



## Seasonal Variation and Health Risk Assessment of Cd, Pb, and Nitrate in Vegetables and Fruits Available in North West of Iran

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### ABSTRACT

**Introduction:** Given the significant role of fruits and vegetables in the Iranian diet, this study aimed to evaluate the health risks linked to the heavy metals in 6+ fruits and vegetables, specifically cadmium (Cd) and lead (Pb), as well as nitrate in household diets.

**Materials and Methods:** A total of 1941 fresh samples were collected from East and West Azerbaijan province in Iran across three seasons. Then, the concentration of Cd, Pb, and nitrate were examined.

**Results:** Analysis using SPSS software version 20 found that all vegetable samples contained heavy metals and nitrates, with Cd levels within permissible limits. Pb concentrations varied by season: 40.4-280.33 ppb in spring, 36.77-283.67 ppb in summer, and 6.21-236.23 ppb in autumn. Pb levels exceeded the national standard organization of Iran (INSO) limit in several samples, including greenhouse cucumbers (48.48%) and conventional cucumbers (62.22%), onions (48.71%). Nitrate concentrations included 45.33-3390 ppb in spring, 9.66-3581 ppb in summer, and 34.28-3281.94 ppb in autumn, with exceedances in samples such as spinach (92%) and leafy vegetables (100%). Despite these exceedances, the target hazard quotient (THQ) for Cd, Pb, and nitrates was below 1, indicating no immediate health risks for the Iranian population.

**Conclusion:** While the presence of Pb and nitrates in certain vegetable samples raises concerns regarding food safety and compliance with health standards, the overall assessment suggests that the levels of these contaminants do not currently pose a significant health risk. Continuous monitoring and adherence to safety standards are essential to ensure public health safety.

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### Introduction

Maintaining high food quality and safety is crucial for public health. It is essential for food in the market to be free of harmful chemical contaminants that could endanger consumers<sup>1</sup>. The consumption of a diverse range of fruits and vegetables is beneficial for addressing nutrient

deficiencies and promoting positive health outcomes<sup>2</sup>. Both the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) recommend the consumption of 400 g of fruits and vegetables each day to help prevent chronic diseases<sup>3</sup>. The consumption level of agricultural food items is a crucial indicator of

community health, particularly in less developed nations<sup>4</sup>. However, contaminated vegetables and fruits can also contribute to the spread of pollutants and pose a threat to human health<sup>5</sup>. The use of both inorganic and organic agrochemicals on crop fields, along with the utilization of untreated sewage for irrigation, can result in the buildup of heavy metals and nitrates in soils and plants, potentially contaminating the food supply<sup>6</sup>.

Heavy metals are naturally occurring elements recognized for their high atomic weight and density<sup>7</sup>. In contrast to organic pollutants, these metals do not easily break down in the environment. These metals present a considerable threat to human health as they can penetrate the body through different routes, including food, water, air, and dermal exposure, and tend to accumulate in organs and living organisms. Heavy metals, such as lead (Pb) and cadmium (Cd), are of particular concern because of their non-biodegradability and adverse effects on humans and animals<sup>8</sup>. Human exposure to Cd and Pb primarily occurs through food consumption, accounting for up to 80–90% of the daily dose<sup>9</sup>. Cd exposure has been associated with lung and prostate cancers and kidney and bone diseases<sup>10</sup>. Pb has been found to impair hematological, cardiovascular, and neurological systems<sup>11</sup>. The International Agency for Cancer Research (IARC) categorizes Cd and its compounds as group 1 carcinogens, whereas Pb and its compounds are considered 'probably' carcinogenic to humans and fall under group 2A. Additionally, the European Food Safety Authority (EFSA) panel on contaminants in the food chain (CONTAM) has set a tolerable weekly intake (TWI) of 2.5 µg per kg of body weight for Cd and 25 µg per kg for Pb<sup>12,13</sup>.

Nitrates are naturally occurring water-soluble inorganic compounds found in various food items, including fruits and vegetables<sup>14,15</sup>. When nitrates in food are converted into nitrites and nitrogen acids in the gastrointestinal tract, they can combine with primary and secondary amines to produce nitrosamines, which are linked to serious health issues such as stomach, intestine, bladder, and mouth cancers, as well as bone cancer in

fetuses<sup>16,17</sup>. Nitrosamines are also associated with methemoglobinemia, commonly known as blue baby syndrome, particularly in children<sup>18</sup>. According to the International Agency for Research on Cancer (IARC), nitrate is classified as a Group 2A carcinogen<sup>16</sup>. Additionally, nitrites can reduce the nutritional value of fruits and vegetables by destroying carotenoids and vitamins A and B<sup>19</sup>. Various factors contribute to the nitrate levels and accumulation in vegetables and fruits, such as the species or genotype of the plant, agronomic practices (including the timing, concentration, and type of nitrogen used), environmental conditions during growth (such as light intensity, spectral quality, photoperiod, air temperature, and carbon dioxide levels), the stage of harvest, and the specific time of day when harvesting occurs<sup>20,21</sup>. In 2002, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) reaffirmed an Acceptable Daily Intake (ADI) of 3.7 mg.kg<sup>-1</sup> for nitrate, equivalent to 222 mg of nitrate per day for a 60 kg adult<sup>22,23</sup>.

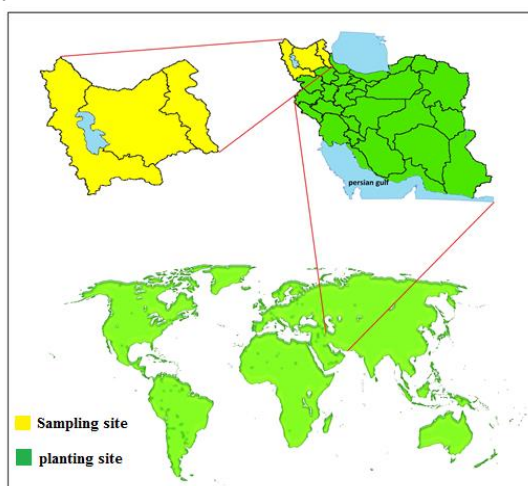
Given the significance of vegetables and fruits in the Iranian diet, this study sought to evaluate the levels of Cd, Pb, and nitrate in samples obtained from East and West Azerbaijan Province in Iran across three seasons and to assess the non-carcinogenic health risks associated with these levels.

## Materials and Methods

### Sampling

A total of 1941 fresh vegetables and fruits from different provinces of Iran were randomly gathered from various local markets, supermarkets, and grocery stores located in East and West Azerbaijan provinces between January 2023 and September 2023. East Azerbaijan is positioned in Iran within the city location category, defined by GPS coordinates of 37° 54' 12.864" N and 46° 16' 5.559" E. Similarly, West Azarbaijan, also in Iran, is categorized within the city location, with GPS coordinates of 37° 27' 18.022" N and 45° 0' 0" E. The region typically experiences cold winters and mild summers. The vegetable and fruit species included in the study consisted of 198 samples of

greenhouse cucumbers, 156 samples of conventional cucumbers, 234 samples of onion, 201 samples of potato, 102 samples of greenhouse tomatoes, 195 samples of conventional tomatoes, 225 samples of spinach, 225 samples of leafy vegetables, 216 samples of apple, and 183 samples of orange. The sampling locations are shown in Figure 1. All samples were stored in paper bags, transported to the laboratory, and kept at 4 °C until analysis.



