Wadi Alshatti University Journal of Pure and Applied Sciences

مجلة جامعة وادي الشاطئ للعلوم البحتة والتطبيقية

Volume 3, No. 2, July-December 2025 Online ISSN: 3006-0877 المجلد 3، الاصدار 2، يوليو - ديسمبر 2025

RESEARCH ARTICLE ENVIRONMENTAL SCIENCES

Assessment of Nitrate Concentration in Some Leafy Vegetables in Brack Alshatti Local Market

Samar Amhemmad Alakhdhari[1], Fatima Mohamed Alzouam[2], Mansour Awiadat Salem[1*]

[1]Environmental Sciences Department, Faculty of Environment and Natural Resources, Wadi Alshatti University, Brack, Libya
[2]Botany Department, Faculty of Sciences, Sebha University, Sebha, Libya

ARTICLE HISTORY

Received 10 April 2025
Revised 05 May 2025
Accepted 10 May 2025
Online 15 May 2025

KEYWORDS

Nitrate;
Leafy vegetables;
Brack;
Spectrophotometer;
Correlation coefficient;
Cluster analysis.

ABSTRACT

This study aimed to determine the concentration of nitrate content in eleven different leafy vegetables collected from the Brack local market in Brack during the year 2023. These leafy vegetables include chard, dill, parsley, lettuce, Spanish, coriander, salad rocket, chives, white cabbage, red cabbage, and Lactuca sativa. Nitrate was determined using the spectrophotometer method; the nitrate concentration was 235.97–249.54, 235.84–238.59, 235.57–249.26, 237.28–249.22, 237.58–245.81, 236.91–241.07, 236.40–247.26, 237.71–24.23, 237.58–238.10, 237.06–249.39, and 236.94–249.23 mg/kg dwt. Their nitrate concentration (mean ± SD) mg/kg dwt. was 245.15±4.06, 242.19±3.81, 240.94±4.73, 240.56±3.72, 240.8±3.13, 239.36±1.43, 237.75±0.85, 243.4±3.86, 237.92±0.18, and 244.2±5.86, respectively. The highest concentration among leafy vegetables was determined in chard (245.15±4.06 mg/kg/dwt), while the lowest nitrate concentration was determined in dill (237.75±0.85 mg/kg/dwt). The results indicate that the nitrate concentration in the leafy vegetables did not vary significantly. The nitrate concentration in leafy vegetable samples was lower than the permissible limits recommended by the FAO/WHO Expert Committee on Food Additives. Multivariate statistics indicated that most vegetable samples exhibited no correlation. Only three principal components were identified to interpret the results, accounting for 100% of the total variation among the eleven leafy vegetables. The cluster analysis produced a dendrogram that grouped the sampled vegetables according to their nitrate concentration dissimilarity. This dendrogram revealed variations among the eleven vegetables studied.

تقدير تركيز النترات في بعض الخضروات الورقية في السوق براك الشاطئ المحلي

سمر أمحمد الاخضري[1]، فاطمة محمد الزوام[2]، منصور عويدات سالم[1*]

الكلمات المفتاحية

النترات
الخضراوات الورقية
براك
مطياف ضوئي
معامل الارتباط
تحليل المجموعات

الملخص

هدفت هذه الدراسة إلى تحديد تركيز النترات في إحدى عشرة خضراوات ورقية مختلفة جُمعت من سوق براك المحلي خلال عام 2023. تشمل هذه الخضراوات الورقية السلق، والشبت، والبقدونس، والخس، والكزبرة الإسبانية، وجرجير السلطة، والثوم المعمر، والكرنب الأبيض، والكرنب الأحمر، ونبات الخس. تم تقدير النترات باستخدام طريقة مطياف الضوء؛ وتراوح تركيز النترات بين 235.97-249.54 ، 235.84-238.59 ، 235.57-249.26 ، 237.28-249.22 ، 237.58-245.81 ، 236.91-241.07 ، 236.40-247.26 ، 237.71-24.23 ، 237.58-238.10 ، 237.06-249.39 ، 236.94-249.23 ملجم/كجم وزن جاف، على التوالي. بينما كان متوسط تركيز النترات ± الانحراف المعياري فيها هذه الخضروات، 245.15±4.06 ، 242.19±3.81 ، 240.94±4.73، 240.56±3.72 ، 240.8±3.13، 239.36±1.43، 237.75±0.85 ، 243.4±3.86 ، 237.92±0.18 ، 244.2±5.86. ملجم/كجم وزن جاف تركيز النترات على التوالي. سُجِّل أعلى تركيز للنترات في الخضراوات الورقية في السلق (245.15±4.06 ملجم/كجم/وزن جاف)، بينما سُجِّل أقل تركيز للنترات في الشبت (237.75±0.85 ملجم/كجم/وزن جاف). تشير النتائج إلى أن تركيز النترات في الخضراوات الورقية لم يتغير بشكل ملحوظ. وكان تركيز النترات في عينات الخضراوات الورقية أقل من الحدود المسموح بها التي أوصت بها لجنة خبراء منظمة الأغذية والزراعة (FAO) ومنظمة الصحة العالمية (WHO) المعنية بالمضافات الغذائية. وأشارت الإحصاءات متعددة المتغيرات إلى عدم وجود أي ارتباط بين معظم عينات الخضراوات. حُددت ثلاثة مكونات رئيسية فقط لتفسير النتائج، تمثل 100% من إجمالي التباين بين الخضراوات الورقية الأحد عشر. وأنتج تحليل المجموعات رسمًا تخطيطيًا شجريًا صنف الخضراوات المأخوذة من العينات وفقًا لاختلاف تركيز النترات فيها. وكشف هذا الرسم عن اختلافات بين الخضراوات الأحد عشر المدروسة.

