RESEARCH ARTICLE

• Arch Hyg Sci 2019;8(4):274-285
• Journal Homepage: http://jhygiene.muq.ac.ir

Potential Health Risk of Heavy Metals Accumulation in Cultivated Mulberry in Urban Landscapes of Arak, Iran: A Case Study

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A-R-T-I-C-L-EI-N-F-O

Article Notes:
Received: Sep 30, 2019
Received in revised form:
Jan 19, 2020
Accepted: Jan 19, 2020
Available Online: Feb 23, 2020

Keywords:
Aluminum
Fruit
Heavy
Industry
Iran
Metals

A-B-S-T-R-A-C-T

Background & Aims of the Study: Heavy metal pollution enters the food chain through industrial, urban, and agricultural sources and due to their capability to be accumulated in food put living beings' health in danger. Although the use of ornamental plants, especially trees and shrubs can be an effective approach in the absorption and uptake of metal contaminants from the soil, it should be noted that the cultivation of edible crops poses a serious threat to the health of people living in industrial and metropolitan cities. Therefore, this study aimed to determine the extent of heavy metal contamination in the fruits and leaves of white mulberry and weeping mulberry in different areas of Arak, Iran.
Materials and Methods: A total of 13 elements, namely aluminum, chromium, cobalt, nickel, copper, zinc, arsenic, cadmium, mercury, lead, vanadium, manganese, and molybdenum, were analyzed in the fruits and leaves of the *Morus alba* var. pendula and *Morus alba* in 13 landscapes of Arak by induced coupled plasma emission spectrometry.
Results: The comparison of average studied accumulated metal concentration with the international standard limit showed that the fruit of mulberry trees in Arak was contaminated with aluminum, nickel, arsenic, cadmium, and lead. The concentrations of aluminum and cadmium 3-6 times and lead 4 times were greater than the permissible limits. The obtained results were compared with their permissible levels set by the Food and Agriculture Organization of the United Nations and World Health Organization. According to results, the concentrations of these metals were much greater in cultivated mulberries in Arak than permissible limits; therefore, they are not safe to consume.
Conclusion: Due to the contamination of mulberries with heavy metals, such as nickel, arsenic, aluminum, and lead, there is a serious warning about their consumption. These heavy metals were accumulated in the leaves of mulberry trees in addition to their fruits.

Please cite this article as: Taghizadeh M, Kazemi A. Potential Health Risk of Heavy Metals Accumulation in Cultivated Mulberry in Urban Landscapes of Arak, Iran: A case study. Arch Hyg Sci 2019;8(4):274-285

Background

There has been an increasing extent of contamination of soils with heavy metals since the beginning of industrialization and urbanization development, which have been brought about serious threats to various ecosystems, surface water, groundwater, food security, and human health (1). Due to the potential effects of heavy metals on human health and their long-term sustainability, food production in contaminated areas is a concern.

Heavy metals can cause soil and

groundwater to be contaminated, generate environmental problems, and put human health at risk even in low concentrations because they are non-degradable and have physiological effects on living beings (2). These pollutants enter the food chain through industrial, urban, and agricultural sources and due to their capability to be accumulated in foods put living beings' health in danger (3). Therefore, it appears necessary to design management strategies to remove them and counteract their effects in environments. In recent years, several technologies have been developed to reduce or eliminate heavy metals contained in the contaminated site, such as phytoremediation method (4).

It has been reported that the absorption of metals by trees can be more effective for the elimination of metals from the soil than hyperaccumulator plants due to their more biomass performance (5). Ang et al. (2010) studied phytoremediation in the soil contaminated with lead and cadmium using tree species (*Acacia mangium, Hopea odorata, Intsia palembanica*, and *Swietenia macrophylla*). The highest amount of accumulated lead was observed in the organs of *A. mangium* (6).

Another study investigated the ability to uptake cadmium, chromium, and nickel from the soil by poplar and mulberry, as well as the accumulation of these metals in their roots, stems, green leaves, and deciduous leaves. The results showed that deciduous leaves accumulated the most amounts of nickel and chromium, compared to other organs. Both plants were identified to be suitable for the phytoextraction of nickel and cadmium based on bioaccumulation factor (BAF) and translocation factor (7).

In a study carried out by Prince et al. (2000) on the amount of copper and cadmium uptake by cultivated white mulberry cuttings, it was demonstrated that the roots had greater ability to accumulate than the leaves, and the roots transmitted these elements less to the leaves. Moreover, their ability to transmit cadmium from the roots to leaves in contaminated soil surface horizons was greater than that of copper translocation due to the easier availability of cadmium in comparison to copper (8).

Although the use of ornamental plants, especially trees and shrubs can be an effective approach in the absorption and uptake of metal contaminants from the soil (9), it should be noted that the cultivation of such plants, which are edible crops, poses a serious threat to the health of individuals living in industrial and metropolitan cities. The reason is that most of the metal contaminants precipitate from the atmosphere to surface soil in a maximum of 2 weeks and then enter into undersurface soil or groundwater through irrigation or rainfall (10).

