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Evaluation of toxic metals content and microbial contamination of parsley (Petroselinum crispum) prepared from local farms in Kushal and Layalestan regions (Lahijan city, north of Iran)

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ABSTRACT

Toxic metals are one of the important environmental contaminants that have been created for humans with the development of societies. These toxic metals with high stability and accumulative properties can cause many problems. Due to the low price of parsley (*Petroselinum crispum*) and the high use of it by local people (North of Iran) in their meals, this study evaluated the contamination of parsley. Therefore, the amount of toxic metals including arsenic (As), cadmium (Cd), lead (Pb), and mercury (Hg) in parsley prepared from local farms in Kushal and Layalestan regions (Lahijan city, North Iran) was evaluated. On the other hand, bacteria and fungi, which are also known as pathogens, are harmful to human health. Hence, all samples were evaluated for microbial contamination (total microbial count). For this purpose, three samples of parsley were prepared from each region (local farm), and the content of toxic metals and microbial contamination was investigated. The results showed that the samples of a farm had toxic metals and microbial contamination beyond the standard due to its proximity to the main road and the mine.

Keywords: Toxic metals, Microbial contamination, Parsley, Environmental contaminants

Introduction

Pollution of resources such as soil, water and air is a major crisis to natural ecosystems and human health that has emerged with industrialization and urbanization (Anastopoulos *et al.* 2017; Li *et al.* 2020). The presence of toxic metals in arable lands and their transfer to crops is a risk to human health. Metals play a main role in many biological and molecular processes, but elements such as As, Pb, Cd, and Hg have not any known functionality in the human body and are very harmful to health more than certain levels (Cannas *et al.* 2020; Domingo and Marquès, 2021). Industrial discharge, agrochemicals, plastics, and mining are the main sources of toxic metal pollution in the environment, and when plants grow in metal-contaminated soils or are irrigated with contaminated water, toxic metals can accumulate in plants (Alinia-Ahandani *et al*. 2020; Edraki *et al*. 2022; Sheydaei 2022; Sheydaei and Alinia-Ahandani, 2021; Yaashikaa *et al*. 2022). Continuous exposure to such toxic metals can cause main problems for human health. For example, Cd poisoning can cause cardiotoxicity and hypertension, or Hg toxicity can cause coronary heart disease, myocardial infarction, carotid artery occlusion, and atherosclerosis (Sevim *et al*. 2020). On the other hand, diseases transmitted from food groups such as meat, fruits, and vegetables continue to have negative effects on public health (Sharma *et al*. 2020; Ibrahim *et al*. 2021). These diseases can be caused by ingesting pathogens or consuming toxins produced by them in food products (Soni *et al.* 2022). The outbreak of food-borne diseases leads to a high rate of mortality, and according to the World Health Organization, this global death rate will increase to 10 million people annually if effective measures are not taken by 2050 (Ibrahim *et al*. 2021). Contamination of products with pathogens can have different sources, such as the use of land-application of raw manure, contaminated irrigation water, use of immature compost, contaminated soil, fecal contamination, and contamination of cutting and packing rooms (Thakali and MacRae 2021). In a previous study, we investigated the amount of toxic metals in parsley (*Petroselinum crispum*) obtained from local farms in Baz Kia Gorab region (Lahijan city, north of Iran), and the results showed that the amount of toxic metals was higher than the standard level (Sheydaei *et al*. 2022). Herein, parsley was chosen for this study because it is widely used by local people in their meals. Also, the study regions (Kushal and Layalestan) were selected because they were a distance from the city and close to the main road and mine. For this purpose, the microbial contamination (total microbial count) as well as the concentration of heavy metals such as Pb, As, Hg, and Cd in parsley obtained from these regions were investigated.

