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MONITORING ENVIRONMENTAL QUALITY VIA NITRATE ACCUMULATION IN GREENHOUSE LETTUCE (*LACTUCA SATIVA* L.)

Irina TOMASU[1], Mihaela DRAGOI CUDALBEANU[1], Simona MARCU SPINU[1], Zina PARASCHIV[2], Augustina Sandina TRONAC[1], Alina ORTAN[1]

[1]Faculty of Land Reclamation and Environmental Engineering, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, 011464, District 1, Bucharest, Romania
[2]Faculty of Biotechnologies, University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, 011464, District 1, Bucharest, Romania

Corresponding author email: mihaela.dragoi@fifim.ro

Abstract

Public health concerns related to nitrate accumulation in vegetables are increasing, as nitrate can be converted to nitrite, which can form harmful N-nitroso compounds. Due to its high capacity to accumulate nitrate, lettuce represents a suitable crop for monitoring the effects of fertilization practices on environmental quality in greenhouse systems. This study evaluated nitrate, nitrite, and ammonium contents in greenhouse-grown lettuce from two sources: supermarkets and local producers. Three samples were collected from supermarkets, four from local producers. Samples were separated into leaves, roots, and growing medium (soil), yielding 21 representative samples. Differences were observed in concentrations and distribution patterns. Supermarket lettuce showed higher leaf nitrate content than local-producer samples. Supermarket samples also exhibited higher nitrite content, particularly in roots, suggesting active nitrate-reduction processes. Leaves were the primary site for nitrate accumulation, followed by roots and soil, whereas nitrite and ammonium were mainly associated with roots and soil. Notably, local producer samples exhibited higher ammonium concentrations in roots and soil compared to supermarket samples, suggesting differences in fertilization practices and nitrogen conversion efficiency. These findings support closer monitoring of fertilization practices to assess environmental quality in protected agriculture and to limit consumer exposure to nitrogen-related compounds.

Key words: *nitrate residue, greenhouse cultivation, lettuce, environmental quality.*

INTRODUCTION

The nitrogen (N) sources are represented by inorganic forms (NH_4^+, NO_2^-, and NO_3^-), which are formed naturally in crops, soil, and water through the N cycle (Gidurus et al., 2012; Martín Leon & Luzardo, 2020). Thus, NH_4^+ and NO_3^- constitute the most important forms of N, as they support plant growth and, when taken up in large quantities, achieve maximum production yields (Colla et al., 2018).

Complex interactions among plant root systems promote efficient N transfer within the soil-plant system, enabling optimal use of available nutrients (Hao et al., 2022). N residues from previous crops also serve as an important energy source for soil bacteria, supporting microbial activity, reducing the need for chemical inputs, and promoting N immobilization processes (Assémien et al., 2019).

N is vital for plant growth, and it circulates between soil and plants, often as an anion (Butnariu, 2018). N fertilizers are the primary artificial N source, with usage rising rapidly. About 50% of applied N is lost via leaching, contaminating surface and groundwater by increasing NO_3^- content. After pesticides, nitrates are the second most concerning water contaminant for environmental and public health protection (Singh et al., 2022).

Plants need enough N to make amino acids, which are vital for cell and tissue formation. Without amino acids, plants can't produce proteins for growth, which harms development. Too much N, however, causes excess biomass, such as stems and leaves, and immature roots (Brady & Weil, 2010). Farmers use N fertilizers to boost crop growth; without them, about a third of food production could be lost. Yet, finding the optimal N level is crucial, as excess N pollutes water and threatens ecosystems (Aczel, 2019). Maintaining the right balance of essential life support elements is crucial. N deficiency in plants causes chlorosis and stunted growth (Britto et al., 2002). Proper N management prevents ecological problems such

as water pollution and soil degradation. Excess N can contaminate groundwater or other water bodies, causing eutrophication that harms aquatic ecosystems and biodiversity (Chislock et al., 2013).

Vegetables are a valuable source of minerals, vitamins, carbohydrates, and biologically active compounds. Adopting a diet rich in vegetables is one of the most effective ways to prevent numerous diseases, including obesity, which is becoming a major public health problem (Luetic et al., 2023). To support a healthy lifestyle that lowers the risk of various diseases and boosts immunity, the World Health Organization (WHO) advises consuming 400-500 g of vegetables daily as part of a balanced diet (Pennington et al., 2010).

Lettuce is a highly nutritious vegetable, abundant in vitamins C, A, K, and B9 (folate). Additionally, it is used medicinally for its high content of flavonoids, tocopherols, and carotenoids, compounds known for their high antioxidant properties. Another advantage is its global use and the fact that it is consumed raw in most cases, thereby retaining its full nutritional value (Popa et al., 2021).

