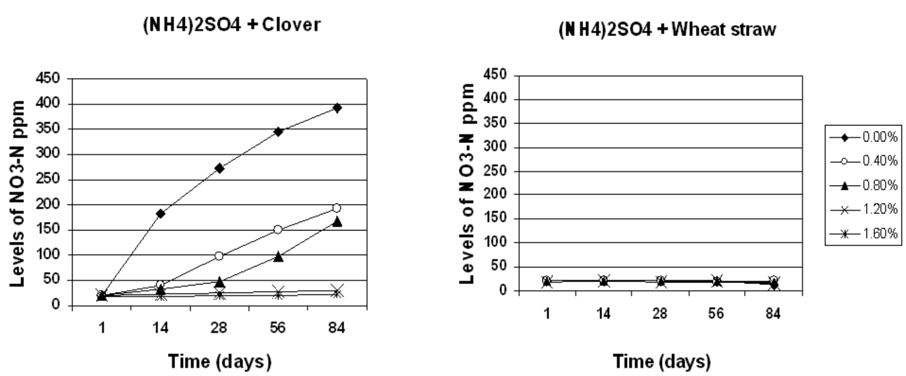

Fig. 3: Levels of No_3-N of $(NH_4)_2$ SO_4 as affected by salinity in presence of organic amendments (ppm-N)

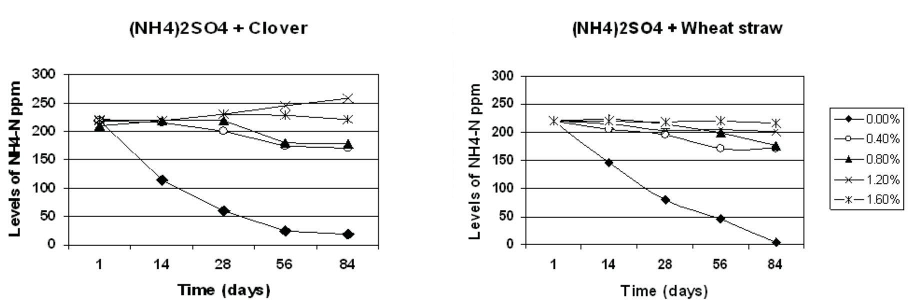

Fig. 4: Levels of NH_4-N of $(NH_4)_2$ SO_4 as affected by salinity in presence of organic amendments (ppm-N)

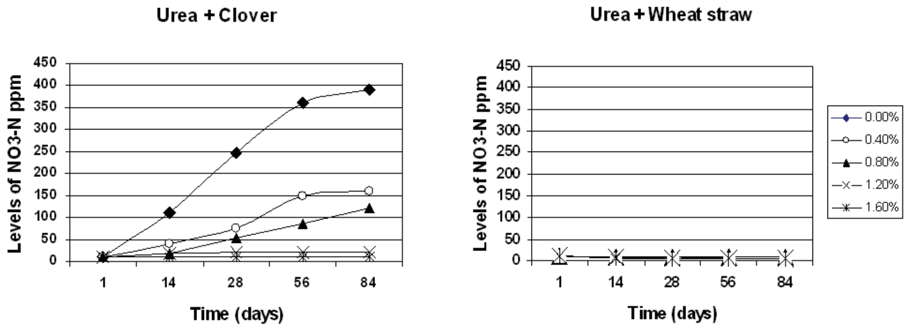

Fig. 5: Levels of NO_3-N of urea as affected by salinity in presence of organic amendments (ppm-N)

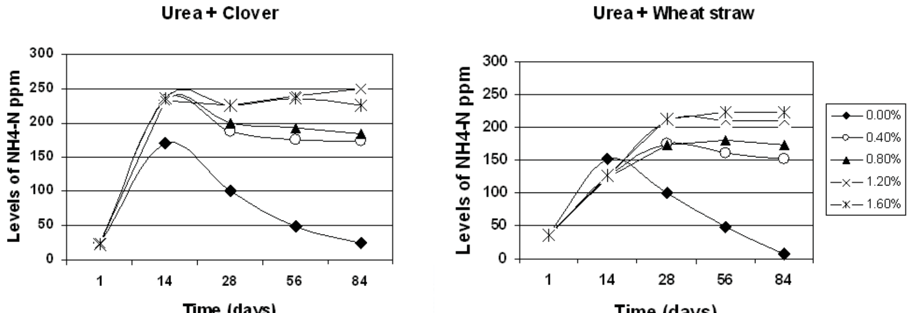

Fig. 6: Levels of NH_4-N of urea as affected by salinity in the presence of organic amendments (ppm-N)

