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Effect of Urea Fertilizer on the Biological Nitrification Process in Soils of Different Textures and Locations

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Abstract. *An experiment was conducted to study the effect of adding different levels of urea fertilizer (ppm Urea-N0,75,150,225,300) on the effectiveness of bio-nitrification in the studied soil. The study treatments for this experiment were (4 soils of different textures and locations × 5 urea levels × 3 replicates) in an experiment in which a completely randomized design (CRD) was used. It was incubated at laboratory temperature (25°C) for 40 days, during which the amount of formed nitrates and remaining ammonium was measured every ten days, noting the replacement of lost water every two days. The highest percentage of formed nitrates (39.8%) was recorded from the ammonium added to Basrah soil after ten days of incubation, followed by Kut soil, which recorded 29.8%, and less than that in Shatra soils (24.2%) and Fajr soils (23.7%). After that, the efficiency of nitrification decreased in the subsequent incubation periods up to 40 days of incubation.The percentage of ammonium remaining from urea added to the soil was recorded after the four incubation periods at 20.0 ppm (when adding 225 ppm urea to the Qurna soil), which differed significantly from the rest of the studied soils.*

Keywords: *Bionitrification, Site, Soil, Tissue, Urea.*

1. INTRODUCTION

Nitrogen is one of the major nutrients necessary for plant growth, as it enters into various vital cellular structures such as chlorophyll, nucleic acids, proteins, phospholipids, etc (Sahi et al., 2022).

Nitrogen is added to the soil in organic or mineral form or is biologically fixed by soil organisms that are symbiotic with plants such as rhizobia or associated with plants such as Azospirillum or in a free form such as Azotobacter (Haran, 2021).

When the environmental conditions are suitable, such as temperature, humidity, light, etc., organic nitrogen is decomposed by microscopic soil organisms into ammonia through the mineralization process. Urea is also decomposed into ammonia by the urease enzyme secreted by many species of bacteria and fungi (Saeedharran and Bustanhanoon, 2020). The result of this process is gases, the most important of which is: nitrogen dioxide gas (N2O), which evaporates from the soil to the stratosphere, where it interacts with the ozone layer and participates in its destruction, or is washed to the lower layers of the soil, passing through the groundwater and then to river water causing it to be polluted with nitrates, It may encourage the growth of algae and aquatic plants and their negative effects on fish wealth, navigation, and tourism (Eutrification) (Haran et al.,2021).

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In recent years, the demand for chemical nitrogen fertilizers has increased due to their importance in increasing the crop, but their excessive use has led to an increase in environmental pollution, so researchers and specialists have resorted to thinking about finding solutions to these environmental problems, such as using organic or biological fertilizers instead of chemical fertilizers or using inhibitors for the nitrification process (Nitrification Inhibitors) to delay or stop the nitrification process and thus preserve nitrogen from deterioration and loss, which reduces the loss of nitrogen fertilizers and enhances its benefit rate on the one hand and reduces the level of environmental pollution on the other hand (Hanoon et al., 2024).

We conclude from the above that expanding the study of the nitrification process is necessary to determine its efficiency and the environmental pollution problems resulting from it. Therefore, this research aims to conduct a study to determine the effectiveness and efficiency of the nitrification process in soils of different textures and from many regions after adding urea fertilizer to them at different levels.

2. MATERIALS AND METHODS

The study used soils taken from many regions with different textures and planted with different crops. Samples were taken from the surface layer at a depth of 0-15 cm. The samples were transferred to the laboratory, dried and ground, then sieved with a 2 mm sieve, and then stored in the refrigerator at a temperature of 4 °C until used.

In order to conduct the experiment accurately, initial analyses were conducted on the studied soils (Table 1). After that, the usual method was followed to determine the efficiency of the biological nitrification process, which depends on adding ammonium nitrogen solution or urea solution and bringing the soil to a moisture close to the field capacity limits (90% of the field capacity) to ensure the availability of appropriate aerobic conditions.

In the experimental units of this experiment, plastic dishes were used, each with a diameter of 20 cm and a depth of 5 cm, each containing 250 g of each of the four studied soils after sifting them with a 2 mm sieve and measuring the saturation capacity of each.

Urea fertilizer was added at five concentrations (0, 75, 150, 225 and 300 ppm N urea), where it was added in the form of a concentrated solution with a concentration of (100 ppm N urea) and in quantities according to the required concentration, and the moisture of the soil treatments was completed to 90% of the field capacity and the total weight of each dish with its contents was taken.

The total number of follicular units in the experiment was 60 experimental units consisting of 4 different soils and treated with five levels of urea fertilizer and with three replicates for each treatment, and a completely randomized design (CRD) was used.

