



## Risk Assessment of Nitrate and Heavy Metals Consumption through Selected Greenhouse Crops on Human Health

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### ABSTRACT

**Background:** The purpose of this research is to investigate the concentration of heavy metals and nitrate in the fruit of pepper, eggplant, cucumber, and tomato cultivated in greenhouses in Kermanshah County.

**Methods:** After sample preparation and extraction, the concentration of heavy metals with an atomic absorption and nitrate (No<sub>3</sub><sup>-</sup>) concentration were determined by a spectrophotometer at a wavelength of 410 nm. Subsequently, Human health risks were assessed using the Health Assessment Index of non-cancer diseases.

**Results:** Pepper exhibited elevated concentrations of nickel (Ni) and cadmium (Cd), while eggplant displayed higher levels of Ni, lead (Pb), and Cd. Cucumbers demonstrated an increased concentration of Cd, and tomatoes exhibited higher copper (Cu) and Cd levels, surpassing the standards set by the World Health Organization (WHO). A significant difference was observed between the studied vegetables and the concentration of heavy metals and nitrate. The highest daily intake rate and risk index of heavy metals and No<sub>3</sub><sup>-</sup> were observed in eggplant and tomato.

**Conclusion:** The risk index of heavy metals was found to be below 0.1. However, the risk index for No<sub>3</sub><sup>-</sup> exceeded 1. Therefore, it is recommended to study the potential sources of heavy metals contamination in the soil, water, and dust of the county to better understand the possible routes of food contamination.

## 1. Introduction

The production of nutritious agricultural products is an issue that is a globally significant concern. In recent years, Iran has also recognized the importance of this matter [1]. Several factors, including the improper use of chemical fertilizers and pesticides, inappropriate harvest time, the proximity of farms to mines, factories, industrial workshops, refineries, and busy rural roads can lead to the accumulation of harmful metals and compounds such as No<sub>3</sub><sup>-</sup> and heavy metals in plants [1]. Chemical pollutants endanger the environment, as well as human and animal health. Among these pollutants, heavy metals are particularly concerning due to their high atomic weight and density above 4 g cm<sup>-3</sup>. Unlike organic pollutants, heavy metals are not easily decomposed by living organisms and remain as stable compounds in nature. Vegetables play a crucial role in the

human diet, as they provide essential nutrients such as carbohydrates, proteins, vitamins, and minerals that have a beneficial effect on human health [2]. The exposure to heavy metals by consuming contaminated vegetables and their toxicity is a serious concern. Of particular significance are Pb and Cd, as they are classified among the most hazardous pollutants, possessing considerable toxicity and exerting detrimental impacts on various forms of living organisms. As a result, the amount of these metals in horticultural products should be under strict control and supervision. The absorption of heavy metals by plants is the main way of entering these metals into the human food chain, which is a great threat to human health. According to the reports, the absorption of heavy metals by plants may be different even in different cultivars of a particular species [3]. Pb and Cd enter the human body through the digestive and respiratory systems and accumulate in tissues. The excessive presence of



