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Potential Health Risk of Nitrate Accumulation in Vegetables Grown in Pol-e Dokhtar County

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KEYWORDS

Daily intake;
Nitrate;
Non-carcinogenic risk;
Total hazard quotient (HQs);
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ABSTRACT: Vegetables as one of the most important sources of nitrate, have positive and negative effects on human health. The goal of this paper was to estimate the nitrate concentration in vegetables production in Pol-e Dokhtar County as one of the main centers of Iranian vegetable production and the possible health risks correlated with high concentration of nitrate in theses crops. Using United States Environmental Protection Agency (USEPA) method, the risk was evaluated and presented as risk diagrams. Additionally, type, amount and method of fertilization in farms with different concentrations of nitrate in vegetables were presented. Almost, all results were within the range of nitrate concentrations in vegetables. Lettuce and Persian leek (716.200 and 378.500 mg NO_3^- kg^{-1} FW, respectively) were the most and least predominant contributors in total NO_3^- uptake, respectively. The risk diagrams showed that all values were lower than the acceptable levels for each of vegetables. Furthermore, in most age groups, this index was greater in women than in men. According to the information in the questionnaires, perhaps one of the most important factors in reducing the nitrate accumulation in the vegetables of Pol-e Dokhtar is due to optimal and timely use of fertilizer. Use of nitrogen fertilizers in form of split in 73% of fields decreased the nitrate concentration in plants with no reduction in yield. Therefore, considering crop yields and nitrate accumulation impacts, improved nitrogen management could provide an opportunity to promote production of vegetables and reducing the effects of negative health in high-risk regions in Lorestan Province.

INTRODUCTION

Nitrate is the main form of nitrogen uptake by plants. A major quality characteristic of vegetables due to its influence on human health is nitrate concentration [1, 2]. Nitrate decomposition products are much more toxic to the human body than nitrate itself [2]. Adequate nitrate concentration reduces the blood pressure, impede the

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platelet aggregation, and improves the endothelial dysfunction [3, 4].

Due to excessive use of nitrate fertilizers, nitrate concentrations in vegetables are higher than past. Approximately 80% of the total nitrate intake by human is provided by vegetables [5]. The contribution of vegetables in the entry of nitrate into the human diet has been shown in various studies [6-9] to develop a database to precisely assess nitrate exposure in different populations, thereby aiding in experiments regarding the health-related outcomes of dietary exposure to nitrate [10].

Minimum daily intake of nitrate for vegetables and fruits in developing countries has been suggested 400 g [11]. So, it is necessary to provide a solution to reduce the amount of nitrate consumed through vegetables [12]. By both the Joint FAO/WHO Expert Committee on Food Additives and the European Communities' Scientific Committee for Food, allowable daily intake (ADI) for nitrate has been set as 0-3.65mg kg^{-1} body weight [13]. According to studies, the concentration of nitrate in vegetables is from less than 1mg $100g^{-1}$ to greater than 1000mg $100g^{-1}$ [14, 15].

According to their nitrate concentration, vegetables can be divided into three groups: low nitrate (< 100 mg/kg), medium nitrate (100–1000 mg/kg) and high nitrate (> 1000 mg/kg) [16]. Research has shown that dark leafy vegetables have higher nitrate and nitrites concentrations than other vegetables [14, 17]. According to a study conduct in Italy, vegetables belonging to the families of *Chenopodiaceaceae*, *Brassicaceae*, *Apiaceae* and *Asteraceae* accumulated more nitrates, while *Convolvulaceae*, *Solonaceae* and *Liliaceae* contained the lowest levels of nitrates [13]. Raczuk et al. (2014) state that the nitrate concentrations of vegetable types varied with a range of 10-4800 mg kg^{-1}, with the highest value in radish (2132 mg kg^{-1}), and the lowest in cucumber (32 mg kg^{-1}) [18].

Obviously, the cumulative effect of nitrate can play a significant role in the development of different carcinogenic and non-carcinogenic diseases [19]. Therefore, risk assessment of these diseases due to consumption of nitrate-contaminated vegetables appears necessary. Risk assessment is the first phase of a set of risk management activities [20]. Risk management is a comprehensive process used to determine, identify, control and minimize the effects and consequences of potential events.

To provide a strategy to manage any risks to human health from dietary nitrate exposure resulting from vegetables consumption, an updated risk assessment considering the present and future amounts of consumption and nitrate concentration is needed. Thus, the current study aimed to evaluate human health risk related to nitrate via consumption of vegetables in Pol-e Dokhtar County as one of the main centers of Iranian vegetable production. For this purpose, at first, samples from vegetables were collected from farm fields, and nitrate concentration in the plant edible parts was measured. Then, the nitrate intake and the associated health risk for different age-sex receptor groups were assessed by the hazard quotient method. Finally, type, amount and method of fertilization in farms with different concentrations of nitrate in vegetables were presented.