The incubation process was carried out in the laboratory for 40 days, then the dishes were weighed and the lost moisture was replaced every two days throughout the incubation period. The amount of formed nitrate nitrogen was measured (Black, 1965) and the remaining ammonium nitrogen was measured every ten days of incubation by adding a little magnesium oxide (MgO) to convert ammonium to ammonia gas. A representative sample of 10 g was taken for each dish and 50 ml of 2M KCl was added. The mixture was then shaken for half an hour and filtered. Both nitrate nitrogen and ammonium nitrogen were measured in the filtrate using the Kjeldahl apparatus (Black, 1965), Then the nitrification rate of urea fertilizer was calculated according to the following equation:

Nitrate- produced in the treated soil- Nitrate- N produced in the control

%Nitrification rate =×100

Concentration of the added urea- N from urea- N only

Table 1. Shows Some Of The Physical, Chemical And Biological Properties Of The Studied Soils

Properties		**Fajr soil**	**Basrah soil**	**Shatra soil**	**Kut soil**
Clay	$g.kg^{-1}$	178	179	192	269
Silt		520	435	535	460
Sand		302	386	273	271
Texture		Mixed clay	Mixed	mixed	clay mixed
Moisture at field capacity %		22	28	29	34
pH		7.73	8.11	7.98	7.91
$E.CedS.m^{-1}$		3.24	2.93	3.4	3.05
lime	$g.kg^{-1}$	122	308	296	201
M.O		25.5	35.3	31.4	20.6
$CECCmol.kg^{-1}$		28.4	24.8	27.8	30.4
Total N %		0.175	0.160	0.115	0.127
$N– NH_4$ $mg.kg^{-1}$		39	46	29	43
$N– NO_3$ $mg.kg^{-1}$		23	17	20	27
Ready P $mg.kg^{-1}$		7.6	7.2	4.7	4.9
Ready K $mg.kg^{-1}$		0.92	0.051	0.066	0.073
Total bacteria	$Cfu.gm^{-1}$ Soil Dry	10^7×1.9	10^7×1.6	10^7×2.7	10^7×3.0
Total fungi		10^4×41	10^4×34	10^4×50	10^4×55

All results were estimated in saturated soil paste extract.

3. RESULTS AND DISCUSSION

Estimation of the amount of nitrates formed in the soil (NO3-N ppm):

Table No. 2 shows the amount of nitrates formed by the nitrification process in the studied soils during the four incubation periods (without adding urea). The results show a slight increase in the amount of nitrates in the soils of Al-Fajr, Basra and Kut during the first ten days of incubation, as it was 23, 17 and 27 NO3-N ppm at the beginning of the experiment and became 27.3, 22.7 and 32.2 NO3-N ppm after incubation, respectively. This increase may come from the oxidation of part of the ammonium in these soils to nitrate by nitrifying bacteria, while in the Shatra soil the amount decreased by half and the reason may be the lack of available ammonium in this soil compared to other soils or it may be due to the consumption of part of the nitrates formed by soil bacteria and fungi. Also, increasing the incubation period by another ten days led to a noticeable increase in the amount of nitrates formed, as it reached 52, 51.8, 57.7 and 53.3 NO3-N ppm for the Fajr, Basra, Shatra and Kut soils, respectively. The reason for this increase is attributed to the oxidation of part of the available ammonium in this soil in addition to the oxidation of part of the ammonium resulting from the decomposition of organic materials originally present in the soil by heterotrophic soil organisms, whose numbers increased as a result of the availability of moisture and suitable temperature (Merzlaya, 2024).

Table 2. Shows The Amount Of Nitrates Formed NO3-N Ppm In The Soil By The Nitrification Process During The Four Incubation Periods (Without Adding Urea).

The Soils	Amount of nitrates formed (NO3-N ppm)			
	Incubation period 10 days	Incubation period 20 days	Incubation period 30 days	Incubation period 40 days
Fajr	27.3	52.0	25.3	41.3
Basra	22.7	51.8	12.5	33.3
Shatra	10.0	57.7	39.0	29.7
Kut	32.2	53.3	37.0	28.7

We also find from the results of Table (2) that the amount of nitrates in the third incubation period decreased significantly, especially in Basra soil, where it reached 12.5 NO3-N ppm, while in Al-Fajr, Al-Shatra and Al-Kut soils, it reached 25.3, 39 and 37 NO3-N ppm, respectively. The reason for this decrease is attributed to the representation of a portion of the nitrates by bacteria and fungi and its conversion into a vital protein, in addition to the fact that a portion of it may have been subjected to reduction by the Denitrification process or by the Nitrate respiration process, although the soil conditions are aerobic (humidity 90% of the field capacity), anaerobic conditions may form in it (Chen & Wang, 2015). Regarding the last incubation period (40 days), the amount of nitrates in the soils of Al-Fajr and Basra increased significantly and reached 41.3 and 33.3 NO3-N ppm, respectively. This may be due to the death of many microorganisms in the soil due to the lack of available carbon, and thus the decomposition of part of the biological nitrogen into ammonium, and then the ammonium is oxidized into nitrates (Sahi et al., 2022).