**Figure 1:** Map of the study area showing the sampling sites.

### Heavy metal determination

#### Standard and reagent solutions

High-purity analytical-grade chemicals with 99.9% purity (Merck Darmstadt, Germany) were used for both sample preparation and analysis. The Pb and Cd standard solutions were prepared by diluting the specified 1000 mg.L<sup>-1</sup> stock solutions (Merck KGaA). The accuracy and precision of the digestion were verified using blank reagents and standard reference materials. Satisfactory recoveries of Cd (101 ± 4.5%) and Pb (93 ± 7.3%) were achieved for these reference materials. Each digested sample was analyzed in triplicate under standard conditions to ensure data quality within a 95% confidence level.

#### Sample preparation and analysis

Before analysis, the gathered samples were thoroughly washed with double deionized water to remove any dust, dirt, potential parasites, or their eggs. Subsequently, the non-edible portions were

discarded following standard household procedures. The edible sections of the samples were dried in an oven at 65 °C for a period ranging from 48–72 h, depending on the sample size, until a consistent weight was reached. After drying, the samples were powdered using an electric grinder and stored in appropriately labeled paper bags intended for acid extraction and subsequent heavy metal analyses <sup>21</sup>.

Each 1 g sample was digested using 5 mL of 65% nitric acid and 2 mL of 30% hydrogen peroxide in a digestion tube. The tube was then placed in a digestion block and heated at 40 °C for one hour, followed by an increase in temperature to 140 °C for three hours. After cooling to room temperature, the mixture was filtered through a 42 µm Whatman filter paper and subsequently diluted with deionized water to achieve a final volume of 25 mL, as described by Farshidi et al. (2023) <sup>24</sup>. The sample analysis was subsequently conducted using a graphite furnace atomic absorption spectrophotometer (Model 2080, Yanglin, South Korea) for total Pb and Cd. The specific conditions for spectroscopic analysis are listed in the [supplementary materials \(Table 1\)](#).

### Nitrite and nitrate determination

#### Reagents and standards

All reagents used in this study were of analytical grade. Disodium tetraborate (Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub>·10H<sub>2</sub>O) was prepared by dissolving 50 g of the compound in 1000 mL of water. The potassium hexacyanoferrate (K<sub>4</sub>Fe(CN)<sub>6</sub>·3H<sub>2</sub>O) solution was prepared by dissolving 106 g of the compound in 1000 mL of deionized water. A zinc acetate (Zn(CH<sub>3</sub>COO)<sub>2</sub>·2H<sub>2</sub>O) solution was prepared by dissolving 220 g of the compound in 1000 mL of water, along with the addition of 30 mL of acetic acid (100%). Sulfanilamide (C<sub>6</sub>H<sub>8</sub>N<sub>2</sub>O<sub>2</sub>S), N-(1-naphthyl) ethylenediamine dihydrochloride (C<sub>10</sub>H<sub>7</sub>NHCH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub>·HCl), acetic acid (100%), and ammonium chloride (analytical grade) from Merck were also employed. Hydrochloric acid (37% v/v) was used in the experiments. The ammoniacal buffer solution was prepared by dissolving 37.4 g of the compound in 1000 mL of

water and adjusting the pH to 9.6 using a concentrated ammonia solution. Solution I was prepared by dissolving 0.4 g of sulphanilic acid in a mixture of 20 mL of HCL and 200 mL of water, followed by filling the flask to the mark with water. Solution II was prepared by dissolving 0.1 g of 1-naphtylamine in 100 mL of water. Solution III was prepared by diluting 445 mL of hydrochloric acid to a final volume of 1000 mL. After preparation, the solutions were stored in a refrigerator and remained usable for three days.

### Sample preparation

Following the sampling process, fresh fruits and vegetables were thoroughly cleaned, rinsed, and properly labeled. The inedible parts of each sample were removed, and the remaining portions were homogenized using a grinder. Subsequently, the homogenized samples were dried in an oven at 70 °C and ground into a fine powder. Next, suitable amounts of the different samples were weighed in triplicate using 100 mL Erlenmeyer flasks. To each flask, 50 mL of a 2% acetic acid solution was added, and the mixture was stirred for 30 min before filtering. The resulting extract was passed through the same filter paper multiple times until a completely smooth extract was obtained.

A 10 mL portion of the filtered extract was transferred to a 200 mL Erlenmeyer flask and heated to boiling. Subsequently, 100 mL of deionized distilled water, previously heated to 80 °C, was added. Next, 5 mL of disodium tetraborate was added to the mixture. The flask was then placed in a boiling water bath for 15 min with occasional shaking. After this heating period, 2 mL of potassium hexacyanoferrate (II) solution and 2 mL of zinc acetate solution was added sequentially, shaking the mixture after each addition. The mixture was allowed to cool.

The cooled mixture was then quantitatively transferred to a 200 mL volumetric flask, rinsed, and filled to the mark with distilled water. The flask was shaken, and the contents were filtered through a fluted filter paper. If necessary, the filtration process was repeated using a 0.45 µm syringe filter until a clear solution was obtained. It

is important to note that all samples were promptly analyzed within one hour after their preparation to ensure accurate results <sup>20</sup>.

### Nitrite determination

Ten milliliters of solution were added to a 50 mL volumetric flask and diluted to 30 mL with purified water. Subsequently, 5 mL of solution I and 3 mL of solution III were added to the flasks. The mixture was thoroughly combined and allowed to stand for 1 min at room temperature, away from light. Next, 1 mL of solution II was added gradually to the flask. The solution was gently mixed and left to rest for 3 min at room temperature, shielded from light. After completing these steps, the flask was filled to the mark with water, and the solution was mixed. Within 15 min of preparation, the absorbance of the solution was measured at 538 nm using a spectrometer. Nitrite (NO<sub>2</sub>) levels were calculated as follows:

$$\text{NO}_2 \text{ (mg/kg)} = m_1 \times \frac{200}{V_1 \times m_0} \quad (1)$$

where  $m_0$  is the weight of the sample,  $m_1$  is the mass (µg) of nitrite from the calibration curve, and  $V_1$  is the portion of the filtrate (mL) <sup>25,26</sup>.

### Nitrates Determination

Ten milliliters of the prepared extract were transferred to a 50 mL Erlenmeyer flask containing approximately 2 g of Cd and 5 mL of buffer solution. The flask was placed on a vertical mechanical agitator and shaken for 5 min. The solution was then filtered through filter paper, and the filtrate was collected in a 50 mL volumetric flask. The determination was carried out in a similar manner to the total nitrites analysis (Section 2.3.3) <sup>25,27</sup>. The results of nitrate (NO<sub>3</sub>) determination are as follows:

$$\text{NO}_3 \text{ (mg/L)} = 1.35 \times \left( \frac{m_2 \times 10000}{V_3 \times V_2 \times m_0} - \text{NO}_2 \right) \quad (2)$$

where  $m_2$  (µg) represents the total mass of NO<sub>2</sub> present in the volume ( $V_2$ ) of the test solution, obtained from the calibration graph,  $V_2$  (mL) is the volume of the test solution used for the spectrometric measurement, and  $V_3$  (mL) is the volume of the aliquot portion of the filtrate used



for the preparation of the test solution. The variables  $m_0$ ,  $m_1$ , and  $V_1$  have the same implications as in Equation (1). The ratio of the relative molecular masses of the  $\text{NO}_3^-$  and  $\text{NO}_2^-$  ions is 1.348<sup>25,27</sup>.

During the determination of nitrate and nitrite content in the samples, ISO guidelines were followed by preparing calibration curves during the method development and routine testing. This process utilizes a series of solutions containing sodium nitrite or potassium nitrate. A calibration curve was created by plotting the absorbance against the concentration. Additionally, duplicate blank tests for nitrate and nitrite were conducted by substituting the test sample with water, while keeping all reagents intact. This was done to ensure the accuracy of the results and to account for any interference. If the nitrate levels exceeded the linear range, the filtrates were reanalyzed using a smaller sample amount.