Consequently, consumer exposure to nitrate (NO_3^-) varies depending on the types and amounts of vegetables consumed. In this context, leafy vegetables occupy a central place, as they have a high capacity for NO_3^- accumulation, favored by both the accelerated growth rate specific to these species and the concentration of nitrogen ions predominantly in the leaves, the edible parts of these plants (Martín Leon & Luzardo, 2020).

In leafy vegetable cropping systems, such as lettuce, a shallow root system limits access to N in deeper soil layers, resulting in low N use efficiency, estimated at an average of only 25% (Pujol et al., 2017). This inefficiency contributes to excessive N accumulation in the soil. Unabsorbed N can be leached as NO_3^- into groundwater, contaminating drinking water resources, or emitted into the atmosphere as nitrous oxide (N_2O), a particularly potent greenhouse gas (Cassman et al., 2002). Lettuce crops respond positively to N fertilization by increasing yields and improving product quality. In practice, high N fertilizer rates are often used to maximize production, but this can cause negative effects such as excess NO_3^- in leaves, which impacts food security, and N leaching, which harms the environment. Lettuce's response to N depends on variety, soil, and climate, and thus requires tailored fertilization to improve N efficiency and minimize risks (de Barros Sylvestre et al., 2019).

In lettuce, shallow roots, frequent high-rate N fertilizers, and intensive irrigation increase N leaching, raising environmental loss risks and reducing sustainability (Korsaeth, 2008). A strategy to reduce N leaching is to lower N fertilizer rates in lettuce. This reduces NO_3^- leaching with minimal impact on yield, while maintaining water quality and vegetable standards (Aragão et al., 2012).

Excessive N in vegetables poses ecological and health risks. Increased NO_3^- intake from vegetables is a primary dietary source. The type, dose, and application method of N fertilizers affect NO_3^- buildup in edible tissues. Rational N management is crucial to safeguard health and the environment (Coimbra et al., 2012).

The maximum allowable limit for NO_3^- in lettuce is set by both the European Union and Romanian national regulations. Thus, the maximum levels in raw greenhouse-grown lettuce are as follows: for lettuce harvested from October 1 to March 31, the limit must not exceed 5,000 mg NO_3^-/kg; for lettuce harvested from April 1 to September 30, the limit must not exceed 4,000 mg NO_3^-/kg (EU No 915/2023).

This study aimed to assess NO_3^- content in greenhouse lettuce, emphasizing the plant's tendency to accumulate NO_3^- under intensive fertilization. Additionally, it focused on the identification and quantification of related inorganic nitrogen species, like nitrite (NO_2^-) and ammonium (NH_4^+), which pose higher toxicity risks. The analysis facilitates the assessment of safety, quality, and potential environmental impact.

MATERIALS AND METHODS

Lettuce sampling

To determine nitrate content and related inorganic nitrogen species (nitrite and ammonium), greenhouse lettuce was sampled in May 2025 from two sources: supermarkets and local producers. Three lettuce samples were collected from supermarkets (SP), and four from local producers (LP). The samples were divided

into the plant's components and the growing medium (leaves, roots, and soil). This resulted in a total of 21 representative samples.

Extraction assessment of nitrate and related inorganic nitrogen species

After sampling, the lettuce samples were frozen and then lyophilized. Following lyophilization, the lettuce samples were ground to a fine powder. Nitrate and related inorganic nitrogen species were extracted using microwave-assisted extraction (MAE), with ultrapure water as the solvent (Novaes et al., 2009). This method is effective for releasing nitrate and related inorganic nitrogen species from leaves, roots, and soil samples (Hao et al., 2015; Zandvakili et al., 2024). From each sample type, 1.5 g was weighed, and 60 mL of ultrapure water was added (plant-to-solvent ratio of 1/40). The extraction parameters were set as follows: extraction power of 500 W for 15 minutes at 100°C. After extraction, the samples were allowed to cool to room temperature for about 10 minutes. The mixture was centrifuged at 10000 rpm for 20 minutes, then filtered through a 0.45 µm cellulose acetate (CA) syringe filter to obtain a clear extract, as shown in Figure 1. The extracts were carefully stored at 4°C, ready for analysis. This step helps ensure everything stays fresh and accurate for the results. The extraction parameters and results are presented in Table 1.