these metals in food leads to an increase in many diseases of the cardiovascular, respiratory, and nervous systems and also plays a role in carcinogenesis and mutagenesis [3]. If the concentration of Cd in the soil is  $0.3 \text{ mg kg}^{-1}$ , the amount of Cd in the tissue of vegetables is higher than the allowed limit [4]. According to the research conducted, the number of heavy metals (Cd and Pb) in onion (*Allium cepa* L.) samples collected from West Azerbaijan, Kurdistan, Hormozgan, Isfahan, and Zanjan provinces was higher than WHO standard [5]. Rice (*Oryza sativa*) is one of the staple foods in Malaysia. According to the research conducted, the amount of heavy metals including arsenic, Cu, and Cr in this product in the state of Perak is higher than the WHO standard and has shown a risk of non-cancerous diseases in adults and children [6]. In tomatoes (*Solanum lycopersicum*), the highest concentrations of Cu, Ni, Cr, manganese (Mn), and Pb were in root > leaf > stem > fruit, respectively, and the amount of Cu was higher than Ni, manganese, and Pb [7]. Nitrogen is the main limiting factor for most crops and  $\text{NO}_3^-$  is the main form of nitrogen absorbed by crops. Farmers often use nitrogen fertilizers to increase crop yield. As a result, many vegetables and forage crops accumulate high levels of  $\text{NO}_3^-$ .  $\text{NO}_3^-$  and nitrite are the essential metals for plant protein synthesis and play an important role in the nitrogen cycle [8].  $\text{NO}_3^-$  is a natural form of nitrogen and is formed from fertilizers, decaying plants, animal manure, and other organic residues [9]. Due to the increased use of synthetic nitrogen fertilizers and animal manure in agriculture, vegetables, and drinking water may now contain higher concentrations of  $\text{NO}_3^-$  than in the past. According to the type of diet, vegetables make up 72 to 94 % of the total absorbed  $\text{NO}_3^-$  [10].  $\text{NO}_3^-$  content of vegetables can be affected by food processing, fertilizer use, and growing conditions, especially soil temperature and light intensity (day). Vegetables whose roots, stems, and leaves are consumed have been reported to have high  $\text{NO}_3^-$  accumulation, unlike vegetables whose fruits are consumed [11]. The researchers listed the plant organs in order of decreasing  $\text{NO}_3^-$  content as follows: petiole > leaf > stem > root > inflorescence > tuber > bulb > fruit > seed [12]. In Kermanshah County, the amount of  $\text{NO}_3^-$  accumulation and human health risk assessment in radish (*Raphanus sativus*), basil (*Ocimum basilicum*), and coriander (*Coriandrum sativum*) vegetables with different irrigation sources including well water, treated sewage and the Qarasu river water were investigated. The results showed that the water of the Qarasu River leads to the accumulation of  $\text{NO}_3^-$  in the mentioned vegetables more than the other two sources. The best source of irrigation for vegetable cultivation in Kermanshah is well water, but all its physical, chemical, and microbial parameters should be investigated [13].  $\text{NO}_3^-$  accumulation in vegetables is influenced by multiple factors, including genetic characteristics (species and varieties), agricultural conditions (planting, growing and harvesting, soil type, fertilizer, etc.), and weather conditions [14, 15]. With the increase of greenhouse crops and the use of chemical fertilizers, it is important to check the concentration of heavy elements and  $\text{NO}_3^-$  in greenhouse products. So far, the concentration of  $\text{NO}_3^-$  and heavy metals,

as well as the risk assessment of consumption of greenhouse products in Kermanshah, have not been studied. Therefore, the purpose of this research is to study pollutants including  $\text{NO}_3^-$  and heavy metals in some greenhouse products of Kermanshah County.

## 2. Materials and Methods

### 2.1 Study area and sample collection

To investigate the concentration of  $\text{NO}_3^-$  and heavy metals in some greenhouse products, the present research was carried out in 2022 at the level of greenhouses in Kermanshah County (Figure 1). The study involved the sampling of cucumber (*Cucumis sativus*), pepper (*Capsicum annum*), tomato, and eggplant (*Solanum melongena*), taking into account the statistical information obtained from the Agricultural Jihad Organization of Kermanshah Province and the active greenhouse operations in the region. To ensure minimal weight loss and preserve tissue water content, the collected samples were immediately transferred to the physiology laboratory located at Razi University Campus of Agriculture and Natural Resources. After being transferred to the laboratory, the samples were washed with distilled water and then dried in an oven for 48 h at a temperature of  $75^\circ\text{C}$ . Once thoroughly dried, the samples were powdered with an electric mill.

### 2.2 $\text{NO}_3^-$ measurement

For the preparation of the extract, 40 mL of 0.025 M aluminum sulfate was added to 0.4 g of ground samples. To enhance sample transparency, approximately 0.5 g of activated charcoal was added to each sample. The samples were then shaken for 30 min by a shaker at 200 rpm. Finally, the resulting extracts were filtered [16]. In order to measure the  $\text{NO}_3^-$  concentration of the samples, 0.8 mL of sulfosalicylic 5 % was added to 1.5 mL of the prepared extract. This step resulted in a temperature increase within the samples. After allowing the samples to cool for 20 min, 17.7 mL of 2 M sodium was added. After the formation of a yellow color, the amount of  $\text{NO}_3^-$  in the samples was determined in terms of  $\text{mg kg}^{-1}$  DW of each sample with a spectrophotometer (Kerry 100 model, Varian, America) at a wavelength of 538 nm.

### 2.3 Measurement of heavy metals

The concentration of heavy metals, namely Ni, Cu, Pb, Zinc (Zn), Fe, and Cd in the samples was measured by the Seilsepour method [1]. The milled samples were poured into a China dish and placed in a furnace at  $500^\circ\text{C}$  for 4 h until a grey or white ash residue was obtained. Then, 10 mL of 2 M  $\text{HNO}_3$  was added to 0.3 g of ash of each sample, and placed in a bain-marie with a temperature of  $70\text{--}80^\circ\text{C}$  to be completely digested. After cooling, the samples were filtered and made up to 50 mL with deionized water. Next, the measurements of heavy metals in samples were performed with an atomic absorption spectrometer (model Phoenix 986. Biotec).