MATERIAL AND METHODS

Study area

Lorestan Province is located in the west of Iran. Pol-e Dokhtar County is a county in Lorestan Province in Iran. In the 2016 census, its population was 73744.

Sample collection

Selection of a suitable size for sample is one of the most important factors in conducting such research. The stratified random sampling with an appropriate allocation is a relevant method for sampling in this study. In the case where we do not have the basic information about our target population and its characteristics, we can use the Cochran formula to estimate the total sample size based on the following formula (1):

$$n = \frac{NZ_{\frac{\alpha}{2}}^{2} pq}{(N-1)d^{2} + Z_{\frac{\alpha}{2}}^{2} pq} \quad (1)$$

Where n= sample size, N= population size, Z= the critical value of the normal distribution at α/2, p= the sample proportion, d= the margin of error. Since the population is not classified with the same size in each subpopulation, the proportional allocation method is used to assign a specified number of samples to each class as Equation 2 and was shown in Table 1.

$$n_i = \frac{N_i}{\sum N_i} n \qquad (2)$$

Table 1. Appropriate sample number based on the cultivated area for each vegetable with a 5% error rate (The cultivation area was obtained from the Organization of Agriculture Jahad Lorestan, 2018).

Vegetable	**Lettuce**	**Spinach**	**Cress**	**green onion**	**Radish**	**Persian Leek**
Area (ha)	60.2	96.2	105.8	91.95	111.8	37.15
Number of samples	10	16	16	15	17	6
Vegetable	Coriander	Parsley	Fenugreek	Dill	Mint	
Area (ha)	94.3	82.7	19	34.85	17.9	
Number of samples	15	14	3	6	2	

The total estimated sample size was 120 samples from edible parts of vegetables collected from different areas of Pol-e Dokhtar (including: Babazeid, Gol Gol, Murani, Paran Parviz, Vareh Zard, Khersdar,) (Figure 1) based on the predominant crop distribution and sizes of agricultural area, at the harvest time. Different types of vegetables in five groups were collected from each plot were mixed to obtain a sample. Fields were randomly chosen considering their size and crops. All samples were stored in polyethylene bags for transport at a constant temperature of 4^0C.

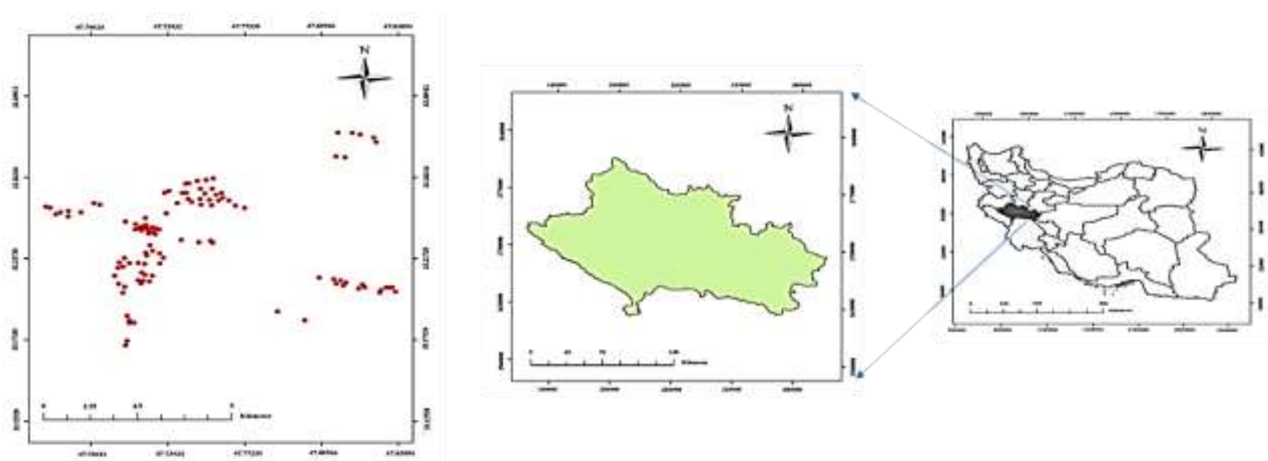


Figure 1. Location of sampling points

In this research, vegetables included lettuce (*Lactuca sativa L*), Persian leek (*Allium cepa*،), spinach (*Lepidium sativum*،), parsley (*Petroselinum crispum*،), cress (*oleracea Spinacia*), dill (*graveolens Anethum*), coriander (*Coriandrum sativum*), fenugreek (Trigonella foenum-graecum), mint (*Mentha Piperita*), radish (*Raphanus sativus var. sativus*) and green onion (*Allium fistulosum*).