#### Limit of detection (LOD) and limit of quantification (LOQ) determination

The LOD refers to the minimum value or concentration of a component that an analytical instrument or specific method can detect. In this context, the analyte must generate a signal that is two to three times greater than the control signal. Conversely, the LOQ represents the lowest concentration of an analyte that can be accurately determined with acceptable uncertainty using a specific method. The standard deviation of the blank signal ( $S_b$ ) was computed to derive the LOD. This was achieved by conducting 10 repetitions of the blank sample, followed by calculating the standard deviation based on the resulting signals. Subsequently, the standard deviation of the reference signal was tripled, and the resulting value was divided by the slope of the standard curve ( $m$ ) using Equation 3 (see below).

$$\text{LOD} = \frac{3S_b}{m} \quad (3)$$

The LOQ was calculated using Equation 4, as follows: Following the determination of the LOD, the obtained value was multiplied by 3.3 to ascertain the LOQ<sup>27</sup>.

$$\text{LOQ} = 3.3 \times \text{LOD} \quad (4)$$

The LOD and LOQ results for the three methods are documented in the [supplementary materials \(Table 1\)](#).

#### Health risk assessment: non-carcinogenic risk

To assess the non-carcinogenic risks associated with heavy metals and nitrate, the average daily dose (ADD) and target hazard quotient (THQ) were determined for each metal and nitrate, along with the calculation of the total TTHQ using Equations 5-7:

$$\text{ADD} = \frac{C \times \text{IR} \times \text{EF} \times \text{ED}}{\text{BW} \times \text{AT}} \quad (5)$$

$$\text{THQ} = \frac{\text{ADD}}{\text{RfD}} \quad (6)$$

$$\text{TTHQ} = \sum \text{HQ}_1 + \text{HQ}_2 + \dots \quad (7)$$

where ADD represents the average daily intake (mg/kg/day), C is the concentration of metal and nitrate in an environmental medium (mg/kg), IR is the intake rate of the environmental medium (kg/day), EF denotes the exposure frequency (365 days/year), ED signifies the exposure duration (70 years), BW indicates the average body weight (70 kg), and AT represents the average time (25550 days)<sup>28</sup>. THQ serves as the THQ, and RfD is the oral reference dose (mg/kg/day) with reference doses for Cd = 0.001, Pb = 0.0035, and nitrate = 1.6 mg/kg/day<sup>20,29</sup>. If the calculated THQ exceeds 1, it may indicate a potential hazardous effect on individuals, whereas values below 1 suggest that no significant risk is likely to arise from heavy metal and nitrate exposure over a lifetime.

#### Statistical analysis

The results were evaluated using SPSS software version 20, with data expressed as mean, standard deviation, and 95% confidence interval. To determine the average concentrations of heavy metals and nitrates in the selected vegetables and fruits, one-way ANOVA and one-sample t-tests were performed at a significance level of 0.05. The results were then compared with the established standard.

## Results

#### Method validation

The optimized and validated analytical method was employed to determine the concentrations of Cd, Pb, and nitrate in the vegetable and fruit samples in the [supplementary materials \(Table 1\)](#).

#### ***Cd, Pb, and nitrate concentration in fruits and vegetables***

Detailed mean and standard deviation values for vegetables and fruits sourced from Iran are provided in the [Supplementary Materials \(Tables 2-4\)](#). Notably, all samples exhibited detectable levels of heavy metals and nitrates. [Table 5 \(supplementary materials\)](#) presents the utilization of a one-way ANOVA test to compare the mean concentrations of Cd, Pb, and nitrate across sampling seasons. Furthermore, a one-sample t-test was employed to compare the mean concentrations of Cd, Pb, and nitrate with the maximum permissible limits (MPL) specified by the National Standard Organization of Iran (INSO) ([Table 6, supplementary materials](#)).

## **Discussion**

### ***Greenhouse and conventional cucumbers***

Cucumbers are among the most widely grown vegetables in the world and are highly valuable economically. The mean levels of Cd ranged from  $5.07 \pm 1.02$  (Urmia) to  $26.33 \pm 1.52$   $\mu\text{g kg}^{-1}$  (Urmia) in greenhouse cucumbers, and from  $4.21 \pm 1.52$  (Tabriz) to  $38.66 \pm 1.15$   $\mu\text{g kg}^{-1}$  (Varzeqan) in conventional cucumbers ([Table 2-4](#)). Similarly, Hosseinpour et al. (2024) indicated that Cd is a common heavy metal contaminant in the area around Lake Urmia. This contamination can be attributed to various factors, such as soil pollution from dust, dry lakebeds, agricultural methods, vehicle traffic, and industrial operations <sup>30</sup>. A statistically significant ( $P < 0.05$ ) difference in sampling seasons was observed in conventional cucumbers, with higher Cd concentrations in the samples collected during the summer season ([Table 5](#)). In contrast, Alimohammadi et al. (2018) found that the mean Cd concentration was  $1 \mu\text{g kg}^{-1}$  in supermarket cucumbers from Tehran, and higher concentrations were found in autumn samples <sup>31</sup>. Despite variations across samples, the mean Cd levels were notably lower than the MPL

set by the INSO ( $P < 0.05$ ) ( $50 \mu\text{g kg}^{-1}$ ) ([Table 6](#)). Similarly, Jalali et al. (2020) reported that Cd levels in greenhouse production systems in Iran were lower than the standard levels <sup>32</sup>.

The concentrations of Pb ranged from  $36.57 \pm 2.85$  (Jiroft) to  $210 \pm 1.24$  (Varamin)  $\mu\text{g kg}^{-1}$  in greenhouse cucumbers and from  $12.64 \pm 3.05$  (Tabriz) to  $204.66 \pm 0.51$  (Shendabad)  $\mu\text{g kg}^{-1}$  in conventional cucumbers ([Table 2-4](#)). The Pb concentrations in conventional cucumbers were significantly dependent on the sampling season, with the highest levels recorded in spring ([Table 5](#)). Conversely, Alimohammadi et al. (2018) reported that cucumbers in autumn ( $98 \mu\text{g kg}^{-1}$ ) were higher in Pb contamination than those in summer ( $40 \mu\text{g kg}^{-1}$ ), which was lower than the findings of this study ( $129.24 \mu\text{g kg}^{-1}$ ). Moreover, the mean Pb value ( $104.95 \mu\text{g kg}^{-1}$  for greenhouse cucumber and  $91.46 \mu\text{g kg}^{-1}$  for conventional cucumber) did not significantly deviate from the limits established by the INSO ( $100 \mu\text{g kg}^{-1}$ ) ( $P > 0.05$ ). Of the mean values observed across nine months, 14 samples, pertaining to both greenhouse and conventional cucumbers, surpassed the maximum limit suggested by the INSO and WHO/FAO ( $100 \mu\text{g kg}^{-1}$ ). The highest recorded Pb concentration ( $210 \mu\text{g kg}^{-1}$ ) was identified in greenhouse cucumbers from Varamin in the first quarter of 2023. This trend aligns with the findings of Mansour et al. (2009), who reported Pb violations in the majority of their greenhouse cucumber samples <sup>33</sup>. Zafarzadeh et al. (2015) noted that the mean levels of Pb in cucumber samples from Golestan province in northern Iran, collected during the spring and summer of 2012, surpassed the maximum permissible thresholds. Specifically, the mean Pb concentrations in cucumbers from Gonbad and Gorgan were recorded at 1470 and 1400  $\mu\text{g kg}^{-1}$ , respectively <sup>34</sup>.

The mean nitrate concentrations were  $9.66 \pm 4.50$  (Urmia) and  $210.86 \pm 5.82$  ppm (Yazd) in greenhouse cucumbers, and  $34.62 \pm 4.46$  (Urmia) and  $188.33 \pm 1.56$  ppm (Tabriz) in conventional cucumbers ([Table 2-4](#)). Rezaei et al. (2014) demonstrated that the mean nitrate concentration in cucumbers from Arak, Iran, was 42.7 ppm <sup>35</sup>.