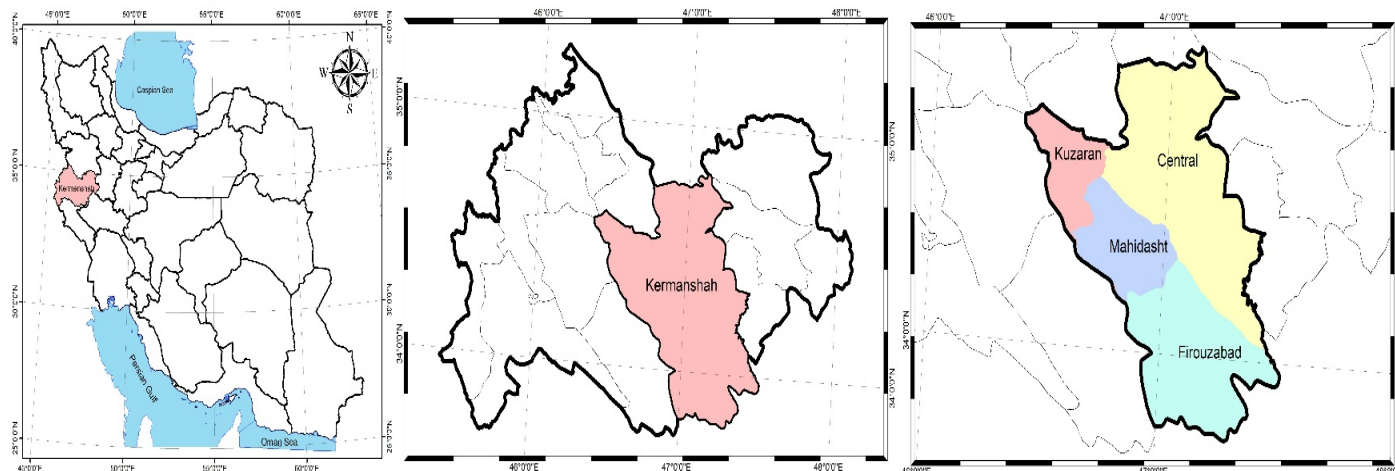


Figure 1. Location of the study area

## 2.4 Non-carcinogenic diseases risk index

Daily intake of vegetables is one of the main ways by which people are exposed to heavy metals [17]. The daily intake of heavy metals and  $\text{NO}_3^-$  by consuming vegetables is calculated from the following formula:

$$\text{EDI} = C \times \text{DC} / \text{BW}$$

EDI = Daily intake of heavy metals and  $\text{NO}_3^-$  in  $\text{mg kg}^{-1}$  of body weight (Estimated Daily Intake).

C = Concentration of heavy metals in  $\text{mg kg}^{-1}$

DC = Daily consumption of vegetables (the average daily consumption of vegetables in Iran is 100 g) [18].

BW = Average body weight for adults 65 kg [19].

This index is used as a tool to evaluate the threats caused by the consumption of food contaminated with heavy metals and  $\text{NO}_3^-$ .

$$\text{HQ} = \text{DIM} / \text{RFD}$$

HQ = Hazard Quotient

DIM = daily intake of heavy metals and  $\text{NO}_3^-$  in  $\text{mg kg}^{-1}$  of body weight (Estimated Daily Intake).

RFD = Reference Concentration (Reference Oral Doses) [20] A health assessment index greater than 1 indicates the risk of consuming food contaminated with heavy metals and  $\text{NO}_3^-$  for consumers [21].

In order to validate the analytical method, IDL (Instrumental Detection Limits), LOD (Limit of Detection), and LOQ (Limit of Quantity) values were calculated according to the following formulas [22].

$$\text{IDL} = \mu + 3\text{SD}$$

Where  $\mu$  is the average value produced by measuring blanks with the same reagent and SD is the standard deviation value.

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$$\text{LOD} = 3.3 \text{ SD} / \text{Slope}$$

$$\text{LOQ} = 10 \text{ SD} / \text{Slope}$$

SD = Standard deviation and slope = The slope of the regression line.

## 2.5 Statistical analysis

The statistical analysis of the data collected in this research was done using SAS software (version 1.9). The data were reported in terms of the average, standard deviation, and 95 % confidence interval. To compare the mean concentrations of heavy metals and  $\text{NO}_3^-$  in pepper, eggplant, cucumber, and tomato, one-way analysis of variance (ANOVA) and one-sample T-tests were performed at a significant level of 0.05. Finally, the obtained numbers were compared with the standard. Data normality test was performed before analysis.