Sample preparation

The plant samples were cleaned and oven-dried at 70°C for 48 h to a constant weight and ground in the centrifugal mill.

The difference between wet and dry weight of plant samples divided by dry weight was considered as the moisture content of the samples. Irrigation water was also analyzed. The water sample was put into polyethylene containers and filtered by filter paper No. 42, and then by using ion chromatography nitrate concentration were measured.

Nitrate analysis

Diazo method [21] which is based on nitrate recovery to nitrite in the presence of zinc powder and hydrogen ion was used to measure nitrate concentration in plant tissues. In this way, nitrite ions formed with sulfanilamide salts produce diazonomic compounds which is formed in the presence of N-1-naphthyethelenediamine dihydrochloride, the amino-azo complex. The color intensity of the color complex was measured at 540 nm by a spectrophotometer (Rayleigh, model UV1601).

Risk assessment methodology

Daily exposure assessment

Using the equation provided by the USEPA (1989) [22], average daily intake rates of nitrate were calculated. The daily intake by ingestion of each vegetable was estimated as Equation 3:

$$\text{Intake } (\mu g kg^{-1} day^{-1}) = (CF \times IR \times FI \times ED) / (BW \times AT) \quad (3)$$

Where CF = concentration of nitrate in food (mg g^{-1}), IR =ingestion rate (g d^{-1}) or (g L^{-1}), FI =fraction ingested from contaminated source (unit less), EF =exposure frequency (d y^{-1}), ED =exposure duration (y), BW =bodyweight (kg), and AT =averaging time that is the period over which exposure is averaged and calculated (d) as: AT =365 (d y^{-1}) ×ED (y).

The fraction ingested from the contaminated source (FI) represents the fraction of consumed food stuffs entered to the blood. The average FI value is 0.25, and the "worst-case" value is 0.4. The value 0.4, which can represent the upper 95th percentile, was used.

Risk assessment

According to the USEPA (1989), non-cancer risks were evaluated [22] (4): HQ = intake/RfD (4)

where HQ = non-cancer hazard quotient, RfD = reference dose (mg $kg^{-1}d^{-1}$). Table 2 presents the values of the parameters used in the above equations. A HQ ratio lower than 1 indicates an exposure that is likely to be without an appreciable risk of adverse health effects during a lifetime [23].

In this study, it is assumed that the maximum concentration of nitrate by the body weight to be ingested daily is less than 3.65 mg/kg and the average concentration of consumption vegetable for Iranian people is approximately 400 g day^{-1}. According to the information in Table 2, the ingestion rate (g d^{-1}), exposure frequency (d y^{-1}) and exposure duration (y) were suggested by other researchers as the basis of the calculation health risk assessment.

Table 2. Factors used in risk assessment

Parameters	Girls 7-14	Boys7 -14	Girls 14-18	Boys 14-18	Women 18-54	Men 18-54	Women >55	Men >55	references
ED (y)	7	7	4	4	27	27	15	15	[24]
EF (d y^{-1})	365	365	365	365	365	365	365	365	[23]
Body weight (kg)	39	35	56	59.1	61	76.4	60.6	65.1	[25]
IR (g d^{-1}) lettuce	7.3	7.3	7.3	7.3	29.3	29.3	29.3	29.3	[26]
IR (g d^{-1}) mint	2	2	2	2	9	9	9	9	[27]
IR (g d^{-1}) Parsley	2	2	2	2	9	9	9	9	[26]
IR (g d^{-1}) spinach	3.56	3.56	3.56	3.56	3.56	3.56	3.56	3.56	[26]
IR (g d^{-1}) dill	2.19	2.19	2.19	2.19	2.19	2.19	2.19	2.19	[26]

IR (g d^{-1}) leek	2.19	2.19	2.19	2.19	2.19	2.19	2.19	2.19	[26]
IR (g d^{-1}) Coriander	0.11	0.11	0.11	0.11	0.11	0.11	0.11	0.11	[26]
IR (g d^{-1}) Cress	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	[26]
IR (g d^{-1}) Fenugreek	0.01	0.01	0.01	0.01	0.3	0.3	0.3	0.3	[26]
IR (g d^{-1}) Radish	0.208	0.208	0.208	0.208	0.208	0.208	0.208	0.208	[26]
IR (g d^{-1}) Green onion	0.212	0.212	0.212	0.212	0.212	0.212	0.212	0.212	[26]

RESULT AND DISCUSSION

Nitrate concentration

The results showed that nitrate concentration of the irrigation water on farms was 18.6 mg L^{-1} (Table 3). Table 4 presents the standard deviation, minimum, average and maximum of nitrate concentrations in the studied samples were compared to the critical value (Table 4). In this study, the results were compared to the critical limits of the Iranian National Standards Organization (INSO) [28] and Welch (2003) [29].

