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Original Article

Pb and Cd in medicinal plants (Case study: Shirazi thyme, sweet violet, pennyroyal and jujube)

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Abstract

Introduction: Medicinal plants are a major source of drugs for the majority of people in the world. Unfortunately, little data is available on the safety of medicinal plants, especially in terms of heavy metal contamination. Therefore, this study was conducted to evaluate the potential risk of Pb and Cd in Shirazi thyme, sweet violet, pennyroyal and jujube marketed in Hamedan City in 2015.
Methods: After preparation of 3 samples of each medicinal plant and acid digestion of the samples according to standard methods, the concentration of elements in samples were determined using atomic absorption spectrophotometer (AAS) in 3 replicates. Also, all statistical analyses were performed in the SPSS software.
Results: The results showed that the maximum mean concentrations of Pb and Cd in specimens were 1420±30 and 40±30 µg/kg for thyme and viola, respectively. The results of health index for all samples were smaller than one for all samples. Also the mean concentrations of Pb and Cd in all samples were lower than WHO permissible limits.
Conclusion: Controlled consumption of medicinal plants has no adverse effects on the consumers' health. However, given the increased use of pesticides, chemical fertilizers, sewage sludge and wastewater by farmers, it is recommended that medicinal plants be regularly monitored for chemical pollutants especially heavy metals.

Introduction

The identification of medicinal plants has a key role in improving the health of the society and herbal treatments are considered an alternative and complementary approach to the improvement of the health level (1). According to the World Health Organization (WHO), 70-80% of the world use medicinal plants and traditional medicine to treat their diseases (2). There are 8000 plant species in Iran, 1800 of which have medicinal properties and are mainly indigenous to this country and cannot grow in other regions (3). The problems with modern pharmaceutics, including the heavy costs imposed, the use of nonrenewable resources such as fossil resources, the environmental pollution caused by pharmaceutical industries and the inability of humans to develop some of the therapeutic substances that naturally exist in plants, have led to a further emphasis on medicinal plants. The desire to produce medicinal and aromatic plants and the demand for natural products are therefore increasing in the world (4). Medicinal plants can be contaminated by heavy metals via root uptake or by the direct absorption of the contaminants deposited on their surface from the atmosphere (5). The use of organic fertilizers such as compost has increased in recent years due to their lower costs, rich organic materials, ability to control erosion, reduce density and increase cation exchange as well as porosity (6,7). Composts, however, are contaminated by heavy metals due to the presence of paint, batteries, electrical appliances, cosmetics and pharmaceutical waste in municipal solid waste (8 and 9). Due to their bioaccumulation and biomagnification potential in food chains and the bodies of living organisms and also their biodegradable nature, heavy metals are considered a major environmental pollutant that may cause severe or chronic toxicity due to its increasing discharge into the environment as a result of agricultural and industrial activities and urban development (10).

Lead is classified as a class 2B carcinogen by the International Agency for Research on Cancer and its toxic effects on the body depend on the individual's metabolic characteristics and diet. These effects are particularly noticeable in four systems in the body, including the gastrointestinal tract, the central nervous system, the peripheral nervous system and the hematopoietic system (11,12). Lead poisoning prevents hemoglobin synthesis and damages kidney function, the reproductive system, the joints and the cardiovascular system and causes acute and chronic injury to both the central and peripheral nervous systems. In children, Pb poisoning causes the poor growth of gray matter and therefore leads to a low IQ (13 and 14). Cadmium is classified as a carcinogen whose excessive accumulation in the body causes bone diseases, pulmonary edema, liver and kidney failure, cardiovascular diseases and hypertension. This toxic metal is also retained in the placenta and prevents from the transmission of Cu and Zn to the fetus (15).

Shirazi thyme is an aromatic plant of the Lamiaceae family that is used as an antiseptic, anesthetic and antispasmodic agent in traditional Iranian medicine (16). Sweet violet is a widely-used medicinal plant in traditional Iranian medicine that is known as an antipyretic, expectorant, diuretic and vulvovaginitis medication that is also used in the treatment of sore throat and rheumatism (17). Pennyroyal is a member of the Lamiaceae family whose aerial parts are commonly used in the treatment of cold, sinusitis, food poisoning, bronchitis and tuberculosis. This plant is also carminative, expectorant, diuretic and antitussive and suppresses menstruation (18). Jujube has long been used as a medicinal plant, especially in East Asia, where it was used to treat disorders such as liver dysfunction, anemia and asthma (19).

