



## Original Article



# Investigating the Concentration of NO<sub>3</sub><sup>-</sup> and Heavy Metals in Some Leafy Vegetables in the Greenhouses of Kermanshah County

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**Abstract**

**Background:** Vegetables are an important part of healthy diet. This study investigated the concentration of heavy metals and nitrate (NO<sub>3</sub><sup>-</sup>) in leafy vegetables cultivated within selected greenhouses in Kermanshah County.

**Methods:** The concentration nickel (Ni), copper (Cu), zinc (Zn), lead (Pb), iron (Fe), and cadmium (Cd) were determined using an atomic absorption spectrometer. NO<sub>3</sub><sup>-</sup> content was measured utilizing a spectrophotometer at a wavelength of 410 nm. To assess the potential health risks associated with vegetable consumption, the health risk index (HRI) for non-cancerous diseases was employed.

**Results:** A significant difference was evident among the three examined vegetables regarding their concentrations of heavy elements and NO<sub>3</sub><sup>-</sup>. Spinach exhibited the highest NO<sub>3</sub><sup>-</sup> content at 203.33 mg/kg, while mint demonstrated the lowest at 113 mg/kg. Among the studied vegetables, spinach exhibited the highest concentrations of NO<sub>3</sub><sup>-</sup> (203.33 mg/kg) and Fe (177.66 mg/kg). Coriander showed the highest concentration of Cd (0.366 mg/kg) and Ni (1.900 mg/kg), while mint displayed the highest concentration of Cu (40.0 mg/kg). The concentration of Ni, Pb and Cd in all three studied vegetables was higher than the World Health Organization (WHO) limit. The HRI for non-cancerous diseases was less than one for the studied heavy metals, and it was higher than one for NO<sub>3</sub><sup>-</sup> in spinach (1.080).

**Conclusion:** Given that prolonged consumption of vegetables poses a risk of carcinogenesis to consumers, it is imperative to implement corrective measures and maintain ongoing monitoring of heavy metal and NO<sub>3</sub><sup>-</sup> levels in crops cultivated within greenhouses in Kermanshah.

**Keywords:** Food safety, Organic agriculture, Inorganic chemicals, Vegetables

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**Introduction**

Heavy metals in vegetables are a serious and direct threat to human health. The significance of heavy metal contamination in vegetables cannot be underestimated due to their pivotal role in the human diet. Vegetables, being a fundamental component of dietary intake, are susceptible to absorbing heavy metals and other toxins from polluted water and soil through their root systems, subsequently transferring these contaminants to humans. Thus, the assessment of food quality, particularly that of vegetables, is imperative.<sup>1</sup> Based on the findings, it is evident that vegetables may accumulate levels of heavy metals exceeding permissible limits for human consumption, even when the concentrations of these metals in soil or water remain within acceptable thresholds.<sup>2</sup>

The escalation of heavy element concentrations in urban environments of developing countries, such as Egypt and

Iran, can be attributed to rapid and unregulated urban and industrial expansions. This phenomenon is exacerbated by human activities and continues to intensify over time.<sup>3</sup> Important sources of heavy metals can be thermal power plants, coal mines, gold mines, urban runoff, etc. The soil contaminated with heavy elements acts as a source of heavy metals and transmits them to water, vegetables, animals and humans.<sup>4</sup> Heavy metals have garnered considerable attention due to their ubiquitous presence, capacity for toxicity at low concentrations, and extended persistence in the environment. Plants, including vegetables, exhibit a pronounced propensity to accumulate heavy metals, posing significant risks to human health even at minimal concentrations. Compounding this issue is the human body's limited capacity to eliminate these metals, thereby impeding the absorption of other essential elements.<sup>5</sup> The circumstances in underdeveloped and developing nations



are particularly acute owing to inadequate oversight of environmental regulations.<sup>6</sup> Despite the well-known health risks, farmers in many developing countries use municipal wastewater for irrigation. Indeed, the scarcity of freshwater resources has led to a significant rise in the utilization of municipal or industrial wastewater, both treated and untreated, for agricultural purposes. This practice has increasingly become a viable option for farmers, particularly in irrigating arid and semi-arid regions.<sup>7</sup> The absorption of heavy metals by vegetables leads to many problems, including reducing the immune system, the high prevalence of upper gastrointestinal cancer, and etc. Cd and Pb are the most toxic elements for humans that lead to disruption to the nervous system, bone softness and reproduction. Other elements such as Cr, Co and Ni are essential for men, but at high concentrations may cause metabolic disorders.<sup>8</sup>