Introduction

Leafy vegetables are essential to the human diet, providing the necessary vitamins and fibre. However, using chemical fertilizers in large quantities and poor agricultural practices led to high levels of nitrates (NO_3) in these vegetables, especially leafy varieties. [1, 2]. Nitrate accumulates in

*Corresponding author

https://doi.org/10.63318/waujpasv3i2_05

vegetables under certain conditions when it exceeds the plant's ability to absorb, reduce, and assimilate nitrate ions. This accumulation is affected by many factors, including the composition of the soil in which the plants grow, the type of crop production methods and fertilization techniques used, seasonal changes, and environmental conditions that affect crop growth [3, 4]. Nitrate content can vary significantly between different plant species and varieties. Leafy vegetables, in particular, tend to accumulate nitrates due to their rapid growth, and nitrates are mainly concentrated in the leaves, which are the edible parts of these vegetables. Thus, consumers may be exposed to different levels of nitrates depending on the types of vegetables they consume [5, 6]. Growing population and food demand have led to increased use of fertilizers, which may lead to higher plant nitrate levels. In general, nitrates are not harmful to humans except sensitive individuals. Once ingested, nitrates turn into nitrites in the stomach, posing health risks, especially when interacting with specific proteins [7, 8] Nitrate contamination caused by chemical fertilizers and pesticide residues in fruits and vegetables has become a significant concern globally, with studies indicating that approximately 80% of dietary nitrate intake comes from vegetables, raising health concerns about nitrate accumulation in food [9 - 11]. Studies have indicated a relationship between consuming large amounts of nitrates and nitrites and the incidence of stomach cancer, and therefore, measuring the level of the unobserved harmful effect of chronic consumption of vegetables is necessary to ensure safety standards for food chemicals [12, 13]. Vegetables have been classified according to their nitrate content into three categories: low, containing 0.5-50 mg/kg, including berries, fruits, grains, and corn vegetables, medium, containing 50-1,000 mg/kg, including potatoes and some vegetables, and vegetables containing high nitrates > 1,000 mg/kg, including lettuce, beets, spinach, and other green leafy vegetables and herbs [14]. The Food and Agriculture Organization (FAO) and the World Health Organization on Food Additives (JECFA) have set an acceptable daily intake of 3.7 mg/kg body weight/day, taking into account sources of nitrate other than food additives, such as drinking water, when assessing nitrate intake [15, 16]. Nitrates found in vegetables are not inherently harmful compared to the risk-to-benefit ratio. Studies indicate that nitrates act as effective nitric oxide (NO) donors, providing many benefits to the heart and blood vessels. These include lowering blood pressure, reducing platelet aggregation, and protecting against damage caused by ischemia. In addition, dietary nitrates improve insulin sensitivity, glucose tolerance, and lipid profiles by reducing triglyceride levels. It may also help increase muscle strength [17 - 19]. Despite the benefits of nitrates, excessive exposure can have harmful effects. Nitrates can react with amines and/or amides to form N-nitroso compounds, which are known carcinogens. For this reason, the International Agency for Research on Cancer (IARC) classifies nitrates and nitrites as likely carcinogenic to humans under certain conditions [20]. Many studies have linked excessive exposure to nitrates to an increased incidence of various types of cancer. Including cancer of the stomach, colon, rectum, oesophagus, thyroid, kidney, and breast [21], exposure to high levels of nitrate is associated with various health problems, including cancer, hypothyroidism and methemoglobinemia, especially in children [22]. Vegetables vary widely in nitrate concentrations, ranging from less than 1.0 mg to more than 100 mg and might exceed 1000 mg per 100 grams. It has been mentioned that celery samples contain 10,800 mg kg-1 [23, 24]. In addition, the concentration of nitrates in organically grown vegetables is generally higher than that of conventionally grown vegetables [25]. A study conducted by [12] in Greece found that the most consumed vegetables contain low levels of nitrates and nitrites, with exceptions such as spinach, celery, and lettuce. Artichokes, Cabbage, cauliflower, celery, turnips, and leeks usually contain a high percentage of nitrates, while asparagus, chicory, and onions contain a lower percentage. A study conducted in the United Kingdom [26] found that lettuce, spinach, celery, and beets contain relatively high concentrations of nitrates, exceeding 1000 mg/kg. Meanwhile, potatoes, cabbage and spring vegetables contain lower nitrate levels, ranging from 100 to 1000 mg/kg. Tomatoes have the lowest nitrate concentrations, measuring less than 100 mg/kg. In a study conducted by [27, 28] in Egypt to estimate the levels of nitrates and nitrites in sixteen types of commonly consumed vegetables, including leafy vegetables, legumes, and root vegetables, it was found that leafy vegetables contained the highest levels of nitrates, with spinach containing 5830 mg/kg. In contrast, celery, lettuce, and Cabbage contained the lowest concentration of nitrates. While the concentration of nitrates in legumes ranged from 30 - 600 mg/kg in the rootstock, the classic red radish contained the highest nitrate content. Many varieties of leafy vegetables are sold in Brack. Although nitrates are considered compounds that threaten human health, there is a lack of available information about nitrate concentration levels in vegetables. Therefore, this study aimed to (1) determine the nitrate concentration in various fresh vegetables frequently consumed in Brack City and (2) determine the health risks of nitrate content of vegetable consumption.