The Estimated Average Daily Intake (EADI) of different elements through food is calculated to determine the long-term risks to consumers (20 and 21). The health index can also be calculated by dividing the AEDI of each element by the Acceptable Daily Intake (ADI) of the element. A health index below one indicates that the controlled consumption of the food item in question does not adversely affect the health of the consumers and vice versa (20).

Several studies have been conducted to date for investigating the accumulation of heavy metals in medicinal plants. The results of a study conducted on heavy metal contamination in some medicinal plants in Baba Ali and Ahangaran mines in Hamadan, Iran, revealed mean Pb and Cd concentrations of 138 and 4.4 mg/kg in the plants of this region (22). In another study on the medicinal plants collected from Northwest India, the mean Pb and Cd concentrations were found to be 0.94 and 0.03 mg/kg (23). In a study on the leaves of native medicinal plants, the mean Pb and Cd concentrations were 18.7 and 0.46 mg/kg in Islamabad, Pakistan, and 26.82 and 0.52 in the Rawalpindi region of this country (24). In a study conducted to investigate the relationship between Pb and Cd concentrations in medicinal plants and urban and industrial pollution in Portugal, a significant positive correlation was found between the mean concentrations of these heavy metals in the plants and their concentrations in the soil (25). A study conducted to investigate the heavy metal content of medicinal plants in high-traffic areas in Nigeria found a concentration range of 1.55-10.47 mg/kg for Pb and a range of 0.02-1.44 mg/kg for Cd (26).

Given the growing discharge of heavy metals into the environment and their consequent accumulation in plants, the present study was conducted in 2015 to evaluate the health risks of Pb and Cd in medicinal plant species including Shirazi thyme, sweet violet, pennyroyal and jujube supplied in Hamadan's consumer market in Iran.

Materials and Methods

To investigate the concentration of Pb and Cd in Shirazi thyme, Sweet violet, Pennyroyal and jujube, first, the researchers ensured that the plants' cultivation area differed and then calculated the sample size as 36 using Cochran's formula; then, they purchased three samples of each plant species from three herbalist's shops that were wholesalers of the medicinal plants in Hamadan, Iran, and the plant samples were then transferred to the laboratory. The samples were washed with distilled water and then with double-distilled water and dried in the shade at room temperature, (22-25 ˚C). The dried samples were ground and homogenized in a porcelain mortar. A 10-ml solution was obtained by adding nitric acid 65% and hydrogen peroxide 30% at a 2:1 volume ratio to 1 g of each of the samples. The solution volume was brought to 3 ml with double-distilled water after being heated and then cooled and after passing through a Whatman filter paper 42 into a 25-ml beaker. In the final stage, after making the stock solution and the standard lead salt with concentrations of 1000, 5000 and 10000 µg/l and the standard Cd salt with concentrations of 5, 50 and 100 µg/l and calibrating the atomic absorption spectrophotometer (Shimadzu model AA-680), the concentrations of Pb and Cd in the medicinal plants were measured in triplicate at wavelengths of 283.3 nm and 228.8 nm (27). It is worth noting that the acetylene flow rate was 1.8 l/min, the air flow rate 15 l/min, the hollow cathode lamp current 6 mA and the slit width 0.7 nm for measuring the concentrations.

Equations 1 and 2 were used to calculate the ADI and the health index of each element (20):

$$EADI = \frac{C \times F}{W \times D} \qquad \text{Equation 1}$$

C: The mean accumulated concentration of an element in the food item under study in mg/kg
D: The number of days in a year (365)
F: The mean annual consumption of the food item by each person in kg
W: The mean body weight (70 kg for adults and 15 kg for children)

$$HI = \frac{EADI}{ADI} \qquad \text{Equation 2}$$

EADI: The Estimated Average Daily Intake of each element in mg/kg/day

ADI: The Acceptable Daily Intake of each element in mg/kg/day, which was calculated as 0.0036 for Pb and 0.001 for Cd (28-30).

The data obtained were analyzed in SPSS-19. The Kolmogorov-Smirnov test was used to examine the normal distribution of the data, the one-sample t-test to compare the mean concentrations of the elements under study using the WHO guidelines, the one-way ANOVA to compare the mean concentrations of the elements in the plants and Pearson's correlation coefficient to investigate the correlations between the mean accumulated concentrations of the elements in the samples.