Numerous studies have investigated the levels of heavy metals, including chromium (Cr), cadmium (Cd), lead (Pb), nickel (Ni), copper (Cu), zinc (Zn), iron (Fe), and manganese (Mn), in various vegetables.<sup>9,10</sup> Mi et al,<sup>11</sup> studied the accumulation of Pb, Cr, Cd, mercury (Hg) and arsenic (As) in 35 genotypes of Chinese cabbage (*Brassica rapa* L.). The results of this research showed that the amount of Cd in these genotypes was higher than the World Health Organization (WHO) limit. The accumulation and absorption of heavy metals in each vegetable depends on weather conditions, soil type, irrigation method, atmospheric sediments, concentration of heavy metals in the soil and maturity level of the vegetable at the time of harvest.<sup>12-14</sup> The investigation focused on assessing the concentrations of Pb, Cu, Cd, and Zn in three types of leafy vegetables (basil, leek, and lettuce) irrigated with both polluted water and fresh water in Hamadan city. The results of this research showed that, the concentration of investigated heavy metals was different in all three vegetables and the amount of Cd and Pb was higher than the WHO limit.<sup>7</sup> Also, the risk of these two heavy metals for non-cancerous diseases was above 1. So, regular monitoring of the content of heavy metals in the vegetables of this city is highly recommended.<sup>7</sup> Due to the high importance of vegetables and the increase in consumption and demand for vegetables in the market, intensive cultivation methods and excessive use of chemical fertilizers, especially nitrogen fertilizers, have increased. Mineral fertilizers such as ammonium nitrate ( $\text{NO}_3^-$ ), urea, and ammonium sulfate are the most widely used fertilizers, which often replace animal manure due to their ease of application and high solubility.<sup>15</sup>  $\text{NO}_3^-$  is absorbed by plant roots and transferred to aerial organs. According to research results, the leaf contains more  $\text{NO}_3^-$  than the root.  $\text{NO}_3^-$  accumulation is different in different plant species and some families such as Brassicaceae, Amaranthaceae, Chenopodiaceae, Asteraceae are known as  $\text{NO}_3^-$  accumulators. The amount of  $\text{NO}_3^-$  in vegetables is largely influenced by environmental (humidity, temperature, length of lighting period and light intensity)

and agricultural factors (levels and forms of nitrogen and availability of other minerals).<sup>16</sup> While  $\text{NO}_3^-$  itself is not inherently toxic to humans, its metabolites, namely nitrite and active nitrogen mediators, pose potential risks to human health. Upon ingestion,  $\text{NO}_3^-$  is absorbed by the digestive system and enters the bloodstream, where it combines with endogenously produced  $\text{NO}_3^-$  from various cellular sources within the body. Although the majority of  $\text{NO}_3^-$  is excreted via urine, approximately 25% is absorbed by saliva. Within the oral cavity, salivary  $\text{NO}_3^-$  is enzymatically converted into nitrite by bacteria. Subsequently, in the acidic environment of the stomach, nitrite can further metabolize into nitric oxide and other reactive nitrogen species, including N-nitrosamines, which are known carcinogens. Additionally, nitrite or nitric oxide can oxidize hemoglobin in red blood cells, converting it into methemoglobin, a form incapable of oxygen transport. Consequently, the metabolism of  $\text{NO}_3^-$  and nitrite may lead to adverse health effects.<sup>17</sup> Apart from its potential adverse effects,  $\text{NO}_3^-$  serves as a precursor of nitric oxide (NO), which is generated through the  $\text{NO}_3^-$ ,  $\text{NO}_2$ , NO pathway within the acidic environment of the stomach (Figure 1). Nitric oxide plays a pivotal role in human physiology, particularly in regulating cardiovascular functions and preserving nerve functionality.<sup>18</sup>

Food quality has emerged as a paramount global concern. In recent decades, heightened awareness and demand for nutritious foods have propelled research into the hazards associated with the consumption of foods contaminated with heavy metals.<sup>19</sup> In Iran, leafy vegetables are not only consumed fresh but also incorporated into various culinary preparations such as soups and stews. Moreover, research indicates that the accumulation of heavy metals and  $\text{NO}_3^-$  in vegetable leaves tends to be higher compared to other parts of the plant. The excessive application of chemical fertilizers, particularly nitrogen-based fertilizers, along with the utilization of pesticides and herbicides in greenhouse vegetable production, are significant contributors to the accumulation of  $\text{NO}_3^-$  and heavy metals. Given the adverse effects of these compounds on human health, it is imperative to establish ongoing monitoring protocols to assess the concentrations of  $\text{NO}_3^-$  and heavy metals in vegetables. Therefore, the present study aimed to determine the contamination of some leafy vegetables (spinach, coriander and mint) grown in the greenhouses of Kermanshah county with heavy metals and  $\text{NO}_3^-$  and evaluate their health risks.