Table 3. Some irrigation water parameters in the farms of Pol-e Dokhtar County

pH	EC (µS cm^{-1})	Ca^{+2}	Mg^{+2}	NO_3^-	HCO_3^-	SO_4^{2-}	Na^+	Cl^-
		mg L^{-1}						
7.79	603.7	35.8	21.3	18.6	127.6	59.7	19.8	43.5

Table 4. Standard deviation, minimum, average and maximum of the nitrate concentration in vegetable samples.

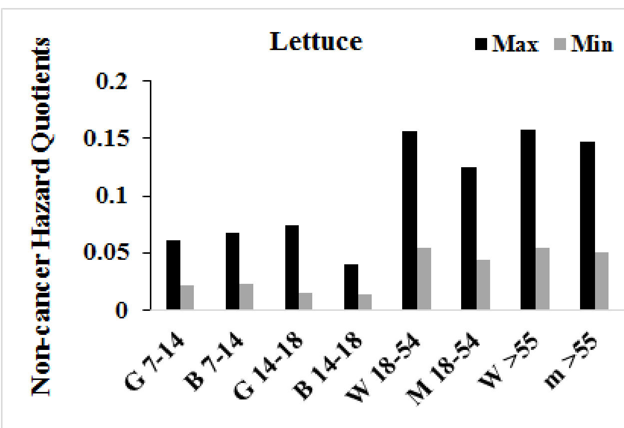
Vegetables		Nitrate concentration (mg kg^{-1} FW)				Critical value (mg NO_3^- kg^{-1} FW)	
		standard deviation	Min	mean	Max	Welch 2003 [29]	INSO [28]
Leafy vegetables	Lettuce (95%UCL)	71.5	452	716.200	1306	1500	1500
	Mint (95%UCL)	34.7	415	488	561	1000	1000
	Parsley (95%UCL)	25.1	356	453.571	579	1000	1000
	Spinach (95%UCL)	42.8	285	473.313	654	2000	2000
	Dill (95%UCL)	18.9	258	462.833	582	1000	1000
	Persian Leek (95%UCL)	15.2	278	378.500	613	1000	1000
	Coriander (95%UCL)	30.4	289	416.864	541	1000	1000
	Cress (95%UCL)	33.6	298	484.063	705	1000	1000
	Fenugreek (95%UCL)	29.2	296	534.667	681	1000	1000
Root vegetables	Radish (95%UCL)	64.3	267	574.471	951	1500	500
	Green onion (95%UCL)	59.3	246	516.133	705	1500	500

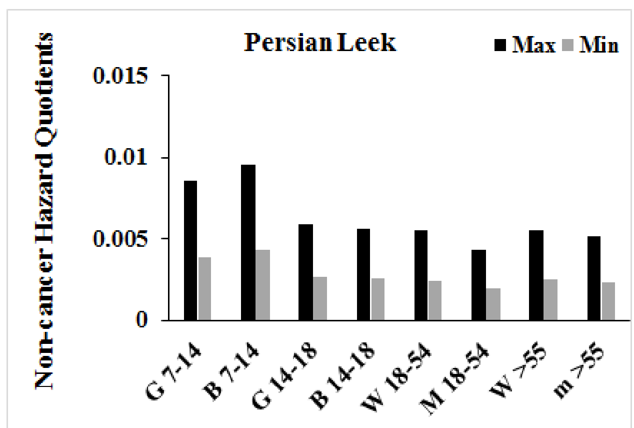
The highest and lowest mean of the nitrate concentrations were observed in lettuce (716.200 mg NO_3^- kg^{-1} FW) and Persian leek (378.5 mg NO_3^- kg^{-1} FW), respectively (Table 4). Based on Table 4, the minimum and maximum of the nitrate concentrations in lettuce (452 and 1306 mg kg^{-1} FW, respectively) were lower than the critical level of 1500 mg NO_3^- kg^{-1} FW. The acceptable level of spinach was 2000 NO_3^- mg kg^{-1} FW that even the maximum of the nitrate concentration in spinach samples was lower than it (Table 4). Generally, the maximum nitrate concentration in all of vegetable samples except radish and green onion was lower than the critical levels reported by welch and INSO (Table 4). The mean of the nitrate concentrations in radish and green onion was lower and higher than the critical level reported by welch (1500 mg NO_3^- kg^{-1} FW) and INSO (500 mg NO_3^- kg^{-1} FW), respectively.