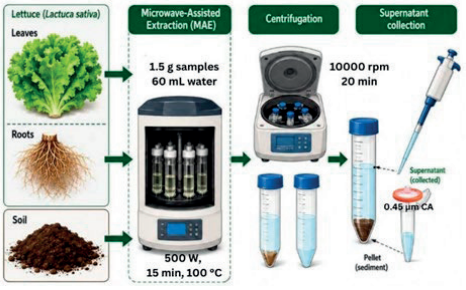


Figure 1. Procedure for extracting nitrate and related inorganic nitrogen species from lettuce samples

Assessment of nitrate content in lettuce samples

The nitrate content assessment of the extracts was performed using a modified ISO 7890-1:1986 spectrophotometric protocol based on 2,6-dimethylphenol (DMP) reagent (ISO, 1986). To 100 µL of each extract, 50 µL of DMP reagent and 100 µL of a mixture of sulfuric and phosphoric acids (9/1) were added. The resulting mixture was incubated at room temperature in the dark for 30 minutes. After incubation, the absorbance of the samples was measured at 345 nm. As a control, the extract was replaced with ultrapure water under the same conditions. The calibration curve was prepared using sodium nitrate ($NaNO_3$) at concentrations ranging from 0 to 20 mg/L. The results were expressed as milligrams of NO_3^- per kilogram of sample (mg NO_3^-/kg).

Assessment of nitrite content in lettuce samples

The nitrite content assessment of the extracts was performed in the presence of the Griess reagent according to a modified spectrophotometric protocol (ISO 6777:1984; Moorcroft et al., 2001). 50 µL of Griess reagent was added to 150 µL of each extract. Griess reagent was a 1:1 mixture of sulfanilic acid and α-naphthylamine acetic solutions. The resulting mixture was incubated at room temperature, in the dark, for 30 minutes. After the incubation period, the absorbance of the samples was read at 520 nm. As a control, the extract was replaced with ultrapure water under the same conditions. The calibration curve was performed using sodium nitrite ($NaNO_2$) in concentrations ranging from 0 to 10 mg/L. The results were expressed as milligrams of NO_2^- per kilogram of sample (mg NO_2^-/kg).

Assessment of ammonium content in lettuce samples

The ammonium content assessment of the extracts was determined using a modified ISO 7150-1:1984 spectrophotometric protocol in the presence of two reagents (ISO, 1984). Reagent A was a mixture of trisodium citrate, sodium salicylate, and sodium nitroprusside, and reagent B was a mixture of sodium hydroxide and sodium dichloroisocyanurate. 25 µL of reagent A and 25 µL of reagent B were added to 200 µL of each extract. The resulting mixture was incubated at room temperature, in the dark, for 60 minutes. After the incubation period, the absorbance of the samples was read at 650 nm. As a control, the extract was replaced with ultrapure water under the same conditions. The calibration curve was performed using ammonium chloride (NH_4Cl) in concentrations

ranging from 0 to 25 mg/L. The results were expressed as milligrams of NH_4^+ per kilogram of sample (mg NH_4^+/kg).

Table 1. Extraction parameters of nitrate and related inorganic nitrogen species

Sampling site	Sample cod	Sample type	Sample/ volume ratio	Sample mass (g)	Initial volume (mL)	Final volume (mL)
Super-market 1	SP1L	leaves	1/40	1.50	60	53
	SP1R	roots	1/160	0.06	10	7.5
	SP1S	soil	1/40	1.5	60	55
Super-market 2	SP2L	leaves	1/40	1.50	60	53
	SP2R	roots	1/40	1.50	60	57
	SP2S	soil	1/40	1.50	60	59
Super-market 3	SP3L	leaves	1/40	1.50	60	52
	SP3R	roots	1/40	1.50	60	58
	SP3S	soil	1/40	1.50	60	57
Local producer 1	LP1L	leaves	1/40	1.50	60	56
	LP1R	roots	1/40	1.50	60	56
	LP1S	soil	1/40	1.50	60	57
Local producer 2	LP2L	leaves	1/40	1.13	45	44
	LP2R	roots	1/80	0.31	25	23
	LP2S	soil	1/40	1.50	60	59
Local producer 3	LP3L	leaves	1/40	1.50	60	58
	LP3R	roots	1/40	1.30	51	47
	LP3S	soil	1/40	1.50	60	50
Local producer 4	LP4L	leaves	1/40	1.50	60	51
	LP4R	roots	1/40	0.80	33	31
	LP4S	soil	1/40	1.50	60	59

Assessment of dietary exposure and chronic health risk

The Estimated Daily Intake (EDI, mg NO_3^- /kg BW/day) and Hazard Quotient (HQ) were calculated according to European Food Safety Authority (EFSA) risk assessment guidelines in order to evaluate the consumer safety implications of nitrate accumulation in edible lettuce leaves (EFSA, 2008):

$$EDI = (C_{leaf} \times FIR) / BW$$
$$HQ = EDI / ADI$$

Where C_{leaf} is the measured nitrate concentration in edible lettuce leaves (mg NO_3^- /kg fresh weight), FIR is the daily consumption rate of leafy vegetables (0.06 kg/day for adults), and BW is the standard adult body weight (70 kg, EFSA, 2012). The Acceptable Daily Intake (ADI) established by EFSA for nitrate is 3.7 mg NO_3^- /kg BW/day. An HQ < 1.0 indicates that chronic exposure remains within safe limits, whereas an HQ > 1 indicates potential chronic health risk.