In metropolises, metal contaminants have been often reported in plants cultivated in landscapes and parks (11). The number of contaminants, namely lead and cadmium, accumulated by the plane tree (*Platanus* species) and paper mulberry (*Broussonetia papyrifera*) were considered high in a different area of Tehran, Iran. The observations showed that plane trees were able to uptake larger amounts of contaminants than paper mulberry (12).

Based on the literature, researchers investigated the ability to extract heavy metal contaminants from the soil and possibility of accumulation of lead, copper, zinc, and cadmium in the root system and aerial organs of mulberry trees. The obtained results showed zinc with the highest amount of accumulation in mulberry organs. Fruits and green leaves were organs with the highest accumulation of heavy metals (13).

Aims of the study

Food consumption is considered to be the main path of human exposure to heavy metals, which can endanger human health. Plants are exposed to pollution in cities due to the immobility structure. Therefore, they are more at risk of contaminants and environmental stresses than any other organism. On the other hand, plants can absorb contaminants from the air, soil, and water simultaneously. Plants at different heights, such as trees, shrubs, and grasses, can absorb metal pollutions. The more plants can accumulate metal contaminants in their aerial organs, the more it is possible to exploit nonedible plants to remove contaminants through the implementation of proper management strategies.

It should be noted that if the plants exposed to smoke and dust are fruit-bearing trees, their fruits will also play a significant role in the absorption of contaminants. Although it is now possible to measure the amount of some air contaminants using mechanical devices, it cannot be said how much of these elements are absorbed by trees and stored in their edible organs.

Due to the problem of pollution caused by metals, such as chromium, cadmium, nickel, and lead, in Arak as an industrial city, it is likely that there will be high uptake of these contaminants by cultivated plants. Therefore, this study aimed to determine the extent of heavy metal contamination in the fruits and leaves of white mulberry (*Morus alba*) and weeping mulberry (*Morus alba* pendula) precipitated onto the organs in different areas of Arak.

Materials & Methods

Study Area

Arak is located in the southern part of Markazi province to the center of Iran (Figure 1). Arak is considered to be an industrial city and one of the industrial centers of Iran. Industrial regions in Arak have been restarted since1989. Arak is located on the 34°5' 30"N and 49°41' 30". In Arak, the average rainfall is around 341 mm, and the climate is cold and semiarid. Moreover, the average yearly temperature is reported as 13.9°C. Arak has a 7,178.98 km^2 surface area and is the eighteenth most populous city of Iran (14).

Plant Sampling

According to the time when the fruits of mulberry trees are ripening, the sampling time was within May to June 2017. In this study, the species of *Morus alba* (white mulberry) and *Morus alba* var. pendula (weeping mulberry) cultivated in the landscapes of Arak were selected for the investigation. In this study based on the reticulation of Arak, 13 regions were chosen in Arak, and 1 region in Hezaveh village located 18 km northwest of Arak was selected as the control region (Figure 2). Because this rural area is far enough from polluting industrial centers, as well as Mighan Wetland, this region was considered a control site.

Figure 1) Location of the assessed areas in Arak, Markazi province, Iran

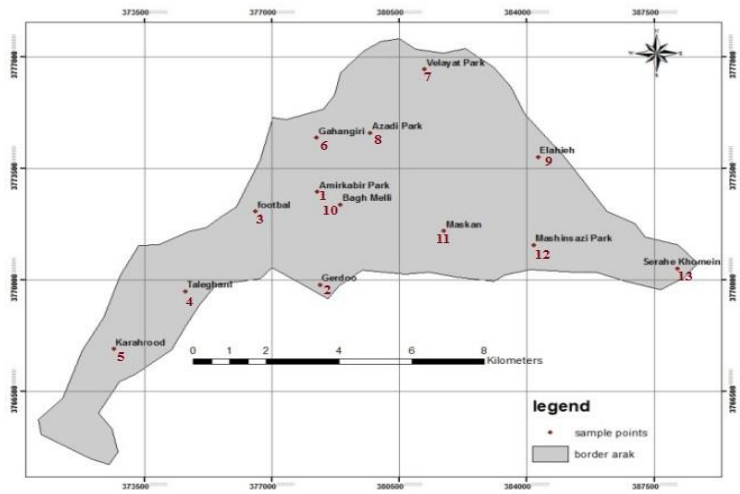

Figure 2) Distribution of sampled areas in Arak (Hazaveh village as the 14th area located 18 km northwest of Arak)

The landscapes and parks inside the selected regions were explored leading to the detection of two common mulberry genotypes. Three replications of any genotype in each region were selected, and from each replication, 10 leaves and 10 fruits were picked up from all parts of canopy cover of the relatively same age and isodiametric trees. The leaves with 10-15 cm long were collected to ensure the same age. All picked up leaves were separately stored in sampling containers with minimal handling and then transferred to the laboratory.