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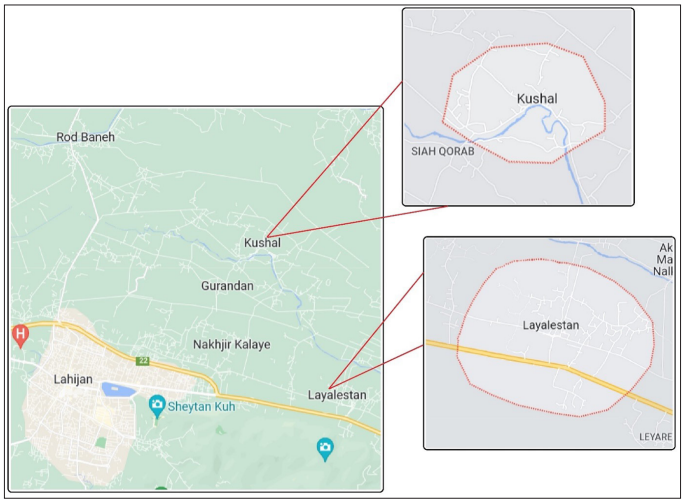

Figure 1.
Regions selected for evaluation (https://www.google.com/maps/place/Layalestan,+Gilan+Province/@37.2124763,50.0605327,13.75z/data).

Experimental

Materials

The samples were prepared from Kushal and Layalestan regions in Lahijan (north of Iran). A total of 6 samples of parsley were gathered from local farms. The samples were gathered from their original positions and packed in clean-checked polyethylene bags. Then, we started labeling, carried them to the lab, and kept them in the refrigerator until analysis. Nitric acid (65%), hydrochloric acid (37%), hydrogen peroxide (30%), potassium chloride, and all other solvents and chemicals were purchased from Merck Chemicals Co. (Germany) and used as received.

Preparation and analysis of samples

Digestion and statistical analysis of the samples were performed according to the method described in the previous study (Sheydaei *et al*. 2022). The concentrations of heavy metals were investigated on a flame atomic absorption spectrometer (SavantAA, GBC). Also, microbial contamination (total microbial count) was performed according to the procedure which was described in the literature and Iran's national standard (Institute of Standards of Iran 2014). Briefly, the desired dilutions prepared with sterile physiological serum were carried out in plate count agar (Scharlau) and incubated for 72 h at 30 °C (Soltan Dallal *et al*. 2016). In addition, the following equation was used to calculate microbial contamination (Institute of Standards of Iran 2014).

$$a = \frac{\Sigma C}{V(N + 0.1n) \times d}$$

Where, ΣC, V, N, n, and d are the total number of microorganisms in the plate, the volume used of the desired dilution, the number of primary dilution plates, the number of higher dilution plates, and the initial dilution exponent, respectively.

Results and Discussion

In this study, two regions were selected (see Fig. 1) and samples were prepared from three local farms in each region (see Fig. 2).

The contents of toxic metals in the samples are summarized in Tables 1 and 2. The evaluation results of the Layalestan region

Figure 2.
The location of selected farms in each region for evaluation)https://satellites.pro/Iran_map#37.202493,50.081692,15 ; https://satellites.pro/Iran_map#37.232531,50.054955,15).

Figure 3.
The quarry in the Layalestan region)https://satellites.pro/Iran_map#37.195523,50.078988,17(.

show that sample 1 contains more toxic metals than the standard level (see Table 1). In fact, the investigated farm (sample 1) is located very close to the main road and there is also a mine there (see Fig. 3). The pollution of resources by heavy metals from automobile has been proven (Chen *et al*. 2005). In fact, these metals are released from burning fuel, corrosion of batteries, wear of brake linings, wear of tires, and leakage of oils (Blok 2005). On the other hand, many pesticides contain As and Cd, and their excessive use causes the pollution of resources (Ahandani *et al*. 2022). It can be said that the suburbs are highly polluted by toxic metals due to their proximity to mines, industrial town, hospitals, and main road where thousands of automobile travel daily. This fact can be clearly seen in the results of samples 2 and 3, which contain toxic metals less than the standard level due to being far from the location of sample 1. According to the

Table 1. Toxic elements concentrations in parsley samples from the Layalestan region

