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Original article

Concentration and exposure assessments of cadmium and lead in pumpkin, sunflower, watermelon, and jabooni seeds collected in Iran

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Summary

Introduction – **Heavy metals such as lead (Pb) and cadmium (Cd) are known to have high human toxicity. Their presence at various rates in oilseeds has gained more importance during the last few years in the absence of regulation in seeds. The consumption of seeds has been recently reported to increase in Iran, especially among adolescents and young people, so that heavy metal contamination in these seeds could become a great problem of public health.** *Materials and methods* – **This study investigated the Cd and Pb levels present in 168 seed samples (pumpkin, sunflower, watermelon and jabooni seeds) collected over the country, using graphite furnace atomic absorption spectrometry after microwave digestion. The data were used to estimate the weekly intake of Cd and Pb in Iranian consumers.** *Results and discussion* – **The highest concentrations of Pb and Cd were found in pumpkin shells and sunflower kernels, respectively. The concentrations of Pb and Cd detected in the seed samples averaged 77±28 µg kg^{-1} and 264±177 µg kg^{-1}, respectively. The Pb content in 39% seed samples was exceeding the limit of 100 µg kg^{-1} established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The seeds with the highest Pb contamination were pumpkin seeds (67%), followed by sunflower (30%), watermelon (25%), and jabooni seeds (15%). None of the watermelon and jabooni seed samples were contaminated with Cd. The highest concentration (731 µg kg^{-1}) and the highest number of samples (35%) contaminated with Cd were recorded in the sunflower seeds. The differences in mean value of Pb and Cd among the seeds collected in various Iranian cities were not found statistically significant. Similarly, the mean intake of Pb and Cd estimated from the seed samples was much lower than the tolerable weekly intake (25.0 and 2.5 µg kg^{-1} $week^{-1}$ for Pb and Cd, respectively) estimated by the JECFA.** *Conclusion* – **Although the mean dietary exposure of cadmium and lead was much lower than the reference doses, the high incidence of Pb and Cd in widely consumed oilseeds in Iran, and the toxic effects of Pb and Cd, indicate the necessity for regular surveillance to reduce intake of heavy metals by Iranian population.**

Significance of this study

What is already known on this subject?

- Heavy metals are systemic toxicants that can accumulate in the human body and in the food chain thus causing various diseases and disorders even at low concentrations.
- Heavy metal contents and chemical forms in edible oilseeds are contingent upon the genotypes, weather and soil conditions in the region in which the seeds are grown.

What are the new findings?

- High incidence of Pb and Cd was found in the analyzed seeds.
- Sunflower seeds had a greater tendency to accumulate Cd in the kernels than the other seeds (pumpkin, watermelon, jabooni). None of the watermelon and jabooni seed samples were contaminated with Cd.
- Pumpkin seeds had a greater tendency to accumulate Pb than other seeds. In pumpkin seeds, Pb accumulated in shell more than in kernels.

What is the expected impact on horticulture?

- Monitoring pollution by heavy metals of wastewater and sewage sludge can prevent contamination of soil and water.
- Decreasing the accumulation of heavy metals in plants through recommendations and policies.
- Using accumulating plant species to decontaminate some polluted sites.

Keywords
Iran, edible oilseeds, *Helianthus annuus*, *Cucurbita pepo*, *Citrullus lanatus*, *Citrullus vulgaris*, biochemical composition, heavy metals, food safety

Résumé

Évaluation de teneur et mesures d'exposition au cadmium et au plomb dans les graines de citrouille, de tournesol, de pastèque et de jabooni collectées en Iran.