Additionally, Dezhangah et al. (2022) reported a mean nitrate content of 72 ppm in cucumbers collected from Zanjan, Iran <sup>20</sup>. Moreover, a study conducted in 2008 in Ahwaz, located in southern Iran, revealed nitrate levels reaching 999 ppm <sup>36</sup>, exceeding the values reported in this study for Ahwaz (106.93 ppm). Both observations exceeded the permissible limit of 90 ppm. The seasonal variation in nitrate content was also significant ( $P \leq 0.05$ ), with samples from autumn containing the highest nitrate percentage (134.72 ppm) (Table 5). This finding could be attributed to the influence of light and temperature conditions, as validated by Tammé et al. (2010) <sup>37</sup>. Notably, nitrate levels in both greenhouse (89.53 ppm) and conventional cucumbers (84.33 ppm) did not significantly differ from the INSO limit ( $P > 0.05$ ), with 15 samples of both greenhouse and conventional cucumbers exceeding the permissible limit of 90 ppm. The primary reason for the elevated nitrate concentrations can be attributed to poor nutritional management and excessive application of manure and chemical fertilizers in farming practices <sup>38</sup>.

### Onion

Onions are used worldwide as a seasoning to enhance the flavor and aroma of food. Furthermore, onions possess medicinal properties, including anticancer, antimicrobial, antiviral, and antifungal properties, and the extracts and essential oils of these plants have demonstrated efficacy in treating cardiovascular diseases <sup>39</sup>.

In this study, the mean concentrations of Cd in onion samples varied from  $3.54 \pm 1.52 \mu\text{g kg}^{-1}$  (Ahvaz) to  $29.38 \pm 5.03 \mu\text{g kg}^{-1}$  (Urmia) (Table 2-4). In particular, the mean Cd concentration in onions under investigation was lower than that observed by Heshmati et al. (2020) in Hamedan, Iran ( $106 \mu\text{g kg}^{-1}$ ) <sup>40</sup>. Shokri et al. (2020) revealed that Cd concentrations in onion samples from Kurdistan and West Azerbaijan provinces were 274.49 and  $526.49 \mu\text{g kg}^{-1}$ , respectively, exceeding the INSO recommended limit of  $50 \mu\text{g kg}^{-1}$  by 2 to 5 times <sup>41</sup>. Amini et al. (2005) also reported an average total Cd amount of  $1790 \mu\text{g kg}^{-1}$  in the fields of Isfahan province <sup>42</sup>. Furthermore, the

harvest season significantly affected Cd content ( $P \leq 0.05$ ), with onions from the summer season exhibiting higher Cd levels ( $19.01 \mu\text{g kg}^{-1}$ ) (Table 5). This could be attributed to the use of phosphorus fertilizers, which have been identified as a significant pathway for Cd entry into agricultural fields <sup>42</sup>. Additionally, the availability of wastewater enables small-scale farmers to utilize wastewater irrigation during the dry season <sup>43</sup>. In a study conducted by Shokri et al. (2022), a comparison between winter and autumn sampling for Cd content did not yield significant differences ( $P > 0.05$ ). The mean Cd content ( $15.43 \mu\text{g kg}^{-1}$ ) was significantly lower than the maximum limit stipulated by INSO ( $50 \mu\text{g kg}^{-1}$ ) ( $P \leq 0.05$ ; Table 5). Mohajer et al. (2014) in Isfahan reported an onion Cd content of  $150 \mu\text{g kg}^{-1}$ , which was higher than the results obtained in the present study ( $20.15 \mu\text{g kg}^{-1}$ ) <sup>44</sup>.

The mean concentrations of Pb in onion samples varied from  $55.03 \pm 6.16$  (Qom) to  $159.77 \pm 12.58 \mu\text{g kg}^{-1}$  (Ajabshir) (Table 2-4). Furthermore, an investigation across several provinces, including Hamedan, Kurdistan, and Kermanshah in 2020–2021 revealed Pb concentration levels ranging from  $53.44 \pm 32$  (Kurdistan) to  $296.50 \pm 35 \mu\text{g kg}^{-1}$  (Isfahan) <sup>41</sup>. Mohajer et al. (2014) also reported Pb concentration of  $1300 \mu\text{g kg}^{-1}$  in onions from Isfahan, which exceeds the findings of this study ( $117.75 \mu\text{g kg}^{-1}$ ). Conversely, the concentration of Pb in onion samples from Hamadan Province was  $16 \mu\text{g kg}^{-1}$  <sup>40</sup>. The influence of seasonal variations on the Pb content in onions was not significantly different ( $P > 0.05$ ; Table 5), aligning with similar findings reported for onion samples collected during winter and autumn <sup>41</sup>. The mean Pb concentration ( $96.39 \mu\text{g kg}^{-1}$ ) did not significantly differ from the maximum limit set by the INSO ( $P > 0.05$ ; Table 6). However, the ratio of Pb concentration in onion samples from various regions, including Ajabshir, Ilkhchi (second quarter 2023), Dezful (first quarter 2023), Ilkhchi (third quarter 2023), Dezful (third quarter 2023), Bonab, Isfahan, and Ahvaz was  $159.77 > 158.47 > 151.33 > 148.22 > 142.80 > 132.07 > 117.75 > 107.28 \mu\text{g kg}^{-1}$ , respectively,

exceeding the INSO tolerable range ( $100 \mu\text{g kg}^{-1}$ ), but remained below the MPL established by WHO/FAO ( $300 \mu\text{g kg}^{-1}$ ). This pollution can be attributed to municipal and industrial effluents in the area <sup>44</sup>.

The mean nitrate concentration in onion samples ranged from  $16.11 \pm 3.21$  ppm (Zanjan) to  $213.57 \pm 8.8$  ppm (Isfahan) (Table 2-4). Nitrate concentrations in onions from Isfahan <sup>45</sup>, Shiraz <sup>46</sup>, Kermanshah <sup>47</sup>, Ravansar <sup>47</sup>, Bisotun <sup>47</sup>, Fars <sup>48</sup>, and Mashhad <sup>49</sup> were reported to be 354, 36.9, 98.02, 65.16, 60.69, 118, and 11.90 ppm, respectively. The results indicated that nitrate concentrations across different seasons (Table 5), and in comparison with INSO (Table 6), did not significantly differ ( $P > 0.05$ ). The ratio of nitrate concentration in onion samples from various locations, such as Isfahan ( $213.57$  ppm)  $>$  Qom ( $144.55$  ppm)  $>$  Dezful ( $140.91$  ppm, third quarter 2023)  $>$  Dezful ( $121.33$  ppm, first quarter 2023)  $>$  Urmia ( $117.94$  ppm)  $>$  Bonab ( $113.66$  ppm)  $>$  Bandar Abbas ( $111.66$  ppm) and  $>$  Tabriz ( $103.40$  ppm), exceeded the tolerable range set by the INSO ( $90$  ppm) and WHO/FAO ( $80$  ppm). In conclusion, onions, as bulbous vegetables, tend to accumulate a substantial amount of nitrate in their edible parts. This accumulation is often attributed to the excessive application of nitrogen fertilizers by farmers <sup>50,51</sup>. However, Haftbaradaran et al. (2018) demonstrated that there was no discernible correlation between the quantity of nitrogen fertilizer applied by farmers and the level of nitrate accumulation in the edible parts of onions. They affirmed that the timing and method of nitrogen fertilizer application are more influential factors than dosage in determining the accumulation of nitrates in food crops and the associated human health risks <sup>45</sup>.