Metal (mg/kg)	Range	Mean ± SDd
Pb	[a]0.80 - 1.60	[a]1.10 ± 0.10
	[b]0.20 - 0.31	[b]0.26 ± 0.10
	[c]0.16 - 0.22	[c]0.20 ± 0.11
Cd	[a]0.36 - 0.94	[a]0.62 ± 0.10
	[b]0.14 - 0.18	[b]0.15 ± 0.05
	[c]0.13 - 0.14	[c]0.11 ± 0.05
Hg	[a]0.06 - 0.11	[a]0.086 ± 0.05
	[b]0.009 - 0.01	[b]0.01 ± 0.05
	[c]0.006 - 0.01	[c]0.008 ± 0.05
As	[a]0.12 - 0.20	[a]0.16 ± 0.10
	[b]0.04 - 0.10	[b]0.06 ± 0.05
	[c]0.03 - 0.08	[c]0.05 ± 0.05

Note: * aSample 1, bSample 2, cSample 3, **SD = Standard deviation *** The international standard for Pb, Cd, Hg, and As is 0.3, 0.2, 0.01, and 0.1, respectively (Codex 1995; Islam and Hoque, 2014).

Table 2. Toxic elements concentrations in parsley samples from Kushal region

Metal (mg/kg)	Range	Mean ± SDd
Pb	[a]0.10 - 0.20	[a]0.14 ± 0.05
	[b]0.10 - 0.20	[b]0.14 ± 0.05
	[c]0.11 - 0.24	[c]0.18 ± 0.10
Cd	[a]0.06 - 0.10	[a]0.08 ± 0.10
	[b]0.04 - 0.10	[b]0.07 ± 0.05
	[c]0.07 - 0.10	[c]0.08 ± 0.05
Hg	[a]0.004 - 0.006	[a]0.005 ± 0.03
	[b]0.004 - 0.006	[b]0.005 ± 0.03
	[c]0.004 - 0.007	[c]0.006 ± 0.10
As	[a]0.04 - 0.06	[a]0.05 ± 0.10
	[b]0.04 - 0.07	[b]0.06 ± 0.10
	[c]0.05 - 0.08	c0.07 ± 0.10

Note: * aSample 1, bSample 2, cSample 3, **SD = Standard deviation ***The international standard for Pb, Cd, Hg, and As is 0.3, 0.2, 0.01, and 0.1, respectively (Codex 1995; Islam and Hoque, 2014).

Table 3. Microbial contamination in parsley (Layalestan region)

Total microbial count	Maximum
Sample 1	1.05×10^5
Sample 2	2.09×10^4
Sample 3	7.7×10^3

Note: Iran's national standard for the total microbial count is 105 (Institute of Standards of Iran 2014).

Table 4. Microbial contamination in parsley (Kushal region)

Total microbial count	Maximum
Sample 1	4×10^3
Sample 2	2.2×10^3
Sample 3	4.5×10^3

Note: Iran's national standard for the total microbial count is 105 (Institute of Standards of Iran 2014).

obtained results, it can be said that the planting of vegetables in region 1 should not be done because the toxic metals are more than the standard and the sources of production of these toxic metals (the main road and mine) are out of the control of the local people. So it can be said that the only way is not to grow the crop or to build a greenhouse and isolate it from the environment.

The result of the evaluation of Kushal region shows that all the samples contain toxic metals less than the standard level (see Table 2). These results show the fact that the regions that are still spared from industrial expansion are less polluted. Heavy metals have accumulative properties, high stability, and toxic effects, and on the other hand, these compounds are not metabolized in the body, their high accumulation can be very harmful to humans (Sheydaei *et al*. 2022) Heavy metals can cause mental retardation, hearing impairment, immune system dysfunction, brain diseases, blindness, muscle weakness, and cancer (Alinia-Ahandani