## Materials and Methods

The current study was conducted in 2023 at the greenhouse level in Kermanshah County to examine the concentration of  $\text{NO}_3^-$  and heavy metals in select leafy vegetables. Initially, we determined a roster of greenhouse proprietors engaged in the cultivation of leafy vegetables. Subsequently, through direct engagement with these greenhouse facilities, the specific varieties of cultivated

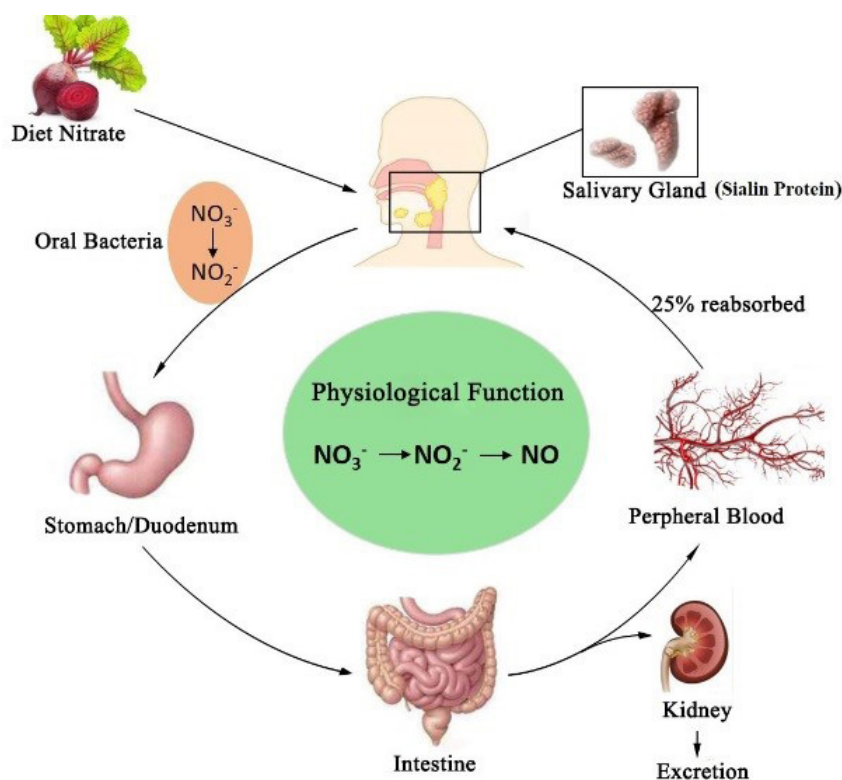


Figure 1.  $\text{NO}_3^-$  Fate in the Body

vegetables were ascertained. Given that leafy vegetables are predominantly cultivated during the winter months within greenhouse settings, there exists a constraint on the variety of vegetables available. Thus, we selected greenhouses that featured comparable vegetable varieties, including spinach (*Spinacia oleracea* L.), coriander (*Coriandrum sativum* L.), and mint (*Mentha piperita* L.). Ultimately, three greenhouses meeting these criteria were chosen for the study. It is noteworthy that certain greenhouse operators declined participation in the study. The collected samples after being transferred to the laboratory were washed with distilled water and then dried in an oven for 48 hours at a temperature of 75 °C. After drying, the samples were powdered with an electric mill. Powdered samples were used for Ni, Cu, Zn, Pb, Fe, Cd and  $\text{NO}_3^-$  concentration analysis.

#### Measurement of Heavy Metals

The extraction of heavy elements from the vegetables was conducted utilizing a digestion method employing three acids namely nitric acid, perchloric acid, and sulfuric acid, with a ratio of 1:1:5, as described in literature.<sup>16</sup> The concentration of heavy metals was determined using atomic absorption spectrophotometer (model Varian SpectraAA 220).<sup>20</sup>

#### $\text{NO}_3^-$ Measurement

To determine the concentration of  $\text{NO}_3^-$  in the samples, a method involving the addition of 40 mL of 0.025 M aluminum sulfate to 0.4 g of ground samples was employed. In order to render the samples transparent, approximately

0.5 g of activated charcoal was introduced into each sample. Subsequently, the samples underwent agitation for 30 minutes using a shaker operating at 200 rotations per minute. Finally, the resulting extracts were subjected to filtration.<sup>21</sup> Next, 0.8 mL of 5% sulfosalicylic acid was introduced to 1.5 mL of the prepared extract. Throughout this step, the samples underwent a temperature elevation followed by a subsequent cooling period. After 20 minutes, 17.7 mL of 2 M sodium hydroxide solution was incorporated into the samples. Once a yellow coloration appeared, the concentration of  $\text{NO}_3^-$  in the samples was determined in terms of mg/kg dry weight (DW) for each sample using a spectrophotometer (Kerry 100 model, Varian, America) operating at a wavelength of 538 nm.