Findings

Table 1 presents the Pb and Cd concentrations observed in the samples of Shirazi thyme, sweet violet, pennyroyal and jujube in µg/kg and Table 2 shows the health index reported for the consumption of these medicinal plants.

The maximum and minimum mean concentrations of Pb were 720±170 in jujube and 1420±30 in Shirazi thyme and the maximum and minimum mean concentrations of Cd were 13±7 in jujube and 40±30 in sweet violet. As shown in Table 2, the health index has a value less than one for all the medicinal plants, suggesting the lack of health risks to the consumers if the plants' consumption is controlled.

The Kolmogorov-Smirnov test revealed a normal distribution for the data on the accumulated concentrations of Pb and Cd in all the medicinal plant samples. The mean accumulated concentrations of Pb and Cd have a significance level less than 0.05 for all the plant samples assessed; according to the one-sample t-test, the concentrations obtained in the present study were significantly lower than the standard thresholds proposed by the WHO, i.e. a mean accumulated concentration of 10,000 µg/kg for Pb and 300 µg/kg for Cd (31) ($P<0.05$). Duncan's multiple range test also revealed statistically significant differences between the Shirazi thyme and Pennyroyal samples and the sweet violet and jujube samples in terms of the mean accumulated concentrations of Pb ($P<0.05$); however, there were no statistically significant differences between the plant samples in terms of the mean accumulated concentration of Cd.

Pearson's correlation test found no statistically significant correlations between the mean accumulated concentrations of Pb and Cd in the plant samples.

Table 1. Concentrations of heavy metals (Pb and Cd) in the medicinal plant samples (µg/kg)

Medicinal Plant	Element	Sample			Mean Concentration ±SD
		1	2	3	
Shirazi thyme	Pb	1396.0	1447.0	1413.0	1420±30
	Cd	41.0	19.0	9.0	23.0±16.0
Sweet violet	Pb	1093.0	714.0	1081.0	960±210
	Cd	65.0	28.0	13.0	40.0±30.0
Pennyroyal	Pb	1651.0	1037.0	1371.0	1350±310
	Cd	16.0	13.0	14.0	14.0±2.0
Jujube	Pb	524.0	774.0	859.0	720±170
	Cd	12.0	7.0	20.0	13.0±7.0

Table 2. Estimated daily intakes and health index for metals found in medicinal plant samples in terms of the potential risk of Pb and Cd

Medicinal Plant	Element	EADI and Health Index			
		EADI (children) (mg/kg/day)	EADI (adults) (mg/kg/day)	HI (children)	HI (adults)
Shirazi thyme	Pb	6.48×10-6	1.38×10-6	1.80×10-3	3.83×10-4
	Cd	1.05×10-7	3.25×10-8	1.05×10-4	2.25×10-5
Sweet violet	Pb	4.38×10-6	9.39×10-7	1.22×10-3	2.61×10-4
	Cd	1.83×10-7	3.91×10-7	1.83×10-4	3.91×10-4
Pennyroyal	Pb	6.15×10-6	1.32×10-6	1.71×10-3	3.67×10-4
	Cd	6.39×10-4	1.37×10-8	6.39×10-1	1.37×10-5
Jujube	Pb	3.29×10-6	7.04×10-7	9.13×10-3	1.96×10-4
	Cd	5.94×10-8	1.27×10-4	5.94×10-5	1.27×10-1

Discussion

Owing to their natural active ingredients and other substances, medicinal plants create ecological balance and prevent the excess accumulation of pharmaceutical substances in the body. In recent decades, the entire world along with researchers have been drawn to herbal medicines due to their few or no side-effects (32). Medicinal plants have always been particularly valuable in societies for meeting the public health requirements in terms of the treatment and prevention of diseases. These plants are in fact valuable natural resources that have recently become of interest in developed countries as the raw materials required for medicines that are not harmful to humans. Iran provides one of the richest sources of medicinal plants in the world with the highly diverse habitat conditions it offers for the growth of various species of these plants (33).

The spatial variability of heavy metals in agricultural surface soils may be affected by parent materials and human activities. In other words, although these metals are naturally created in the soil by nonhuman activities such as parent rock weathering (34), human activities can also contribute to them in the soil (35). In fact, human activities, including industrial development, metal melting, mining and the use of chemical and organic fertilizers containing heavy metals may lead to the accumulation of heavy metals in the soil (36).

