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Evaluation of Lead, Cadmium and Nickel Contamination in *Lactuca Sativa* Cultivated in Savadkooh Region, Iran

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To investigate the level of heavy metal contamination in the lettuce cultivated in
Place: Savadkooh County in Mazandaran, Iran.
Methodology: five samples were taken from the lettuce produced in this area and also from the soils in which they were cultivated, and the concentrations of Nickel, Lead, and Cadmium were measured by atomic absorption spectrometry. Descriptive and inferential statistics were used to analyze the measured data. Data analysis was performed by SPSS ver.16 at the 0.05 significance level.
Results: The highest lead concentration, 0.07ppm, was observed in the sample No. 5 (Lettuce 5), the highest cadmium concentration, 0.008ppm, was measured in the sample No. 2 (Lettuce 2), and the highest nickel concentration, 0.07ppm, was observed in the samples No. 1 and 4 (Lettuce 1 and Lettuce 4).
Conclusion: The results of this study show that while all the lettuces cultivated in the Savadkooh region contain some amounts of nickel, cadmium, and lead, these amounts are much lower than the limits specified in Iran's national standard, and therefore these products are perfectly safe to

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consume. Also, the concentration of each heavy metal in the lettuces was found to be directly correlated with the corresponding concentration in the soil in which they were grown, indicating that the heavy metal content of the products increases with the increasing heavy metal content of the soil.

Keywords: Nickel; Lead; Cadmium; contamination; lettuce.

1. INTRODUCTION

Given the limitations of natural food resources, ensuring the food security of a growing population has long been an important global subject of discussion. One of the major topics of this discussion is the contamination of food resources with heavy elements, which are among the most important non-point sources of pollution. Indeed, heavy metal contamination is a very serious environmental problem. Heavy metals can enter our food chain through plants, causing significant health damages [1]. With the rapid industrialization of the world, environmental issues like heavy metal contamination are also growing at a rapid pace. The combination of this rapid industrialization with ill-managed urbanization and long-term use of massive amounts of pesticides and chemical fertilizers is accelerating the accumulation of toxic substances in our soil and air [2]. Heavy metals are widely dispersed in our environment and count among the most important chemical contaminants of our food. The term “heavy metals” refers to two groups of elements. The first group includes elements like iron, manganese, copper, zinc, and molybdenum, which are essential for normal metabolic processes and therefore called micronutrients. These elements are more harmful to plants than to humans and animals when absorbed in excess. The second group includes elements like mercury, lead, and cadmium, which have a fairly limited impact on plant growth but do significant damage to humans and animals even at low concentrations [3]. In terms of their toxicity to living organisms, heavy metals can be arranged in the following order: Hg> Cu> Zn> Ni> Pb> Cd> Cr> Sn> Fe> Mn> Al [4]. Exposure to heavy metals through the consumption of contaminated vegetables could be a major health threat. The intake of high concentrations of certain elements is an especially serious problem for vegetables grown near industrial areas and busy roads and those exposed to municipal and agricultural wastewater. This may lead to an imbalance in the concentration of elements, which in turn may significantly affect their availability and interaction with other nutrients and metals [5]. Exposure to cadmium, lead, and nickel compounds is believed to be especially dangerous during pregnancy, childhood, and adolescence, as it causes irreversible changes in the central nervous system. Lead is also known to cause cardiovascular diseases and impair hemoglobin biosynthesis, vitamin D metabolism, kidney and liver function, immune and reproductive systems, and iron, zinc, and copper metabolisms. Cadmium is carcinogenic and toxic, causes skeletal disorders, liver damage, cardiovascular diseases, and gonadal dysfunction, and disturbs the body’s mineral balance [6]. The plants grown in soil with excess nickel content tend to have higher nickel contents. Research has shown that at a concentration of 100 mg/kg, nickel is an essential element for plant metabolism, but the presence of higher concentrations of nickel causes the leaves to grow yellow and reduces the yield [7]. Lettuce (*Lactuca sativa*) is one of the most important leafy vegetables, which is widely used in fresh foods and salads, although some types of it are cooked before consumption [8]. With about 1.7 million hectares of cultivated land dedicated to lettuce production, this plant is one of the world’s most highly produced vegetables. The main origin of lettuce is the Mediterranean region and southwest Asia. But the world’s most important centers of lettuce production are currently North and Central America, Europe, China, Spain, Italy, India, and Japan [9]. Mazandaran province is one of Iran's most important centers of vegetable production including lettuce. In Mazandaran, the area under lettuce cultivation is approximately 5000 hectares (both as the second crop after rice in paddy fields and as the primary crop in other fields). Lettuce yield in this province is about 20 t ha-1, which is lower than Iran’s average yield of 27 t ha-1. Given the toxicity, stability, and non-biodegradability of heavy metals, this type of contamination has become a major source of environmental concern, especially in urban areas [10]. The goal of this study was to investigate the health of *Lactuca sativa* produced in the Savadkooh County of Mazandaran in terms of contamination with lead, cadmium and nickel.