#### Non-carcinogenic Diseases Risk Index

Consumption of vegetables on a daily basis constitutes one of the primary avenues through which individuals are exposed to heavy metals.<sup>22</sup> The daily intake of heavy metals and  $\text{NO}_3^-$  from vegetable consumption can be computed using equation 1.

$$\text{EDI} = C \times \text{FIR} / \text{WAB} \quad (1)$$

Where, EDI represents the estimated daily intake of  $\text{NO}_3^-$  and heavy metals in mg/kg of body weight; C denotes the concentration of heavy metals in mg/kg, and FIR is the daily consumption of vegetables. It should be noted that the mean daily consumption of vegetables is 100 g in Iran.<sup>23</sup> Additionally, in Equation 1, WAB represents the average body weight for adults, which is 65 kg. The

index used in Equation 2 serves as a tool to assess the risks associated with the consumption of food which has been contaminated with  $\text{NO}_3^-$  and heavy metals.<sup>24</sup>

$$\text{HQ} = \text{EDI}/\text{RFD} \quad (2)$$

Where, HQ denotes the hazard quotient, and RFD stands for reference concentration (reference oral doses).<sup>25</sup>

An assessment index with a value of higher than 1 indicates a potential risk linked to the food consumption which has been contaminated with  $\text{NO}_3^-$  and heavy metals.<sup>26</sup>

### Statistical Analysis

The statistical analysis of the data was conducted using SAS (version 1.9) software, developed by an American company based in the USA. The data were reported as average and its standard deviation along with 95% confidence interval. To compare the average concentrations of  $\text{NO}_3^-$  and heavy metals and in the vegetables, one-sample *t* test and one-way ANOVA were conducted at a significance level of 0.05. Subsequently, the obtained values were compared with the established standards. Additionally, the data were assessed for normality using SAS software.

### Results and Discussion

Table 1 represents average, maximum, minimum, standard deviation along the T-test statistics related to the Pb, Cd, Ni, Fe, Cu, Zn, and  $\text{NO}_3^-$  in coriander, spinach,

and mint. In the table, the standard limit of the WHO has been presented as well. Based on the one-sample T-test, a significant difference was observed between the concentration of  $\text{NO}_3^-$  and heavy metals compared to the standard limit introduced by the WHO across all four types of vegetables ( $P=0.05$ ). In spinach, the concentrations of  $\text{NO}_3^-$ , Ni, Pb, and Cd were observed to exceed the standard limits established by the WHO. Similarly, in coriander, elevated concentrations of Ni, Cu, and Cd were detected, while in mint, concentrations of Pb and Cd surpassed the WHO standards (Table 1).

Based on the results of the analysis of variance, it was determined that the type of vegetable exerted a statistically significant influence on the concentrations of Ni, Zn, Fe, and Cd at a probability level of 1%. Additionally, Cu, Pb, and  $\text{NO}_3^-$  concentrations were found to be significantly impacted by the type of vegetable at a probability level of 5% (Table 2). The results of the analysis of variance revealed a significant impact of the type of vegetable on the daily absorption of Zn, Fe, and Cd at a probability level of 1%. Furthermore, Ni, Cu, Pb, and  $\text{NO}_3^-$  concentrations were also significantly influenced by the type of vegetable at a probability level of 5% (Table 2).

Also, the type of vegetable exhibited a noteworthy effect on the risk index values of Zn and Fe. Similarly, the risk index values of Cd, Ni, Cu, Pb, and  $\text{NO}_3^-$  were significantly affected by the type of vegetable at a probability level of 5% (Table 2).

Table 1. Average Concentration of Heavy Metals (mg/kg) in Spinach, Coriander and Mint

Vegetable Type	Heavy Metals	Frequency	Average Concentration	Standard Deviation of Concentration	Minimum Concentration	Maximum Concentration	WHO Standard Limit	t*
Spinach	Ni	3	1.700	1.000	0.700	2.700	1.5	0.35
	Cu	3	30.000	10.000	20.000	40.000	40	-1.73
	Zn	3	25.000	10.000	15.000	35.000	60	-6.06
	Pb	3	0.300	0.100	0.200	0.400	0.2	0.00
	Fe	3	177.666	10.016	168.000	188.000	425	-44.77
	Cd	3	0.133	0.057	0.100	0.200	0.3	1.00
	$\text{NO}_3^-$	3	203.333	105.039	100.00	310.000	200	0.05
Coriander	Ni	3	1.900	1.000	0.900	2.900	1.5	0.69
	Cu	3	40.000	10.000	30.000	50.000	40	0.00
	Zn	3	32.000	10.000	22.000	42.000	60	-4.85
	Pb	3	0.200	0.100	0.100	0.300	0.2	-1.73
	Fe	3	151.000	11.532	140.000	163.000	425	-41.15
	Cd	3	0.366	0.057	0.300	0.400	0.3	8.00
	$\text{NO}_3^-$	3	140.333	10.016	130.000	150.000	200	-10.32
Mint	Ni	3	1.500	0.100	1.400	1.600	1.5	0.00
	Cu	3	36.000	11.532	25.000	48.000	40	-0.600
	Zn	3	35.000	10.000	25.000	45.000	60	-4.33
	Pb	3	0.366	0.152	0.200	0.500	0.2	0.76
	Fe	3	60.000	10.000	50.000	70.000	425	-63.22
	Cd	3	0.133	0.057	0.100	0.200	0.3	1.00
	$\text{NO}_3^-$	3	113.000	11.269	100.000	120.000	200	-13.37

