



Probabilistic non-carcinogenic risk assessment of nitrate in vegetables: A cross-sectional study in Minab city

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ABSTRACT

The aims of this study were to determine the concentration levels of nitrates in different types of vegetables, as well as the probabilistic non-carcinogenic risk assessment of nitrate in vegetables. Vegetables were classified into three groups included leafy (lettuce and cabbage), tuberous (potato and onion), and bush (tomato and cucumber) products. Three samples of each 20 vegetable types were obtained from agricultural lands and the nitrate levels were measured using a High-Performance Liquid Chromatography (HPLC) system (KNAUER) equipped with a UV detector (UV-Detector-K2500). The mean nitrate concentration levels in leafy, bush and tuberous vegetables were 685.87 ± 52.87 , 153.48 ± 20.15 and 63.58 ± 27.32 mg/kg, respectively. The mean of hazard quotient (HQ) in adults and children in region 1 were 0.028 and 0.096; for region 2 were as 0.027 and 0.096; and for region 3 were 0.028 and 0.090, respectively (Figure 1-3). The HQ values due to nitrate for adults and children in three regions were <1 , hence consumers are in acceptable health risk. While nitrates in vegetables were within acceptable health risk range, ongoing monitoring and management of nitrate levels are necessary to mitigate potential health hazards.

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INTRODUCTION

Vegetables are indeed a rich source of vitamins, minerals, and antioxidants, which contribute significantly to their health benefits and a diet rich in vegetables can significantly contribute to reducing the risk of both cancer and cardiovascular disease by providing essential nutrients and bioactive compounds (Dias, 2012; Sadeghi et al., 2015). Nitrate content in vegetables is a significant concern for human health due to its potential harmful effects (Huang et al., 2019). Nitrate is indeed an anion that plays a crucial role in the nitrogen cycle. The association between nitrate consumption and health risks, including methemoglobinemia and certain cancers, is a significant concern (Ding et al., 2018; Hmelak Gorenjak & Cencič, 2013). Nitrites can indeed bind with food amines to form N-nitrosamines, which are associated with an increased risk of various cancers, including esophageal and stomach cancers (Chung et al., 2003; Ding et al., 2018). Nitrate present in a wide range of vegetables, fruits, and processed foods (Hosseini et al., 2023; Sanja Luetic et al., 2023; Merusi et al., 2010; Sadeghi et al., 2015). Nitrate levels in vegetables can indeed vary significantly based on several factors, including the type of

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vegetables, the nitrogen content in the soil, temperature, sunlight, and other factors (Taghipour et al., 2019). The excessive use of nitrogen-containing fertilizers has significantly increased nitrate levels in vegetables, which now contribute a high percentage of nitrates to the human diet (Hassani Moghaddam et al., 2019). The consumption of vegetables and other kitchen produce that absorb and retain large amounts of nitrate can indeed pose health risks to humans (Qasemi et al., 2024). Nitrate is converted to nitrite in the human and animal digestive system through bacterial activity, primarily in the oral cavity and stomach, and Nitrite can react with amines in the acidic environment of the stomach to form nitrosamines, which is a carcinogenic chemical (Carlström et al., 2020; Ma et al., 2018; Pereira et al., 2013). The International Agency for Research on Cancer (IARC) has categorized ingested nitrate and nitrite, under conditions that result in endogenous nitrosation, as “probably carcinogenic to humans” (Group 2A) (Humans & Cancer, 2010). Nitrates are indeed essential nutrients in both human and animal diets, playing a crucial role in plant growth and metabolism. However, while nitrates themselves are not inherently toxic, their metabolites and byproducts, such as nitrite, nitric oxide, and nitrous compounds, can pose health risks (Bondonno et al., 2015; Bryan & Loscalzo, 2017; Panel et al., 2020). Research conducted in Golestan and Isfahan provinces shows that there is a direct relationship between the use of nitrogenous chemical fertilizers and the prevalence of stomach cancer (Moradnia et al., 2019; Semnani et al., 2009). These fertilizers may pollute the environment and contribute to an increased risk of stomach cancer due to the presence of nitrogen and other chemicals. The prevalence of gastrointestinal cancers, particularly stomach cancer, in the eastern part of Minab city aligns with broader trends observed in Iran. Reports indicate that gastrointestinal cancers, including gastric cancer, are among the most common and fatal cancers in the country (Khaksar et al., 2024). Therefore, managing nitrate accumulation in vegetables is crucial for ensuring food safety and reducing potential health hazards. The concern over nitrate and nitrite in vegetables has indeed become a significant public health issue due to soil and agricultural product contamination with nitrous compounds. This study is the first to conduct a probabilistic non-carcinogenic risk assessment of nitrate exposure from commonly consumed vegetables in Minab city, Iran. Unlike previous studies, which primarily report mean nitrate concentrations or deterministic risk estimates, this research applies a probabilistic approach to better characterize the range and likelihood of potential health risks associated with nitrate intake from local vegetables.