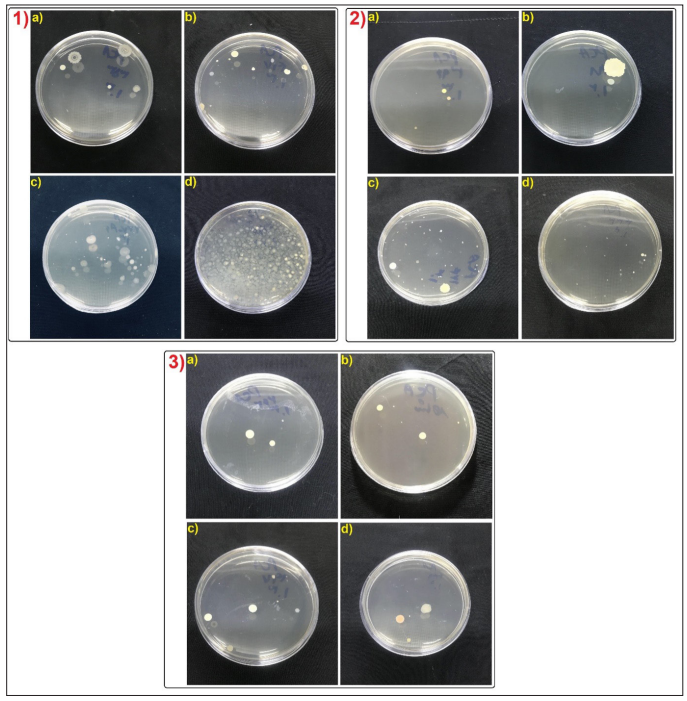

Figure 4.
Observations of the total microbial count of each sample in Layalestan region: (a and b) sample with 10^4 dilution (c and d) sample with 10^3 dilution.

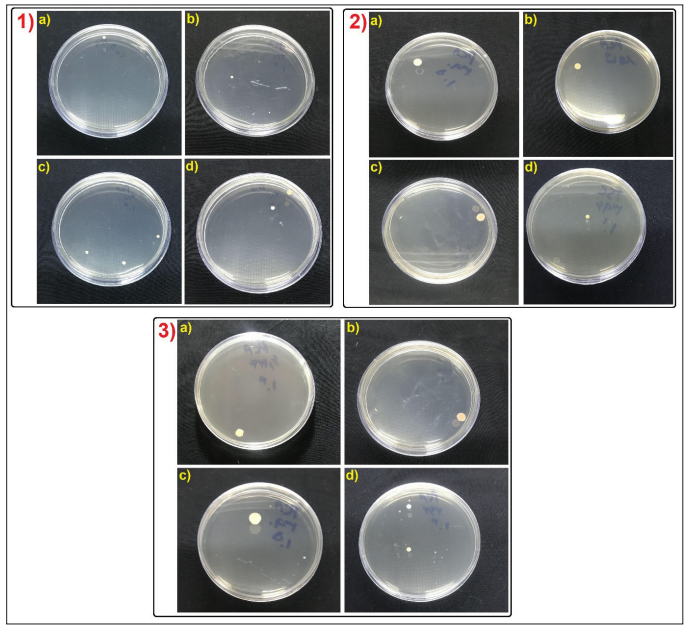

Figure 5.
Observations of the total microbial count of each sample in Kushal region: (a and b) sample with 10^4 dilution (c and d) sample with 10^3 dilution.

et al. 2021; Sheydaei *et al*. 2020). Therefore, the contamination of resources with heavy metals can be an important problem.

In this study, microbial contamination (total microbial count) was also investigated, the results of which can be seen in Tables 3 and 4 as well as Figures 4 and 5. Due to the lack of proper disinfection, vegetables can be a source of bacterial contamination, and

the presence and content of microorganisms vary depending on many factors such as the type of product, agricultural activities, and geographic region (Ibenyassine *et al*. 2007). Moreover, excessive use of pesticides and fertilizers also causes crop contamination, and farmers should receive the necessary training in this field (Beuchat 2002). Although pathogens can be reduced by washing vegetables, but the presence of pathogens inside the plant tissue and the hydrophobicity of the plant surface limit its effectiveness to some extent (Olaimat and Holley, 2012). As the results show (Tables 3 and 4), according to the national standard of Iran (standard 5272), except for sample 1 of Layalestan region, the rest of the samples are acceptable (they are free of contamination).

Conclusions

In summary, the amount of toxic metals in parsley (*Petroselinum crispum*) collected from local farms in the Kushal, and Layalestan regions were investigated. The results showed that the sample of a farm in the Layalestan region had more toxic metals than the standard level. High levels of toxic metals can have major harmful effects on humans. Also, investigation of microbial contamination of vegetables showed that only 1 sample from Layalestan region was unacceptable. This study shows that crops should be continuously investigated. Since the assessment of heavy metals and microbial contamination is a specialty and also relatively expensive, governments should periodically investigate these contaminations and investigate crops in local farms and markets.

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