ISSN : 3105-0794

الهيئة الليبية للبحث العلمي

Libyan Authority For Scientific Research

(LIJNS) Libyan International Journal of Natural Sciences

VOLUME 2 ISSUE 1 MAY 2026

Bi-Annual, Peer- Reviewed, and Open Accessed e-Journal

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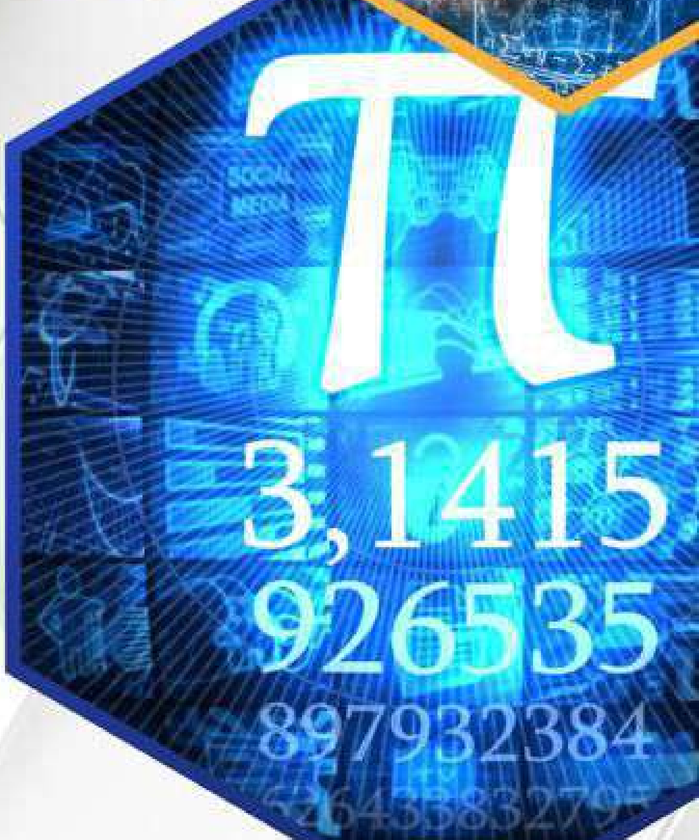

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Libyan International Journal of Natural Sciences (LIJNS)
Vol. 2 Issue No 1 May 2026

Estimation of Nitrate and Nitrite Levels in Soils Collected from Farms Surrounding Al-Khoums City

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ARTICLE INFORMATION

Article history:
Received 29 December 25
Accepted 15 March 26
Published 3 April 2026

Keywords:
Nitrate, Nitrite, Environmental

ABSTRACT

Nitrate compounds commonly occur in various environmental media, including water, soil, and vegetables. Although nitrate itself does not pose a significant risk to human health, its conversion into nitrite represents a major threat to human health and life. This study focused on areas surrounding the city of Al-Khoums (Kaam, Aimsilata, and Ghoneima). 27 soil samples were analyzed using a spectrophotometer to estimate nitrate and nitrite concentrations. Sample analysis showed that soil nitrate concentrations ranged between 27.85 and 42.21 ppm, whereas very low nitrite concentrations were detected in these samples (3.97–9.01 ppm).

1. Introduction

Previous studies indicate that almost 72–94% of the total nitrate intake comes from consumed vegetables (Pietro, 2006). Nitrate is a key component of the nitrogen cycle and is also an essential nutrient for protein synthesis in plants. Several studies indicate that nitrate primarily originates from fertilizers and decaying plant matter and that intensive use of synthetic nitrogen fertilizers has increased nitrate levels in soils and irrigation water (Zhao-hui, 2015). Nitrate itself does not pose a direct threat to human health; however, the most significant effects occur when it is reduced to nitrite in the digestive system, as shown in Figure 1.

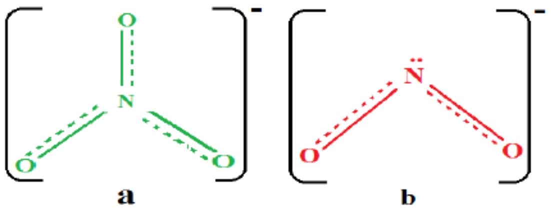

Figure 1: Structural forms of (a) nitrate and (b) nitrite

Nitrate is a fully oxidized form of nitrogen and is chemically stable because its structure is a nitrogen atom and three oxygen atoms, where all of them are in the same plane. They are arranged in a symmetrical trigonal resonance hybrid with bond angles of 120 degrees. Nevertheless, it can accept electrons and thereby act as an oxidizing agent, being itself reduced to nitrite (Alfred, 1999).