Statistical analysis

Statistical analysis of the results was performed using GraphPad (version 11.0.2). The correlation between nitrate, nitrite, and ammonium contents was determined using the Pearson correlation coefficient.

RESULTS AND DISCUSSIONS

Nitrate content in lettuce samples

Limited research has investigated the influence of source of origin on NO_3^- content in lettuce. In this study, a quantitative analysis of greenhouse lettuce samples from different sources, including supermarkets and local producers, was conducted. The results showed significant differences based on source, plant parts, and growing medium (soil).

NO_3^- distribution was uneven among leaves, roots, and soil, with higher concentrations accumulating mainly in the leaves. Comparing supermarket and local producer samples, notable differences emerged: supermarket lettuce had NO_3^- content ranging from 73.99 ± 2.87 mg NO_3^-/kg (soil) to 5340.81 ± 80.95 mg NO_3^-/kg (leaves), while local producer samples ranged from 71.02 ± 1.45 mg NO_3^-/kg (soil) to 3212.92 ± 50.89 mg NO_3^-/kg (leaves) (Figure 2). Notably, leaves, roots, and soil samples differed, with leaves showing the highest absorption because they are the primary tissues that store NO_3^-.

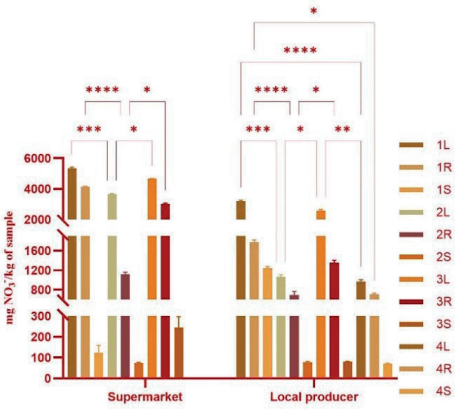


Figure 2. Nitrate content in different lettuce samples collected from supermarkets and local producers. Significant differences were noted at the following levels: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, and **** = $p < 0.0001$

Regarding European standards for leafy vegetables, the higher NO_3^- content in

supermarket samples raises food safety concerns. EU No 915/2023 sets a maximum limit of 4000 mg/kg for NO_3^- in greenhouse-grown lettuce from April 1 to September 30. Conversely, local producer samples are significantly lower; thus, exposure to high concentrations of NO_3^- from food is a major risk factor for human health. In the analyzed samples, the high values in lettuce leaves suggest increased accumulation of these compounds in the plant's edible parts. Because lettuce is often consumed raw in large quantities, even a moderate exceedance of the limit can lead to significant exposure, especially when combined with other dietary risk factors.

Therefore, the results underscore the importance of rigorous monitoring of agricultural inputs and compliance with the maximum permissible limit for NO_3^- in fresh vegetables.

Nitrite content in lettuce samples

The evaluation of NO_2^- content in lettuce samples revealed significant variation between supermarket and local producer samples. The supermarket samples showed much higher concentrations, especially for SP1R (roots), where content exceeded 2500 mg NO_2^-/kg, followed by SP3R (roots) at approximately 2000 mg NO_2^-/kg. In contrast, local producers' samples recorded considerably lower values, ranging from approximately 10 to 55 mg NO_2^- /kg, with the highest in the LP4R (root) sample (Figure 3).

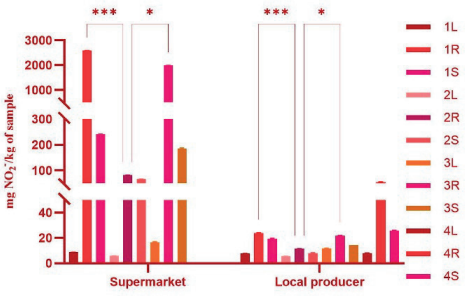


Figure 3. Nitrite content in different lettuce samples collected from supermarkets and local producers. Significant differences were noted at the following levels: * = $p < 0.05$, and *** = $p < 0.001$

The data show an opposite trend compared to NO_3^-. NO_2^- is significantly accumulated in the roots, whereas NO_3^- is accumulated mainly in the leaves. This may indicate a partial enzymatic conversion of NO_3^- to NO_2^-. At the same time, soil samples (SP1S, SP4S) indicate a notable presence of NO_2^-, which may suggest the presence of specific microorganisms that favor the conversion of NO_3^- to NO_2^-.

This uneven distribution suggests a significant difference between the two types of sources in post-harvest stages. Thus, lettuce collected from supermarkets is susceptible to the biochemical conversion of NO_3^- to NO_2^-, a process favored by long-term storage, temperature fluctuations, and local anaerobic conditions in closed packaging. This process is significantly less pronounced in lettuce grown and sampled directly from local producers, which explains the lower NO_2^- values.