## 3. Results and Discussion

IDL ranged from 0.0008 to 0.01  $\text{mg kg}^{-1}$ , which were below LOD indicating good sensitivity of the measuring instrument for analysis. For heavy elements, LOD ranged from 0.019 to 0.25  $\text{mg kg}^{-1}$ . The LOQ, value ranged from 0.063 to 0.83  $\text{mg kg}^{-1}$ . The amount of LOD and LOQ for  $\text{NO}_3^-$  was equal to 9.5 and 31.5, respectively (Table 1). The result shows both the LOD and LOQ values were higher than the IDL, indicating that the results of the analysis can be considered reliable [22].

Table 1. Validation Parameters

Metals	IDL (mg kg <sup>-1</sup> )	LOD (mg kg <sup>-1</sup> )	LOQ (mg kg <sup>-1</sup> )
Ni	0.002	0.13	0.43
Cu	0.001	0.12	0.40
Zn	0.0008	0.22	0.73
Pb	0.01	0.043	0.14
Fe	0.003	0.25	0.83
Cd	0.005	0.019	0.063
No <sub>3</sub> <sup>-</sup>	0.001	9.5	31.5

The results of mean, minimum and maximum, standard deviation, relative standard deviation, and T-test statistical number of the concentration of Ni, Cu, Pb, Zn, Fe, Cd, and No<sub>3</sub><sup>-</sup> in pepper, eggplant, cucumber, and tomato and the standard limit of the WHO of these heavy metals in Table 1 are reported. According to the results of the one-sample T-test, there was a significant difference between the concentration of heavy metals (Ni, Cu, Pb, Zn, Fe and Cd) and No<sub>3</sub><sup>-</sup> with the standard limit of the WHO ( $P \leq 0.05$ ) (Table 2). A diet containing vegetables is essential for maintaining optimal human health due to their significant contribution of fiber, vitamins, minerals, and antioxidants. Therefore, vegetables should be free of toxic factors such as heavy metals [23]. The presence of heavy metals not only affect the nutritional value of fruits and vegetables but also poses detrimental effects on consumers' well-being. For this reason, regulatory standards for acceptable limits of heavy metal content have been established at both national and international levels. Among the heavy metals, Ni and Cd are potentially dangerous as they offer no essential benefits to human health and even very low concentrations of these metals pose a cancer risk. In addition, high No<sub>3</sub><sup>-</sup> concentration is also a serious risk to human health, which leads to blue baby syndrome or methemoglobinemia [24]. The WHO has established recommended standard limits for Ni, Cu, Zn, Pb, Fe, Cd, and No<sub>3</sub><sup>-</sup> at 1.5, 40, 60, 0.3, 425, 0.1, and 200 mg kg<sup>-1</sup> respectively [25]. According to the obtained results, the concentration of Ni (1.5 mg kg<sup>-1</sup>) and Cd (0.32 mg kg<sup>-1</sup>) in pepper, the concentration of Ni (1.93 mg kg<sup>-1</sup>), Pb (0.32 mg kg<sup>-1</sup>) and Cd (0.38 mg kg<sup>-1</sup>) in eggplant and the concentration of Cu (44.50 mg kg<sup>-1</sup>) and Pb (0.27 mg kg<sup>-1</sup>) in tomato exceed the WHO standard limits (Table 2). It should be noted that the consumption of pepper, eggplant, cucumber, and tomato is not the only possible way of entering these heavy metals into the body, as they can also be ingested through other food sources and polluted air. According to the results obtained (Figure 2), there was a significant difference between pepper, eggplant, cucumber, and tomato in terms of the concentration of Ni, Cu, Zn, Cd, No<sub>3</sub><sup>-</sup> (at the probability level of one percent), lead and iron (at the probability level of five percent). Eggplant had the highest concentration of Ni (1.93 mg kg<sup>-1</sup>), Pb (0.32 mg kg<sup>-1</sup>), and Cd (0.38 mg kg<sup>-1</sup>), while cucumber had the lowest concentration of Ni (0.63 mg kg<sup>-1</sup>), Pb (15.0 mg kg<sup>-1</sup>) and Cd (0.17 mg kg<sup>-1</sup>). The results showed that the highest amount of Cu (44.50 mg kg<sup>-1</sup>), Zn (48.80 mg kg<sup>-1</sup>), Fe (142.00 mg kg<sup>-1</sup>), and No<sub>3</sub><sup>-</sup> (86.70 mg kg<sup>-1</sup>) was in tomato (Figure 2). The lowest concentration of Cu (21.70 mg