Preparation and elemental analysis

All samples of organs were dried in an oven at 70°C for 48 h. Plant materials were ground with an electric grinder to fine powders and saved as dried powders to be measured for heavy metals analysis. The 0.5 g portions of sieved plant samples were digested with a mixture of HCl/HNO_3 (3:1, v/v) on the hot block digester at 130°C for 1 h. Then, 40 cc sodium hypochlorite was added to the flasks. After completion, the specimens were filtered into 50 cc volumetric flasks through filter paper. The volumes of filtrates increased to 50 cc by adding deionized water. The resulted solutions were stored in plastic containers.

The amounts of 13 elements, including aluminum, chromium, cobalt, nickel, copper, zinc, arsenic, cadmium, mercury, lead, vanadium, manganese, and molybdenum, in the leaves and fruits of weeping mulberry and white mulberry were measured by induced coupled plasma emission spectrometry at 228/802 nm, and the concentration of cadmium in all treatments were calculated and recorded in ppm (15).

Data analysis

This experiment was implemented in a completely randomized design with three replicates of each treatment. Firstly, the normality of data was investigated, followed by assuring the accuracy of normal distribution, and then the obtained data about the content of accumulated elements were analyzed using the SAS software (version 9.1). The means of three replicates for all chemicals and physical analyses were subjected to a one-way analysis of variance. Duncan's Multiple Range test was used to compare the mean and determine the significance of statistical differences in treatments at $P \leq 0.05$. The data of each treatment in this study were represented as mean±standard deviation. The metal accumulation of the elements (i.e., Al, Cr, Co, Ni, Cu, Zn, As, Cd, Hg, Pb, V, Mn, and Mo) belonging to the white mulberry and weeping mulberry of the study area were correlated by the Spearman's rank correlation coefficient and analyzed using SPSS software (version 22). Firstly, the normality of the data was studied, and then the Spearman's rank correlation coefficient was calculated.

Results

Although most of the heavy metals were observed in trees in all areas (even in Hezaveh as a control site), the extent of these 13 elements uptake by leaves and fruits of the two mulberry species was very different from one area to another (tables 1 and 2). According to all results of metal accumulation in white mulberry and weeping mulberry in different regions of Arak, it can be concluded that in general there was no significant difference between the two species in terms of metals

Table 1) Mean±standard deviation of heavy metals in two species of mulberry trees (Wh: white mulberry [*Morus alba*] and We: weeping mulberry [*Morus alba* Var. pendula]) cultivated in different areas of Arak, Iran (ND: none detected)