MATERIALS AND METHODS

This cross-sectional study was performed in 2023. Vegetables were classified into three groups included leafy (lettuce and cabbage), tuberous (potato and onion), and bush (tomato and cucumber) products. Three samples of each 20 vegetable types including green cucumber, onion, tomato, pumpkin, eggplant, potato, hot pepper, bell pepper, green bean, cabbage, lettuce, local vegetable (turnip greens), cilantro, celery, spinach, dill, basil, parsley, and leeks were obtained from agricultural lands in three steps. Sampling was done in three different regions. Considering that most of the vegetables consumed by the residents of Minab County in the fall, winter, and spring are grown and produced in the city itself, three regions were determined for sampling, including Region 1: High cancer risk (a region with high cultivation of agricultural products, which includes the districts of Tokhur-Hashtbandi, Cheraghabad, Sandrak, and Rawang, and also has a human population of 85,072 people). Region 2: A region with average cultivation of agricultural products, which includes the districts of Tirur, Ghorband, and Haj Khadmi, and also has a human population 25,618 people. Region 3: The area without agricultural crops, which includes the central and coastal parts of this city and also has a human population of 65,090 people. The sampling procedure involves collecting 10-15 samples of each type of vegetable, grinding them with a Moulinex shredder, and storing them in sterile plastic bags inside an ice box

for transportation to the laboratory. The sample preparation and extraction methods referenced in studies by Hongsibsong et al (Hongsibsong et al., 2014) and Ghaffari et al (Ghaffari et al., 2019). The nitrate levels were measured using a High-Performance Liquid Chromatography (HPLC) system (KNAUER) equipped with a UV detector (UV-Detector-K2500). The procedure includes three repetitions for each sample, and the average of these values was considered the nitrate level.

Sample analysis

In order to extracting nitrate from vegetables the standard protocols used in scientific studies for nitrate determination was used. So that, 50 mL of distilled or deionized water was added to 1 g of homogenized vegetable sample. Then, the mixture was heated at 70–80°C for 15 minutes to extract nitrate efficiently. After heating, the sample is cooled and filtered through a membrane (0.45 µm) to remove particulates. The filtrate was then analyzed using HPLC. The injection was done immediately after extraction. The mobile phase consisted of 50% methanol, 50% distilled water and 0.01 moles of octylamine. As well as, pH was adjusted to 7 using phosphoric acid.

Probabilistic non-carcinogenic risk assessment

Estimated Daily intake was calculated, as (Cai et al., 2023; Özlü, 2024; Zhang et al., 2024):

$$EDI = \frac{C \times IR}{BW} \quad (1)$$

Where EDI is estimated daily intake (mg/kg-d); C is the concentration of nitrate in vegetables (mg/kg); IR is the ingestion rate of vegetables (0.712 kg/d)(Helgilibrary, 2024); and BW is body weight for children and adults is 20 kg and 70 kg, respectively (EPA, 2015).

The hazard quotient was calculated, as(Huang et al., 2025; Özlü, 2024):

$$HQ = \frac{EDI}{RfD} \quad (2)$$

Where, EDI is the estimated daily intake (mg/kg-d) and RfD is oral references dose of nitrate (1.6 mg/kg-d). The HQ was calculated in 3 regions of Minab based on concentration levels of nitrate in vegetables. To identify the uncertainties in the health risk assessment, the Monte Carlo simulation (MCS) model was applied with 5000 repetitions. The distribution type of C and IR variables were selected as log-normal (Bounar et al., 2020; Gao et al., 2022), and BW as the normal distribution.