### Potato

Potato (*Solanum tuberosum* L.), a root vegetable, is one of the most intensively cultivated and profitable crops, recognized for its high energy content, starch, vitamins C, B, E, and K, organic acids, and mineral substances, which confer significant therapeutic value in the human diet <sup>52</sup>.

The concentration distribution of Cd in potatoes varied from  $6.55 \pm 3.32 \mu\text{g kg}^{-1}$  (Tabriz, third quarter 2023) to  $81.02 \pm 0.45 \mu\text{g kg}^{-1}$  (Tabriz, second quarter 2023) (Table 2-4). Jafarian et al. (2013) reported the mean concentrations of Cd in potatoes, both with and without skin, from various sampling sites in Isfahan. According to the findings, potatoes with skin from Dorche, Isfahanak, Dashti, and Ilchi farms, the Valiasr retail market, and the Soroush retail market exhibited undetectable, 2910, 670  $\mu\text{g kg}^{-1}$ , and undetectable concentrations, respectively. Similarly, potatoes without skin from these locations displayed contents of 300, 670, and 670  $\mu\text{g kg}^{-1}$  and undetectable, respectively <sup>53</sup>. In a recent study involving potatoes harvested in Hamedan, the Cd concentration was reported to be  $22 \mu\text{g kg}^{-1}$ , which is consistent with the present study (average  $25.79 \mu\text{g kg}^{-1}$ ) <sup>40</sup>. Furthermore, potato samples from Lorestan Province demonstrated Cd content within the range of 97 to 198  $\mu\text{g kg}^{-1}$  <sup>54</sup>. The results indicated significant seasonal variability ( $P \leq 0.05$ ), with higher Cd accumulation observed during the summer (Table 5). However, it is noteworthy that the Cd concentration in potatoes ( $16.23 \mu\text{g kg}^{-1}$ ) remained below the MPL established by the INSO ( $100 \mu\text{g kg}^{-1}$ ) and WHO/FAO ( $200 \mu\text{g kg}^{-1}$ ) (Table 6).

The analysis of Pb content in potato samples revealed a variance from  $20.03 \pm 5.12$  (Tabriz) to  $260.47 \pm 4.58 \mu\text{g kg}^{-1}$  (Urmia) (Table 2-4). Particularly, a recent study conducted in Lorestan province demonstrated a Pb range of 168–300  $\mu\text{g kg}^{-1}$  <sup>54</sup>. Moreover, potatoes with and without skins from various sampling sites in Isfahan exhibited a content range from undetectable to 7140  $\mu\text{g kg}^{-1}$  <sup>53</sup>. Heshmati et al. (2020) reported the mean Pb concentration in potatoes consumed in Western Iran as  $29 \mu\text{g kg}^{-1}$ , whereas Beversad et al. (2014) documented the mean Pb content in potatoes marketed in Isfahan province as  $70 \mu\text{g kg}^{-1}$  <sup>55</sup>. Similar to Cd results, the Pb content also displayed seasonal variations ( $P \leq 0.05$ ), with higher concentrations observed during the summer season (Table 5). Moreover, a comparison of Pb levels in potato samples ( $146.26 \mu\text{g kg}^{-1}$ ) with the INSO



(200  $\mu\text{g kg}^{-1}$ ) limit revealed that the mean levels of Pb in potato samples were significantly lower than the MPL ( $P \leq 0.05$ ; Table 6). It is important to emphasize that adherence to proper farming practices, which preclude the use of fertilizers that pose a risk of Cd and Pb contamination, can ensure that the concentrations of these metals in vegetable products remain low <sup>56</sup>.

The assessment of nitrate values in potato samples demonstrated a range from  $74.69 \pm 4.50$  (Kermanshah) to  $289.65 \pm 9.08$  ppm (Tabriz) (Table 2-4). According to the literature, the nitrate concentration in potatoes was documented as 160.66 ppm in Mashhad, Iran <sup>57</sup>; 73.40 ppm in Shiraz, Iran <sup>46</sup>; 350 ppm in Kermanshah, Iran <sup>58</sup>; 520 ppm in Tehran, Iran <sup>59</sup>; 137 ppm in Zanjan, Iran <sup>20</sup>; and 130 ppm in Hamedan, Iran <sup>15</sup>. Nitrate levels in potatoes were not significantly dependent on the season ( $P > 0.05$ , Table 5). Moreover, the mean nitrate level (171.56 ppm) did not significantly differ from the MPL established by the INSO (170 ppm) ( $P > 0.05$ ; Table 6). Samples from Tabriz (289.65 ppm), Ardabil (271.79 ppm), Hamedan (228.89 ppm), Gorgan (213.33 ppm), and Bostan Abad (180.09 ppm) revealed nitrate levels exceeding the MPLs of INSO and WHO/FAO (250 ppm). Gathungu et al. (2000) noted that among various factors affecting nitrogen content in potato plants, the late application of nitrogen fertilizers led to greater accumulation in potato tubers <sup>60</sup>.

#### Greenhouse and conventional tomato

Tomato (*Solanum lycopersicum L.*) stands out as one of the most widely consumed and nutritious vegetables, serving as a significant source of essential micronutrients such as vitamin C, calcium, magnesium, and potassium <sup>61</sup>. Tomato consumption has been associated with a reduced risk of cardiovascular diseases and certain types of cancer, including prostate, lung, and stomach cancer <sup>62</sup>.

The mean levels of Cd in greenhouse tomatoes ranged from  $6.10 \pm 2.08$  (Azarshahr) to  $33.66 \pm 8.06$   $\mu\text{g kg}^{-1}$  (Urmia), while for conventional tomatoes, the range was from  $4.45 \pm 1.15$   $\mu\text{g kg}^{-1}$  (Urmia) to

$40.22 \pm 2.08$   $\mu\text{g kg}^{-1}$  (Hormozgan) (Table 2-4). Previous reports on Cd content from various regions of Iran indicated lower than detection limit in Fars province <sup>48</sup>, 3  $\mu\text{g kg}^{-1}$  in Hamedan <sup>40</sup>, 100  $\mu\text{g kg}^{-1}$  in Hamedan province <sup>32</sup>, 280  $\mu\text{g kg}^{-1}$  in Tabriz <sup>63</sup>, and undetectable to 7700  $\mu\text{g kg}^{-1}$  in Golestan province <sup>64</sup>. Furthermore, the p levels of Cd in greenhouse tomatoes exhibited seasonal variations ( $P \leq 0.05$ ), with higher levels observed in spring (Table 5). Importantly, none of the tomato samples were found to be contaminated with Cd levels higher than the MPL set by the INSO (50  $\mu\text{g kg}^{-1}$ ) ( $P \leq 0.05$ ; Table 6).