Materials and methods

Collection of vegetable samples

Eleven leafy vegetables, including dill, coriander, parsley, lettuce, onion leaves, spinach, Eruca sativa, white cabbage, red cabbage, Lactuca sativa, and lettuce, were collected in polyethene bags from the Brack local market in 2024. Their scientific names were *Petroselinum crispum, Lactuca sativa, Spinacia oleracea, Coriandrum sativum, Allium cepa, Romaine Lettuce, Brassica oleracea convar. Capitata var. Alba, Brassica oleracea var. capitata f. rubra, Beta vulgaris sub sp, Anethum graveolens, Eruca Sativa*.

Assessment of nitrate content in vegetable samples

Samples of leafy vegetables were thoroughly cleaned with tap water before being rinsed with distilled water to eliminate impurities from the surface, including dust, dirt, and spray residue. They had been sliced into slices of uniform size that were dried for 24 hours at 70°C. The samples were ground after dilution manually and sieved with a 2 mm sieve [28]. 0.1 g of each sample was taken separately in a 50 ml test tube, and 10 ml of distilled water was added to it and placed in a water bath at 80°C for 30 minutes. The contents were cooled to room temperature, the solution was filtered through the Whatman 41 filter paper, and nitrate was then measured.

Preparation of the standard curve

The standard curve was prepared as described by [29]. A standard stock solution of KNO_3 was created with a concentration of 500 mg/L by dissolving 0.7221 g of KNO_3 in 200 ml of deionised water, and the solution was stored at 4°C. 0.1 ml of working standard solutions containing 0.1 to 1.0 mg/L of NO_3 were taken (see Figure 1). In a 30 ml tube, 0.4 ml of a salicylic acid solution (5% salicylic acid dissolved in sulfuric acid) was added. The mixture was left at room temperature for 20 minutes, after which 9.5 ml of 2M NaOH solution was slowly incorporated. Nitrate concentration was determined spectrophotometrically at 410 nm.

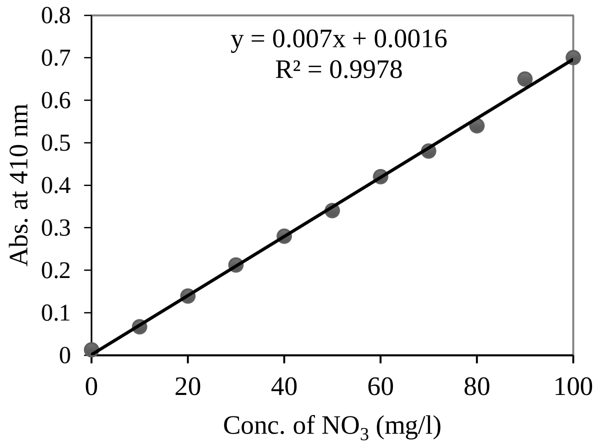

Fig.1: Standard curve of nitrate (NO_3)

Assessment of nitrate in the leafy vegetable samples

The determination of nitrate in the samples was calculated according to [30]. 0.1 ml of plant extract was taken in a 30 ml test tube, and 0.4 ml of salicylic acid solution was added. Leave the mixture at room temperature for 20 minutes, then slowly add 9.5 ml of 2M sodium hydroxide solution. The nitrate concentration in the sample was measured using a colourimetric spectrometer (Jenway 2705) [31]. Sample-free water was used as a control, and nitrate was expressed in milligrams per kilogram of dry weight (mg/kg dry weight). The nitrate concentration was calculated through the straight-line equation according to the equation:

$$Y = 0.008x + 0.1176 \quad (1)$$

And, $Y = \frac{C\,V}{W}$

Where: Y = Nitrate concentration (mg/kg)

C = Nitrate concentration calculated with OD410 using the regression equation.

V = The sample's total volume extracted. (ml)

W = sample weight (g)

Statistical analysis

A multivariate statistical approach was employed to analyse the data and understand the variation in nitrate content of leafy vegetables. This approach is helpful for modelling and interpreting large datasets to reduce the dimensionality of data and extract useful information for soil quality assessment and management [32]. The present study applied CA and PCA to a results dataset. CA is a technique that assembles objects based on their similarity or dissimilarity by forming a dendrogram. PCA reduces the dimensionality of a large number of datasets by transforming them into a new set of variables called principal components (PCs). Principal components are non-correlated and arranged in decreasing order of importance. A correlation matrix of eleven leafy vegetables was computed, and factor loadings defined the nature of variation. Any principal component with an eigenvalue greater than 1 was considered significant. Dendrograms, presented in graphical form, provide a visual summary by dramatically reducing the dimensionality of the data [33]

Method Validation

Method validation was evaluated, by considering the calibration curve, limit of detection (LOD), and limit of quantification (LOQ). A series of standard nitrate solutions, ranging in concentration from 10 to 100 mg/L, was prepared to create calibration curves. The limit of detection and quantification were estimated to be three times and ten times, respectively, the ratio of the raw sample solution standard deviation to the calibration curve slope [34],

Results and discussion

The calibration curve exhibited a linear relationship, characterized by a strong determination coefficient (R^2) of 0.9978. The derived linear equation for the standard solution, which ranges from 1.0 to 100 mg/L, is y = 0.0075x + 0.0016. This high regression coefficient reflects excellent linearity in the analytical response of the proposed sample. The limits of detection (LOD) and limits of quantification (LOQ) for nitrate were determined to be 0.033 mg/L and 0.099 mg/L, respectively.