The presence of NO_2^- at high concentrations in lettuce samples, especially those from trade, represents a significant health risk. NO_2^- are directly involved in the formation of nitrosamines, compounds with carcinogenic potential that can also induce methemoglobinemia, a serious condition in infants and vulnerable people (Popa et al., 2021).

Unlike NO_3^-, for which there are clear legislative thresholds in leafy vegetables, there are no maximum permitted limits for NO_2^- in lettuce, but any high concentration is concerning. The concentration of 2500 mg NO_2^-/kg detected in SP1R (root) is markedly higher than NO_2^- content typically reported in the literature for fresh lettuce, generally below 10-50 mg/kg in leaves and below 100 mg/kg in roots (Chung et al., 2003). This anomaly may indicate either intensive post-harvest NO_3^- reduction or methodological interference at 520 nm from root pigments. This warrants further investigation, including validation against an independent analytical method.

The localization of NO_2^- in the roots of the lettuce samples shows that NO_2^- accumulation is blocked in the lower parts of the plant, possibly due to reduced foliar absorption. This pattern indicates that the agronomic strategies applied in the greenhouse (ventilation, N dose limitation, short time between harvest and analysis) help maintain low NO_2^- content in the edible part of the plant. Regarding NO_2^- accumulation, the data highlight a clear contrast between lettuce samples from supermarkets and those from local

producers. The problem is more pronounced in supermarket samples, with worryingly high values.

Ammonium content in lettuce samples

In the supermarket lettuce samples, NH_4^+ values varied significantly depending on the plant parts analyzed, as illustrated in Figure 4. The maximum concentration was recorded in the SP1R (root) sample, with a value of 30.93 ± 1.08 mg NH_4^+/kg, followed by SP2R with approximately 25.56 ± 0.50 mg NH_4^+/kg and SP3R with approximately 20.77 ± 0.77 mg NH_4^+/kg. In contrast, the soil samples (SP1S - SP3S) showed lower concentrations, ranging from 3.5 to 5.5 mg NH_4^+/kg. These results suggest that NH_4^+ ions tend to be present in higher amounts in the roots and absent in the leaves.

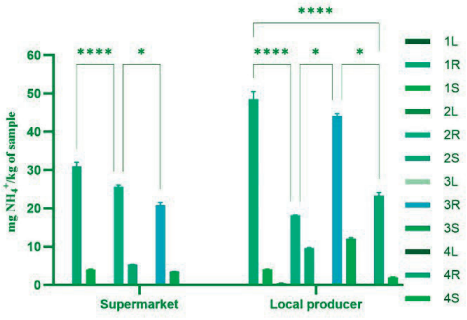


Figure 4. Ammonium content in different lettuce samples collected from supermarkets and local producers. Significant differences were noted at the following levels: * = $p < 0.05$, and **** = $p < 0.0001$

Samples from local producers showed a clear trend toward higher NH_4^+ accumulation in both the roots and the soil. The maximum values were recorded in samples LP1R (48.55 ± 2.00 mg NH_4^+/kg) and LP3R (44.11 ± 0.66 mg NH_4^+/kg). These concentrations are nearly double those observed in the supermarket lettuce samples. Soil samples LP2S and LP3S also recorded significant concentrations (from 9.5 to 12 mg NH_4^+/kg), while samples LP1S and LP4S showed values from 2 to 4 mg NH_4^+/kg. This pattern suggests progressive NH_4^+ accumulation, possibly favored by localized application of N fertilizers, high temperatures, increased humidity, and poor aeration. Additionally, the low NO_3^- content in some of these samples may indicate incomplete conversion of NH_4^+ to NO_3^- at physiological levels.

The presence of NH_4^+ can have significant implications for the plant's internal ionic balance and the nutritional quality of the product. Although the NH_4^+ ion is a natural and metabolically active form of N, its accumulation in tissues indicates physiological stress and can lead to the formation of secondary compounds (such as biogenic amines) that contribute to bitter taste or accelerate post-harvest spoilage. From a consumer health perspective, high concentrations of NH_4^+ are not strictly regulated, but when present alongside NO_3^- and NO_2^-, they can contribute to metabolic imbalances, digestive discomfort, or cumulative effects with frequent consumption. Thus, concentrations of approximately 50 mg/kg should be viewed with caution, especially in the context of unprocessed consumption (raw plant), which is characteristic of this type of vegetable.

Analysis of NH_4^+ concentrations by source and plant part revealed uneven distribution and a high potential for accumulation under protected cultivation conditions (e.g., greenhouses). In all cases, the root was the dominant accumulation compartment, reflecting active translocation of reduced N to metabolically active areas of the plant. Differences between samples from local producers and those from the supermarket suggest that, in commercial crops, there may be stricter control of the dose and form of applied NH_4^+, whereas samples from local producers point to a potential N overapplication, likely combined with environmental conditions that suppress nitrification.