HM.	Genus	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Aluminum	Wh.	50.3±14.8	37.6±11.0	29±0	26.7±0.4	22.1±2.8	29.1±1.7	16.6±1.8	15.9 ±0.8	24.3±2.0	28.5±6.0	21.1±3.7	22.1±6.0	31±3.4	19.2±5.0
	We.	51.0±10.0	68.2±18.6	35±11.5	31.9±1.0	20.5±3.2	24.6±1.3	29.1±1.8	14.9±1.4	24.3±1.6	18.5±0.5	41.2±8.4	22.1±1.3	28.7±1.5	29.2±4.5
Chromium	Wh.	ND	ND	ND	ND	0.2	ND	ND	0.02	ND	ND	0.07	0.05	0.11	ND
	We.	0.25	0.09	ND	ND	ND	0.07	ND	ND	0.09	ND	ND	ND	0.09	ND
Cobalt	Wh.	0.05±0.0	0.03±0.0	ND	ND	0.04±0.0	0.04±0.0	0.03±0.0	0.03±0.0	ND	0.04±0.0	0.03±0.0	0.03±0.0	ND	0.09±0
	We.	0.07±0.1	0.03±0.0	ND	0.04±0.0	ND	0.03±0.0	0.03±0.0	0.03±0.0	0.03±0.03	ND	0.03±0.0	0.03±0.0	ND	0.07±0.0
Nickel	Wh.	0.8±0.9	0.4±0.5	ND	ND	1.0±0.6	0.6±0.7	0.6±0.7	0.7±0.8	ND	1.08±1.20	1.02±1.19	0.93±1.09	ND	1.09±0.1
	We.	1.11±1.3	0.8±1.0	ND	0.5±0.6	ND	0.6±0.7	0.7±0.8	0.4±0.5	0.5±0.6	ND	0.75±0.88	0.60±0.71	ND	1.0±0.2
Copper	Wh.	2.7±3.04	2.0±2.3	ND	ND	2.5±2.8	2.9±3.2	1.5±1.7	2.7±3.1	ND	1.58±3.17	2.26±2.65	2.16±2.53	ND	5.3±1.1
	We.	3.03±3.5	2.8±3.3	ND	2.6±2.8	0±0	2.3±2.7	1.6±1.9	1.3±1.5	2.2±2.6	ND	2.62±3.08	2.09±2.46	ND	4.4±0.9
Zinc	Wh.	10.5±12.1	11.3±13.2	ND	ND	9.2±10.9	6.5±13.1	5.6±6.6	10.1±11.9	ND	12.1±14.0	11.4±13.3	11.9±13.9	ND	20.9±2.0
	We.	10.4±12.3	9.9±11.7	ND	8.01±8.7	ND	10.2±12.0	5.2±6.1	3.3±3.9	10.8±12.6	ND	9.4±11.1	7.09±8.32	ND	20.4±5.3
Arsenic	Wh.	ND	0.09±0.1	ND	ND	0±0	0.07±0.0	ND	ND	ND	ND	ND	0.05±0.06	ND	0.1±0.0
	We.	0.2±0.2	ND	ND	ND	0.2±0.2	ND	ND	0.02±0.0	0.09±0.10	ND	0.07±0.08	ND	ND	0.09±0.0
Cadmium	Wh.	0.4±0.4	0.67±0.3	0.9±0	0.9±0	0.6±0.3	0.45±0.4	0.67±0.37	0.6±0.3	0.9±0	0.45±0.49	0.6±0.3	0.6±0.3	0.9±0	0.005±0.0
	We.	0.67±0.3	0.67±0.3	0.9±0	0.45±0.4	0.9±0	0.6±0.3	0.6±0.3	0.6±0.3	0.6±0.3	0.9±0	0.6±0.3	0.6±0.3	0.9±0	0.22±0.3
Mercury	Wh.	0.02±0.0	0.02±0.02	ND	ND	0.01±0.0	0.01±0.0	0.003±0.0	0.01±0.0	ND	0.008±0.0	0.003±0.0	0.008±0.0	ND	0.02±0.0
	We.	0.03±0.0	0.02±0.03	ND	0.02±0.0	ND	0.04±0.0	0.003±0.0	0.003±0.0	0.003±0.0	ND	0.003±0.0	0.007±0.0	ND	0.01±0.0
Lead	Wh.	1.5±1.0	1.07±0.3	1.2±0.5	2.7±0.2	1.1±0.3	1.2±0.4	2.35±2.63	1.5±0.2	1.8±0.0	1.3±0.1	1.03±0.2	1.33±0.4	1.6±0.1	1.3±1.2
	We.	1.39±0.1	1.7±0.6	0.9±0	0.88±0.01	1.1±0.3	0.85±0.1	0.7±0.1	0.7±0.2	0.8±0.0	0.9±0	0.95±0.0	0.9±0.0	0.9±0	0.7±0.1
Vanadium	Wh.	0.08±0.1	0.07±0.0	ND	ND	ND	0.08±0.0	0.02±0.03	ND	ND	0.07±0.08	ND	ND	ND	0.04±0.0
	We.	ND	ND	ND	0.08±0.0	ND	ND	0.06±0.0	0.03±0.0	ND	ND	0.31±0.6	0.04±0.0	ND	0.06±0.0
Manganese	Wh.	23.8±28.4	27.3±32.0	ND	ND	ND	ND	9.9±11.6	ND	ND	12.9±14.8	ND	ND	ND	12.8±15.0
	We.	ND	ND	ND	17.6±19.3	ND	ND	9.2±10.8	3.7±4.3	ND	ND	17.8±20.9	12.1±14.2	ND	14.9±17.5
Molybdenum	Wh.	0.4±0.4	0.2±0.2	ND	ND	ND	0.4±0.4	0.1±0.1	ND	ND	0.32±0.3	ND	ND	ND	0.07±0.0
	We.	ND	ND	ND	0.2±0.2	ND	ND	0.16±0.1	0.09±0.1	ND	ND	0.65±0.7	0.05±0.1	ND	0.5±0.6

Table 2) Mean±standard deviation of heavy metals in organs of mulberry trees (Le: leaf and Fr: fruit) cultivated in different areas of Arak, Iran (ND: none detected)