2. MATERIAL AND METHODS

Samples were taken from the lettuce fields and their soils in the Savadkooh county at five fields. Five samples with 3 replication were taken from five fields. The samples named Lettuce 1 and Lettuce 2 were taken from two different fields in the Shirgah district, the Lettuce 3 was taken from the Pol-e- Sefid district, and Lettuce 4 and Lettuce 5 were taken from two different fields in the Zirab district. All samples were tested with 3 replications. After sampling, all soil and lettuce samples were transferred to the laboratory of the University of Tehran, where their Lead, Cadmium, and Nickel contents were measured.

2.1 Preparation of Lettuce Samples

To prepare the lettuce samples for measurements, 20g of the collected lettuce was weighed on a laboratory scale to the nearest 0.001g, and then placed inside a crucible and heated at 500°C for 8 hours to dry. After turning the sample completely into ash, 50ml of 6M hydrochloric acid was added to the crucible in a way that its content was entirely impregnated with the acid. The crucible was then placed on a water bath to let the acid evaporate. Next, 30ml of 0.1M nitric acid was added to the crucible to dissolve its remaining contents. Finally, lead, cadmium, and nickel contents of the sample were measured by optical atomic absorption spectroscopy. The wavelength, gas flow intensity, temperature configuration, and other settings of the spectroscopy machine were adjusted according to the instructions provided by the manufacturer [11].

2.2 Preparation of Soil Samples

The collected soil samples were air dried, compacted with a plastic mallet, and then passed through a 4 mm sieve. The soil collected under the sieve was used for chemical analysis. The absorbable concentration of heavy metals in the soil was measured by the use of DTPA, calcium chloride, and Tri ethanolamine, with the pH of the extract ant solution adjusted to about 7.3 [12]. The concentration of heavy metals was then measured by a Perkin Elmer AAS4100 atomic absorption spectrometer. The collected data were analyzed using descriptive and inferential statistical methods. Descriptive statistics was used to obtain descriptive coefficients for the collected data and inferential statistics was used to test the research hypotheses. The one-sample Kolmogorov-Smirnov test was used to check the normality of the data. The one-sample t-test was used to compare the mean heavy metal concentrations with the corresponding standard limits. A one sample test of means compares the mean of a sample to a pre-specified value and tests for a deviation from that value. Pearson's correlation coefficient was used to check whether there is a relationship between heavy metal concentrations in soil and those in lettuce. Data analyses were performed using SPSS version 16, with $P = .05$ considered statistically significant.

3. RESULTS AND DISCUSSION

According to Smirnov-Kolmogorov test Table, if the significant level for all independent and dependent variables is greater than the test level (0.05), the data distribution is normal.

3.1 Lead

As the results presented in Table 2 show, the significance statistics obtained for all lettuce samples are less than 0.05. In other words, the lead concentrations in all lettuce samples were significantly lower than the standard limit with $P = .05$. Considering the mean lead concentration of the samples, it can be concluded that the lead content of all of the five lettuces is lower than the maximum allowable limit and falls in the acceptable range.

As shown in Fig. 1, the five lettuces contain roughly the same amount of lead.

After establishing the normality of the data using the Kolmogorov-Smirnov test, Pearson's correlation coefficient was used to investigate whether the concentration of lead in the soil is correlated with the concentration of lead in the lettuces. The results of this investigation are provided in Table 2.

As the results of Table 3 show, the significance statistic obtained in the correlation test for Lettuce 1 is less than 0.05, which means lead concentration in the soil has had a significant impact on lead concentration in this lettuce sample. The positive sign of the correlation statistic shows that the amount of lead in Lettuce 1 has increased with the amount of lead in the soil.