2. Research aim

This study aims to quantitatively assess and compare nitrate concentrations in soils from selected areas surrounding Al-khoums city.

3. Solutions & Chemicals

All solutions were prepared using deionized (DI) water obtained from a desalination plant in Al-khoums city. All reagents and solvents were of analytical grade and used without further purification, as listed in Table 1.

Table 1: List of the most important chemicals and solvents used in this study with their sources and purities.

Chemical name	Formula	MW (g/mol)	Purity
Calcium chloride	$CaCl_2$	110.98	88%
Cadmium sulphate	$CdSO_4.\ 8H_2O$	352.47	74%
N-(1-Naphthyl) ethylene di-amine	$C_{12}H_{14}N_2$	186.25	98%

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N-(1-Naphthyl) ethylene di-amine di-hydrochloride	$C_{12}H_{16}Cl_2N_2$	259.17	98%
Ammonium chloride	NH_4Cl	53.49	87%
Ammonium hydroxide	NH_4OH	35.03	93%
Hydrochloric acid	HCl	36.46	96%
Acetic acid	CH_3COOH	60.05	99%
Sulfanilic acid	$C_6H_7NO_3S$	173.19	98%
Zinc sulphate	$ZnSO_4 .7H_2O$	287.53	94%
Zinc metal	Zn	65.38	99%

4. Reagents

4.1 Ammonium chloride buffer solution

Dilute 20ml of HCl (12M) to 500ml with water and mix it with 50ml of NH_4OH, then dilute this mixture to one liter with water and mix well (Kharicha, 2018). This process should give a buffer solution (pH 9.6–9.7).

4.2 Zinc sulphate solution

Dissolve 120g of $ZnSO_4.7H_2O$ in distilled water and dilute to one liter, and so you get 0.42M of zinc sulphate solution.

4.3 Cadmium sulphate solution

Dissolve 37g of $CdSO_4.8H_2O$ in distilled water and dilute to one liter, in order to get 0.14M of cadmium sulphate.

4.4 N-(1-Naphthyl) ethylene di-amine di-hydrochloride

This reagent remains stable for at least one week and can be prepared by adding 1% of it to 60% acetic acid, then storing this solution in a glass-stoppered Erlenmeyer flask in a freezer (Kamalinia, 2009).

4.5 Colour reagent

Mix similar values from the sulfanilic acid solution & N-(1-naphthyl) ethylene diamine, immediately before use.

4.6 Zinc metal

Cut 10 cm of zinc metal to get sticks.

5. Cadmium electrode

The following technique was used to prepare a cadmium electrode (a modified Jones reductor) (Makkar, 2007).

5.1 Cadmium electrode preparation

- In two 800-mL beakers, 3-5 Zn sticks were placed in 500 mL of $CdSO_4$
- solution. After a thick layer of Cd was formed, the Zn sticks were removed, and the spongy metallic Cd layer was scraped off by rubbing the sticks against each other while kept under an aqueous layer.
    - After 6–8 hours, the product was collected and washed twice with 500 mL of water.
    - Cd scrapings were transferred to another beaker and blended well.
    - The blended material was then sieved through (8 - 40 mesh) sieves while it was still wet.
    - Washed particles were placed in a beaker with 0.1N HCl, stirring occasionally with a glass rod.
    - The product was left overnight in an acid.
    - The final product was stirred for degassing, and transported to the small column, then rewashed with (500ml) of water (twice).
    - The modified reductor was filled with (DI) and plugged with clean cotton. Cd particles were then added up to 8 - 10cm in the column (see Figure 2).
    - During the filling process, the liquid level was not allowed to fall below the Cd surface, just as when the column was drained by gravity.
    - Bubbles in the Cd column were eliminated by tapping the sides of the column, and then the column was washed with 25ml of NH_4Cl buffer.