HM.	Org.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Aluminum	Le.	52.3±14.1	53.6±21.9	27±4.3	29.7±3.5	21.5±2.1	27.3±3.6	23.7±6.9	15±1.4	24±1.4	25.7±7.7	28.2±11.3	23.1±4.7	30±3.9	26.4±6.4
	Fr.	49.0±10.8	52.2±23.9	37±8.7	29±2.1	21.1±3.9	26.5±1.8	22.0±7.1	15.9±0.8	24.6±2.1	21.2±5.0	34.1±13.3	21.1±3.6	29.7±1.1	22.0±7.2
Chromium	Le.	ND	0.09±0.1	ND	ND	ND	0.07±0.08	ND	ND	ND	ND	ND	0.05±0.06	ND	0.10±0.02
	Fr.	0.25±0.28	ND	ND	ND	0.2±0.2	ND	ND	0.02±0.02	0.09±0.1	ND	0.07±0.08	ND	ND	0.09±0.03
Cobalt	Le.	0.05±0.0	0.06±0.0	ND	0.04±0.0	ND	0.07±0.0	ND	ND	ND	ND	ND	0.03±0.0	ND	0.09±0
	Fr.	0.07±0.1	ND	ND	ND	0.04±0.0	ND	0.06±0.0	0.06±0.0	0.03±0.0	0.04±0.0	0.06±0.02	0.03±0.0	ND	0.07±0.0
Nickel	Le.	0.85±0.9	1.3±0.6	ND	0.5±0.6	ND	1.3±0.3	ND	ND	ND	ND	ND	0.9±1.0	ND	1.04±0.1
	Fr.	1.1±1.3	ND	ND	ND	1.07±1.2	ND	1.3±0.4	1.2±0.4	0.5±0.6	1.08±1.2	1.7±0.6	0.6±0.7	ND	1.1±0.3
Copper	Le.	2.7±3.0	4.9±1.7	ND	2.6±2.8	ND	5.3±1.1	ND	ND	ND	ND	ND	2.1±2.5	ND	4.6±0.2
	Fr.	3.0±3.5	ND	ND	ND	2.5±2.9	ND	3.2±0.9	4.06±1.9	2.2±2.6	2.5±3.1	4.8±1.5	2.0±2.4	ND	5.1±1.5
Zinc	Le.	10.5±12.1	21.3±6.5	ND	8.0±8.7	ND	22.0±6.3	ND	ND	ND	ND	ND	11.9±13.9	ND	21.3±3.2
	Fr.	10.4±12.3	ND	ND	ND	9.2±10.9	ND	10.8±3.2	13.5±8.6	10.8±12.6	12.1±14.0	20.8±6.6	7.09±8.3	ND	20.0±4.6
Arsenic	Le.	0.5±0.3	0.50±0.3	0.9±0	0.55±0.3	0.9±0	0.41±0.2	0.9±0	0.9±0	0.9±0	0.9±0	0.9±0	0.7±0.3	0.9±0	0.2±0.1
	Fr.	0.8±0.0	0.9±0	0.9±0	0.9±0	0.6±0.3	0.9±0a	0.4±0.3	0.4±0.3	0.69±0.3	0.4±0.4	0.53±0.3	0.6±0.3	0.9±0	0.3±0.3
Cadmium	Le.	0.4±0.4	0.4±0.3	0.9±0	0.45±0.4	0.9±0	0.23±0.3	0.9±0	0.4±0.4	0.9±0	0.9±0	0.9±0	0.6±0.3	0.9±0	0.005±0.0
	Fr.	0.6±0.3	0.9±0	0.9±0	0.9±0	0.6±0.3	0.9±0	0.4±0.3	0.9±0	0.6±0.3	0.4±0.4	0.4±0.3	0.6±0.3	0.9±0	0.2±0.3
Mercury	Le.	0.02±0.0	0.05±0.0	ND	0.02±0.0	ND	0.06±0.0	ND	ND	ND	ND	ND	0.008±0.0	ND	ND
	Fr.	0.03±0.0	ND	ND	ND	0.01±0.0	ND	0.007±0.0	0.01±0.0	0.003±0.0	0.008±0.0	0.007±0.0	0.007±0.0	ND	0.01±0.0
Lead	Le.	1.4±1.0	1.5±0.7	1.2±0.5	1.81±1.8	1±0.1	0.87±0.1	0.7±0.1	1.1±0.2	1.1±0.4	1.14±0.2	0.9±0.1	0.9±0.3	1.2±0.3	0.7±0.1
	Fr.	1.5±0.3	1.3±0.5	0.9±0	1.8±0.9	1.31±0.3	1.25±0.4	2.09±2.4	1.1±0.6	1.0±0.5	1.07±0.1	1.009±0.1	1.3±0.3	1.3±0.4	1.39±1.20
Vanadium	Le.	0.08±0.1	0.07±0.0	ND	0.08±0.0	ND	0.08±0.0	ND	ND	ND	ND	ND	ND	ND	0.1±0.0
	Fr.	ND	ND	ND	0±0b	ND	ND	0.09±0.0	0.03±0.0	ND	0.07±0.0	0.3±0.6	0.04±0.0	ND	ND
Manganese	Le.	23.8±28.4	27.3±32.0	ND	17.6±19.3	ND	ND	ND	ND	ND	ND	ND	ND	ND	27.8±8.6
	Fr.	ND	ND	ND	ND	ND	ND	19.1±5.7	3.7±4.3	ND	12.9±14.8	17.8±20.9	12.1±14.2	ND	ND
Molybdenum	Le.	0.4±0.4	0.2±0.2	ND	0.2±0.2	ND	0.4±0.4	ND	ND	ND	0.3±0.3	ND	ND	ND	0.6±0.5
	Fr.	ND	ND	ND	ND	ND	ND	0.2±0.0	0.09±0.1	ND	ND	0.6±0.7	0.0.5±0.19	ND	ND

Table 3) Results of comparison of heavy metal concentrations in mulberry trees with those of the Food and Agriculture Organization of the United Nations, World Health Organization, and other permissible values