kg<sup>-1</sup>) and Zn (31.40 mg kg<sup>-1</sup>) was observed in cucumber. Pepper had the lowest amount of Fe (71.00 mg kg<sup>-1</sup>) and eggplant had the lowest amount of No<sub>3</sub><sup>-</sup> (27.00 mg kg<sup>-1</sup>) (Figure 2). The results of this research showed that the type of vegetable had a significant effect on the amount of heavy metals and No<sub>3</sub><sup>-</sup>, so eggplant (Ni, Pb and Cd) and tomato (Cu, Zn, Fe and No<sub>3</sub><sup>-</sup>) had the highest amount of heavy metals (Figure 2). Ni is one of the toxic heavy metals that leads to cancer and many other diseases in humans, unfortunately, the amount of Ni in eggplant was higher than the standard of the WHO. A high concentration of Cu is associated with liver and kidney damage. The concentration of Cu in tomato was higher than the standard (Figure 2). Cd exists in nature in very small quantities, but this amount causes toxicity. A high concentration of Cd causes bone defects and high blood pressure. Pb is absorbed in mineral forms, and lead poisoning causes kidney and reproductive system dysfunction and it damages the central nervous system as well [26]. Pepper, tomato, and eggplant are from the Solanaceae family. Since different genera of a family have common nutritional needs, this can be used to predict the amount of heavy metal accumulation in vegetables of the family [27]. Fruit vegetables play an important role in human health in terms of No<sub>3</sub><sup>-</sup> absorption. It seems necessary to train farmers to comply with the principles of fertilization, including the appropriate time, amount, and composition of fertilizers, and to cultivate genotypes with low No<sub>3</sub><sup>-</sup> accumulation capacity and harvest crops at the right time [14]. The amount of No<sub>3</sub><sup>-</sup> in tomato was higher than the other studied vegetables, and eggplant had the lowest amount of No<sub>3</sub><sup>-</sup> (Figure 2). According to the obtained results, the type of vegetable had a significant effect on the daily absorption of heavy metals and No<sub>3</sub><sup>-</sup> (Table 3). The highest amount of absorption of Ni, Pb, and Cd was observed in eggplant. While the maximum daily absorption of Cu, Zn, Fe, and No<sub>3</sub><sup>-</sup> was in tomatoes (Table 3). Based on the obtained results, the effect of the type of vegetable on the HQ was significant. The highest level of HQ was with the consumption of eggplant (Ni, Pb and Cd) and tomato (Cu, Zn, Fe and No<sub>3</sub><sup>-</sup>). In the studied heavy metals, the HQ was less than 1, while in No<sub>3</sub><sup>-</sup>, this rate was higher than 1 in all four studied vegetables (Table 4). According to the results obtained from the concentration of heavy metals in the studied vegetables (Figure 2), the maximum daily absorption rate (Table 3) and risk index (Table 4) of heavy metals and No<sub>3</sub><sup>-</sup> were observed in eggplant and tomato. The high concentration of heavy metals and No<sub>3</sub><sup>-</sup> in pepper, eggplant, cucumber, and tomato is associated with an increase in the daily absorption of heavy metals, which leads to an increase in the risk index of heavy metals with the consumption of these vegetables. If the level of HQ is less than 0.1, there is no risk due to vegetable consumption, while if this level is between 0.1 and 1, there is a small risk due to vegetable consumption. According to the obtained results (Table 3), the risk index of heavy metals in the studied vegetables is less than 0.1, which indicates that the risk is within the acceptable range and consuming the studied vegetables is not a problem for the consumer.



Table 2. The mean concentration of heavy metals and  $\text{NO}_3^-$  ( $\text{mg kg}^{-1}$ ) in the fruit of some greenhouse crops