When there are large quantities of heavy metal ions in the environment, they are absorbed by plant roots and transferred to the aerial parts to cause metabolic disorders in the plants and disrupt their growth (37). Large amounts of heavy metals in the soil are also a serious threat, as they may destroy the soil structure, reduce the biological activities of the soil and its performance and productivity, compromise the quality of the soil products, increase the accumulation of heavy metals in agricultural products and damage human health by entering the food chain (38). In addition, the contamination of medicinal plants and their products with heavy metals is known to be potentially caused by water, soil and air pollution during their period of cultivation, through processing (unwanted or unintentional contamination during the drying, storage, transportation, maintenance and production of the processed products) or through the intentional addition of certain compounds to the herbal products for enhancing their therapeutic properties (39).

In Iran, medicinal plants are often collected from wild (natural) plants and usually unknown places and their use therefore entails health and safety risks to the consumers due to the likelihood of their removal from potentially-contaminated areas (40). The periodic monitoring of these plants seems essential due to the increasing use of these products by the public and their wide application in traditional medicine.

The present study was conducted to investigate the concentration of heavy metals including Pb and Cd in the medicinal plants Shirazi thyme, sweet violet, Pennyroyal and jujube. The maximum mean concentrations of Pb and Cd were 1420±30 µg/kg in Shirazi thyme and 40±30 µg/kg in sweet violet, which are both below the WHO threshold. Nonetheless, it is important to also note the maximum acceptable intake of heavy metals through medicinal plants by the Provisional Tolerable Intake, which has been determined by the FAO/WHO as 25 µg/kg of body weight for Pb and 7 for Cd (41). Kulhari et al. (2013) reported a mean concentration of 940 µg/kg for Pb and 30 µg/kg for Cd in the medicinal plants collected from northwest India, which are nearly consistent with the figures obtained in the present study (23). Mahmood et al. (2013) reported the mean concentration of Pb and Cd as 18700 and 460 µg/kg in the leaves of medicinal plants native to Islamabad, Pakistan, and as 26820 and 520 µg/kg in the leaves of the plants native to the Rawalpindi region of this country (24); these values are larger than those obtained in the present study. Rehman et al. (2013) found a concentration range of 990-15460 µg/kg for Pb and 100-1410 µg/kg for Cd in eight medicinal plant species collected from different locations in Pakistan; their reported mean Pb concentration was less than the WHO threshold (42). Lokhande et al. (2010) found the mean concentration of Pb and Cd to be 2942 and 2325 µg/kg in the leaves and roots of six medicinal plant species collected from India; these figures are higher than those reported in the present study, particularly in the case of Cd (43). Hussain et al, (2006) reported the mean concentration of Pb in four medicinal plant species used in Pakistan as 217, 125 and 94 µg/kg for the root, stem and leaves, in respective order; all of these figures were also less than the WHO threshold (44). Linger et al. (2002) found the mean concentration of Cd in the leaves of Cannabis sativa cultivated in Germany as 3920 µg/kg, which is significantly higher than the results obtained in the present study (45).

According to the results obtained, the health index for the intake of Pb and Cd through the consumption of medicinal plants is less than one. The controlled consumption of these plants therefore has no adverse health effects on humans. Similar results were also reported in other studies. A study conducted to investigate the effects of using municipal sewage and wastewater in Shahrekord, Iran, on the growth, functioning and accumulation of Pb and Cd in the medicinal plant Dracocephalum found the mean concentrations of these elements to be far less than the WHO thresholds (46). Another study conducted to investigate non volatile toxic heavy metals in the medicinal plants collected from the Punjab region of Pakistan also found the mean concentrations of Zn, Pb, Cd, Cr and Cu in the plants to be less than the WHO thresholds (47).

Conclusion

The lower mean accumulated concentrations of Pb and Cd in the study samples may be attributed to the effect of different removal times (48), the presence of large quantities of organic materials in the soil (49), the high capacity of some plants such as medicinal plants for absorbing and storing Pb in their root and preventing its transfer to their aerial parts (50-52) and the conversion of lead to insoluble compounds such as chloropyromorphite, pyromorphite and lead carbonate in the vicinity of phosphate in the soil (53). Although the controlled consumption of the medicinal plants under study does not entail any health risks for the consumers, the accumulation of heavy metals in commonly-consumed food products, especially medicinal plants, is recommended to be carefully monitored to ensure food safety, mainly because of the increasing use of organic fertilizers, particularly composts (54) and sewage sludge as fertilizers containing heavy metals, the indiscriminate use of pesticides and chemical fertilizers, the poor management of waste and the steering of waste and leachate into water sources.

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