Reference	Aluminum	Chromium	Cobalt	Nickel	Copper	Zinc	Arsenic	Cadmium	Mercury	Lead	Vanadium	Manganese	Molybdenum
Iran's national standard limit								0.05-0.1		0.1-0.3			
US EPA,2015					0.04			0.001		0.04			
FAO/WHO,2011		2.3	0.01		20	100		0.1-0.2		0.3		500	40
SEPA,2005				10	20	100		0.1-0.2					
WHO,1999		1.2	2	0.5	2	1.5	0.1	0.2	0.05	0.5		5	
China standard limit		1	1	0.6	10	50	0.7	0.1	0.02	0.2		6.61	
Mean permissible limits	5-10	1-2.3	0.01-1	0.2-0.6	20-50	10-100	0.1-0.7	0.1-0.2	0.02-0.05	0.3			
Present study	29	0.046	0.03	0.7	2	8.2	0.7	0.6	0.008	1.2	0.039	4.7	0.078
Critical value in mulberry (times)	3-6	Permissible	Low	Low	Permissible	Permissible	Low	3-6	Permissible	4	Permissible	Permissible	Permissible

FAO: Food and Agriculture Organization of the United Nations
WHO: World Health Organization
SEPA: State Environmental Protection Administration

Table 4) Spearman's correlation coefficients between some metals with aluminum, chromium, cobalt, nickel, copper, zinc, arsenic, cadmium, mercury, lead, vanadium, manganese, and molybdenum in *Morus alba*

	Aluminum	Chromium	Cobalt	Nickel	Copper	Zinc	Arsenic	Cadmium	Mercury	Lead	Vanadium	Manganese	Molybdenum
Aluminum													
Chromium	0.35												
Cobalt	0.15	.683**											
Nickel	0.13	.615*	.907**										
Copper	0.15	.643*	.959**	.883**									
Zinc	0.14	.613*	.935**	.884**	.963**								
Arsenic	0.10	-0.43	-.902**	-.774**	-.895**	-.922**							
Cadmium	0.00	-0.52	-.941**	-.781**	-.919**	-.925**	.983**						
Mercury	.560*	.539*	.656*	.555*	.674**	.579*	-0.49	-.570*					
Lead	0.38	-0.09	0.01	-0.09	-0.07	-0.18	0.10	0.05	0.26				
Vanadium	0.23	0.14	.703**	.705**	.681**	.675**	-.726**	-.731**	.576*	0.31			
Manganese	0.50	0.32	.685**	.672**	.640*	.687**	-.626*	-.636*	0.46	0.41	.799**		
Molybdenum	0.23	0.33	.719**	.729**	.711**	.716**	-.690**	-.731**	0.50	-0.02	.866**	.657*	

accumulation (Table 1). There was also no significant difference between the fruits and leaves; however, a great variation was observed in the amount of metal accumulation according to regions (Table 2). The order of metal accumulation in mulberry trees in a different area was as follows:

Al > Mn > Zn > Cu > Pb > Ni > As > Cd > Mo > Co > Cr > V > Hg

Mulberry trees in regions 1, 2, 11, and 13 were identified as areas with the highest accumulation of heavy metals. In contrast, region 3 was categorized in a clean area for the accumulation of heavy metals. According to the obtained results of this study, the accumulation of aluminum concentrations was observed in the mulberry trees of all regions. Mulberry trees contaminated with aluminum were identified in Hezaveh, an area outside Arak. However, there is a long distance between Hezaveh and the city, and no clean area was observed regarding aluminum contaminants (Table 1). Therefore, it can be noted that the problem about the toxicity of aluminum in Arak as an industrial city is serious and needs to be managed.

Iranian Aluminum Company (IRALCO), located in northeastern Arak, is the main contributor to aluminum contamination of Arak. In this study, the comparison of the measured lead amount in the fruits of mulberry trees in different areas of Arak with concentration permissible limits of lead for edible products showed that lead accumulation in the fruits was four times higher than the concentration permissible limits (Table 3).

Table 4 shows the calculated Pearson correlation coefficients between metals in the sample organs of mulberry in this study. Table 4 tabulates the correlation between accumulated elements in the organs of mulberry. Accumulated cobalt was positively correlated with accumulated nickel, copper, and zinc (r=0.9, r=0.95, and r=0.93, respectively). Furthermore, significant negative correlations were observed between accumulated cobalt with accumulated arsenic and cadmium (r=0.9 and r=0.94, respectively).

Accumulated copper was positively correlated with accumulated zinc (r=0.96) and negatively correlated with accumulated cadmium (r=0.91) in the organs of cultivated mulberry in Arak. In addition, accumulated zinc showed significant negative correlations with accumulated arsenic and accumulated cadmium (r=0.92). A significant negative correlation was determined between accumulated arsenic and accumulated cadmium (r=0.98).

Discussion

To date, a limited number of studies have been conducted on aluminum pollution in Arak and other cities. Amini et al. (2015) reported the adverse effects of aluminum contamination on the anatomical traits of *Robinia pseudoacacia* and *Ailanthus altissima* leaves close to IRALCO (16). This finding confirms the existence of the aluminum pollution source in Arak. Salari et al. (2011) also reported the content of aluminum in the plants of an industrial site in Kerman, Iran, higher than that in the control region and over standard Environmental Protection Agency permissible limits (between 31 and 160 ppm) (17).