The mean level of Pb ranged from  $35.52 \pm 3.21$  (Azarshahr) to  $280.33 \pm 19.65$  (Urmia)  $\mu\text{g kg}^{-1}$  in greenhouse tomatoes and from  $13.22 \pm 3.58$  (Shiraz) to  $236.23 \pm 31.57$   $\mu\text{g kg}^{-1}$  (Kashan) in conventional tomatoes (Table 2-4). Previous studies have indicated that the Pb content in Isfahan <sup>53</sup>, Hamedan province <sup>32,40</sup>, Golestan province <sup>64</sup>, and Tabriz <sup>63</sup> varied from undetectable to 7140, 7 to 30370, undetectable to 390, and 280  $\mu\text{g kg}^{-1}$ , respectively. In greenhouse tomatoes, Pb content showed significant seasonal variations ( $P \leq 0.05$ ), with spring samples exhibiting the highest levels (Table 5). Comparison of Pb levels with the MPL established by the INSO (100  $\mu\text{g kg}^{-1}$ ) did not show significant differences in either greenhouse or conventional tomatoes ( $P > 0.05$ ; Table 6). Samples that exceeded the MPLs set by the INSO and WHO/FAO (100  $\mu\text{g kg}^{-1}$ ) for Pb were from Urmia (280.33  $\mu\text{g kg}^{-1}$ , first quarter 2023), Varamin (220  $\mu\text{g kg}^{-1}$ ), Tabriz (192.66  $\mu\text{g kg}^{-1}$ ), Isfahan (189.57  $\mu\text{g kg}^{-1}$ ), and Urmia (152.24  $\mu\text{g kg}^{-1}$ , third quarter 2023) for greenhouse tomatoes. For conventional tomatoes, samples that exceeded the MPLs were from Urmia (206.66  $\mu\text{g kg}^{-1}$ ), Kazerun (150  $\mu\text{g kg}^{-1}$ , first quarter 2023), Tarom (201.66  $\mu\text{g kg}^{-1}$ ), Varzeqan (240.66  $\mu\text{g kg}^{-1}$ ), Marand (140.44  $\mu\text{g kg}^{-1}$ ), Kazerun (129.51  $\mu\text{g kg}^{-1}$ , second quarter 2023), Kashan (236.23  $\mu\text{g kg}^{-1}$ ), Saveh (233.64  $\mu\text{g kg}^{-1}$ ), and Bandar Abbas (109.42  $\mu\text{g kg}^{-1}$ ). The current study found that tomatoes from greenhouse agriculture had a higher Pb content (123.94  $\mu\text{g kg}^{-1}$ ) than those from conventional agriculture (101.46  $\mu\text{g kg}^{-1}$ ). This could be attributed to several factors,

including the use of chemical fertilizers for soil fertilization, sewage sludge, and various human activities, especially land transport <sup>65</sup>.

The nitrate content ranged from  $13.66 \pm 3.78$  (Shabestar) to  $182.64 \pm 3.09$  (Azarshahr) ppm in greenhouse tomatoes and from  $45.32 \pm 3.01$  (Varzeqan) to  $205 \pm 5.9$  (Urmia) ppm in conventional tomatoes (Table 2-4). West Azerbaijan and Urmia regions serve as significant agricultural centers. The sources of nitrate pollution primarily stem from biological factors and chemical fertilizers, largely resulting from the discharge of untreated wastewater and farming activities <sup>66</sup>. Rezaei et al. (2014) conducted a study in Arak, Iran, and reported a nitrate concentration of 7.82 ppm in tomatoes. Another study by Dezhangah et al. (2022) in Zanjan, Iran, found a mean concentration of 31 ppm of nitrate in tomatoes. In 2008, a study in Ahwaz, Iran, reported nitrate levels of 1644 ppm in tomatoes <sup>36</sup>. In Isfahan province, Iran, the mean value of nitrate in tomatoes was found to be 15 ppm <sup>67</sup>. Atefi et al. (2021) discovered that tomatoes from local markets in Mashhad, Iran, had a nitrate level of 11.75 ppm. In Fars province, Iran, Mohammadpour et al. (2022) studied 83 tomato samples for nitrate content, which varied from 0 to 154.40 ppm. Significant seasonal variations in the nitrate content of conventional tomatoes were observed ( $P \leq 0.050$ ), which were higher in spring (Table 5). This effect is likely due to sunlight intensity, with lower intensity in the spring leading to higher nitrate accumulation, whereas shading increases nitrate accumulation by discouraging the activity of nitrate reductase <sup>68</sup>. The mean nitrate level in greenhouse tomatoes (106.60 ppm) did not significantly differ from the MPL set by INSO (120 ppm) ( $P > 0.05$ ), whereas in conventional tomatoes, it was significantly lower (95.01 ppm) than the MPL set by INSO ( $P \leq 0.05$ ; Table 6). Similar to the present study results, Hosseini et al. (2023) presented findings from a comprehensive global systematic review, meta-analysis, and meta-regression on the levels of nitrate and nitrite in vegetables and fruits. They reported that vegetables grown in greenhouse environments

exhibited higher nitrate levels than those cultivated in open fields <sup>69</sup>. Samples from Jolfa (186.50 ppm) > Azarshahr (182.64 ppm) > Tabriz (166.66 ppm, first quarter 2023) > Tabriz (152.30 ppm, third quarter 2023) > Urmia (138 ppm) > and Marand (133.28 ppm) for greenhouse tomatoes and Urmia (205 ppm) > Shiraz (184.66 ppm, second quarter 2023) > Shiraz (163 ppm, first quarter 2023.) > Marand (156.70 ppm) > Dezful (133.38 ppm) for conventional tomatoes exceeded the MPL set by INSO but fell within the safety limit set by the WHO/FAO (300 ppm).

### Spinach

Spinach (*Spinacia oleracea* L.) is a member of the *Amaranthaceae* family and is widely cultivated as a leafy green vegetable in Central and Western Asia. These vegetables provide minerals, vitamins, fiber, and other nutrients essential for a healthy diet <sup>70</sup>.

Regarding Cd content, the analysis results revealed a range from  $9.17 \pm 1.52$  (Qom) to  $32.49 \pm 2.64$  (Ahvaz)  $\mu\text{g kg}^{-1}$  (Table 2-4). Shahryari et al. (2012) reported a undetectable mean Cd concentration in spinach samples from five farms around Gorgan in north Iran <sup>71</sup>. Jafarian et al. (2013) conducted a survey of Cd concentrations in spinach from four major cities in Isfahan province, reporting levels ranging from 670 (Dorche farms) to 2910 (Sorush retail market)  $\mu\text{g kg}^{-1}$ . Furthermore, Souri et al. (2018) noted a Cd level of  $520 \mu\text{g kg}^{-1}$  in spinach samples from the southern suburbs of Tehran, Iran <sup>72</sup>. The spinach in Ahvaz city contained 290  $\mu\text{g}$  of Cd per  $\text{kg}^{-1}$  as reported by Bahrami et al. (2019) <sup>73</sup>, which is higher than the present study results ( $32.49 \mu\text{g kg}^{-1}$ ). The Cd content in spinach was not significantly dependent on the season ( $P > 0.05$ ) (Table 5). Importantly, none of the spinach samples exceeded the limits set by the INSO (100  $\mu\text{g kg}^{-1}$ ) and WHO/FAO (200  $\mu\text{g kg}^{-1}$ ) (Table 6).

The reported concentrations of Pb ranged from  $74.33 \pm 4.04$  (Urmia) to  $233.46 \pm 3.57$  (Tabriz)  $\mu\text{g kg}^{-1}$ , according to the data in Table 2-4. Previous studies have noted Pb levels in spinach from various regions, such as Isfahan province <sup>53</sup>,

Markazi province <sup>74</sup>, and Kahrizak in the southern part of Tehran, Iran <sup>72</sup>, ranging from undetectable to 7140, 1733.62, and 4700  $\mu\text{g kg}^{-1}$ , respectively. Interestingly, the Pb levels in spinach samples did not display seasonal variations, as indicated in [Table 5](#) ( $P > 0.05$ ). Furthermore, the mean Pb level (150.89  $\mu\text{g kg}^{-1}$ ) was significantly lower than the MPL established by the INSO, which is 200  $\mu\text{g kg}^{-1}$ , as shown in [Table 6](#). However, it is worth noting that samples from Tabriz (233.46  $\mu\text{g kg}^{-1}$ ) and Kashan (240  $\mu\text{g kg}^{-1}$ ) exceeded the standard limits set by the INSO but remained below the safety limit recommended by the WHO/FAO (300  $\mu\text{g kg}^{-1}$ ). The elevated levels of Pb in spinach could be attributed to pollutants present in irrigation water, farm soil, or environmental pollution, possibly from highway traffic emissions, as highlighted by Qiu et al. (2000) <sup>75</sup>.