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

According to the obtained results in Figure 2, a significant difference between spinach, coriander and mint in terms of the concentration of Ni, Cu, Zn, Cd, Pb, Fe and NO<sub>3</sub><sup>-</sup> was observed. The absorption rate of studied heavy metals was different in all three vegetables. In spinach, the highest concentrations were observed for NO<sub>3</sub><sup>-</sup> (203.33 mg/kg) and Fe (177.66 mg/kg). In coriander, the highest concentrations were recorded for Cd (0.366 mg/kg), Ni (1.900 mg/kg), and Cu (40.0 mg/kg). Mint exhibited the highest concentrations of Pb (0.366 mg/kg) and Zn (35.00 mg/kg) (Figure 2).

In the county of Kermanshah, there is a high consumption of leafy vegetables, and the increasing trend of greenhouse cultivation is evident due to limited land availability and water resources. The findings of this research indicate variations in the concentrations of heavy elements and NO<sub>3</sub><sup>-</sup> across different families of spinach, coriander, and mint. This suggests that the absorption of heavy elements and NO<sub>3</sub><sup>-</sup> by roots varies among different plant families. The use of nitrogen fertilizer is one of the most important things related to fertilizing vegetables, especially leafy vegetables. It is worth mentioning that the

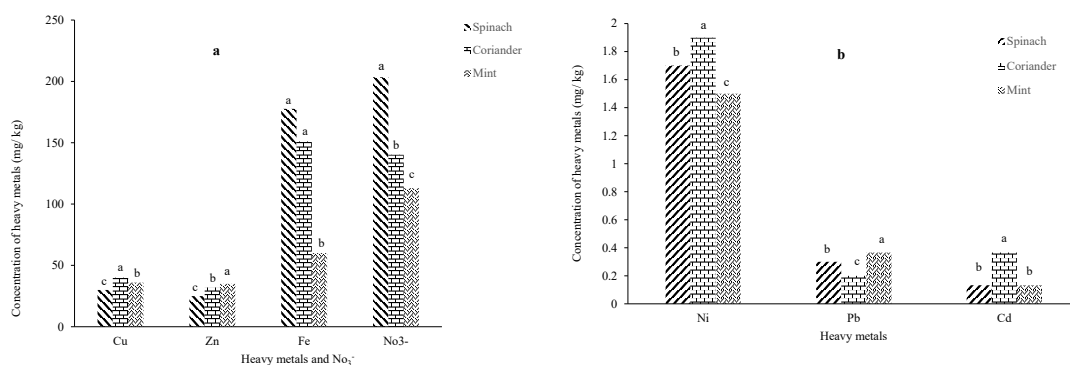
type, amount and time of chemical fertilizers, especially nitrogen fertilizer, have an effect on NO<sub>3</sub><sup>-</sup> accumulation in the plant.<sup>27</sup> NO<sub>3</sub><sup>-</sup> absorption by plant roots also depends on NO<sub>3</sub><sup>-</sup> concentration in the soil and soil pH. In addition to environmental factors (light, temperature, etc), genetic factors also affect NO<sub>3</sub><sup>-</sup> accumulation in plants.<sup>28</sup> It can be said that the lack of fertilizer management (nitrogen fertilizer) and the excessive use of pesticides and herbicides are the main reasons for the increase in the concentration of heavy elements and NO<sub>3</sub><sup>-</sup> in spinach, coriander and mint. Light intensity affects the amount of NO<sub>3</sub><sup>-</sup> accumulation in the plant. High light intensity typically decreases the accumulation of NO<sub>3</sub><sup>-</sup> in plants. However, in greenhouses, particularly during the colder seasons when leafy vegetables are cultivated, light intensity tends to be lower compared to outdoor conditions. Consequently, it is anticipated that vegetable tissues may contain elevated levels of NO<sub>3</sub><sup>-</sup>.<sup>29</sup> In Kermanshah, leafy vegetables are primarily cultivated in greenhouses during the cold seasons, characterized by low light intensity. Consequently, alongside the extensive use of chemical fertilizers, pesticides, and herbicides, the