Moreover, the contamination zoning of 22 areas of Tehran, using mulberry leaves showed a high level of aluminum contamination in central and eastern Tehran (18). The main source of aluminum is naturally inside the soil, air, and water and might be disseminated in the environment by nature and human activities. Aluminum atoms release from plants, biofuels, and other fuels and could bond with tiny particles inside the air and soil (19).

Among the important environmental factors of aluminum, toxicity can increase the ratio of rainfall to evapotranspiration, rainfall to soil transpiration ratios, soil sulfides oxidation, soil acidification, industrial activities, application of nitrate and ammonia fertilizers in agriculture, and reaction of soil clay particles with hydrogen ions. The above-mentioned factors cause to release aluminum ions from soil particles and accordingly increase its concentration in the environment (20).

Aluminum solubility in the soil aquatic environment provides plants to absorb it leading to the increased accumulation of aluminum in plant tissues, and humans might either be exposed to this toxic metal directly by consuming contaminated agriculture crops or indirectly via releasing aluminum (21). Several studies have been carried out on heavy metal contamination in vegetables and fruits in which the amount of lead, cadmium, chromium, nickel, iron, zinc, and manganese in most cases exceeded the maximum permissible level (22-25).

Contamination with lead and cadmium was reported to be the most common contamination in these crops, and the toxicity with these metals caused by the consumption of crops, especially vegetables was emphasized in most studies. The use of chemical fertilizers, irrigation by sewage, proximity to industrial centers, and precipitation of atmospheric pollutants have been considered the sources of contamination. Arak has been reported to be contaminated with metal pollutants, including lead and cadmium (26). It seems that the contamination of mulberry trees with lead in Arak is probably due to the precipitation of atmospheric lead onto the foliage and soil and then its absorption by the tree root and transfer to upper organs.

Similarly, other researchers believe that lead particle emission from the exhaust of vehicles fueling gasoline is the most important and most common source of contamination of the soil, atmosphere, plants, and water (27). According to the literature, the origin of 81% of the total atmospheric lead particles released into the atmosphere is attributed to this source (28). Contamination intensity of the soil and roadside plants, as well as the vegetation alongside the streets, by lead emission from vehicle exhausts depend on traffic volume and distance from the road. Moreover, lead salts are flushed into the soil by rainfall up to 51% after precipitation onto the roadside plants (29).

In addition, among other heavy metals,

cadmium contamination is noteworthy, because this metal is easily absorbed by the root of plants, and its toxicity is 20 times higher than other heavy metals. Therefore, it is possible for cultivated plants in different areas of Arak, including mulberry trees, to contain some concentrations of these metals. The results of the present study are close to the findings indicative of the presence of some concentrations of heavy metals in the organs of cultivated trees in metropolitan cities.

Zarasoundi and Pourkassab (2011) demonstrated that *Eucalyptus camaldulensis* dehnh. species has a high potential to absorb vanadium, copper, zinc, and lead. *Conocarpus erectus* L. has a high potential to absorb chromium and cobalt, and *Ficus religiosa* L. has a high potential to absorb strontium, arsenic, and zirconium (30). Sardabi et al. (2013) assessed the ability of various *Eucalyptus camaldulensis* dehnh. species to uptake heavy metals, especially lead to be at a high level (31). The accumulation of cadmium, chromium, and nickel in *Populus alba* and *Morus alba* has been studied in contaminated soil. The determination of the BAF and translocation factor showed that *Populus alba* and *Morus alba* were introduced for the accumulation of cadmium and nickel (7).

A number of other studies on the uptake of heavy metals by mulberry trees have been conducted under contamination simulation (12) (32-34); however, none of them reflects the conditions to which these species are naturally exposed to absorb metals in real contaminated places. In studies conducted by Prince et al. (2000) and Wang (2003), it was shown that most of the cadmium in mulberry trees is accumulated in the roots, compared to that in the leaves (8). Ashfagh et al. (2009) also demonstrated that zinc was primarily accumulated in the leaves of the mulberry tree (32).

Zhao et al. (2013) proved that *Morus alba* L. is capable of extracting heavy metals from the soil and concentrating them on the root-to-leaf path, and that is why they introduced mulberry trees as an appropriate alternative for phytoextraction (35). In plants, such as mulberry trees, high biomass, strong root system, rapid growth in contaminated soils, increased evaporation, and transpiration enable contaminants to vertically immigrate from the soil; therefore, it is possible to reduce the concentration of metals in the soil and decrease the risk of pollution in the groundwater after harvesting and removing aerial organs (13).

A more extensive study was carried out by Nikolova (2015) on the concentration of metals in the roots, trunk, foliage, and fruits of mulberry cultivated in contaminated areas of Bulgaria. The results of the aforementioned study showed that the leaves are preceded by fruits for accumulating the highest amount of zinc, lead, cadmium, and copper, respectively. Therefore, they warned about the unsuitable consumption of mulberry fruits cultivated in contaminated areas (13). Similarly, there were also large amounts of accumulated heavy metals in the leaves and fruits of mulberry trees in the present study.