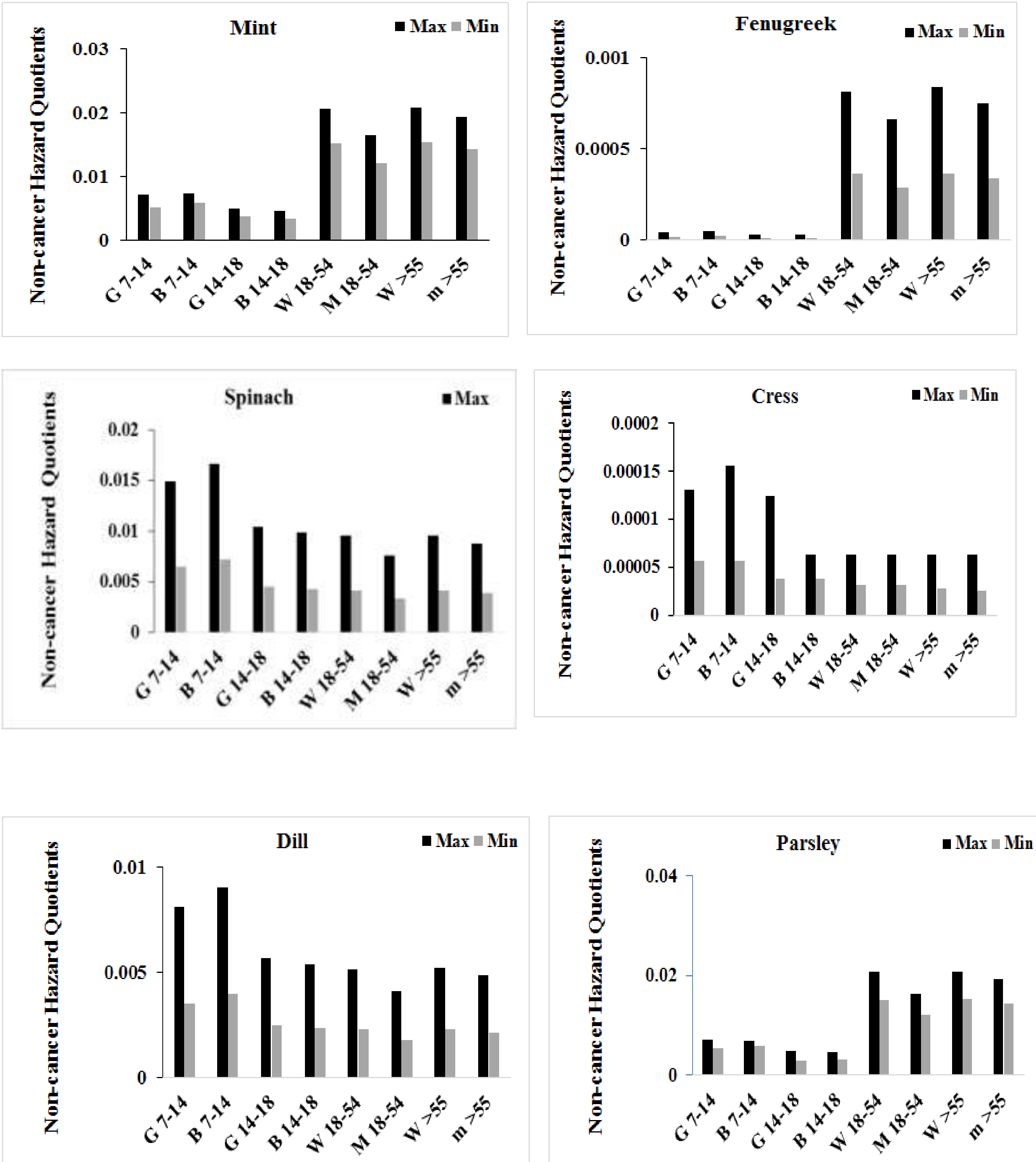
Non-cancer risk assessment

In this study, the risk related to nitrate in vegetables for different age groups was evaluated. Furthermore, the non-cancer hazard quotient (HQs) values is a better index compared to a traditional single-based critical concentration to monitor the possibility of harmful effects of nitrate accumulation in food crops. Figure 2 shows non-cancer risk diagrams regarding different vegetables ingestion in different age and sex groups under minimum and maximum nitrate concentrations. According to these results, total hazard quotient for all receptor groups is much smaller than 1 indicating that the potential non-cancer risk due to dietary intake of nitrate via vegetables consumption is acceptable. The highest non-cancer hazard quotient in spinach, dill, persian leek, radish, cress and green onion was observed in boys (7-14 years). However, in mint, lettuce and fenugreek, the highest non-cancer hazard quotient was observed in adults (women≥55 years).