Estimation of nitrate content in leafy vegetables

Vegetables are one of the most important food sources of nitrates. Therefore, in this study, the vegetables most consumed in the Brack Alshatti city were analyzed, and samples of eleven leafy vegetables were studied (Table 1), which are often sold in the local market. These leafy vegetables include chard, dill, parsley, lettuce, Spanish, coriander, salad rocket, chives, white Cabbage, red Cabbage, and Lactuca sativa. Nitrate was determined using the spectrophotometer method; the nitrate concentration was 235.97 - 249.54, 235.84 - 238.59, 235.57 -249.26, 237.28 - 249.22, 237.58 - 245.81, 236.91–241.07, 236.40 - 247.26, 237.71 - 24.23, 237.58 - 238.10, 237.06 -249.39, and 236.94 - 249.23 mg/kg dwt. Their nitrate concentration (mean ± SD) mg/kg dwt. was 245.15±4.06, 242.19±3.81, 240.94±4.73, 240.56±3.72, 240.8±3.13, 239.36±1.43, 237.75±0.85, 243.4±3.86, 237.92±0.18, and 244.2±5.86, respectively. The results showed that nitrate content in the leafy vegetables are within the allowable limit [34], does not vary considerably among them (Fig. 2). Comparable nitrate concentration levels have been observed in leafy vegetables like mint, coriander, and dill, with reported values of 317.5, 360, and 402 mg/kg of fresh weight, respectively, in Tehran, Iran [35]. In contrast, findings from Bel Dokhtar Province in Iran indicate that the nitrate concentrations were notably higher: 716 mg/kg in salad, 473 mg/kg in mint, 476 mg/kg in dill, and 416 mg/kg in coriander [36, 37]. in their study estimating the nitrate concentration in some vegetables in the city of Varzagan in north-western Iran, also indicated that the nitrate concentration in both Cabbage, salad, coriander, spinach, and dill recorded 161, 781, 707, 441, 501, and 772 mg/kg dry weight, respectively, as indicated by [37]. The concentration of nitrate in vegetable samples measured in Greece was 209, 282, and 1250 mg/kg wet weight in kale, salad, and spinach, respectively [38, 39]. The concentration of nitrates in spinach and coriander taken from greenhouses in Kermanshah province was 203.33 and 140.37 mg/kg wet weight, respectively. (40) also indicated that there was a variation in the concentration of nitrates as a result of adding organic or chemical fertilizers to salad, spinach, and Cabbage, where the average concentration of nitrates when adding organic fertilizer was 809, 482, and 882, while the average concentration of nitrates when adding chemical fertilizer was 1074, 577, and 573 mg/kg. Wet weight, respectively [40]. In contrast, many studies have indicated that vegetables contain high concentrations of nitrates [41]. The concentration of nitrates in vegetables collected from Croatia reached 1035, 3184, 2351, and 1040 mg/kg/fwt in salad, spinach and Cabbage, respectively [42, 43]. The nitrate concentration reached 5136, 5628, and 4504 mg/kg/dwt in salad, mint, and Cabbage, respectively [44]. The concentration of nitrates in Arugula, salad, lettuce, spinach, and chard in vegetables collected from the Canary Islands in Spain recorded 3266, 607.5, 819.7, 1494, and 1750 mg/kg/fwt [45]. indicated that the average concentration of nitrates in Cabbage, salad, spinach, Cabbage, and Cabbage in Korea reached 1059, 1386.5, 2124, 2012, and 2336 mg/kg/fwt [46], The absorption and accumulation of nitrates in vegetable tissues are affected by several factors, including the intensity of daylight, soil type, temperature, moisture and frequency of plants in the field, greenness, genetics, resistance to delay, vegetative unit size, storage time and nitrogen source [47 - 49]. Nitrogen fertilization is among the main factors affecting the presence of nitrates. The enzyme that reduces nitrate (nitrate reductase), which works during the day, can reduce nitrate in the plant, indicating that the variation in the nitrate content of one type of vegetable may be due to different agricultural practices, types of fertilizers (chemical or organic), and their quality and quantities added [41].

Statistical analysis

Correlation statistics

The relationship between different leafy species and their nitrate content is presented in Table 2. The results indicate a strong positive correlation between parsley and dill (r = 0.740**), whereas a positive correlation was occurred between lettuce and parsley (r = 0.621*); Lactuca. Sativa and lettuce (0.889*); red cabbage and coriander (r = 0.843*). The degree of correlation among the leafy vegetable samples was analyzed by establishing the relationship between the two variables.

The value of the correlation coefficient “r” provides information about the association between two species of leafy vegetables; however, the sign indicates whether the association is positive or negative. A positive strong correlation shows a similar origin, the way of nitrate accumulation in the vegetable, the irrigation process; however, the week association between vegetables suggests that these vegetables are independent from each other [50, 51].