Statistical analysis

Statistical analyses were performed using SPSS version 26 and Excel version 2016. The Shapiro test was used to determine the normality of the data. The ANOVA test was used to comparison the nitrate levels among three vegetable groups. The statistically significant level was considered at p-value<0.05.

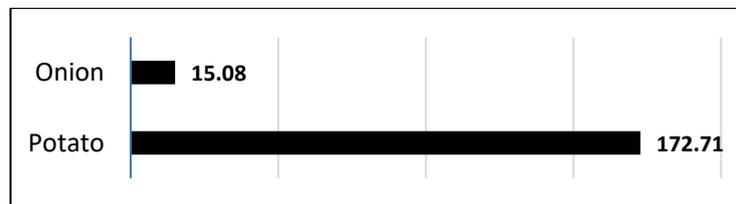
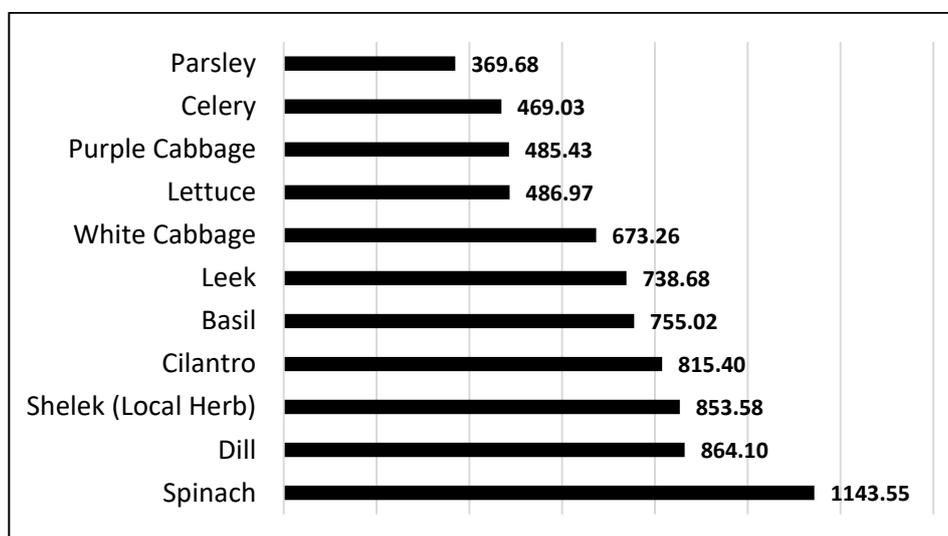
RESULTS AND DISCUSSION

The nitrate concentration levels (mg/kg) in bush vegetables

The mean nitrate concentration levels (mg/kg) in bush vegetables are presented in Table 1. Among bush vegetables, the highest nitrate concentration was observed in zucchini, with 296.23 mg/kg, while the lowest concentration was found in tomatoes, with 17.09 mg/kg. The chart illustrating the nitrate concentration for each bush vegetable is presented in the table below.

Table 1. The mean nitrate concentration levels (mg/kg) in bush vegetables

Product	Zucchini	Eggplant	Green Beans	Bell Pepper	Cucumber	Chili Pepper	Tomato
Nitrate Conc. (mg/kg)	296.23	275.78	205.97	189.35	118.77	102.17	17.09

**Fig. 1.** Nitrate concentration (mg/kg) in tuber vegetables**Fig. 2.** Mean nitrate concentration (mg/kg) in leafy vegetables

Nitrate concentration in tuber vegetables

The mean nitrate concentration levels (mg/kg) in tuber vegetables are presented in Fig. 1. Among tuber vegetables, the highest nitrate concentration was recorded in potatoes, with 172.71 mg/kg, while the lowest was in onions, with 15.08 mg/kg.

Nitrate concentration in leafy vegetables

The mean nitrate concentration levels (mg/kg) in leafy vegetables are presented in Fig. 2. Among the leafy vegetables, the highest nitrate concentration was found in spinach, with 1143.55 mg/kg, while the lowest was in parsley, with 369.68 mg/kg.