Figure 2: modified Jones reductor

5.2 Cadmium electrode efficiency

- The column was washed with 25 mL of NH_4Cl buffer, just before use & drained to the top of Cd.
- Sodium nitrate (6.0 mL, working solution) was mixed with 5 mL NH_4Cl buffer solution and poured onto the column.
- The flow rate was adjusted to 3-5 mL/min, and the effluent was collected in a 50 mL volumetric flask.
- The empty column was washed with 15mL of distilled water, and then the washings were collected in the same flask.
- Add 5.0 mL of acetic acid (60%) to 10 mL of the colour reagent, then dilute to volume and mix well with distilled water. Keep it in the dark for 25 min.
-
- The reference column was prepared in the same way, using 6 mL of water instead of 6 mL of nitrate solution.

- The absorbance for (both reference & samples) has been recorded at 550 nm.
- Before taking the reading of absorbance, the instrument was standardized to zero absorbance using 10 mL of colour reagent solution (no nitrite) after being diluted to 50 mL with distilled water.
- The blank column absorbance reading was subtracted from that of the standard, and the concentration of $NaNO_2$ was determined from the standard calibration curve.

Note: If column efficiency is 90%, recondition the column by passing 25 mL of 0.1M HCl followed by two portions of water (50 mL each), and then 25 mL of NH_4Cl buffer solution.

6. Sample collection

To obtain representative results, three separate areas surrounding Al-khoums city were selected from three directions. These locations are geographically distant from each other, and for each one, three farms were identified. At each farm, three sites were chosen, at which a number of three samples were collected, as shown in Figure 3.

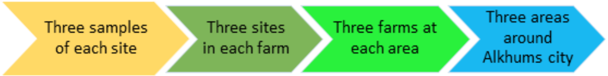

Figure 3: The sequencing of sampling steps

7. Soil Samples

Twenty-seven soil samples cover the whole three areas targeted by the study.

- Kaam (location 1).
- Aimsilata (location 2).
- Ghoneima (location 3).

All samples were collected from these three different sites around Al-khoums city, and because of this, a mechanism had to be developed for numbering samples (a clear way to differentiate between samples), as shown in the next diagram.

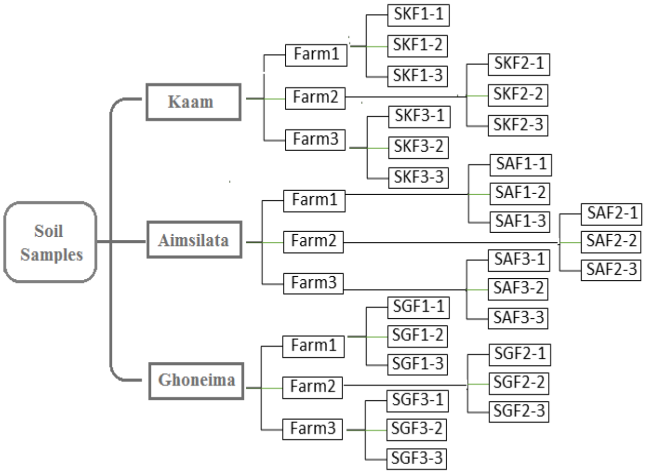

Figure 4: Schematic diagram showing the number of coding soil samples, where (F) farm, (S) soil, (K) Kaam, (A) Aimsilata, and (G) Ghoneima.

The above figure shows the selected method of coding soil samples, which numbered twenty-seven samples distributed evenly over the three study regions, with three farms for each region and three soil samples taken randomly from separated locations within each farm.

It is worth mentioning that soil samples were collected from the surface layer (15 cm depth), where most crops are usually grown (Williams, 1989). The crucible dried at 89 - 100°C for 30 min, then cooled in a desiccator and weighed.