The significance statistics obtained in the correlation test for Lettuce 3 and Lettuce 5 are more than 0.05. Therefore, the amount of lead in the soil has had no significant effect on the concentration of lead in these two samples.

3.2 Cadmium

As shown in Table 4, the significance statistics obtained for cadmium concentrations in Lettuce 1, Lettuce 4, and Lettuce 5 are lower than 0.05. These results show that cadmium concentrations in all of the five lettuces are lower than the maximum allowable limit and therefore acceptable.

Since the raw data obtained from the measurements made in Lettuce 2 and Lettuce 3 were identical in all three replications, the tests could not be done for these samples. In the next step, Duncan's test was used to compare the amount of cadmium in the five lettuces. The results of this test are illustrated in the diagram below.

In Fig. 2, it can be seen that there is a significant difference between the concentrations of cadmium in different lettuces (P= .05). Lettuce 2 has a significantly higher cadmium content than other samples (P= .05). However, there is no significant difference between other samples in this respect (P= .05).

Again, after checking and confirming the normality of the data using the Kolmogorov-Smirnov test, Pearson's correlation coefficient was used to examine the relationship between cadmium concentrations in the soil in the lettuces. The results of this test are provided in Table 4.

As shown in Table 5, the significance statistic obtained in the correlation test for Lettuce 1 is lower than 0.05. This suggests that cadmium concentration in the soil has had a significant effect on cadmium concentration in Lettuce 1. The positive sign of the correlation statistic indicates that the concentration of cadmium in Lettuce 1 has increased with the concentration of this metal in the soil.

3.3 Nickel

In Table 6, it can be seen that the significance statistics obtained in this test are higher than 0.05. This means that there is no significant difference between the nickel contents of the five lettuce samples.

As shown in Fig. 3, the five lettuces have similar amounts of Nickel.

The national standard of Iran does not specify a specific amount of nickel in lettuce, but FAO_ WHO has set the permissible limit of this metal in lettuce 0.2 ppm (FAO / WHO, 1984) that the results obtained in the present study are less than This is the amount.

As the results presented in Table 7 show, the significance statistics obtained in the correlation test for Lettuce 3 and Lettuce 4 are lower than 0.05. Therefore, nickel concentration in the soil has had a significant impact on nickel concentration in these two samples. The correlation statistics for these relationships are positive, which means the concentration of nickel in these lettuces has increased with the concentration of nickel in the soil.

Contamination of soil and plants with heavy metal, especially through irrigation with municipal and industrial wastewater or sewage sludge, has been the subject of numerous studies around the world [3]. The goal of the present study was to investigate the concentration of heavy metals in the lettuce being grown in the Savadkooh county of Mazandaran and the soil in which they are being cultivated.
The main anthropogenic sources of heavy metal contamination include:

1- Transportation vehicles and their fuels (linear emission).
2- Fuel combustion processes and industrial units that release pollution into the air through an emitter (point emission).
3- House heating equipment in urban and domestic sectors (surface emission) [13].

Numerous studies conducted in recent years have shown the bold role of street dust in urban soil pollution near roads. The primary source of heavy metals is the exhaust gas of motor vehicles. However, these elements can also be produced by those vehicle parts that are exposed to abrasion such as tires and brakes as well as catalytic converters. Resuspension of metal-rich road dust because of traffic may also be a major source of road pollution, especially on roads with heavy traffic and those with a high percentage of heavy vehicles [14].