Dietary exposure and chronic health risk

The EDI of NO_3^- varied considerably among the lettuce samples, ranging from 0.831 to 4.578 mg/kg BW/day, as seen in Table 2. The highest EDI values were obtained for the supermarket samples SP1L (4.578 mg/kg BW/day) and SP3L (3.997 mg/kg BW/day), both exceeding the ADI of 3.7 mg/kg BW/day. Accordingly, their HQ values were above unity (HQ = 1.237 and 1.080, respectively), indicating that the estimated exposure exceeded the selected health-based guidance value. In contrast, SP2L showed an EDI of 3.145 mg/kg BW/day and an HQ of 0.850, while all samples obtained from local

producers had substantially lower EDI (0.831–2.754 mg/kg BW/day) and HQ values (0.225–0.744). Thus, only SP1L and SP3L were identified as samples of potential concern based on the adopted EFSA ADI.

These findings are consistent with previous dietary exposure assessments showing that lettuce can represent an important source of nitrate intake. Luetic et al. (2023) reported that, assuming consumption of a 100 g portion, one lettuce sample contained sufficient NO_3^- to exceed the ADI, demonstrating that individual high-NO_3^- lettuce samples may substantially increase dietary exposure. Similarly, Brkić et al. (2017) estimated that consuming 150 g of lettuce would account for 47.5% of the ADI in spring and as much as 85.4% in autumn, emphasizing that NO_3^- exposure from lettuce strongly depends on its NO_3^- concentration. More recently, Dodocioiu et al. (2025) reported an EDI of 3.65 mg/kg BW/day for adults, whereas the estimated EDI for children reached 8.11 mg/kg BW/day. Lettuce with elevated NO_3^- concentrations may represent a substantial contribution to daily NO_3^- exposure and, under specific exposure scenarios, may exceed health-based guidance values (Brkić et al., 2017; Luetic et al., 2023; Dodocioiu et al., 2025).

Table 2. Estimated Daily Intake and Hazard Quotient of nitrate in supermarket and local producer lettuce samples

Sample code	NO_3^- (mg/kg)	EDI (mg/kg BW/day)	HQ	Risk
SP1L	5340.81	4.578	1.237	Risk exceeded
SP2L	3669.32	3.145	0.850	Acceptable
SP3L	4663.58	3.997	1.080	Risk exceeded
LP1L	3212.92	2.754	0.744	Acceptable
LP2L	1069.20	0.916	0.248	Acceptable
LP3L	2593.25	2.223	0.601	Acceptable
LP4L	969.98	0.831	0.225	Acceptable

Pearson Correlation and Heatmap Matrix

To evaluate potential relationships among the N forms accumulated in lettuce, Pearson correlations were computed for the NO_3^-, NO_2^-, and NH_4^+ contents across all samples examined (Figure 5).

Weak positive correlations were observed between NO_3^- and NO_2^- (r=0.35) and between NO_2^- and NH_4^+ (r=0.31), while a very weak negative correlation was found between NO_3^- and NH_4^+ (r=-0.06). These low values indicate weak associations among the reactive N forms, suggesting that NH_4^+, NO_2^-, and NO_3^- behave largely independently in lettuce rather than through direct biochemical coupling. This finding indicates that the accumulation of each N form is driven by multiple physiological and environmental factors, such as sample origin and post-harvest conditions, highlighting the need for integrated monitoring of all N forms to accurately assess product quality.

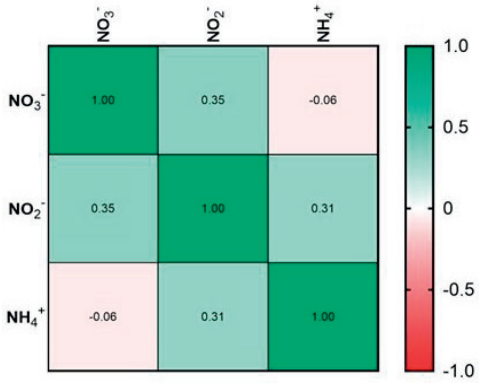


Figure 5. Pearson correlation between nitrate, nitrite, and ammonium contents in lettuce samples

In soil, N circulates through the biogeochemical N cycle, which includes essential processes such as biological N fixation, nitrification, and denitrification, each regulated by specialized microbial communities. These processes are mediated by functional genes that encode enzymes involved in the transformations of N compounds. In biological fixation, molecular nitrogen (N_2) is reduced to ammonia (NH_3) or other N-containing compounds by enzymes produced by N-fixing bacteria, enabling plants to absorb it. Non-symbiotic N fixation, carried out by free-living diazotrophs, is a major source of N for terrestrial ecosystems (Hao et al., 2022). Nitrification occurs under aerobic conditions and is mediated by nitrifying bacteria, which oxidize reduced inorganic forms of N, such as NH_4^+, to NO_2^- and then to NO_3^-. NH_4^+ and NO_3^- are taken up by plant cells through transport across the plasma membrane (Figure 6).