It is noteworthy that in this study, the HQs values were not calculated for children under seven years of age, since vegetables are not an important source of nitrate exposure in children's diets (under 7 years) and contribute to the intake of less than 10% of nitrate. In this study, non-cancer risks were measured according to the USEPA (1989). In this method, owing to low weight and exposure time of children, it causes unrealistic predictions of the non-cancer hazard quotient. In this method, due to low weight and placement time, it causes unrealistic predictions. Therefore, these predictions were removed.






Mint
Max Min
Non-cancer Hazard Quotients
0.03
0.02
0.01
0
G 7-14
B 7-14
G 14-18
B 14-18
W 18-54
M 18-54
W >55
m >55
Fenugreek
Max Min
Non-cancer Hazard Quotients
0.001
0.0005
0
G 7-14
B 7-14
G 14-18
B 14-18
W 18-54
M 18-54
W >55
m >55
Spinach
Max
Non-cancer Hazard Quotients
0.02
0.015
0.01
0.005
0
G 7-14
B 7-14
G 14-18
B 14-18
W 18-54
M 18-54
W >55
m >55
Cress
Max Min
Non-cancer Hazard Quotients
0.0002
0.00015
0.0001
0.00005
0
G 7-14
B 7-14
G 14-18
B 14-18
W 18-54
M 18-54
W >55
m >55
Dill
Max Min
Non-cancer Hazard Quotients
0.01
0.005
0
G 7-14
B 7-14
G 14-18
B 14-18
W 18-54
M 18-54
W >55
m >55
Parsley
Max Min
Non-cancer Hazard Quotients
0.04
0.02
0
G 7-14
B 7-14
G 14-18
B 14-18
W 18-54
M 18-54
W >55
m >55