The mean nitrate concentration (mg/kg) in all consumed vegetables and crops

Based on the results of conducted experiments, leafy vegetables exhibited the highest mean nitrate concentration, at 685.87 ± 52.87 mg/kg, followed by bush vegetables with 153.48 ± 20.15 mg/kg. The lowest nitrate concentration was observed in tuber vegetables, at 63.58 ± 27.32 mg/kg. The mean concentration levels of nitrate among the three types of vegetables (tuber, bush and leafy) were significantly different (p -value < 0.001).

Our results showed that leafy vegetables generally contain higher nitrate levels than root or fruit vegetables. Shahbaz zadegan et al (Shahbazzadegan et al., 2010) and Pourmoghimi

Table 2. Mean nitrate concentration (mg/kg) in three vegetables groups

Vegetables type	Nitrate Concentration (mg/kg)	Standard Deviation
Tuber vegetables	63.58	27.32
Bush vegetables	153.48	20.15
Leafy vegetables	685.87	52.87
<i>p-value (among three vegetables types)</i>		<0.001

et al (Pourmoghim et al., 2010) reported that the order of nitrate content was highest in leafy vegetables, followed by roots and tubers, which is consistent with the results of the present study. In another, lettuce has significantly higher nitrate levels compared to tomatoes (Ram et al., 2022; Salehzadeh et al., 2020). Leafy greens such as rucola (*Eruca sativa*), Swiss chard (*Beta vulgaris*), spinach, and lettuce tend to have the highest nitrate concentrations among vegetables. For instance, Rucola showed nitrate levels as high as 4526.3 mg/kg in some studies, with 70% of samples exceeding the acceptable daily intake (ADI) if 100 g were consumed daily (S. Luetic et al., 2023). Swiss chard also exhibited high nitrate levels, with some samples exceeding 3000 mg/kg (S. Luetic et al., 2023). These vegetables are particularly concerning because they are often consumed raw, retaining their nitrate content. Leafy vegetables often have nitrate levels ranging from 1,533 to 15,394 mg/kg in dry matter, with spinach and rucola among the highest (Sanja Luetic et al., 2023; Salehzadeh et al., 2020; Tamme et al., 2010). Root vegetables typically have lower nitrate levels, averaging around 3,709 mg/kg in dry matter (Salehzadeh et al., 2020). Fruit vegetables have the lowest nitrate levels, averaging about 3,332 mg/kg in dry matter (Salehzadeh et al., 2020). This difference arises because nitrates are absorbed and transported through the plant's xylem to the leaves, where they accumulate more than in storage organs like roots or fruits. Studies have consistently shown that nitrate concentrations in vegetables vary significantly across countries and even within different regions of the same country (León & Luzardo, 2020; Ranasinghe & Marapana, 2018; Tamme et al., 2006).

Nitrate levels in vegetables can be influenced by factors such as the type of vegetable, environmental conditions, farming methods, and processing techniques (Ram et al., 2022). Kyriacou et al. reported that the effect of radiation is significantly lower in areas with many sunny days compared to the effect of fertilization (Kyriacou et al., 2019). The limitation highlighted in our study is the lack of data on cultivation practices, fertilizer types, and environmental conditions, which their effect are confirmed in previous researches on nitrate accumulation in vegetables (Alemzadeh Ansari et al., 2019; Colla et al., 2018).

The higher nitrate levels in leafy vegetables are a natural part of their growth and development. Understanding these differences can help consumers make informed choices about their diet and appreciate the nutritional benefits of various vegetable types. Consuming a balanced diet with a variety of vegetables can help mitigate potential risks while maximizing nutritional benefits. The scientific literature highlights that leafy greens such as rucola and Swiss chard consistently show the highest nitrate concentrations among vegetables, posing potential health risks when consumed excessively or raw. Cooking methods can mitigate these risks by reducing nitrate content. However, regulatory frameworks need to address not only nitrates but also nitrites, which are currently unregulated despite their potential toxicity.