Table 3. Effect Of Added Urea Concentration Urea-N Ppm On The Rate Of Nitrification Process In Soils Of Different Regions During The Four Incubation Periods

The Soils	Added concentrations of urea (ppm)	Incubation period 10 days		Incubation period 20 days		Incubation period 30 days		Incubation period 40 days	
		Amount of NO_3 formed(ppm)	Nitrification rate %	Amount of NO_3 formed(ppm)	Nitrification rate %	Amount of NO_3 formed(ppm)	Nitrification rate %	Amount of NO_3 formed(ppm)	Nitrification rate %
Fajr	75	15.4	20.9	16.3	21.7	8.0	10.3	2.4	3.2
	150	34.0	22.3	23.3	15.5	13.7	9.5	9.6	6.3
	225	57.5	25.0	37.3	16.6	22.8	10.6	17.7	7.9
	300	69.4	22.6	54.7	18.2	12.3	4.8	21.0	7.0
Average		44.1	22.7	32.9	18.0	14.2	8.8	12.7	6.1
Basra	75	29.3	39.4	14.3	19.1	11.2	14.6	9.4	12.5
	150	62.0	41.0	31.6	21.1	20.5	14.0	19.7	13.1
	225	85.3	37.4	47.0	20.9	17.2	8.2	30.4	13.5
	300	113.3	37.1	62.0	20.7	27.5	9.8	48.0	16.0
Average		72.0	38.8	38.7	20.5	19.1	11.7	26.9	13.8
Shatra	75	23.3	31.4	12.6	16.8	10.4	13.4	9.6	12.8
	150	41.0	27.0	26.0	17.3	4.3	3.2	11.7	7.7
	225	59.7	26.0	42.0	18.7	11.3	5.6	23.6	10.5
	300	92.3	30.1	52.6	17.5	8.5	3.4	34.0	11.3
Average		54.1	28.6	33.3	17.6	8.6	6.4	19.7	10.6
Kut	75	12.7	17.3	16.2	21.0	8.3	10.7	10.2	13.7
	150	37.5	24.6	35.0	23.3	10.0	7.0	16.3	10.9
	225	59.4	25.8	50.0	22.5	21.7	10.2	34.0	15.1
	300	76.7	24.9	80.4	26.8	19.6	7.2	44.3	14.8
Average		46.6	23.2	45.4	23.4	14.9	8.8	26.2	13.6

- LSD 0.05=2.63

-Table numbers after subtracting the value of comparison coefficients.

- Statistical analysis is for the average amount of nitrates formed.

As for the fourth incubation period (40 days), we notice that the amount of nitrates formed in the Shatra and Kut soils has decreased from what it was, becoming 29.7 and 28.7 NO3-N ppm. The reason for this may be attributed to the increased activity of aerobic bacteria and thus their reduction of a portion of the formed nitrates. It may also be greater in these two than their activity in the previous two soils. The results of Table 3 also show that the highest percentage of added nitrogen oxidized to nitrate was in the first ten days of incubation. We also note that the higher the concentration of added nitrogen, the greater the amount of nitrate formed by the biological oxidation of ammonium. Consequently, the amount of nitrate formed in the Basra soil treatment increased to 73.5 NO3-N ppm, and the nitrification rate for the same treatment was about 39.8% during the first incubation period (10 days).

However, after the second and third incubation periods, the amount of nitrate formed decreased in all the soils studied, then the amount of nitrate formed increased again during the fourth incubation period (40), with the exception of the Fajr soil treatment, where the amount of nitrate formed continued to decrease. Referring to the results of Table 1, we find that there is a direct relationship between the amount of organic matter and the amount of nitrates formed. This clearly indicates the existence of a direct relationship between organic matter and the speed of the nitrification process in these soils. This may be explained by the fact that moistening the soil, increasing its ventilation, and providing sources of nutrition for soil organisms through the presence of organic matter in appropriate quantities have helped increase the numbers of heterotrophic bacteria and fungi, and thus increased the decomposition of organic matter as well as the oxidation of ammonium to nitrate by these organisms (Zhao et al., 2023).