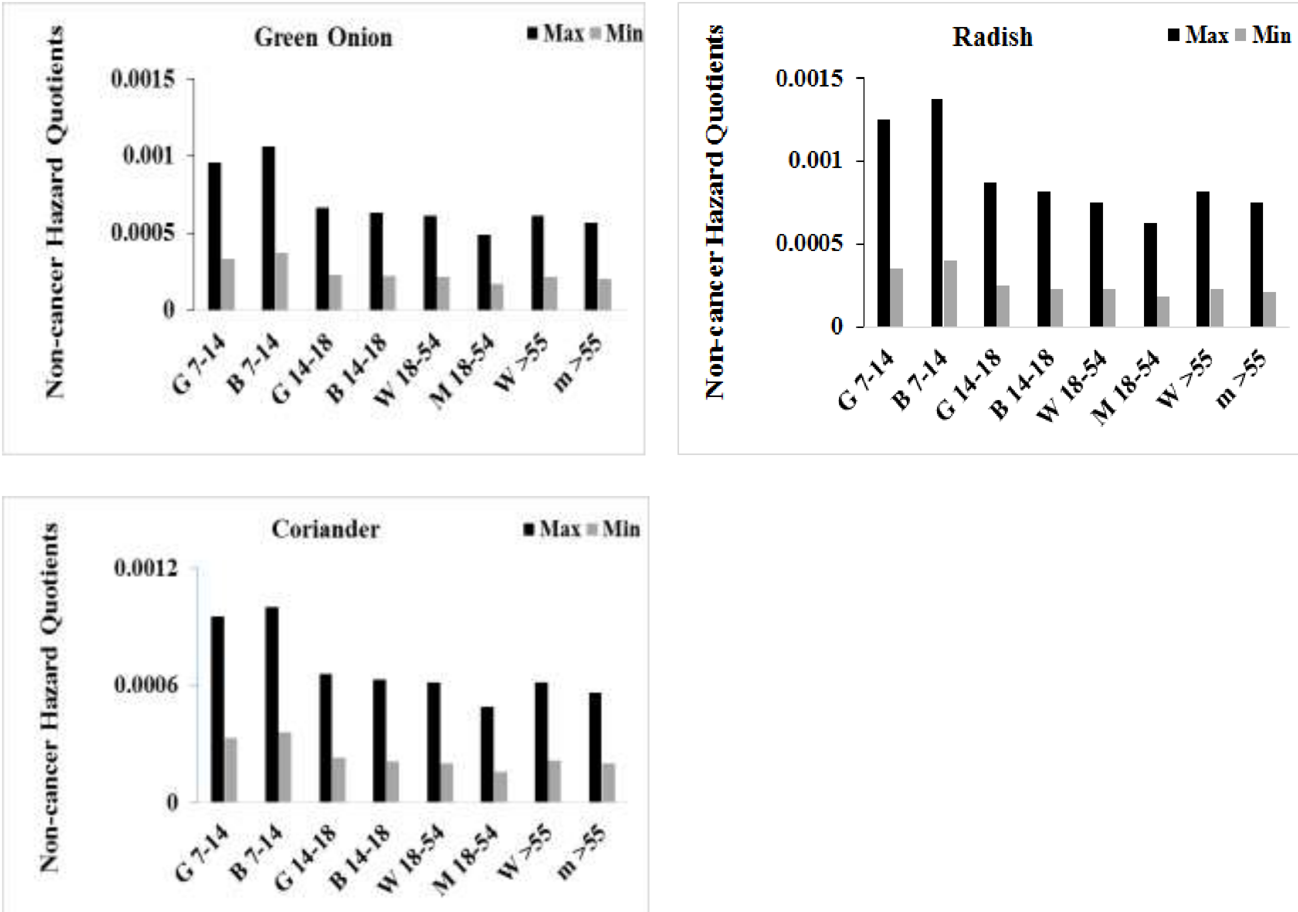


Figure 2. Maximum and minimum non-cancer hazard quotient of nitrate through ingestion of different vegetables (G: girls, B: boys, W: women, M: men)

Figure 3 presents the total non-cancer hazard quotient. Our results indicated that the THQ values for consuming vegetables together in this study for all different age groups were lower than 1. Thus, due to ingestion of this compound via consumption of vegetables, consumers are not at non-carcinogenic risk.

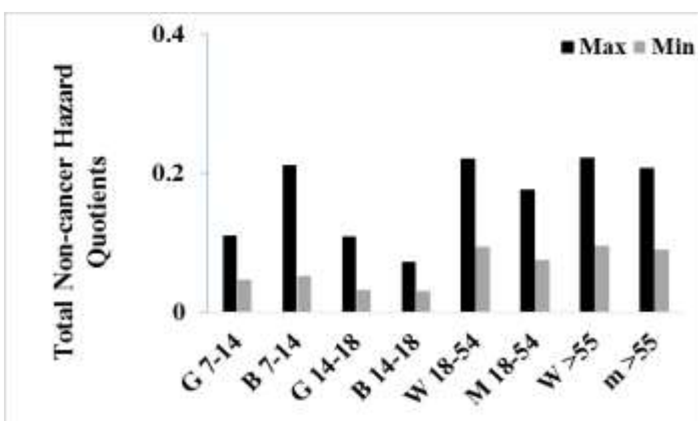


Figure 3. Maximum and minimum total non-cancer hazard quotient of nitrate through ingestion of vegetables (G: girls, B: boys, W: women, M: men)

Management questionnaire results

In the present study, the agricultural management of farmers in the analyzed fields was evaluated by a questionnaire on 42 farms, based on type, amount and method of fertilization. Table 4 presents a summary of the questionnaire results. According to Table 5, some reasons for the nitrate depletion in the vegetables of Pol-e Dokhtar County can be described as follows:

Table 5. Summary of the management questionnaire results (there was not enough information about fertilization in 5% of farms)

Nitrate concentration (mg kg^{-1} FW)	Percentage of fields	Type, amount and method of fertilization					
		Urea (kg h^{-1})	triple super phosphate (kg h^{-1})	potassium sulfate (kg h^{-1})	zinc sulfate (kg h^{-1})	micronutrient fertilizer (kg h^{-1})	Organic fertilizer (ton h^{-1})
<300	45%	<100 In three split	40- 70 pre-plant	60-100 One month after planting	20-30 one application after planting	10-20 one application after planting	5-7 pre-plant
300-400	28%	100-200 In three split	40-70 pre-plant	60-100 One month after planting	20-30 one application after planting	10-20 one application after planting	≤5 pre-plant
400-500	15%	200-300 one application immediately after planting	40-70 pre-plant	60-100 One month after planting	-	10≥ one application after planting	-
500≤	7%	300≤ one application immediately after planting	40-70 pre-plant	60≥ One month after planting	-	-	-

Additionally, Table 6 shows the results of the soil analysis of the study area.

Table 6. Physical and chemical properties of soil

parameter	**pH**	**EC**	**N**	**OC**	**Mg**
Quantity	7.44	1.43 (ds m^{-1})	0.173%	1.03 %	4.91 cmol kg^{-1}
parameter	**P**	**K**	**B**	**Mn**	**Zn**
Quantity	6.65 ppm	529.4 ppm	0.39 mg kg^{-1}	5.7 mg kg^{-1}	0.93 mg kg^{-1}
parameter	**Fe**	**Silt: Sand: Clay**	**$CaCO_3$**	**Cu**	
Quantity	3.8 mg kg^{-1}	(39:35: 26)%	22.3%	0.71 mg kg^{-1}	

In this study, a correlation was observed between the amount of nitrate accumulation in the edible parts of the plant, the amount and time of fertilizer application by farmers. The results revealed that the simultaneous use of organic and mineral fertilizers prevented the accumulation of nitrate in vegetables. Division of the nitrogen fertilizer required and application of micronutrient fertilizers improved assimilation in the plant.