Principle of component analysis (PCA)

From the data, only three principal components were us for the interpretation of results. PC1, PC2, and PC3 were retained from the original data because these three principal components accounted for 100% of the total variation among the 11 leafy vegetables. The percentage variations explained by PC1, PC2, and PC3 were 42.96%, 35.60%, and 21.44%, respectively (Table 3). The high loading variables for the first principal component (PC1) explained 42.96% were, Dill (0.909), Parsley (0.996), Lettuce (0.989) and Lactuca Sativa (.866), the second component (PC2) explained 35.60% of the total variation and highly loaded with chard (0.636), Spanish (-0.847), coriander (0.887), onion leaves (-0.875) and red cabbage (0.976), whereas the third component (PC3) which explained 21.44% loaded with salad rocker (0.527) and white cabbage (0.986).

Table 1: Descriptive analysis of nitrate concentration (mean ± SD) in leafy vegetables samples

Vegetable name	Local Name	N	NO_3 (mg/kg/dwt) Min.	Max.	Mean ± SD	Var.	Allowable limit (mg-kg-1. Fwt) *
Parsley	المعدنوس	12	235.57	249.26	240.97±4.70	22.09	2,000
Lettuce	السلطة	12	237.28	249.22	240.56±3.72	13.84	2,0000
Spanish	السبانخ	12	237.58	245.81	240.80±3.13	9.82	3000 - 2000
Coriander	الكسبر	10	236.91	241.07	239.36±1.43	2.06	2,000
Onion leaves	اوراق البصل	10	237.71	249.23	243.40±3.86	14.90	-
Lactuca Sativa	الخس	6	236.94	249.23	244.20±5.86	34.31	2,000
White Cabbage	الملفوف الابيض	6	237.58	238.10	237.92±0.19	0.03	900 – 500
Red cabbage	الملفوف الاحمر	6	237.06	249.39	244.93±4.18	17.44	900 – 500
Chard	السلك	13	235.97	249.54	242.19±3.81	14.50	2,000
Dill	الشبت	8	235.84	238.59	237.75±0.85	0.73	2,000
Salad rocket	الجرجير	10	236.40	247.26	240.02±3.00	8.85	-

(*) = [35]; (-) = not mentioned

Table 2: Correlation coefficients of the relationship between different leafy vegetables and their content of nitrate

	Chard	Dill	Parsley	Lettuce	Spanish	Coriander	S.Rocket	O.Leaves	L.Sativa	W Cabbage	R. cabbage
Chard	1										
Dill	.471	1									
Parsley	.268	**.740****	1								
Lettuce	.029	.278	**.621***	1							
Spanish	-.231	.391	-.076	-.052	1						
Coriander	.398	.098	.422	.149	-.507	1					
S.rocket	.184	.107	-.043	.125	-.271	.281	1				
O.leaves	-.443	-.005	-.024	-.119	.710	-.440	-.405	1			
L.Sativa	.196	.374	.806	**.889***	-.513	-.006	-.651	-.152	1		
W.Cabbage	-.522	-.332	-.205	-.369	-.415	-.009	.014	.660	-.010	1	
R.cabbage	.766	.047	.354	.393	-.762	**.843***	.014	-.790	.407	-.578	1

** Correlation is significant at the 0.01 level (2-tailed)

* Correlation is significant at the 0.05 level (2-tailed)

Table 3: Total Variance Explained

Compo	Initial Eigenvalues Total	% of Variance	Cumulative %	Extraction Sums of Squared Loadings Total	% of Variance	Cumulative %	Rotation Sums of Squared Loadings Total	% of Variance	Cumulative %
Chard	4.824	43.850	43.850	4.824	43.850	43.850	4.725	42.958	42.958
Dill	4.000	36.366	80.216	4.000	36.366	80.216	3.916	35.601	78.559
Parsley	2.176	19.784	100.000	2.176	19.784	100.000	2.358	21.441	100.000
Lettuce	9.767E-16	8.879E-15	100.000						
Spanish	5.563E-16	5.058E-15	100.000						
Coriander	3.592E-16	3.266E-15	100.000						
S. rocket	1.624E-16	1.476E-15	100.000						
O. leaves	-2.934E-17	-2.668E-16	100.000						
L. Sativa	-1.562E-16	-1.420E-15	100.000						
W.Cabbage	-2.304E-16	-2.095E-15	100.000						
R.cabbage	-4.860E-16	-4.418E-15	100.000						

Extraction Method: Principal Component Analysis.