- The crucible was returned to the oven for another 30 min, then cooled, and the weight was recorded again.
- 3 g of the sample was taken into the crucible.
- The crucible was placed in the oven (100 - 105°C) for about 3 hours.
- The crucible was transferred to the desiccator for cooling and weighing, which represented the weight of the crucible plus that of the sample after drying.
- The crucible returned to the oven and dried for one more hour.
- The crucible was allowed to dry before being weighed again.
- The drying, cooling, and weighing processes were repeated until the difference between two successive weights matched.
- Dried samples were kept for different investigations, and the humidity percentage was calculated using equation 1 for each sample:

$$\text{Humidity }\% = \frac{\text{Weight before drying} - \text{weight after drying}}{\text{Weight before drying}} \times 100$$

8. Nitrite determination

- Transfer 10 mL of the filtrate sample into a 50 mL volumetric flask, and then add these additives.
- 10 mL of NH_4Cl buffer solution.
- 5ml, 60% acetic acid.
- 10 mL colour reagent (equal volume of sulfanilic acid solution and N-(1-naphthyl) ethylene diamine).
- Dilute to volume with water, mix, and let stand for 25 min in a dark place. The mixture should be red-pink azo dye, which can be measured by a UV-Vis spectrophotometer (the absorbance of the solution at 550 nm).

9. Nitrite & Nitrate analysis

In the analysis of nitrate, the sample solution was divided into two parts (A and B), so that the first part (A) was analysed directly to obtain the amount of nitrite, and then a reduction process was performed on the second part (B) to convert any amount of nitrate in the sample into nitrite. Then, this part was analyzed with the same procedure to estimate the total amount of nitrite, and from it, the amount of nitrate calculated indirectly by taking the difference between these results (Merino, 2017).

10. Reduction of nitrate into nitrite

For converting nitrate into nitrite, one can follow these steps:

- Take 10 mL of the portion sample that needs to be reduced.
- Mix it with 5 mL of NH_4C1 buffer solution.
- Pass the mixture through the cadmium column.
- Wash the column with 25 mL of NH_4C1 buffer solution.
- Wash the column with 15 mL of water and collect the effluent in a 50 mL volumetric flask.

11. Results and discussion

Soil humidity was calculated according to Equation 1 and presented as a percentage for all soil samples in Table 2.

Table 2: Values of humidity in soil samples (%)

Location	Soil sample	Natural sample (g)	Humidity (%)
Kaam	KS1	1	31
	KS2	1	29
	KS3	1	30
Aimsilata	AS1	1	16
	AS2	1	14
	AS3	1	16
Ghoneima	GS1	1	20
	GS2	1	22
	GS3	1	21

Results show slight differences in the relative humidity values between the three regions, where the highest moisture value was recorded in Kaam (29 - 31%) and then in *the Ghoneima* area (20 - 21%), while the soil of *Aimsilata* had the lowest humidity (14-16%), see Table 2.

12. Nitrate & nitrite

The washing extract of soil samples can be analyzed for nitrite, while in the case of nitrate, one can indirectly calculate the amount of nitrate after it's reduced to nitrite. Table 3 represents the amount of nitrite and nitrate in all samples collected from different locations.

Table 3: Values of nitrite and nitrate in soil samples

sample	A (ppm)	B (ppm)	C = B-A (ppm)
KS1	4.13	42.38	38.25
KS2	6.20	41.75	35.55
KS3	9.01	50.21	41.20
AS1	5.17	33.02	27.85
AS2	7.15	38.53	31.38
AS3	3.97	37.02	33.05
GS1	7.10	36.75	29.65
GS2	8.25	27.46	42.21
GS3	6.11	43.11	37.00

Where (A) is the initial value of nitrite before reduction treatment in (ppm), (B) is the total value of nitrite after the reduction process of the existing nitrate in the sample (ppm), and (C) is the original value of nitrate calculated in the sample (ppm). In general, the results of the analysis of soil samples showed that the amount of nitrate in the soil was at the normal level, ranging from (27.85–42.21 ppm), yet nitrate is still within the acceptable range compared with standard values (20-40ppm) set up by specialized scientists (Coviello, 2020). However, the amount of nitrite in the soil was significantly smaller (3.97-9.01ppm).

13. Conclusion

Nitrate concentrations in the studied soils did not exceed internationally recommended limits.

I. The amount of nitrate in the soil of the studied areas did not exceed the permissible limits according to international specifications for healthy soils.
II. Nitrite concentrations in the soil samples were sufficiently low to be considered negligible.
III. The amount of nitrate in the soil is not a definitive indicator of its level in plants.
IV. Finally, continuous monitoring of nitrate levels in soil is recommended, along with expanding future research to include other areas.

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