**Table 2.** Results of Analysis of Variance Concentration, Daily Absorption and Risk Index Values of Heavy Metals and NO<sub>3</sub><sup>-</sup> in Spinach, Coriander and Mint

df	Mean Square							
	Ni	Cu	Zn	Pb	Fe	Cd	NO <sub>3</sub> <sup>-</sup>	
<b>Concentration of Heavy Metals and NO<sub>3</sub><sup>-</sup></b>								
Block	2	1.47 <sup>*</sup>	111.00 <sup>ns</sup>	300.00 <sup>**</sup>	0.007 <sup>ns</sup>	33.77 <sup>*</sup>	0.001 <sup>ns</sup>	4044.44 <sup>*</sup>
Treat	2	0.12 <sup>**</sup>	76.00 <sup>*</sup>	79.00 <sup>**</sup>	0.021 <sup>*</sup>	11418.77 <sup>**</sup>	0.054 <sup>**</sup>	6438.11 <sup>*</sup>
Error	4	0.27	111.00	0.012	0.017	149.77	0.004	3608.11
CV	-	10.56	9.81	15.09	16.15	9.44	11.57	19.46
<b>Daily Absorption of Heavy Metals and NO<sub>3</sub><sup>-</sup></b>								
Block	2	44.872 <sup>ns</sup>	0.8012 <sup>ns</sup>	0.570 <sup>**</sup>	0.000056 <sup>*</sup>	0.244 <sup>ns</sup>	0.000008 <sup>ns</sup>	29.221 <sup>ns</sup>
Treat	2	3.663 <sup>*</sup>	0.549 <sup>*</sup>	2.167 <sup>**</sup>	0.00015 <sup>*</sup>	82.500 <sup>**</sup>	0.000393 <sup>**</sup>	46.515 <sup>*</sup>
Error	4	8.241	0.8019	0.002	0.000128	1.082	0.000032	26.06
CV	-	10.56	19.81	10.09	16.15	9.44	11.57	19.46
<b>Risk Index Values of Heavy Metals and NO<sub>3</sub><sup>-</sup></b>								
Block	2	0.00915 <sup>ns</sup>	0.00050 <sup>ns</sup>	0.000024 <sup>**</sup>	0.00000351 <sup>*</sup>	0.00000050 <sup>ns</sup>	0.0000080 <sup>ns</sup>	0.1141 <sup>ns</sup>
Treat	2	0.1121 <sup>*</sup>	0.00034 <sup>*</sup>	0.0000063 <sup>**</sup>	0.0000095 <sup>*</sup>	0.000168 <sup>**</sup>	0.00039 <sup>*</sup>	0.1817 <sup>*</sup>
Error	4	0.0206	0.00050	0.002	0.0000080	0.0000022	0.000032	0.1018
CV	-	10.56	19.81	11.70	16.15	9.44	11.57	19.46

CV, Coefficient Variation

ns; non-significant effect; \* significant effect at the 0.05 level and \*\* significant effect at the 0.01 level.



**Figure 2.** Concentration of Cu, Zn, Fe, NO<sub>3</sub><sup>-</sup> (a), Ni, Pb and Cd (b) (mg kg<sup>-1</sup>) in spinach, coriander and mint

diminished light exposure serves as an additional factor contributing to the accumulation of heavy elements and NO<sub>3</sub><sup>-</sup> in spinach, coriander, and mint. Moreover, genetic factors, including plant type and species, play a pivotal role in NO<sub>3</sub><sup>-</sup> accumulation, attributable to the plant's capacity to produce the NO<sub>3</sub><sup>-</sup> reductase enzyme. This enzyme, serving as the principal catalyst in nitrogen metabolism, is inversely associated with NO<sub>3</sub><sup>-</sup> accumulation, with higher enzyme levels correlated with decreased NO<sub>3</sub><sup>-</sup> accumulation.<sup>30</sup> According to the findings of the current study, spinach leaves exhibited the highest concentration of NO<sub>3</sub><sup>-</sup> compared to coriander and mint (spinach > coriander > mint), surpassing the threshold established by the WHO, as illustrated in Figure 2. This observation aligns with the conclusions drawn by Luo et al.<sup>31</sup> In addition to the type of vegetable, the duration of the vegetable's growth period also influences the accumulation of NO<sub>3</sub><sup>-</sup> in its tissues. Generally, early vegetables tend to exhibit higher levels of NO<sub>3</sub><sup>-</sup> accumulation.<sup>32</sup> The growth period of spinach is shorter compared to that of coriander and mint. Therefore, the observed higher accumulation of NO<sub>3</sub><sup>-</sup> in spinach is consistent with expectations (Figure 2). Leafy vegetables, characterized by numerous mesophyll cells and vacuoles, serve as sites for NO<sub>3</sub><sup>-</sup> accumulation.<sup>28</sup> According to the studies, about 80% of the NO<sub>3</sub><sup>-</sup> that enters the body is through vegetables and fruits.<sup>33</sup> The WHO and the Scientific Committee of the European Union recommend a permissible daily intake of NO<sub>3</sub><sup>-</sup> through food and water ranging from 0 to 3.7 mg/kg of body weight per day. Therefore, an individual with an average weight of 70 kg should not exceed an intake of 259 mg of NO<sub>3</sub><sup>-</sup> per day.<sup>34</sup> In the current study, the daily absorption of NO<sub>3</sub><sup>-</sup> in spinach, coriander, and mint was found to be below the permissible limit, as outlined in Table 3. Based on the consumption levels of these vegetables, no adverse effects are anticipated. Conversely, research conducted in Shanghai, China, has indicated that the NO<sub>3</sub><sup>-</sup> content