When NO_3^- absorption greatly exceeds plant assimilation, NO_3^- can accumulate in plant

tissues. The type of fertilizer used plays a key role in the NO_3^- accumulation in the edible parts of vegetables. N can be supplied not only as NO_3^- but also as NH_3, urea ($CO(NH_2)_2$), or other organic compounds. Choosing the appropriate fertilizer, depending on the plant part intended for consumption, can help reduce the concentration of NO_3^- in the final product (Popa et al., 2021).

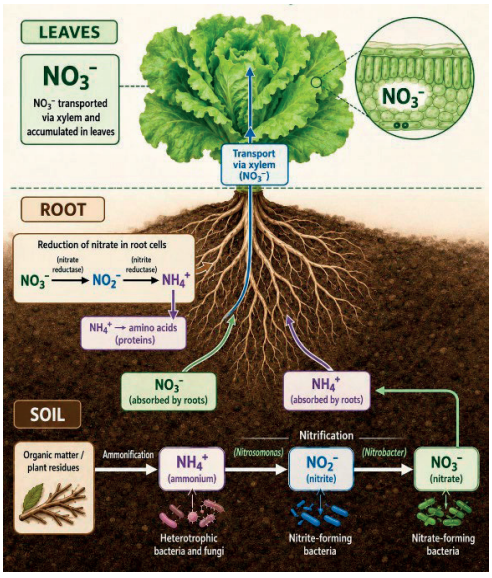


Figure 6. Formation and conversion of N forms in lettuce (Source: Authors-generated using AI)

The NO_3^- accumulation is influenced by multiple factors, including soil composition, cropping system, fertilizer application regime, and climatic and seasonal conditions during the growing season (Martín Leon & Luzardo, 2020). In non-legume crops, higher NO_3^- concentrations tend to accumulate in the leaves, whereas lower concentrations accumulate in the roots. For this reason, leafy vegetables, e.g., arugula, spinach, lettuce, celery, and parsley, are considered prominent species that accumulate NO_3^- (Colonna et al., 2016).

The effect of N forms (NH_4^+, NO_2^-, and NO_3^-) on plant growth depends not only on the cultivated species but also on their concentrations and relationships. Usually, the form in which N is applied influences the anion-to-cation absorption ratio, thereby affecting dry matter production and root nutrient uptake. The quantity and quality of applied fertilizers are determining factors in the growth, production, and quality of leafy vegetables (Cencič et al., 2013).

It is emphasized that N accumulation varies significantly not only between species but also among varieties within the same species, with these differences influenced by environmental factors. Additionally, the N content depends on the specific plant part consumed (Jakabová et al., 2022).

The current results clearly show that the source of the lettuce sample significantly affects NO_3^- buildup. On average, the leaf NO_3^- contents were about 2.3 times higher in the supermarket samples compared to the local producer ones. This suggests that the sample origin, along with related cultivation methods and N management practices, plays a key role in NO_3^- accumulation. This source-dependent variation aligns with previous research indicating that NO_3^- contents in lettuce are strongly influenced by factors such as N availability, fertilization approach, growing system, environmental conditions, and plant physiology. For example, Aires et al. (2013) found higher NO_3^- concentrations in conventionally grown baby-leaf vegetables than in organic ones, highlighting how production systems impact NO_3^- contents. Similarly, Pinto et al. (2014) linked soil inorganic N contents to differences in NO_3^- and NH_4^+ contents in lettuce. More recently, Martínez-Moreno et al. (2024) demonstrated that nutrient solution management effectively controls NO_3^- accumulation, while Nguyen et al. (2026) revealed that N assimilation and the resulting NO_3^-, NO_2^-, and NH_4^+ concentrations are also strongly affected by light intensity and plant genotype.

Interestingly, the NO_3^- contents in soil at harvest did not directly match the pattern seen in lettuce leaves. Although the supermarket samples had the highest leaf NO_3^- contents, the soil NO_3^- concentrations were relatively low, except for LP1 soil, which contained 1243.48 mg NO_3^-/kg. This discrepancy indicates that lettuce NO_3^- accumulation reflects the plant's entire history of N availability, uptake, transport, and assimilation. Pinto et al. (2014) also showed that soil physicochemical properties affect inorganic N mobility and that differences in NO_3^- leaching and plant availability can significantly influence N transfer from soil to lettuce. Moreover, Zhang

et al. (2025) found that just five days of nutrient deprivation before harvesting reduced NO_3^- contents by 57.49% in leaves and 50.77% in roots, highlighting how short-term pre-harvest N availability strongly impacts tissue nitrate concentrations.