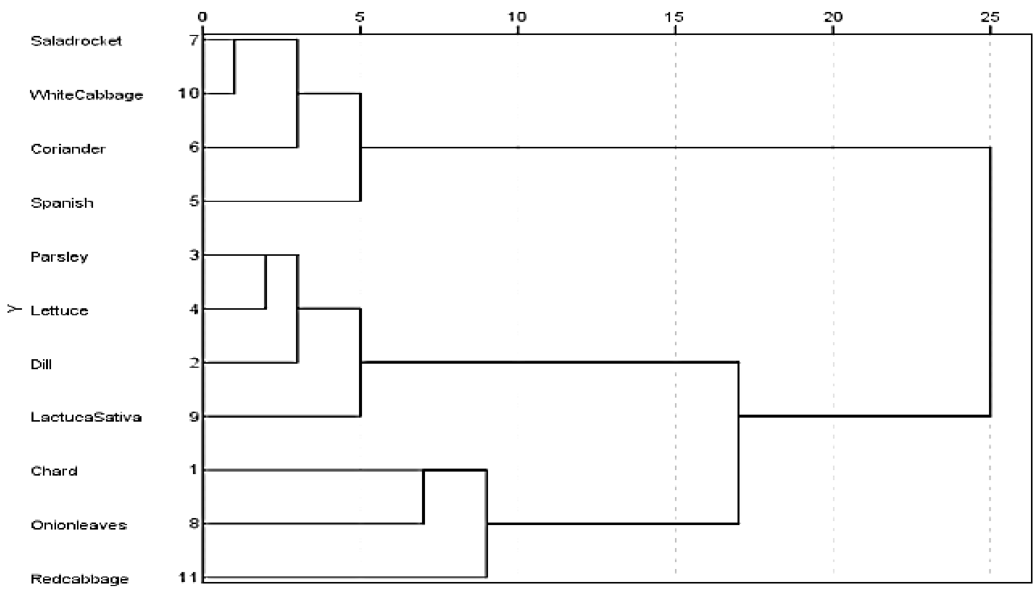

Fig. 2. Dendrogram based on the cluster analysis of 11 different leafy species

Table 4: Rotated Component Matrix

Leafy vegetables	Components 1	2	3
Chard	.478	.636	-.606
Dill	.909	-.361	-.208
Parsley	.996	.078	-.034
Lettuce	.989	-.056	.136
Spanish	-.028	-.847	-.530
Coriander	-.014	.887	-.461
Salad rocket	-.820	-.011	.572
Onion leaves	.483	-.875	.033
Lactuca Sativa	.866	.362	.343
White. Cabbage	.109	-.130	.986
Red cabbage	.171	.976	-.132

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
a. Rotation converged in 6 iterations.

Cluster analysis (CA).

Cluster analysis is a valuable tool for solving classification problems, aiming to place variables into groups where the degree of association is substantial between members of the same cluster and weak between members of different clusters [52]. The cluster analysis (Fig. 2) was applied to standardized datasets sorted by nitrate concentration in the leafy vegetable samples to understand the mutual dissimilarities among them, which separated the eleven samples into three statistically significant clusters: cluster I contains salad rocket, white cabbage, coriander, and Spanish; cluster II contains parsley, lettuce, dill, and Lactuca sativa; and cluster III contains chard, red cabbage, and onion leaves. The dataset was treated using Ward’s linkage method with squared Euclidean distance as a dissimilarity measure. CA generated a dendrogram, grouping the sampled vegetables based on the percentage of dissimilarity in nitrate concentration. The dendrogram showed dissimilarity among the eleven study vegetables.

Conclusions

This study aimed to assess nitrate concentration in eleven leafy vegetables collected from the Brack local market for the first time. The results demonstrated that the mean nitrate contents in vegetables are similar to those reported by others, and some of the data were even lower than the recommended levels adopted by the WHO. Therefore, nitrate intake through vegetable consumption is safe for consumers. Although the nitrate content in the studied vegetables is within the permissible limit, systematic monitoring is recommended for their sustainability and safety.

Author Contributions: All authors have made a substantial, direct, and intellectual contribution to the work and approved it for publication

Funding: This research received no external funding

Data Availability Statement: No data were used to support this study.

Conflicts of Interest: The authors declare that they have no conflict of interest.

Acknowledgment: Not applicable.

References.

[1] H. Nakaa, S. Alwaleed, M. Alshatory, A. Alshanokey, and M. A. Salem, "Assessment of the concentration of some heavy metals and their risk index to the health of the population in some vegetables produced in Brack region, Libya," *J. Misurata Univ. Agric. Sci*., vol. 4, no. 2, pp. 338-362, 2024.

[2] M. Kyriacou, G. Soteriou, G. Colla, and Y. Rouphael, "The occurrence of nitrate and nitrite in Mediterranean fresh salad vegetables and its modulation by preharvest practices and postharvest conditions," *Food Chem*., vol. 285, pp. 468-477, Jul. 2019, doi: 10.1016/j.foodchem.2019.02.001.

[3] K. Hakeem, M. Sabir, M. Ozturk, M. Akhtar, and F. Ibrahim, "Nitrate and nitrogen oxides: sources, health effects and their remediation," *Rev. Environ. Contam. Toxico*l., vol. 242, pp. 183-217, 2017.

[4] M. Salem, L. Al-Ethawi, Z. Eldrazi, and A. Noralldien, "A case study of the total and available phosphorus concentration in Libyan agricultural soils in different depths and seasons in long-term chemical and animal manure fertilization," *Int. J. Res. Stud. Biosci*., vol. 2, no. 2, pp. 1-9, 2014.

[5] L. Quijano, et al. "Risk assessment and monitoring programme of nitrates through vegetables in the Region of Valencia (Spain)," *Food Chem. Toxicol*., vol. 100, pp. 42-49, 2017, doi: 10.1016/j.fct.2016.12.010.

[6] V. Kmecl et al. "Nitrate and nitrite contamination of vegetables in the Slovenian market," *Food Addit. Contam.: Part* B, vol. 12, no. 3, pp. 216-223, 2019, doi: 10.1080/19393210.2019.1600589.

[7] M. Iammarino, A. Di Taranto, and M. Cristino. "Monitoring of nitrites and nitrates levels in leafy vegetables (spinach and lettuce): A contribution to risk assessment," J. *Sci. Food Agric*., vol. 94, no. 4, pp. 773-778, 2014, doi: 10.1002/jsfa.6439.