Excessive use of organic and inorganic fertilizers in agriculture can lead to significant nitrate accumulation in soils, posing environmental and health risks (Qasemi et al., 2024). Nitrate is a naturally occurring compound in vegetables, but excessive intake can pose health risks. The probabilistic non-carcinogenic risk assessment of nitrate in vegetables involves evaluating the potential health impacts of consuming vegetables with high nitrate levels. In this study a health risk assessment (HRA) method was used to evaluate the probabilistic non-carcinogenic risk

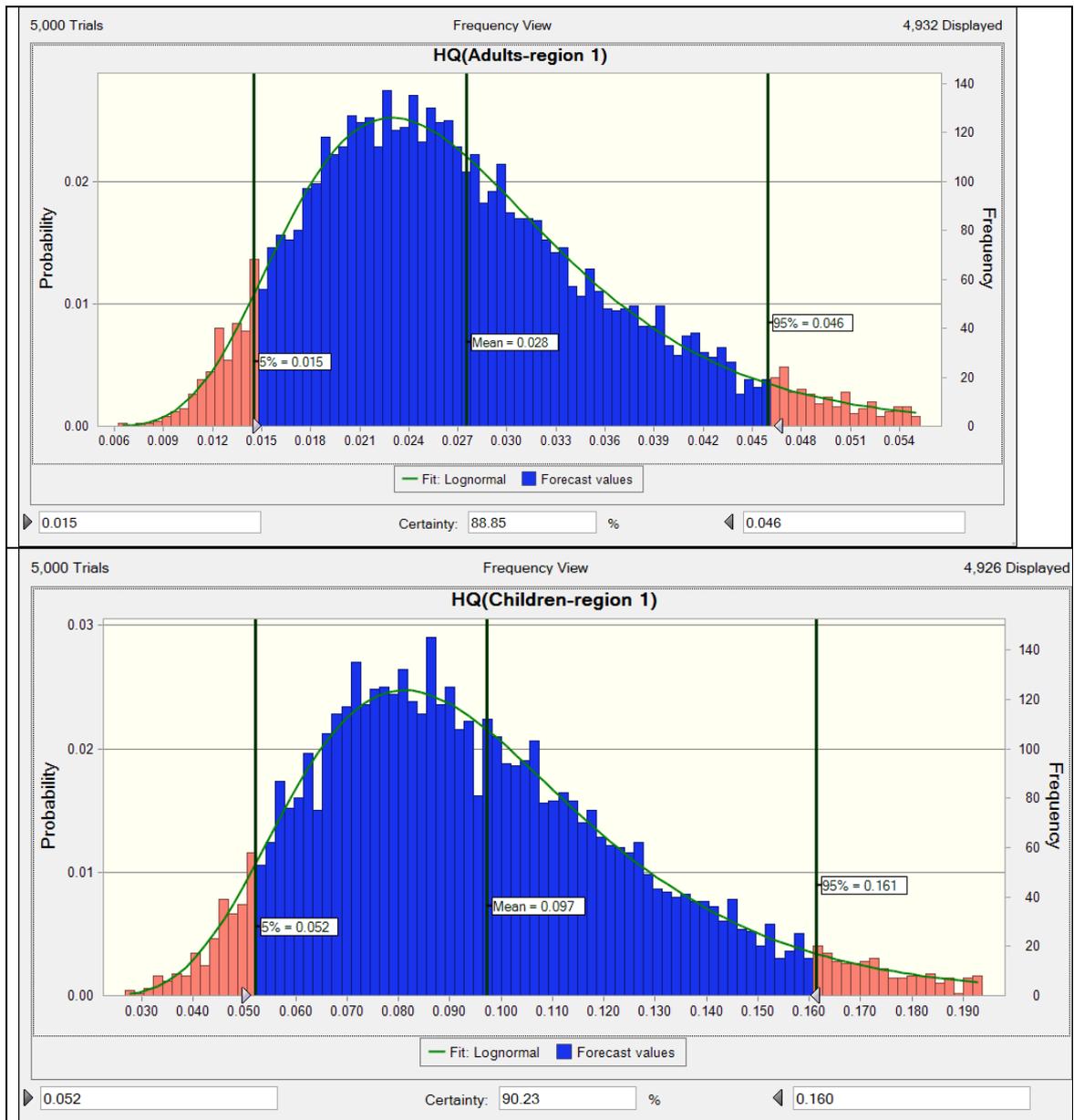


Fig. 3. Non-carcinogenic risk of nitrate in vegetables in adults and children in the region 1 of Minab city