in leafy vegetables exceeds the WHO limit. The primary contributor to elevated NO<sub>3</sub><sup>-</sup> levels in vegetable tissues is the application of chemical fertilizers. Therefore, the utilization of organic fertilizers presents an effective means to mitigate NO<sub>3</sub><sup>-</sup> accumulation. Additionally, it was observed that the highest NO<sub>3</sub><sup>-</sup> accumulation occurs approximately one week after fertilization, with the petiole and leaf exhibiting higher levels than the stems due to their lower metabolic activity.<sup>31</sup> As a result, greenhouse owners were advised to use organic fertilizers instead of chemical fertilizers and harvest leafy vegetables on time. Unfortunately, none of the greenhouse owners had done a soil test before planting, which will play an important role in fertilizer management.

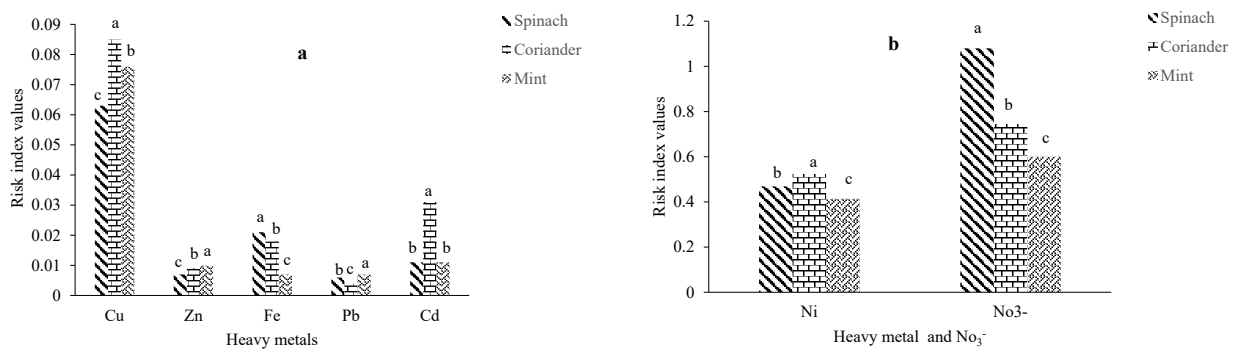
Based on the results provided in Table 3, the elevated concentrations of heavy metals in all three types of vegetables were correlated with an augmentation in the daily uptake of heavy metals and NO<sub>3</sub><sup>-</sup>. Spinach exhibited the highest daily absorption of Fe at 15.101 mg/kg.day, a value not significantly distinguishable from that of coriander at 12.835 mg/kg.day. Coriander displayed the highest daily absorption rates of Ni (10.498 mg/kg.day), Cu (3.400 mg/kg.day), and Cd (0.031 mg/kg.day). Mint demonstrated the highest daily absorption of Zn at 2.975 mg/kg.day. Additionally, spinach manifested the highest daily absorption of NO<sub>3</sub><sup>-</sup> at 17.283 mg/kg.day (Table 3). These findings collectively indicate that the elevated levels of heavy metals in all three vegetable varieties were accompanied by an increase in the daily uptake of heavy metals and NO<sub>3</sub><sup>-</sup>.

Based on the results presented in Figure 3, the NO<sub>3</sub><sup>-</sup> health assessment index value for spinach (1.080) exceeded 1. The heightened concentration of heavy metals and NO<sub>3</sub><sup>-</sup> observed in the leaves of the examined vegetables was correlated with an elevation in the daily uptake of heavy metals and a correspondingly high health evaluation index (Figure 3).

**Table 3.** The Amount of Daily Absorption of Heavy Metals and NO<sub>3</sub><sup>-</sup> (mg/kg.day) by Consuming the Leaf of Some Greenhouse Vegetable

Vegetable Type	Ni	Cu	Zn	Pb	Fe	Cd	NO <sub>3</sub> <sup>-</sup>
Spinach	9.393 <sup>b</sup>	2.550 <sup>c</sup>	2.125 <sup>c</sup>	0.025 <sup>b</sup>	15.101 <sup>a</sup>	0.011 <sup>b</sup>	17.283 <sup>a</sup>
Coriander	10.498 <sup>a</sup>	3.400 <sup>a</sup>	2.720 <sup>b</sup>	0.017 <sup>c</sup>	12.835 <sup>b</sup>	0.031 <sup>a</sup>	11.928 <sup>b</sup>
Mint	8.288 <sup>c</sup>	3.060 <sup>b</sup>	2.975 <sup>a</sup>	0.031 <sup>a</sup>	5.100 <sup>c</sup>	0.011 <sup>b</sup>	9.605 <sup>c</sup>

In each column, the shared letters denote groups that are significantly different at the 5% level, as determined by Duncan's multiple.