DISCUSSION

Despite some reports indicating a high nitrate accumulation rate in vegetables produced in some regions of Iran [10, 30, 31], our results demonstrated that accumulation of nitrate in eatable sections of vegetables and its associated health risk in Pol-e Dokhtar County were below the acceptable level. In addition, there was a significant relationship between nitrate concentration and type of vegetables ($p \leq 0.05$). Among the studied vegetables, lettuce showed the highest nitrate accumulation because it has a greater ability to absorb nitrate by the roots and transfer it to the shoots [14]. Nitrate concentration was lower than in radish bulb than in other vegetables.

We found a downward trend for nitrate in leaves and roots, respectively. These findings are consistent with results of Jafari et al. (2001) in which the nitrate concentration in the tissue of vegetables tissue was in the following orders: leaf > stem > root > grain [32]. It has been shown that the nitrate accumulation and assimilation in vegetables are related to various factors, including genotype of the plant, soil properties, climate, culture density, growth period, and nitrogen source [33, 34].

Generally, the results of this study indicated that the concentration of nitrate in fresh weight in different studied vegetables was in the following order: lettuce > radish> Fenugreek > green onion>other leafy vegetables. In one study conducted by Pirsaheb et al. (2012), the average nitrate concentration in leafy vegetables was higher than root vegetables, and it was the lowest in fruit vegetables [35]. Nitrate anion is transported by the woody vessel and is therefore mainly present in the leaves. Also, fruits and seeds have lower nitrate concentrations than leaves [36]. To accumulate nitrate in the fruit, the amount of nitrate in the plant must be higher than the rate of nitrate assimilation in shoot potential. Generally, secondary compounds like proteins are stored in the fruit [37].

The HQs value of individual NO_3^- through consumption of vegetables should be less than one in order not to have the explicit hazards for the presence of these contaminants in the whole life of an area through consumption of vegetables [38]. In the present study, the risk value less than one for non-cancerous diseases in all cases (Figure. 2), the health risk was close to the limit of the acceptable risk value. This indicates that no significant adverse effects on a person's health will be observed during the period of a person's life due to absorption of nitrate by consuming the studied vegetables [22]. Therefore, the nitrate intake through such foods can be safe for the consumer. Aghili et al. (2009) in one study on the quality of vegetables grown in Isfahan Province reported that the HQ for all population groups via consumption of vegetables was smaller than 1 [24].

Assessment of the fertilization questionnaire demonstrated that the type, amount and method of fertilization affected surplus accumulation of nitrate in the studied vegetables, and the most important reason was often related to excess application of nitrogen fertilizers by farmers. The research results revealed a correlation between the content of nitrogen fertilizer applied by farmers and the nitrate accumulation level in the eatable parts of vegetables. The results of this research are same as those reported by Mehrabi et al. (2017) showing that the concentration of nitrate in soil is directly connection with the amount of nitrogen fertilizer application [39].

The results showed that the combined application of organic and chemical fertilizers prevented the accumulation of nitrate in the studied vegetables, and the application of micronutrients diminish the accumulation of nitrate in the plant. In most fields, the split application of nitrogen fertilizers leads to a decrease in nitrate concentration in vegetables without a reduction in yield and even no increase in the overall economic yield production. Haftbaradaran et al. (2018) showed that Factors such as the type, amount and timing of fertilization have a great impact on the amount of nitrate accumulation in vegetables [31]. According to the Iranian agricultural statistics (2017) [40], owing to increase in the price of nitrogen fertilizers, it appears that farmers' approach to production of vegetables in the farm has been based on more use of organic fertilizers. It can be one of the main reasons for the decreased nitrate concentration in vegetables in Pol-e Dokhtar County.

CONCLUSIONS

Monitoring of the nitrate concentration level in vegetables is extremely important. In the current study, the concentration of nitrate in edible parts of vegetables in farms of Pol-e Dokhtar County was measured. The results of the nitrate content in vegetables revealed that the highest amount of nitrate was found in plants tissue, which was significantly lower than the allowable level of nitrate daily intake. The highest amount of nitrate was found in lettuce (716 mg kg^{-1} FW) and radish (574 mg kg^{-1} FW). In addition, the results showed that despite considerable concern about accumulation of nitrate in vegetables, there was no potential risk for the residence health related to vegetables consumption. Health risks associated with nitrate exposure were not significant because THQ values of nitrate by consumption of vegetables and fruits were less than one. Considering the many benefits that vegetables have for human health, their consumption in terms of nitrates is improbable to be troublesome

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Conflict of interests

The authors declare that there is no conflict of interest regarding the publication of this article.

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