[8] L. Al-Ethawi, and M. Salem. "Study of residual effect of N fertilizer (Total N) on the soil," *J. Phys.: Conf. Ser*., vol. 1294, p. 072001, 2019, doi: 10.1088/1742-6596/1294/7/072001.

[9] P. Knekt, R. Järvinen, J. Dich, and T. Hakulinen. "Risk of colorectal and other gastro-intestinal cancers after exposure to nitrate, nitrite and N-nitroso compounds: A follow-up study," *Int. J. Cancer*, vol. 80, no. 6, pp. 852-856, 1999.

[10] C. Hipkin, D. Simpson, S. Wainwright, and M. Salem. "Nitrification in plants that also fix nitrogen," *Natur*e, vol. 430, pp. 98-101, 2004.

[11] M. Salem, M. Michael, S. Wainwright, and C. Hipkin. "Nitroaliphatic compounds in Hippocrepis Comosa and other legumes in the European flora," *Phytochemistry*, vol. 40, no. 1, pp. 89-91, 1995.

[12] P. Song, L. Wu, and W. Guan. "Dietary nitrates, nitrites, nitrosamines intake and gastric cancer risk: A meta-analysis," *Nutrients*, vol. 7, pp. 9872-9895, 2015.

[13] M. Salem, and N. M. Abuhadara. "An investigation of the occurrence of 3-Nitropropionic acid in some Leguminousae plants in the south east of Libya," *Sebha Univ. J*., vol. 1, no. 1, pp. 55-60, 2010.

[14] H. Salehzadeh, A. Maleki, R. Rezaee, B. Shahmoradi, and K. Ponnet. "The nitrate content of fresh and cooked vegetables and their health-related risks," *PLoS ONE*, vol. 15, no. 1, p. e0227551, 2020.

[15] FAO/WHO, "Nitrate (and potential endogenous formation of N-nitroso compounds)," *WHO Food Additive Series 50*, Geneva: World Health Organization, 2011.

[16] European Commission (EC), "Opinion on nitrate and nitrite," *Reports of the Scientific Committee for Food (SCF)*, 26th Series, pp. 21-28, 1992.

[17] J. Kobayashi, K. Ohtake, and H. Uchida, "NO-rich diet for lifestyle-related diseases," *Nutrients*, vol. 7, no. 6, pp. 4911-4937, 2015, doi: 10.3390/nu7064911.

[18] J. Lovegrove, A. Stainer, and D. Hobbs. "Role of flavonoids and nitrates in cardiovascular health," *Proc. Nutr. Soc*., pp. 1-13, 2017.

[19] M. Jones, C. Thompson, L. Wylie, and A. Vanhatalo. "Dietary nitrate and physical performance," *Annu. Rev. Nutr*., vol. 38, pp. 303-328, 2018.

[20] H. Liu, et al. "Relationship of dietary nitrate intake from vegetables with cardiovascular disease mortality: A prospective study in a cohort of older Australians," *Eur. J. Nutr*., vol. 58, pp. 2741-2753, 2019, doi: 10.1007/s00394-018-1823-x.

[21] International Agency for Research on Cancer (IARC), "Ingested nitrate, nitrite, and cyanobacterial peptide toxins," *IARC Monogr. Eval. Carcinog. Risks Hum.*, vol. 94, pp. 1-464, 2010.

[22] L. Xie, et al. "Association between dietary nitrate and nitrite intake and site-specific cancer risk: Evidence from observational studies," *Oncotarget*, vol. 7, pp. 56915-56932, 2016.

[23] M. Karwowska, and A. Kononiuk. "Nitrates/nitrites in food—Risk for nitrosative stress and benefits," *Antioxidants*, vol. 9, no. 3, p. 241, 2020.

[24] N. Hord, Y. Tang, and N. Bryan. "Food sources of nitrates and nitrites: The physiologic context for potential health benefits," *Amer. J. Clin. Nutr*., vol. 90, pp. 1-10, 2009.

[25] W. Zhong, C. Hu, and M. Wang. "Nitrate and nitrite in vegetables from north China: Content and intake," *Food Addit. Contam*., vol. 19, no. 12, pp. 1125-1129, 2002.

[26] S. De Martin, and P. Restani. "Determination of nitrates by a novel ion chromatographic method: Occurrence in leafy vegetables (organic and conventional) and exposure assessment for Italian consumers," *Food Addit. Contam*., vol. 20, no. 9, pp. 787-792, 2003.

[27] J. Hsu, J. Arcot, and N. A. Lee. "Nitrate and nitrite quantification from cured meat and vegetables and their estimated dietary intake in Australians," *Food Chem*., vol. 115, pp. 334-339, 2009.

[28] M. Salem, and L. Al-Ethawi. "A study of the presence of residual of nitrogenous fertilizer nitrate (NO3-) in some soils of Brack - Ashkada agriculture project," *J. Life Sci. Technol*., vol. 1, no. 1, pp. 84-88, 2013.

[29] T. Abo-Bakr, S. El-Iraqui, and M. Huissen. "Nitrate and nitrite contents of some fresh and processed Egyptian vegetables," *Food Chem*., vol. 19, pp. 265-275, 1986.

[30] E. Ajebe, and T. Bahiru. "Spectrophotometric determination of nitrite and nitrate in some selected vegetables cultivated in Adami Tulu Judo Kombolicha District Farms, Ethiopia," *J. Anal. Bioanal. Tech*., vol. 9, p. 410, 2018, doi: 10.4172/2155-9872.1000410.