Lack of excessive accumulation of zinc and copper was probably due to the insufficiency of contaminant sources for these two metals in Arak. In both mulberry species, there was a similar accumulated metal content in both leaves and fruits. Similar to this finding was also observed in the results of a study by Nikolova (2015). Mulberry leaves have a special ability to trap atmospheric contaminant aerosols, so that the contents of accumulated copper, zinc, lead, and cadmium in both washed and nonwashed leaves were significant, compared to those of the control specimens (13).

As previously mentioned, evaluating the accumulation of metals in mulberry trees showed that contamination with cobalt, nickel, and arsenic (to a low concentration), as well as with aluminum, cadmium, and lead (to a high concentration) was observed in white mulberry and weeping mulberry (especially in their fruits) cultivated within Arak landscapes. Mulberry organs have the distinctive ability to uptake relatively large quantities of heavy metals and hold aerosol pollutants from the air (13).

The problem of contamination with metals, such as chromium, nickel, cadmium, and lead, in Arak as an industrial city has been reported by researchers (26). Since the study was conducted in seasons (i.e., spring and summer) during which there was the issue of the predominance of fine dust dispersion, it can be concluded that metal contaminants have been dispersed, along with dust transportation and their subsequent precipitation.

Metal contamination caused by major industrial factories of Arak, such as Heavy Equipment Production Company, Avangan, Iranian minerals corporation, Azarab Industries, IRALCO, Machine Sazi Arak, Wagon Pars, Iran Combine Manufacturing Company, Lajvar Industrial Group, Arak Petrochemical Company, Imam-Khomeini Oil Refinery, and Arak Heavy Water Production Plant. Moreover, it can be noted that contaminant sources surrounding Arak, such as Mighan wetland, may also cause the contamination of mulberry trees with aluminum, cadmium, lead, nickel, cobalt, and arsenic through adverse dust phenomenon. Some researchers have reported metal contaminants, such as copper, lead, zinc, aluminum, iron, nickel, and chromium, in Mighan wetland, and they have mentioned the sewage outlet of the refinery as an effective factor (14, 36, 37).

In the present study, there was a great variation among the areas of Arak regarding the absorption of metal contaminants by mulberry trees. The species of plants differ in their uptake abilities of heavy metals and subsequent distribution of metals within their organs (7). Furthermore, other factors effective on accumulation include different growth periods in various species, growth rate of plants, and physical and chemical properties of the soil. In other words, the bioavailability of elements in plants depends on various factors, including physicochemical properties of the soil, climate, plant genotype, water source used for irrigation, and agronomic management (38).

The solubility and bioavailability of heavy metal ions are extremely variable for several reasons effective in their concentration in soil solution (39). Plant species are selected based on root depth, contaminant nature, soil conditions, and climate for phytoremediation. Root depth directly affects the depth of the soil to be remediated, which depends on the plant species. Root depth may also differ significantly for one species depending on location conditions, such as soil structure, depth of hard soil layer, soil fertility, and contaminant concentrations (40).

The results showed a strong correlation between accumulated heavy metals in mulberry. A high correlation coefficient between these metals can demonstrate their common sources, codependency, and similar behavior during their transportation (41). The positive correlation may imply a relation between these metals and some origin for them. In addition, as these metals are applied in industries and they have more concentration in urban environments, it can be concluded that they have anthropogenic origins. The poor correlation coefficient for other metals shows that there are multiple and independent contamination sources.

Conclusion

A comparison was made between average studied accumulated metal concentration with Iran national standard limit, international standard limits by the Food and Agriculture Organization of the United Nations/World Health Organization, and directions of health and food organizations of other countries. The results of the comparison showed that the fruits of mulberry trees in Arak were contaminated with aluminum, nickel, arsenic, cadmium, and lead. Contamination with aluminum and cadmium both 3-6 times and lead 4 times were higher than the limits.

According to the comparison of metal limits, for edible crops, such as fruits and vegetables, with metal contents contained in the fruits of mulberry trees in different areas of Arak, it can be noted that the amounts of chromium, copper, zinc, and mercury, in the mulberry fruits are in accordance with the standard limits. However, the contamination of these edible fruits with cobalt, nickel, and arsenic was low, and its contamination with aluminum, cadmium, and lead was high and exceeded the standard limit.

Due to the contamination of mulberry fruits with heavy metals, such as nickel, arsenic, aluminum, and lead, there is a serious warning about their consumption. In addition to fruits, the leaves of mulberry trees also accumulated these heavy metals. It should be noted that if the plants exposed to smoke and dust are fruit-bearing trees, then, their fruits play an important role in the absorption of dust and precipitates.

Mulberry trees have been cultivated in some passageways and as their fruits are skinless they are prone to absorb precipitates and dust, so that passersby and inhabitants may get poisoned by the consumption of these fruits. The wider the leaf area of the plants, the more effective will be the absorption of dust and haze, and Mulberry trees are considered in this class.

Footnotes

Acknowledgements

The authors are grateful to Arak University for supporting this project under the research number of 2355/96.

Funding

This study was conducted and financially funded by Arak University in Arak, Iran.

Conflict of Interest

The authors declare that there is no conflict of interest.

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