Table 1: Changes in nitrifier counts (x $10^2 g^{-1}$) 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
			(NH4)2 SO4				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 + (NH4)2SO4				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 + (NH4)2SO4				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

Conclusion:

The increasing use of unsuitable water for irrigation, specially those of high salinity, to overcome the lack in fresh and appropriate water for agriculture aroused concern about its effect on some biological activities in soil. Therefore, the present investigation which based on a laboratory experiment extended to 12 wks, was undertaken to study the possibility of using high saline water for soil irrigation and its effect on the biological transformations of some ammoniacal (NH4)2SO4, and urea) and organic amendments (Wheat straw and clover) decomposition in soil. In the same time, the study included the effect of presence or absence of organic amendments on alleviation of the detremental effect of salinity on mineral nitrogen transformations in soil.

REFERENCES

Alexander, M. and F.F. Clark, 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.

Azam, F. and S. Farooq, 2003. Nitrification inhibition in soil and ecosystem functioning- An Overview. Pakistan Journal of Biological Science, 6: 528-535.

Azam, F., Gill and S. Farooq, 2005. Availability of CO_2 as a factor affecting the rate of nitrification in soil. Soil Biology and Biochemistry., 37: 2141-2144.

Ballmann, A. and R. Conrad, 1998. Influence of O_2 and 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 series of Agronomy. American Society of Agronomy Incorporation, Madison, Wisconsin, U.S.A. 1179-1237.

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

Broadbent, F.E. and K.B. Tyler, 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 A.S. Mashhady, 1984. Nitrification of ammonium sulphate and urea fertilizers under saline condition. ZentralBlatt Microbiology, 139: 343-347.

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

Gill, S., M. Abid, Z. Ahmed and F. Azam, 2006. Organic amendment accelerates nitrification in soil. Soil and Environ., 25: 35-39.

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

Jin, R., P. Zheng, Q. Mahmood and B. Hu, 2007. Osmotic stress on nitrification in an airlift bioreactor. Journal of Hazardous Materials., 146: 148–154.

Kuenen, J.G. and L.A. Kobertson, 1988. Ecology of nitrification and denitrification. The nitrogen and Sulpher Cycles eds. Cole, J. A. and Ferguson, S. Cambridge University Press, ISBN Cambridg, pp: 162-218.

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 C.D. Wolf, 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 F.E. Clark, 1996. Ammonification and Nitrification part-8., Soil Microbiology and Biochemistry. Academic press. Incorporation, 525 B Street, San Diego, ISBN, California, pp: 181-197

Rankov, V., 1965. Effect of soil salinity on relationship of Azotobacter and other microorganisms. Soil and Fertilizers, 28: 3882.

Rysgaard, S., P. Thastum, T. Dalsgaard, P.B. Christensen and N. Sloth, 1999. Effects of salinity on $NH4^+$ adsorption capacity, nitrification, and denitrification in Danish Estuarine sediments. Estuaries., 22: 21-30.

Saad, O.A.O., S. Lehmann and R. Conrad, 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.

Santoro, A.L., and A. Enrrich-Prast, 2009. Salinity control of nitrification in saline shallow coastal lagoons. Acta Limnol. Bras., 21(2): 263-267.

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

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

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

Singh, J.S. and A.K. Kashyap, 2007. Variations in soil N-mineralization and nitrification in seasonally dry tropical forest and savanna ecosystems in Vindhyan region, India.Tropical Ecology, 48(1): 27-35.

Strauss, E.A., N.L. Mitchell and G.A. Lamberti, 2002. Factors regulating nitrification in aquatic sediments: effects of organic carbon, nitrogen availability, and pH. Can. J. Fish. Aquat. Sci., 59: 554–563.

Subba-Rao, N.S., 1999. Nitrification and denitrification. Soil Microbiology, Fourth Edition, Science publishers, Inc. U.S.A., P.B. 699. Enfield, ISBN. New Hampshire., 03748: 270-273.

Westerman, R.L. and T.C. Tucker, 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.

Wong, V.N.L., R.S.B. Greene, B.W. Murphy, R. Dalal and S. Mann, 2005. Decomposition of added organic material in salt-affected soils. *In:* Roach I.C. ed. *Regolith.* CRC LEME, pp: 333-337.