The nitrate concentrations in spinach samples ranged from  $1415.16 \pm 37.07$  (Qom) to  $3581.99 \pm 14.58$  (Urmia) ppm, based on the data in [Table 2-4](#). Nowrouz et al. (2012) documented a mean nitrate concentration of 201 ppm in spinach from Varzeghan City, northwestern Iran <sup>76</sup>. Shahbazzadegan et al. (2010) identified a nitrate level of 1021 ppm in spinach from Ardabil, Iran <sup>77</sup>. Dezhangah et al. (2022) from Zanjan, Iran revealed nitrate levels in 12 spinach samples within the range of 108-1950 ppm, with an average level of 653 ppm <sup>20</sup>. Moreover, statistical analysis showed that there were no significant differences ( $P > 0.05$ ) in nitrate concentration among the various seasons ([Table 5](#)), a finding consistent with Nowrouz et al.'s (2012) observations in spinach from Varzeghan city, Iran <sup>76</sup>. In comparison to the INSO limits (2000 ppm), the mean concentration of nitrate (2496.73 ppm) was notably higher, as detailed in [Table 6](#). Interestingly, 77% of the spinach samples exceeded the INSO limit, whereas 23% surpassed the safety limit recommended by the WHO/FAO (3000 ppm). Ortega-blu et al. (2020) highlighted that excessive nitrogen fertilization practices can have a direct impact on nitrate concentrations in leafy vegetables <sup>78</sup>.

### Leafy vegetables

Based on the data presented in [Table 2-4](#), the accumulation of Cd in leafy vegetable samples ranged from  $8.44 \pm 1.52$  (Qom) to  $30.55 \pm 1.15$  (Tabriz)  $\mu\text{g kg}^{-1}$ . Notably, these values are lower than those reported in previous Iranian studies by Rahmdel et al. (2018), which documented levels between 200-480  $\mu\text{g kg}^{-1}$  <sup>79</sup>. Furthermore, the mean Cd concentration in leafy vegetables in Tehran, Iran, was found to be 30  $\mu\text{g kg}^{-1}$  <sup>80</sup>, which is consistent with the findings of this study. Conversely, in Hamadan Province, western Iran, the Cd concentration was reported to have an average of 1460  $\mu\text{g kg}^{-1}$  <sup>81</sup>, which was substantially higher than that observed in the present study. An Iranian study found that leafy vegetables in Sanandaj had a mean Cd concentration ranging from undetectable to 6500  $\mu\text{g kg}^{-1}$  <sup>82</sup>. Statistical analysis revealed no significant differences between the seasons ( $P > 0.05$ ; [Table 5](#)). The mean Cd level in the leafy vegetable samples (21.07  $\mu\text{g kg}^{-1}$ ) was significantly lower than the safety limit established by the INSO at 100  $\mu\text{g kg}^{-1}$  ( $P \leq 0.05$ ; [Table 6](#)).

In the analysis of leafy vegetable samples, Pb levels exhibited diverse ranges, varying from  $68.27 \pm 6.55$  (Urmia) to  $283.67 \pm 3.64$   $\mu\text{g kg}^{-1}$  (Tabriz) ([Table 2-4](#)). Literature reviews have demonstrated the extent of Pb accumulation in leafy vegetables found in different regions across Iran. For instance, the Pb accumulation levels were reported as follows: Shiraz 2180-3910  $\mu\text{g kg}^{-1}$  <sup>79</sup>, Tehran 84.5-161.43  $\mu\text{g kg}^{-1}$  <sup>80</sup>, Sanandaj 11230-16990  $\mu\text{g kg}^{-1}$  <sup>82</sup>, Ahvaz 116-166  $\mu\text{g kg}^{-1}$  <sup>73</sup>, and the highest level in Hamadan province ranged from 13500 to 21800  $\mu\text{g kg}^{-1}$  <sup>81</sup>. There was no significant impact of the crop season on Pb accumulation ( $P > 0.05$ ; [Table 5](#)). Samples from Tabriz (283.67  $\mu\text{g kg}^{-1}$ , second quarter 2023) exceeded the INSO safety limits but remained within the WHO/FAO MPL (300  $\mu\text{g kg}^{-1}$ ) ([Tables 3 and 6](#)).

In leafy vegetables, the analysis revealed a broad range of nitrate levels, extending from  $1386.47 \pm 16.21$  (Urmia) to  $3281.94 \pm 8.07$  ppm (Tabriz) ([Table 2-4](#)). In a study conducted in Zanjan, Iran, Dezhangah et al. (2022) recorded a mean concentration of 115 ppm for leafy

vegetables (excluding spinach), which was significantly lower than the levels observed in the current study. Furthermore, in Hamadan province, lettuce and leek displayed nitrate concentrations of 1072 and 1070 ppm, respectively <sup>15</sup>. Rahmani (2022) reported a nitrate value of 1814.2 ppm for leafy vegetables in Isfahan <sup>83</sup>. In autumn, the nitrate levels were significantly higher than those in other seasons ( $P \leq 0.05$ ; [Table 5](#)). In 100% of the leafy vegetables, the nitrate levels exceeded INSO's MPL (1000 ppm) ([Table 2-4](#)), as reported by Dezhangah et al. (2022). The accumulation of nitrate in leafy vegetables is influenced by several factors, including soil type, planting density, environmental conditions such as temperature and humidity, plant maturity, timing of harvest, growing season, and nitrogen application <sup>16</sup>. Nitrate levels can also be affected by different parts of the vegetable and the age of the plant tissue at the time of consumption, with younger and inner leaves generally containing lower levels of nitrate than older and outer leaves <sup>84</sup>.

#### Apple

Fruits contain advantageous constituents such as dietary fiber, vitamins, minerals, and antioxidants. Despite these nutritional benefits, they may also contain various toxic elements present in varying concentrations across a wide spectrum <sup>85</sup>.

The findings indicated that the concentrations of Cd in the collected apple samples ranged from  $2.42 \pm 3.08 \mu\text{g kg}^{-1}$  in Piranshahr to  $34.25 \pm 3.60 \mu\text{g kg}^{-1}$  in Urmia ([Table 2-4](#)). No statistically significant seasonal differences were observed ( $P > 0.05$ ; [Table 5](#)). Furthermore, the Cd levels in apples ( $14.21 \mu\text{g kg}^{-1}$ ) were notably lower than the MPL stipulated by the INSO at  $50 \mu\text{g kg}^{-1}$  ( $P \leq 0.05$ ; [Table 6](#)). To date, no study has been conducted on the amount of Cd in Iranian apples, and these results are presented for the first time.

The mean total Pb concentration in apple samples ranged from  $6.21 \pm 0.1$  (Piranshahr) to  $152.55 \pm 3.69$  (Urmia)  $\mu\text{g kg}^{-1}$  ([Table 2-4](#)). There was no significant association between the Pb concentration and the different seasons ( $P > 0.05$ ;

[Table 5](#)). The mean Pb concentration ( $70.18 \mu\text{g kg}^{-1}$ ) fell within INSO's MPL of  $100 \mu\text{g kg}^{-1}$  ([Table 6](#)). However, the samples from Urmia ( $152.55 \mu\text{g kg}^{-1}$ ), Oshnavieh ( $126.91 \mu\text{g kg}^{-1}$ ), and Siyahrud ( $122.25 \mu\text{g kg}^{-1}$ ) surpassed the INSO and WHO/FAO recommended MPL of  $100 \mu\text{g kg}^{-1}$ , respectively. Plant foliage can be polluted with Pb through the absorption of air deposits, which are pollutants caused by automotive traffic, fossil fuel combustion, and industrial and mining activities <sup>86</sup>. Furthermore, fruits may be contaminated with Pb during manufacture, transportation, storage, and marketing <sup>87</sup>.