In this study, NO_3^- distribution was tissue-dependent, with higher concentrations in leaves than roots, and much lower in soil. This pattern reflects NO_3^- uptake by roots and transport to shoots, where it may be stored or reduced through assimilation. NO_3^- metabolism occurs in both roots and shoots, with their relative roles influenced by N supply and environmental factors. Schiefloe et al. (2023) demonstrated substantial differences in NO_3^- partitioning between lettuce shoots and roots, depending on the NH_4^+:NO_3^- ratio supplied to plants; increasing NH_4^+ availability reduced NO_3^- concentrations in both compartments by approximately 7–8 times. Likewise, Zhang et al. (2025) observed coordinated yet tissue-specific changes in NO_3^- concentration and NO_3^- transporter expression in lettuce leaves and roots during nutrient deprivation. These findings support the marked root–leaf compartmentalization observed in the present study.

The distribution of NH_4^+ differed markedly from that of NO_3^-. NH_4^+ was absent or present only at trace levels in leaves, whereas appreciable concentrations occurred in roots. Soil NH_4^+ concentrations were intermediate but substantially lower than those measured in roots. This preferential accumulation in roots aligns with their primary role in NH_4^+ uptake and assimilation. Schiefloe et al. (2023) reported that lettuce grown under a high-NH_4^+ nutrient regime accumulated almost three times more NH_4^+ in roots than plants receiving a low-NH_4^+ treatment, whereas changes in shoot NH_4^+ were comparatively limited. Pinto et al. (2014) also observed a close relationship between NH_4^+ concentration and glutamine synthetase activity in lettuce, supporting the rapid incorporation of NH_4^+ into organic N compounds. Consequently, the very low NH_4^+ concentrations detected in the leaves of the present samples likely indicate efficient assimilation and limited translocation or storage of free NH_4^+ in edible tissues.

NO_2^- showed a different distribution pattern. Leaf concentrations were low, while some root and soil samples, especially in the supermarket samples, showed higher values. This pattern suggests root-zone N transformations and tissue-specific NO_2^- reduction may contribute to NO_2^- formation. NO_2^- is an intermediate of NO_3^- assimilation, usually rapidly reduced during N metabolism, so its accumulation is lower than NO_3^-. Nguyen et al. (2026) found that the balance among NO_3^-, NO_2^-, NH_4^+, nitrate reductase, and nitrite reductase in lettuce depends on enzyme activity and environmental conditions.

Leaf NO_2^- concentrations also show the large variability reported for commercial lettuce. Aires et al. (2013) reported NO_2^- concentrations ranging from 0.25 to 1.89 mg NO_2^-/kg FW in green and red lettuce from conventional and organic production systems. In contrast, Luetic et al. (2023) reported NO_2^- concentrations ranging from 21.1 to 378.1 mg NO_2^-/kg FW. The authors noted that cultivation, post-harvest handling, and storage affect nitrite levels. While the leaf NO_2^- concentrations in this study were higher than some reports for freshly harvested baby-leaf lettuce, they stayed within the variability seen in commercial leafy vegetables. From a food-safety perspective, NO_3^- accumulation in the edible leaves is the most relevant finding. Luetic et al. (2023) reported NO_3^- concentrations of 169.7–3184.2 mg NO_3^-/kg FW in commercial lettuce. In comparison, LP2 and LP4 in the present study were close to this literature range, whereas the supermarket samples showed considerably higher NO_3^- concentrations. Qureshi et al. (2026) reported approximately 2900 mg NO_3^-/kg FW and 2.29 mg NO_3^-/kg FW in lettuce and considered NO_3^- control an important criterion of nutritional quality.

CONCLUSIONS

The present work aimed to compare the N forms (NO_3^-, NO_2^-, and NH_4^+) in lettuce grown in greenhouses and sold in supermarkets and by local producers. Based on experimental data, clear source- and tissue-dependent differences in the distribution of NO_3^-, NO_2^-, and NH_4^+ in greenhouse lettuce were observed between these two sources in the concentrations and internal distributions of these compounds across the leaves, roots, and soil. These variations suggest different N uptake and assimilation patterns

across the production sources rather than isolated parts of the N cycle.

Supermarket samples generally showed higher NO_3^- concentrations in the edible leaves and higher NO_2^- levels in the roots, whereas NH_4^+ was predominantly associated with the root compartment. Several supermarket leaf samples exceeded the EU maximum NO_3^- content of 4000 mg NO_3^-/kg for greenhouse lettuce harvested between April and September, highlighting a potential food-safety concern.

From an agronomic standpoint, the findings emphasize the importance of N management just before harvest. For food safety, the high NO_3^- contents observed in some edible leaves highlight a need for careful monitoring.

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