Vegetable type	Heavy metals	Frequency	Mean concentration	Standard deviation of the concentration	Relative standard deviation	Minimum concentration	Maximum concentration	WHO standard limit	t*
Pepper	Ni	3	1.66	0.11	0.60	1.55	1.77	1.5	2.52
	Cu	3	20.91	10.00	47.80	10.91	10.91	40	-10.89
	Zn	3	39.20	1.00	2.50	38.20	40.20	60	-36.03
	Pb	3	0.18	0.10	55.55	0.08	0.28	0.2	-2.08
	Fe	3	71.00	1.00	1.40	70.00	72.00	425	-613.15
	Cd	3	0.32	0.01	3.12	0.31	0.33	0.3	38.80
	$\text{NO}_3^-$	3	39.50	1.00	2.53	38.50	40.50	200	-277.99
Eggplant	Ni	3	1.93	0.07	3.62	1.85	2.00	1.5	9.83
	Cu	3	30.35	10.00	32.94	02.35	40.35	40	-10.13
	Zn	3	43.60	1.00	2.29	42.60	44.60	60	-28.41
	Pb	3	0.32	0.10	31.25	0.22	0.42	0.2	0.47
	Fe	3	121.00	10.00	8.26	111.00	131.00	425	-52.65
	Cd	3	0.38	0.01	2.63	0.37	0.39	0.3	50.06
	$\text{NO}_3^-$	3	27.00	1.00	3.70	26.00	28.00	200	-299.64
Cucumber	Ni	3	0.63	0.10	15.87	0.53	0.73	1.5	-15.07
	Cu	3	20.17	10.00	49.57	10.17	30.17	40	-30.17
	Zn	3	31.40	1.00	3.18	31.40	32.40	60	-49.54
	Pb	3	0.05	0.10	200.00	0.05	0.25	0.2	-2.56
	Fe	3	105.00	1.00	0.95	104.00	106.00	425	-554.26
	Cd	3	0.17	0.01	5.88	0.16	0.18	0.3	12.99
	$\text{NO}_3^-$	3	55.80	1.00	1.79	54.80	56.80	150	-249.76
Tomato	Ni	3	0.92	1.00	108.69	0.84	1.00	1.5	-12.29
	Cu	3	40.45	10.00	24.72	30.45	50.45	40	7.8
	Zn	3	48.80	1.00	2.04	47.80	49.80	60	-19.40
	Pb	3	0.27	0.10	37.03	0.17	0.37	0.2	-0.42
	Fe	3	142.00	10.00	7.04	132.00	152.00	425	-49.02
	Cd	3	0.29	0.01	3.44	0.28	0.30	0.3	33.08
	$\text{NO}_3^-$	3	86.70	1.00	1.15	85.70	87.70	200	-196.24

\* t-student statistical number for the difference between the average number of heavy metals and  $\text{NO}_3^-$  with their standard limits

During the survey conducted in Gonabad vineyards, the amount of heavy metals Pb, Ni, Mn, Fe, Cu, Zn, cobalt (CO), arsenic (As), and Cd in grape (*Vitis L.*) was measured. The amount of Pb and Cd in grape samples was more than the standard limit of the WHO, but the HQ of non-cancerous diseases for the measured heavy metals was less than 1 [28]. Rice (*Oryza sativa*) is the main food of Bangladeshi people and they eat rice at least twice a day. As a result, the presence of heavy metals in rice grains has become a major concern in Bangladesh. Therefore, during research, heavy metals in rice grains and HQ were investigated in the Tangail region of Bangladesh. The concentration of Cr, Pb, As, and Cd was higher than the WHO standard. The HQ of non-cancerous diseases of Cu, Ni, As, Cd, and Pb metals was higher than 1, which indicates the risk of non-cancerous diseases [26]. Excessive use of animal manure and chemical fertilizers, pesticides, and irrigation of agricultural land with sewage water are the main reasons for changing the level of heavy metals in the soil, which affects the absorption of heavy metals by the root of the plant and their concentration in the plant [29]. The amount of Cu and Zn in cucumber and pumpkin (*Cucurbita L.*) has been more than the allowed limit

of the Food and Agriculture Organization (FAO), which is due to the high daily consumption of these vegetables so they are not suitable for human consumption [30]. Irrigation of the land where cucumber, tomato, and lettuce are cultivated with treated wastewater has led to an increase in the amount of Cu and Cd in these vegetables, which leads to an increase in the HQ of non-cancerous diseases [31]. In Kyrgyzstan, the amount of heavy metals in a number of leafy vegetables, including parsley (*Petroselinum crispum*), celery (*Apium graveolens*), basil, spinach (*Spinacia oleracea*), red cabbage (*Brassica oleracea* var. capitata f. rubra) and lettuce (*Lactuca sativa*), was investigated. The results showed that Cd, Cu, Fe, Mn, Ni, Pb, and Zn in the studied vegetables were within the allowed limit and only the HQ of parsley for non-cancerous diseases was higher than 1 [32]. The results of investigating the amount of heavy metals in tomato, onion, jute mallow (*Corchorus olitorius*), and spinach in three states of Nigeria showed that the amount of Ni in these vegetables was higher than the standard of the WHO and also Pb the HQ was higher than 1 [33]. The average concentration of Pb in the lands of Isfahan is reported to be  $26 \text{ mg kg}^{-1}$ , and it is because of the use of animal manure and atmospheric subsidence [34].