assessment of nitrate in vegetables across two children and adults age groups. The mean of HQ in adults and children in region 1 were 0.028 and 0.096; for region 2 were as 0.027 and 0.096; and for region 3 were 0.028 and 0.090, respectively (Figure 3-5). The HQ values due to nitrate for adults and children in three regions were lower than 1 value, hence consumers are in acceptable health risk. Most studies indicate that the non-carcinogenic risk from vegetable consumption is generally within acceptable limits (Ali et al., 2021; Uddin et al., 2021), which are in line with the results of the present study.

In the present study, the HQ values for exposure to nitrate from vegetables in children were higher than adults. This suggests that children are the most vulnerable population to nitrate concentrations in vegetables. Consequently, we recommend that future studies prioritize addressing this demographic to better understand and mitigate potential health risks associated with nitrate exposure. The higher risk of nitrate intake for children is also supported by several

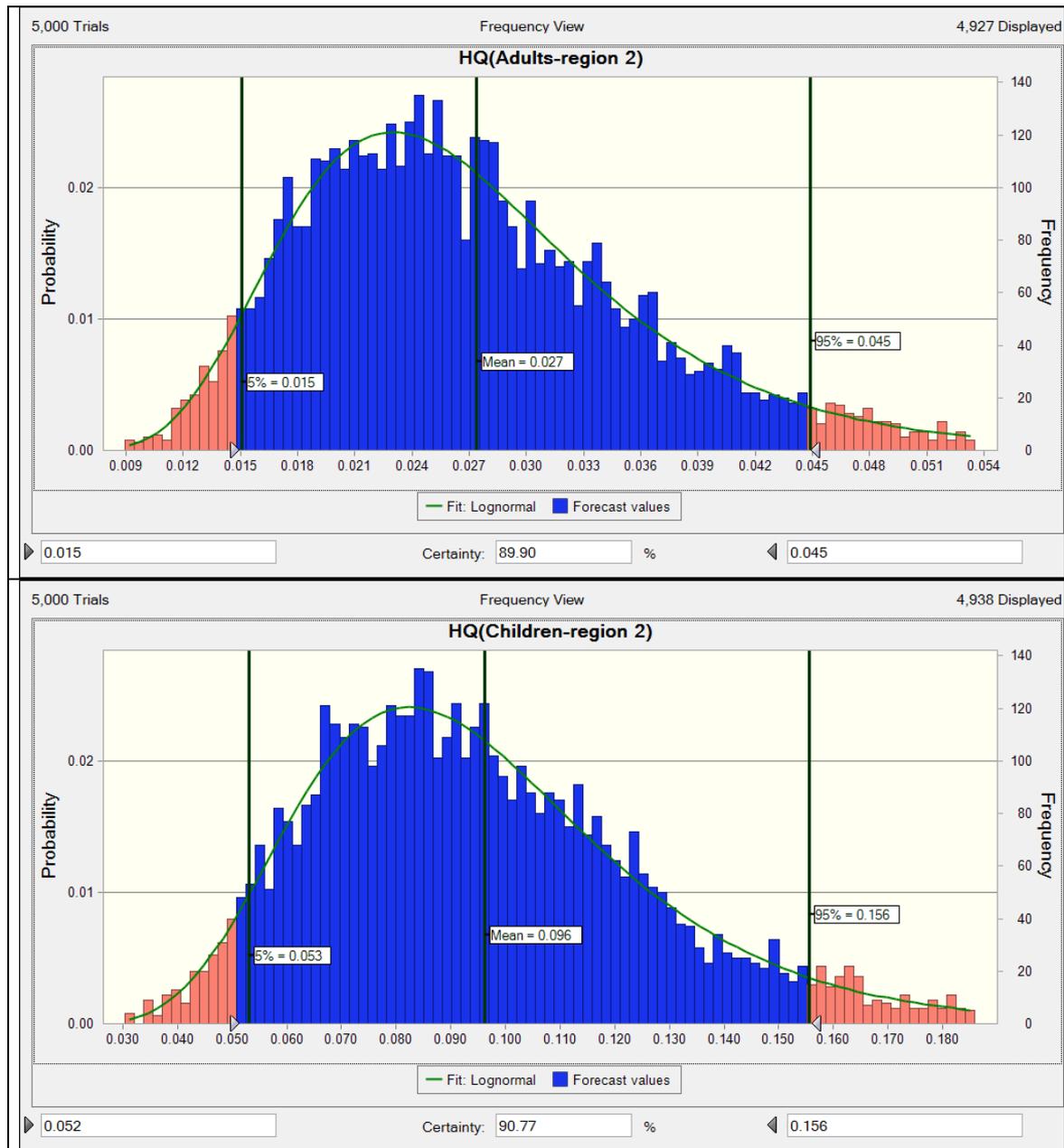


Fig. 4. Non-carcinogenic risk of nitrate in vegetables in adults and children in the region 2 of Minab city

studies (Haftbaradaran et al., 2018; Qasemi et al., 2024). Children are more vulnerable to nitrates due to lower stomach acidity compared to adults.

In recent years, the prevalence of non-communicable diseases (NCDs) has risen significantly (Bahardoust et al., 2023; Bahardoust, Mousavi, Ziafati, et al., 2024), driven by increasing levels of environmental pollutants alongside other contributing factors (Bahardoust, Mousavi, Dehkharghani, et al., 2024). In this study, the calculations were based on the nitrate concentrations of raw vegetables. However, this approach may lead to an overestimation of nitrate intake, as cooking and other forms of food preparation can significantly reduce nitrate concentrations. Previous studies have shown that cooking methods, such as boiling, can result in a substantial loss of nitrates due to leaching (De Martin & Restani, 2003; Sanja Luetic et al., 2023; Vahed et al., 2015). Cooking and frying process can indeed impact nitrate levels in vegetables, often reducing them by about 30–40%. However, the extent of this reduction can