[31] M.Matthew, et al. "Spectroscopic evaluation of nitrate and nitrite concentrations in selected fruits and vegetables," Int. *J. Sci. Eng. Sci*., vol. 3, no. 9, pp. 32-35, 2019.

[32] L. Zhao, and Y. Wang. "Nitrate assay for plant tissues," Bio *Protoc*., vol. 7, no. 2, p. e2029, 2017, doi: 10.21769/BioProtoc.2029.

[33] K. Singh, et al. "Multivariate statistical analysis and geochemical modelling for geochemical assessment of groundwater of Delhi, India," *J. Geochem. Explor*., vol. 175, pp. 59-71, 2017, doi: 10.1016/j.gexplo.2017.01.001.

[34] H. Zhao, et al. "Spatial correlations between urbanization and river water pollution in the heavily polluted area of Taihu Lake Basin, China." J. Geogr. Sci. 23, 735–752 (2013). https://doi.org/10.1007/s11442-013-1041-734.

[35] F. Sayed, and E. Rezvan. "measuring nitrate and nitrite concentrations in vegetables and fruits in Shiraz". Journal of applied science and environmental. vol. 18, no. 3, pp 451-457. 2014.

[36] J. Farsani, Z. Mogharab, and M. Feyzian. "Potential Health Risk of Nitrate Accumulation in Vegetables Grown in Pol-e Dokhtar County". *Journal of Chemical Health Risks*. vol, 11, no, 1, pp 99-111 2021.

[37] S. Oskouei, N. Miri, M. Taghdir, M. Nejatian, M. Sepandi, and S. Abbbaszadeh. "Evaluation of Nitrate and Nitrite Levels in Leafy Vegetables to Assess the Potential Health Risks in Tehran, Iran". Journal of Chemical Health Risks. vol. 14, no, 3. Pp 589-597. 2024.

[38] P. Nowrouz, et al. "Nitrate determination of vegetables in Varzeghan City, Northwestern Iran," *Health Promot. Perspect.*, vol. 2, no. 2, pp. 244-250, 2012.

[39] M. Salem, and A. Noralldien. "Concentration of cadmium, lead and chrome in some vegetables amended with phosphate and urea fertilizers for more than forty years," *CPQ Microbiol*., vol. 1, no. 2, pp. 01-14, 2018.

[40] K. Fytianos, and P. Zarogiannis. "Nitrate and nitrite accumulation in fresh vegetables from Greece," *Bull. Environ. Contam. Toxicol*., vol. 62, pp. 187-192, 1999.

[41] V. Simeonov et al., "Assessment of the surface water quality in northern Greece," *Water Res*., vol. 37, no. 17, pp. 4119-4124, 2003

[42] V. Triantafyllidis, D. Hela, and G. Salachas. "Comparison of nitrate content in leafy vegetables conventionally and organically cultivated in Western Greece," *J. Environ. Prot. Ecol*., vol. 9, no. 2, pp. 301-308, 2008.

[43] S. Luetic, et al. "Leafy vegetable nitrite and nitrate content: Potential health effects," *Foods*, vol. 12, p. 1655, 2023, doi: 10.3390/foods12081655.

[44] V. León, and O. P. Luzardo. "Evaluation of nitrate contents in regulated and non-regulated leafy vegetables of high consumption in the Canary Islands, Spain: Risk assessment," *Food Chem. Toxicol*., vol. 146, p. 111812, 2020, doi: 10.1016/j.fct.2020.111812.

[45] J. Suh, et al. "Risk assessment on nitrate and nitrite in vegetables available in Korean diet," *J. Appl. Biol. Chem*., vol. 56, no. 4, pp. 205-211, 2013, doi: 10.3839/jabc.2013.033.

[46] J. Dich, et al. "Dietary intakes of nitrate, nitrite and NDMA in the Finnish mobile clinic health examination survey," *Food Addit. Contam*., vol. 13, pp. 541-552, 1996.

[47] T. Du, Y. Zhang, and X. Lin. "Accumulation of nitrate in vegetables and its possible implications to human health," *Agric. Sci. China*, vol. 6, no. 10, pp. 1246-1255, 2007.

[48] M. Correia, et al. "Contribution of different vegetable types to exogenous nitrate and nitrite exposure," *Food Chem*., vol. 120, pp. 960-966, 2010.

[49] P. Santamaria. "Review nitrate in vegetables: Toxicity, content, intake and EC regulation," *J. Sci. Food Agric*., vol. 86, no. 14, pp. 10-17, 2006.

[50] V. Wagh, et al. "Influence of hydro-geochemical processes on groundwater quality through geostatistical techniques in Kadava River basin Western India," *Arab. J. Geosci*., vol. 12, no. 1, p. 7, 2019, doi: 10.1007/s12517-018-4136-8.

[51] M. Brogueira, and G. Cabeçadas. "Identification of similar environmental areas in Tagus estuary by using multivariate analysis," *Ecol. Indic*., vol. 6, no. 3, pp. 508-515, 2006.

[52] G. Unnikrishnan, et al. "Prevalence of hypothyroidism in adults: An epidemiological study in eight cities of India," *Indian J. Endocrinol. Metab*., vol. 17, no. 4, pp. 647-652, 201, 2013.