The amount of residual ammonium in the soil (NH4-N ppm):

Table (4) shows the amount of residual ammonium from adding different concentrations of urea fertilizer to different soils after four incubation periods. From the results of the table, it is clear that the amount of residual ammonium after ten days of incubation was directly proportional to the amount of ammonium added in the form of urea for all the soils studied. This applies in general to the second incubation period. As for the subsequent incubation periods, the amount of residual ammonium decreased significantly to the point that it was less than the treatments to which urea was not added. The reason may be the consumption of ammonium by microorganisms, including bacteria and fungi, in the metabolic process. This may be attributed to the fact that adding urea at different concentrations may lead to a significant increase in the number of microscopic soil organisms, especially those that analyze urea, and thus consume a large part of the ammonium (Haran et al., 2021).

Table 4. The Amount Of Remaining Ammonium NH4-N Ppm After Adding Urea To The Studied Soils

The Soils	Added concentrations of urea (ppm)	Residual ammonium NH_4-N ppm			
		Incubation period 10 days	Incubation period 20 days	Incubation period 30 days	Incubation period 40 days
Fajr	75	25.5	17.7	14.6	2.4
	150	51.2	30.7	29.3	2.0
	225	65.2	41.3	35.2	1.4
	300	81.7	49.7	42.5	2.0
Basra	75	23.0	8.00	6.0	8.4
	150	37.1	14.7	7.5	16.7
	225	53.4	19.7	9.6	20.0
	300	83.7	23.3	10.9	16.7
Shatra	75	21.4	10.3	3.3	13.7
	150	50.8	28.0	3.4	16.0
	225	50.1	31.7	2.3	8.00
	300	101.1	46.9	2.0	11.0
Kut	75	16.0	26.0	12.3	6.7
	150	44.4	42.4	17.6	3.6
	225	55.7	55.6	21.0	5.6
	300	63.1	66.9	22.6	18.0

The slight increase observed in the soils of Basra and Shatrah in the amount of residual ammonium during the last incubation period (40 days) may be due to the death of many microorganisms that grew and multiplied during the first incubation periods and consequently the decomposition of part of their organic nitrogen into ammonium, which enhanced the amount of residual ammonium in the studied soil (Merzlaya, 2024).

DAFTAR REFERENSI

Black, C. A. (1965). *Methods of soil analysis: Part 2. Chemical and microbiological properties.* American Society of Agronomy, Inc. https://doi.org/10.2134/agronmonogr9.1

Chen, Y., & Wang, F. (2015). Insights on nitrate respiration by *Shewanella*. *Frontiers in Marine Science, 1*, Article 80. https://doi.org/10.3389/fmars.2014.00080

Feng, J., Wu, X., Zhou, F., & Chi, R. (2024). Role of calcium chloride on the eluting process of residual ammonium from weathered crust elution-deposited rare earth ore tailings. *Minerals, 14*(5), 521. https://doi.org/10.3390/min14050521

Hanoon, M., Haran, M., Ahmed, K., Al-Taey, D., Jasim, A., & Fadhil, M. (2024). Response of sorghum (*Sorghum bicolor* L.) to plant growth hormones produced by *Aspergillus* sp. *Research on Crops, 25*. https://doi.org/10.31830/2348-7542.2024.ROC-1056

Haran, M. (2021). Efficacy of inoculation and potassium fertilizer on mycorrhiza growth of barley (*Hordeum vulgare*) under different levels of water stress. *Indian Journal of Ecology, 48*, 143–147.

Haran, M., & Hanoon, M. (2021). Study of the efficacy of bacterial inoculate carriers and levels of mineral fertilizer on growth and yield of millet (*Pennisetum* L.). *Research on Crops, 16*, 1581–1586.

Merzlaya, G. (2024). Nitrates and heavy metals in agrocenoses with prolonged use of organic fertilizers. *Agrokhimiya, 2024*(8), 95–104. https://doi.org/10.31857/S0002188124080136

Saeedharan, M., & Bustanhanoon, M. (2020). Effect of fungal inoculation and different levels of chemical fertilization on wheat growth and production (*Triticum aestivum* L.). *Ecology, Environment and Conservation, 26*(2), 676–682.

Sahi, M., Haran, M., & Hanoon, M. (2022). Effect of inoculation with *Bacillus* spp., *A. chroococcum*, *P. fluorescens* and phosphorus levels on the amount of major nutrients in maize (*Zea mays* L.) irrigated with saline water. *Caspian Journal of Environmental Sciences, 20*, 581–585. https://doi.org/10.22124/cjes.2022.5686

Zhao, W., Bi, X., Bai, M., & Wang, Y. (2023). Research advances of ammonia oxidation microorganisms in wastewater: Metabolic characteristics, microbial community, influencing factors and process applications. *Bioprocess and Biosystems Engineering, 46*, 1–13. https://doi.org/10.1007/s00449-023-02866-5