#### Orange

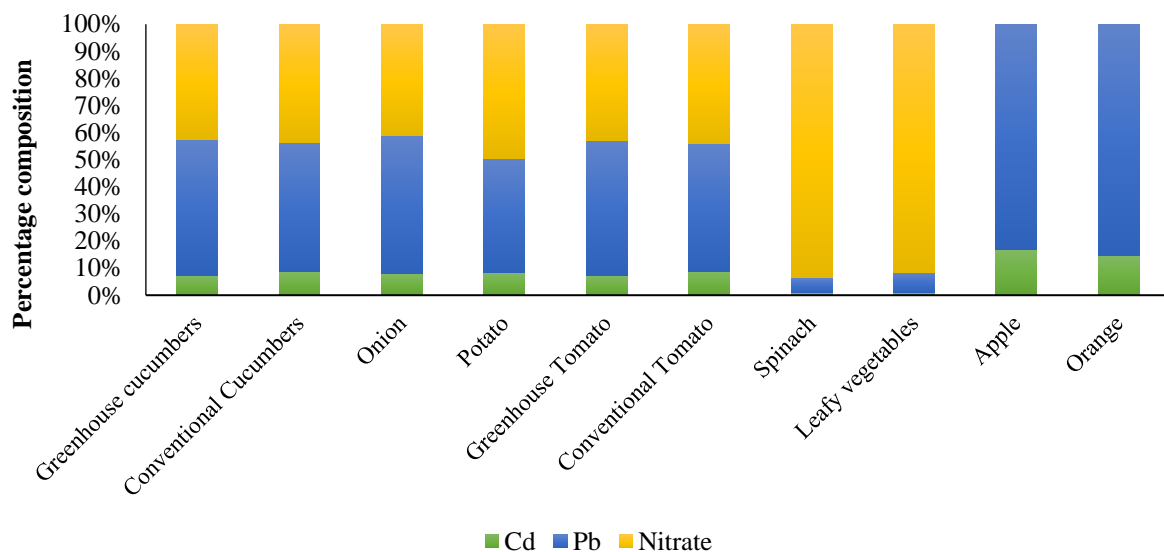
Cd and Pb concentrations were examined in orange samples from various regions. For Cd, levels ranged from  $5.42 \pm 5.24$  (Jannat shahr) to  $18.44 \pm 2.51$  (Jiroft)  $\mu\text{g kg}^{-1}$ , showing variability across locations ([Table 2-4](#)). No significant seasonal differences were observed in the Cd concentrations ( $P > 0.05$ ; [Table 5](#)). Moreover, the mean Cd concentration ( $13.76 \mu\text{g kg}^{-1}$ ) was significantly below the MPL advised by the INSO ( $50 \mu\text{g kg}^{-1}$ ) ( $P \leq 0.05$ ; [Table 6](#)).

The Pb levels in oranges ranged from  $25.29 \pm 2.98$  (Jannat) to  $208.24 \pm 7.21$  (Ramsar)  $\mu\text{g kg}^{-1}$  ([Table 2-4](#)). Similar to Cd, Pb levels did not fluctuate seasonally ( $P > 0.05$ ; [Table 5](#)). Ramsar oranges exhibited the highest mean Pb concentration of  $208.24 \mu\text{g kg}^{-1}$ , surpassing both the INSO and WHO/FAO MPLs ( $100 \mu\text{g kg}^{-1}$ ).

#### Composition percentage of heavy metals and nitrate in vegetables and fruits

Greenhouse and traditional cucumbers, onions, tomatoes, apples, and oranges exhibited a higher proportion of Pb than Cd and nitrate (Figure 2). Conversely, potatoes, spinach, and leafy vegetables displayed a higher proportion of nitrate than Pb and Cd (Figure 2). Statistical analysis revealed that Cd, Pb, and nitrate levels were significantly higher in potatoes, leafy vegetables, and spinach than in other crops ( $P < 0.05$ ).





**Figure 2:** Composition percentage of heavy metals and nitrate in vegetables and fruits

### Non-carcinogenic risk assessment

THQ values are commonly used to evaluate the potential health risks associated with the consumption of food crops. The THQ values for Cd, Pb, and nitrate ranged from 0.116 (cucumbers) to 0.609 (potatoes), 0.198 (cucumbers) to 0.88 (potatoes), and  $3.8 \times 10^{-7}$  (cucumbers) to  $1.9 \times 10^{-5}$  (leafy vegetables), respectively in the [supplementary materials \(Table 7\)](#). The findings indicated that the THQ values for all heavy metals and nitrate fell within the safe range ( $<1$ ) for all vegetables and fruits, suggesting a low health risk associated with exposure to these substances through vegetable and fruit consumption. These results align with previous studies assessing heavy metal and nitrate concentrations and associated health risks in agricultural products in Iran. Alimohammadi et al. (2018) reported that the THQ values of As, Cd, Pb, Cr, Ni, Cu, and Zn in vegetables from Tehran supermarkets were below  $1^{31}$ . Shokri et al. (2022) demonstrated that the non-cancer risk posed by Pb and Cd in Iranian onion samples was below standard limits<sup>41</sup>. Additionally, Mohammadpour et al. (2022) found that the THQ values of nitrate in tomato and onion samples from Fars Province, Iran, were all below  $1^{48}$ . In contrast, a risk assessment study by Tajdar et al. (2024) on exposure to Pb, Cd, and As through the consumption of leafy vegetables in Tehran, Iran,

revealed a risk level exceeding  $1^{80}$ . The results indicated that the TTHQ for potatoes and fruits exceeded 1, suggesting that they may pose greater health risks than other vegetables and fruits ([Table 7](#)). The concentrations of heavy metals and nitrate, along with the average daily intake and THQ from consuming these samples, were higher than those of the other samples. The TTHQ for tomatoes (0.931) and leafy vegetables (0.916) was below 1, but very close to 1, indicating a significant risk associated with exposure to heavy metals and nitrates through the consumption of these crops, warranting further attention in the [supplementary materials \(Table 7\)](#).

### Conclusion

Identifying the importance of including vegetables and fruits in daily diets is essential for monitoring the levels of heavy metals and nitrates in these food items to protect individual health. This study assessed the levels of Cd, Pb, and nitrate in commonly consumed vegetables and fruits in East and West Azerbaijan, Iran. The findings revealed that none of the samples exceeded the permissible limit for Cd, but some samples showed concentrations of Pb and nitrate that raised concerns regarding their safety. Greenhouse and traditional cucumbers, onions, tomatoes, apples, and oranges had higher levels of

Pb than Cd and nitrate, whereas potatoes, spinach, and leafy vegetables had higher nitrate levels than Pb and Cd. The risk assessment indicated that the THQ values were below 1 for all vegetables and fruits, indicating no immediate health risks to the Iranian population. However, the TTHQ for potatoes and fruits exceeded 1, suggesting potentially higher health risks. Continuous monitoring of samples and the implementation of effective solutions for the proper use of chemical fertilizers are recommended to ensure food safety.

### Conflict of Interest

The authors declare that there is no conflict of interest.

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### Code of Ethics

This study was approved by the Ethics Committee of Maragheh University of Medical Sciences, Maragheh, Iran, with ethical code of IR.MARAGHEHPHC.REC.1402.008.

### Authors' contributions

Melika saeedlunia: Data curation, Ali Abdollahnejad: Formal analysis, Farhang Hameed Awlqadr: Writing - review and editing, Maryam Farshidi: Methodology, Mojtaba Pourakbar: Validation, Behzad Ebrahimi: Supervision

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