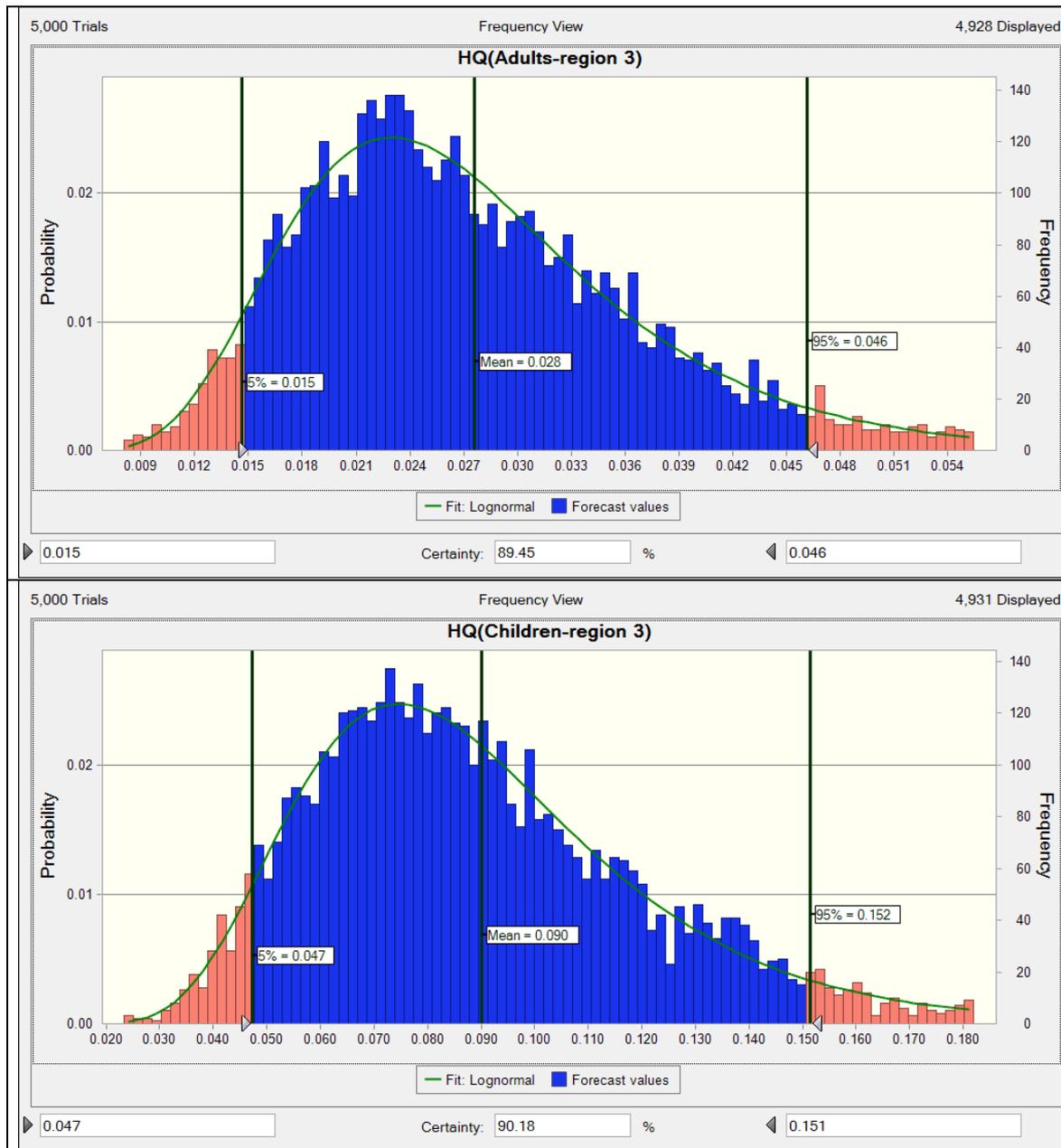


Fig. 5. Non-carcinogenic risk of nitrate in vegetables in adults and children in the region 2 of Minab city

vary based on several factors, including type of vegetable, cooking method, cooking time and temperature (Authority, 2008; Salehzadeh et al., 2020; Wu et al., 2021). To provide a more accurate assessment, it would be beneficial to consider the effects of cooking on nitrate levels or to analyze nitrate concentrations in cooked vegetables. This adjustment could help offer a more realistic estimate of nitrate intake from vegetables in a typical diet.

The overuse of fertilizers undermines agricultural sustainability by degrading soil quality, polluting ecosystems, and reducing nutrient-use efficiency. Balanced fertilization strategies, including precision agriculture and eco-friendly organic amendments, are essential for mitigating these impacts. Optimize fertilization and irrigation to reduce nitrate accumulation, educate consumers about the risks associated with high nitrate intake from certain vegetables, establish and enforce nitrate limits in agricultural products to ensure public health safety are highly recommended. To reduce health risks from nitrate accumulation in vegetables, farmers and

researchers should focus on implementing good agricultural practices and exploring strategies to minimize nitrate concentrations (Qasemi et al., 2024). Nitrate exposure from water, fruits, and processed foods also contributes significantly to daily intake. Future studies should assess these sources alongside vegetables for a holistic understanding of nitrate risks. By adopting these practices and addressing research gaps, it is possible to reduce nitrate levels in vegetables while ensuring food safety and sustainability. The limitations of the present study include the small number of samples, lack of data on cultivation methods, types of fertilizers used, and environmental/seasonal conditions, which should be considered when generalizing the results, as well as previous studies.

CONCLUSION

Based on the results, leafy vegetables exhibited the highest mean nitrate concentration, followed by bush and tuber vegetables. Nitrate levels in the studied regions pose a low health risk to both adults and children. The HQ values for nitrate exposure were consistently below 1 for all both adults and children groups' across the three regions. This suggests that the current nitrate concentrations in vegetables are within acceptable limits and do not present immediate health concerns. However, it's important to note that while the current risk levels are acceptable, ongoing vigilance is necessary. Continuous monitoring and management of nitrate levels in food sources should be maintained to ensure long-term safety. This approach is crucial because nitrate levels can fluctuate due to various factors such as agricultural practices, seasonal changes, and water source contamination. Furthermore, special attention should be given to other vulnerable groups such as infants and pregnant women, who may be more susceptible to nitrate-related health issues. By maintaining proactive monitoring and management strategies, potential future health hazards associated with nitrate exposure can be effectively mitigated, ensuring the continued safety of the food and water supply for all consumers.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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