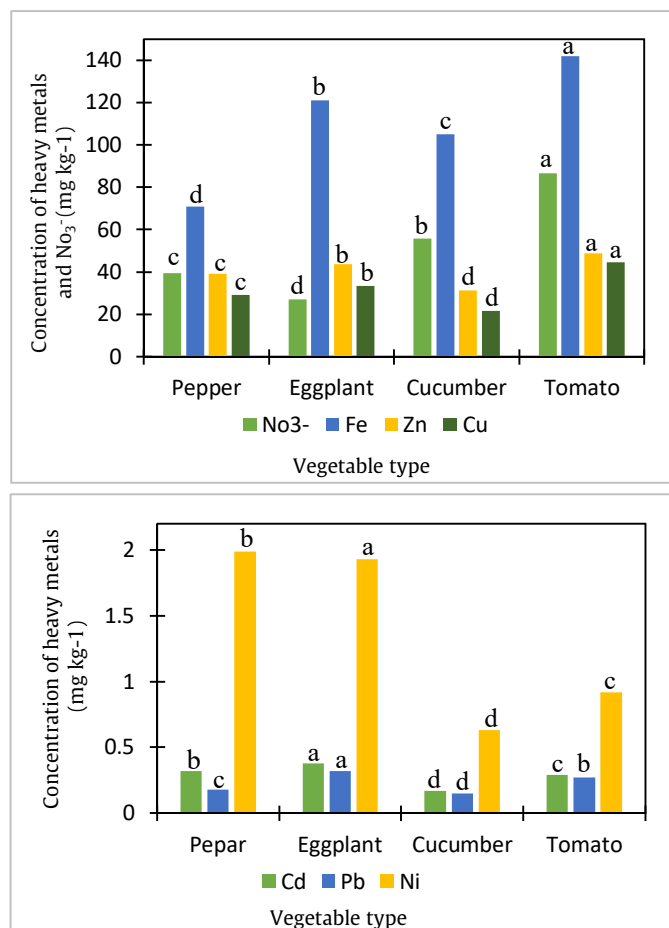


Figure 2. Comparison of the concentration of heavy metals and  $\text{NO}_3^-$  ( $\text{mg kg}^{-1}$ ) in the fruit of some greenhouse crop

The high concentration of Pb and Cd is related to the high consumption of animal manure and chemical fertilizers, especially chemical fertilizers with Cd and Pb impurities [35].  $\text{NO}_3^-$  in the body can turn into  $\text{NO}_2^-$ , which is a toxic substance. Then,  $\text{NO}_3^-$  in the stomach leads to harmful effects such as liver, pancreas, lung, esophagus, bladder, and colon cancers by forming N-nitrosamine [36]. There is a direct correlation between  $\text{NO}_3^-$  toxicity and its daily consumption. The WHO has determined the allowed limit of  $\text{NO}_3^-$  consumption for a 70 kg person at  $3.7 \text{ mg kg}^{-1}$  of body weight per day [37]. According to the obtained results (Table 3), the daily absorption rates of  $\text{NO}_3^-$  for pepper, eggplant, cucumber, and tomato are 3.35, 2.29, 4.74, and  $7.36 \text{ mg kg}^{-1}$  of body weight per day, respectively. As a result, the daily consumption of  $\text{NO}_3^-$  in cucumber and tomato is more than

the allowed limit of the WHO. The frequency of consumption and a person's weight have a great influence on the risk index related to  $\text{NO}_3^-$  and vegetable consumption. According to the results (Table 4), the HQ of pepper, eggplant, cucumber and tomato was 2.09, 1.43, 2.96, and 4.60, respectively, which indicates the risk of non-cancerous diseases by consuming these vegetables. Because the HQ is higher than 1. The risk of  $\text{NO}_3^-$  consumption as a result of vegetable consumption depends on the type of vegetable, environmental conditions, planting method, soil, and the length of the growth period [38]. In the study conducted by Jalali (2008), several vegetables in Hamedan were investigated in terms of  $\text{NO}_3^-$  accumulation. The results showed that the amount of  $\text{NO}_3^-$  accumulation in leek (*Allium porrum*), potato (*Solanum tuberosum*), onion, tomato, lettuce, and melon (*Cucumis melo* var. inodorus) is lower than the allowed limit of the WHO, and the HQ is less than 1, which means that there is no risk of non-cancerous diseases by consuming these vegetables [39]. The main vegetables that are offered in the market of Qom province include lettuce, tomato, onion, and potato. During research, the amount of  $\text{NO}_3^-$  in these four vegetables was investigated. According to the obtained results, the  $\text{NO}_3^-$  concentration in tomato and onion was higher than the allowed limit of the Iranian national standard. The daily consumption of  $\text{NO}_3^-$  is less than the allowed limit ( $3.70 \text{ mg kg}^{-1}$  of body weight per day) and the HQ of non-cancerous diseases is less than 1. As a result, the consumption of these four vegetables is not dangerous for the consumer's health [40]. An investigation on  $\text{NO}_3^-$  accumulation and human health risk assessment in vegetables irrigated with different irrigation sources reported that the highest amount of  $\text{NO}_3^-$  was in the basil that was irrigated with well water and chemical fertilizers were also used. The average daily consumption of vegetables grown in Kermanshah with any irrigation source is less than the allowed limit, which is not dangerous for the health of the consumer [13]. During the survey conducted in Mashhad, the  $\text{NO}_3^-$  content of some vegetables was measured. The results showed that the amount of  $\text{NO}_3^-$  in radish and lettuce was higher than the standard of the WHO, which poses a risk to children and adults [41]. Nitrogen nutritional management plays a role in improving  $\text{NO}_3^-$  concentration in agricultural products. In addition to the type and amount of nitrogen fertilizer used, in order to reduce the concentration of  $\text{NO}_3^-$  in edible vegetables, especially in the cold season of the year when the amount of  $\text{NO}_3^-$  accumulation is higher due to the decrease in the intensity of sunlight, the time of fertilizer use and the type and management of planting are also of particular importance.