**Figure 3.** Risk index values of, Cu, Zn, Pb, Fe, Cd (a) Ni, NO<sub>3</sub><sup>-</sup> (b) in spinach, coriander and mint

Currently, contamination of agricultural products with heavy metals, including fruits and vegetables, has become one of the most important health concerns.<sup>6</sup> The presence of heavy metals not only contributes to environmental concerns but also facilitates increased absorption by plants, thereby resulting in a cascade of complications and diseases in humans. Unlike many organic pollutants, heavy metals are resistant to decomposition by living organisms, with most forming stable compounds within natural environments. These metals exhibit strong affinity for absorption by living tissues, and due to their prolonged biological half-life, they are challenging to eliminate from tissues, leading to their accumulation within the body.<sup>7</sup> In the research conducted by Zheng et al.<sup>35</sup> in the industrial city of Huludao, China, the concentration of heavy metals in vegetables was higher than the permissible limit, and the risk index for non-cancerous diseases was higher than one for both age groups of children and adults. The results of the research conducted by Babaakbari et al.<sup>36</sup> showed that the concentration of Zn and Cu in basil, garden cress (*Lepidium sativum*), radish (*Raphanus sativus* var. *sativus*), leek (*Allium ampeloprasum*), mint, parsley (*Petroselinum crispum*) and savory (*Satureja hortensis*) vegetables was more than the WHO limit, and the health risk index (HRI) of non-cancerous diseases was higher than one. In fact, the accumulation of heavy metals in leafy vegetables is more than tuberous and root vegetables. Consumption of vegetables is the most important route of human exposure to heavy metals that can threaten human health. The risk index for the studied vegetables was less than one, which indicates that the risk is within the acceptable range (Figure 3). There are no adverse effects of non-cancerous diseases for the studied elements from the consumption of spinach, coriander and mint produced in the greenhouses of Kermanshah. In the research conducted in the city of Riyadh, Saudi Arabia, the amount of Fe, Hg and Cd in parsley and spinach was higher than the WHO limit, which was attributed to atmospheric and industrial pollution.<sup>37</sup> In vegetables irrigated with wastewater, the risk index for heavy metals Cd and Mn was reported to be more than one, which means that the consumption of these vegetables seriously threatens the health of consumers.<sup>38</sup> In Hamadan county, the use of unrefined irrigation water has led to an increase in the amount of Pb, Cd, Cu and Zn in basil (*Ocimum basilicum*), leek and lettuce (*Lactuca sativa*), which is associated with high risk of non-cancerous diseases. The irrigation of leafy vegetables with tannery wastewater has resulted in heightened accumulation of heavy metals, particularly exceeding the WHO limit for Cd and Cr. Consequently, the risk index for cultivated vegetables has been reported to surpass a value of 1.<sup>39</sup> According to the results obtained from the examination of the amount of heavy elements in leafy vegetables including spinach, coriander and mint in Bangladesh, the amount of Cr, Cd, Ni and Cu is more than the daily consumption limit, which indicates a potential risk to health with the

consumption of these vegetables.<sup>6</sup> Also, the high amount of heavy elements and  $\text{NO}_3^-$  in the studied vegetables can be due to the high consumption of chemical fertilizers.<sup>40</sup> The high amount of Pb and Cd elements in vegetables can accumulate in the bones, kidneys and liver. They can also adversely affect the reproductive system. Chemical fertilizers containing phosphate typically contain approximately 0.0005 to 0.5 mg/kg of Cd, a heavy metal that accumulates in the soil upon the application of such fertilizers.<sup>41</sup> The high amount of Cd in all three vegetables can be due to the excessive use of insecticides and fungicides containing Cu and Cd, which is also absorbed through the aerial parts (stem and leaf) in addition to the roots.<sup>6</sup>

### Conclusion

This study revealed that the amount of Ni, Pb and Cd in spinach, coriander and mint, as well as the amount of  $\text{NO}_3^-$  in spinach was more than the WHO limit. However, the health risk was more than one only for spinach due to the high accumulation of  $\text{NO}_3^-$ , which indicates the risk of non-cancerous diseases by consuming this vegetable. Therefore, it seems necessary to monitor the amount of heavy metals and  $\text{NO}_3^-$  in leafy vegetables in greenhouses in Kermanshah. Simultaneously, it is crucial to acquaint greenhouse owners with the consequences of excessive use of chemical fertilizers, as well as with the principles of nutritional management and the analysis of soil and water employed within greenhouses.

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### Competing Interests

The author declares no competing interests.

### Ethical Approval

Not Applicable.

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