The risk of heavy metal contamination in vegetables grown near old industrial areas cannot be ignored [15,16], as this type of contamination could be very problematic. The concentration of different elements in vegetables also depends on factors such as the natural amount of elements in the environment, the amount of elements in mineral fertilizers, and the amount of fertilizer used in the cultivation process. For most soils, the natural source of these metals is bedrock. In agricultural fields, some amounts of metals enter the soil along with organic and mineral fertilizers (mainly calcium and phosphates). Another source of metals is plant protection products [17]. In a study published in 2012, high cadmium concentration was associated primarily with the granulometric composition of soils and secondarily with their position with respect to high-traffic roads. This study also showed that the amount of cadmium in the tested vegetables was slightly associated with the topsoil properties, including the total and dissolved cadmium content [18]. This report is consistent with the results of our research. Kabata-Pendias and Pendias (1999) reported that soil pH is the most important factor for the absorption of heavy metals by vegetables [19]. This was confirmed by a study conducted by Bielecka et al. (2009) which showed the lower risk of leaching of heavy metals and their absorption into plants in alkaline soils ($7.1<pH<8.1$) and that the presence of organic matter can inhibit the uptake of metals from soil solution [20]. These soil properties, which determine the solubility of metals in the soil, can be utilized to immobilize heavy metals in the solid phase. Metal mobility and bioavailability can be affected by adding organic matter or minerals to the soil. A basic solution for limiting the mobility of metals is to carry out soil de acidification with lime. The use of phosphate can also affect the mobility of heavy metals and their uptake by plants [21]. In a study by Alexander et al. (2006), they reported high accumulation of cadmium, copper, and zinc in lettuce and spinach and also observed the highest lead accumulation in lettuce and onion. They also reported that the amounts of heavy metals that accumulate in vegetables depend on the plant cultivar [22]. Our study also showed the availability of these elements in lettuce.

Table 1. Results of Kolmogorov-Smirnov test of studied metals

Heavy metals	Number of samples	Mean	Standard deviation	Test statistic K-S	Significance level	Test result
Pb of lettuce	15	0.054	0.0180	0.727	0.666	Normal
Pb of soil	15	10.530	1.5055	0.949	0.329	Normal
Ni of lettuce	15	0.058	0.0182	0.815	0.520	Normal
Ni of soil	15	11.800	1.9712	0.552	0.921	Normal
Cd of lettuce	15	0.005	0.0018	0.524	0.947	Normal
Cd of soil	15	0.007	0.0022	0.645	0.799	Normal

Table 2. Results of the one-sample t-test for comparing the mean lead concentration in the lettuce samples with the standard limit (in ppm)

Sample group ID	Number of samples	Mean ± Standard deviation	Standard limit	t statistic	Degrees of freedom	Level of statistical significance
Lettuce 1	3	0.05 ± 0.01	0.3	-43.301	2	0.001
Lettuce 2	3	undetected	undetected	undetected	undetected	undetected
Lettuce 3	3	0.05 ± 0.02	0.3	-21.651	2	0.002
Lettuce 4	3	0.06 ± 0.01	0.3	-41.569	2	0.001
Lettuce 5	3	0.07 ± 0.03	0.3	-13.279	2	0.006

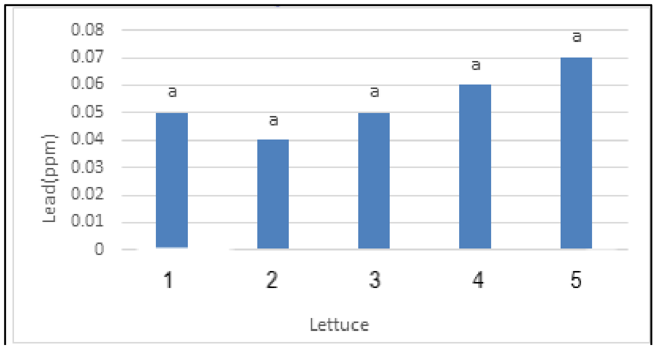

Fig. 1. Lead concentration in the lettuce samples (in ppm)

Table 3. Results of the Pearson's correlation test on the relationship between lead concentration in the soil and lead concentration in the lettuce samples (in ppm)

Sample group ID	Correlation statistic	Level of statistical significance	Result
Lettuce 1	1	<0.001	Significant correlation
Lettuce 2	undetected	undetected	undetected
Lettuce 3	0.5	0.667	No significant correlation
Lettuce 4	undetected	undetected	undetected
Lettuce 5	0.982	0.121	No significant correlation

Table 4. Results of the one-sample t-test for comparing the mean cadmium concentration in the lettuce samples with the standard limit (in ppm)

Sample group ID	Number of samples	Mean ± Standard deviation	Standard limit	t statistic	Degrees of freedom	Level of statistical significance
Lettuce 1	3	0.004 ± 0.002	0.5	-39.837	2	0.001
Lettuce 2	3	undetected	undetected	undetected	undetected	undetected
Lettuce 3	3	undetected	undetected	undetected	undetected	undetected
Lettuce 4	3	0.0053 ± 0.0011	0.5	-67	2	0.001
Lettuce 5	3	0.005 ± 0.002	0.5	-38.71	2	0.001