Table 3. The amount of daily absorption of heavy metals and  $\text{NO}_3^-$  ( $\text{mg kg}^{-1} \text{ day}^{-1}$ ) by consuming the fruit of some greenhouse crops

Vegetable type	Ni	Cu	Zn	Pb	Fe	Cd	$\text{NO}_3^-$
Pepper	0.141 <sup>b</sup>	2.47 <sup>c</sup>	3.33 <sup>c</sup>	0.015 <sup>c</sup>	6.03 <sup>d</sup>	0.027 <sup>b</sup>	3.35 <sup>c</sup>
Eggplant	0.164 <sup>a</sup>	2.84 <sup>b</sup>	3.70 <sup>b</sup>	0.027 <sup>a</sup>	10.28 <sup>b</sup>	0.033 <sup>a</sup>	2.29 <sup>d</sup>
Cucumber	0.053 <sup>d</sup>	1.84 <sup>d</sup>	2.66 <sup>d</sup>	0.012 <sup>d</sup>	8.92 <sup>c</sup>	0.014 <sup>d</sup>	4.74 <sup>b</sup>
Tomato	0.078 <sup>c</sup>	3.78 <sup>a</sup>	4.14 <sup>a</sup>	0.023 <sup>b</sup>	12.07 <sup>a</sup>	0.024 <sup>c</sup>	7.36 <sup>a</sup>
<i>p-value</i>	**	**	**	*	*	*	**

\* In each column means that common letters are significantly different at the 5% level are Duncan's multiple range tests

Table 4. Risk index values of heavy metals and  $\text{NO}_3^-$  with fruit consumption of some greenhouse crops

Vegetable type	Ni	Cu	Zn	Pb	Fe	Cd	$\text{NO}_3^-$
Pepper	0.007 <sup>b</sup>	0.06 <sup>c</sup>	0.011 <sup>c</sup>	0.0038 <sup>c</sup>	0.0086 <sup>d</sup>	0.027 <sup>b</sup>	2.09 <sup>c</sup>
Eggplant	0.008 <sup>a</sup>	0.07 <sup>b</sup>	0.012 <sup>b</sup>	0.0069 <sup>a</sup>	0.0146 <sup>b</sup>	0.027 <sup>a</sup>	1.43 <sup>d</sup>
Cucumber	0.002 <sup>d</sup>	0.04 <sup>d</sup>	0.008 <sup>d</sup>	0.0032 <sup>d</sup>	0.0127 <sup>c</sup>	0.014 <sup>d</sup>	2.96 <sup>b</sup>
Tomato	0.003 <sup>c</sup>	0.04 <sup>a</sup>	0.013 <sup>a</sup>	0.0058 <sup>b</sup>	0.0172 <sup>a</sup>	0.024 <sup>c</sup>	4.60 <sup>a</sup>
<i>p-value</i>	**	**	**	*	*	**	**

\* In each column means that common letters are significantly different at the 5% level are Duncan's multiple range tests

## 4. Conclusion

According to the obtained results, the concentration levels of heavy metals and  $\text{NO}_3^-$  were found to vary depending on the vegetable type. The HQ for heavy metals in pepper, eggplant, cucumber, and tomato were reported to be less than 1, indicating a lower risk associated with the consumption of these vegetables. However, the HQ value for  $\text{NO}_3^-$  exceeded 1, suggesting a potential risk of non-cancerous diseases associated with the consumption of these vegetables.

## Authors' Contributions

Masoomeh Amerian: Project design; field research and laboratory analysis of samples; data analysis; writing manuscript.

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## Conflicts of Interest

The author declares no competing interests.

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## Ethical considerations

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