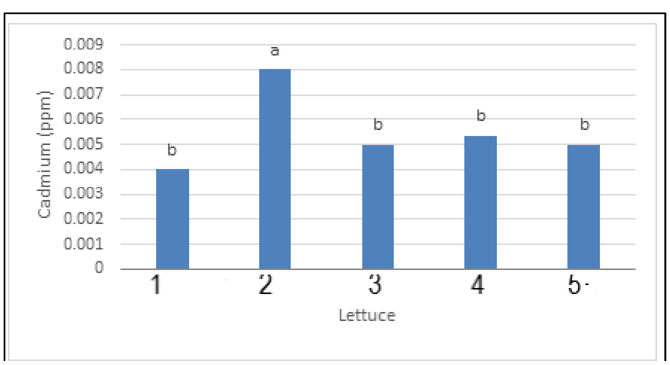

Fig. 2. Cadmium concentration in the lettuce samples (in ppm)

Table 5. Results of the Pearson's correlation test on the relationship between cadmium concentration in the soil and cadmium concentration in the lettuce samples (in ppm)

Sample group ID	Correlation statistic	Level of statistical significance	Result
Lettuce 1	1	<0.001	Significant correlation
Lettuce 2	undetected	undetected	undetected
Lettuce 3	undetected	undetected	undetected
Lettuce 4	undetected	undetected	undetected
Lettuce 5	undetected	undetected	undetected

Table 6. Results of one-way analysis of variance test to compare the amount of Nickel in 5 lettuces in terms of ppm

Level of statistical significance	F statistic	Mean sum of squares	Degrees of freedom	Sum of squares	Source	Metal
		0.001	4	0.002	Intergroup	
0.177	1.962	0	10	0.003	Intragroup	Nickel
			14	0.005	Total	

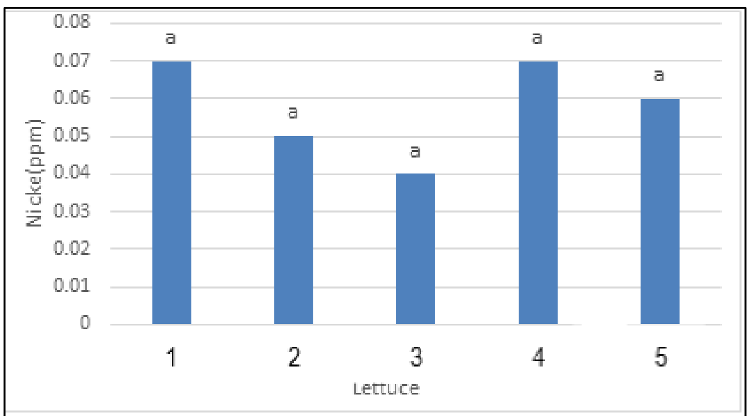

Fig. 3. Nickel concentration in the lettuce samples (in ppm)

Table 7. Results of the Pearson's correlation test on the relationship between Nickel concentration in the soil and nickel concentration in the lettuce samples (in ppm)

Result	Level of statistical significance	Correlation statistic	Sample group ID
undetected	undetected	undetected	Lettuce 1
undetected	undetected	undetected	Lettuce 2
Significant correlation	<0.001	1	Lettuce 3
Significant correlation	<0.001	1	Lettuce 4
undetected	undetected	undetected	Lettuce 5

4. CONCLUSION

Overall, the results of this study showed that while all lettuces in the study area contained some amounts of lead, cadmium, and nickel, the concentration of these metals was much lower than the permitted levels specified by the Institute of Standards and Industrial Research of Iran and FAO-WHO.

These products are completely safe for consumption. The results also suggest that the concentration of lead, cadmium, and nickel in the studied lettuces is directly related to the concentration of these metals in the soil in which they have been grown. From these results, it can be concluded that there need not be any concern about the consumption of lettuce cultivated in Savadkooh region and this product will not endanger the health of consumers in any way. It was also observed that as the amount of heavy metals in the soil increases, so does the concentration of these metals in the product. Therefore, farmers should be educated about the prudent use of chemical fertilizers and replacing them with organic fertilizers in order to prevent heavy metal contamination of soil and plants in the future.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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