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Introduction – **Les métaux lourds tels que le plomb (Pb) et le cadmium (Cd) sont connus pour leur toxicité humaine élevée. Leur présence à diverses teneurs dans les graines oléagineuses a gagné en importance au cours des dernières années en l'absence de réglementation semencière. La consommation de graines a récemment augmenté en Iran, en particulier parmi les adolescents et les jeunes, de sorte que la contamination de ces graines par les métaux lourds pourrait devenir un grave problème de santé publique.** *Matériel et méthodes* – **Cette étude a mesuré les niveaux de Cd et Pb présents dans 168 échantillons de graines (de citrouille, tournesol, pastèque et jabooni) collectés dans le pays, par spectrométrie d'absorption atomique à four graphite après digestion par micro-ondes. Les données ont été utilisées pour estimer la ration hebdomadaire de Cd et Pb chez les consommateurs iraniens.** *Résultats et discussion* – **Les plus fortes concentrations de plomb et de cadmium ont été trouvées dans les coques les graines de citrouille et de tournesol, respectivement. Les concentrations moyennes de Pb et de Cd détectées dans les échantillons de semences étaient de 77 ± 28 µg kg^{-1} et de 264 ± 177 µg kg^{-1}, respectivement. La teneur en plomb dans 39% des échantillons de semences dépassait la limite de 100 µg kg^{-1} établie par le Comité mixte FAO/OMS d'experts des additifs alimentaires (JECFA). Les graines les plus contaminées par le Pb étaient les graines de citrouille (67%), suivies de celles du tournesol (30%), de pastèque (25%), et de jabooni (15%). Aucun échantillon de graines de pastèque ni de jabooni n'a été contaminé par du Cd. La plus forte concentration (731 µg kg^{-1}) et le plus grand nombre d'échantillons contaminés par du Cd (35%) ont été enregistrés pour les graines de tournesol. Les différences de valeur moyenne en Pb et en Cd parmi les graines collectées dans différentes villes iraniennes n'ont pas été trouvées statistiquement significatives. De même, la ration moyenne de plomb et de cadmium estimée à partir des échantillons de semences était nettement inférieure à l'apport hebdomadaire tolérable (25,0 et 2,5 µg kg^{-1} semaine^{-1} pour le plomb et le cadmium, respectivement) estimé par le JECFA.** *Conclusion* – **Bien que l'exposition alimentaire moyenne au cadmium et au plomb soit beaucoup plus faible que les doses de référence, l'incidence élevée en Pb et en Cd dans les graines oléagineuses largement consommées en Iran et les effets toxiques du Pb et du Cd indiquent la nécessité d'une surveillance régulière pour réduire l'ingestion de métaux lourds par la population iranienne.**

Mots-clés
Iran, grains oléagineuses comestibles, *Helianthus annuus*, *Cucurbita pepo*, *Citrullus lanatus*, *Citrullus vulgaris*, composition biochimique, métaux lourds, sûreté alimentaire

Introduction

Heavy metals are systemic toxicants that are known to induce multiple organ damage at lower levels of exposure (Duffus, 2002). According to World Health Organization of the United Nations (WHO), lead (Pb) and cadmium (Cd) are two of the four heavy metals which can lead to negative health effects in humans and experimental animals (Duran *et al.*, 2008; Winiarska-Mieczan and Kiczorowska, 2012). Emerging line research has suggested the primary entrance routes of metals in the human body are from food ingestion and inhalation (Beyersmann and Hartwig, 2008). The drastic increases in existence of toxic metals in foods are now associated with the malignancy and carcinogenesis and toxicity in different organs, including the liver, kidney, heart, nervous system, and cardiovascular system (Cherfi *et al.*, 2014; Karri *et al.*, 2016; Radwan and Salama, 2006; WHO, 2010).

Experimental evidence confirmed a higher usage of oilseeds in vegetarian diets in humans and increased risk of toxic metal contamination (Rodushkin *et al.*, 2008). Oilseeds are a rich source of nutrients, such as iron, phosphorus, magnesium, niacin, and folate. Additionally, oilseeds contain phytoestrogens and vitamin E as an antioxidant, which provide a protective effect against coronary heart disease (CHF) and which lower blood cholesterol levels (McKevith, 2005; Todorov, 1988). The content of metals and their species (chemical forms) in edible seed oils is contingent upon the genotype, weather conditions, and local soil conditions in the region in which the seeds are grown (Farzin and Moassesi, 2014). Thus, a continuous intake of low amounts of Cd over long periods of time could result in metal accumulation in the tissue of the human body, leading to organ damage.

The other problems of heavy metal contamination in oilseeds and other nuts is related to direct exposure to foods that are employed in food formulation and the possible transfer of that contamination to edible oilseeds (Wuana and Okieimen, 2011). Previous studies have shown that the biological half-life of Cd in the liver and the kidney is approximately 7 years and 17 years, respectively; this leads to accumulation and constancy in long periods in human organs, which leads to organ dysfunction (Vanderpool and Reeves, 2001). The use of seeds in animal feeds, confectionery products, and edible oils are most likely the main reasons for human exposure to toxic metals (Fakoor *et al.*, 2011). However, little information came out of the determination of Pb and Cd contents in the muscle and liver tissues of the rainbow trout (*Oncorhynchus mykiss*) from Hamedan Province cold water fish farms (Reyahi *et al.*, 2016).

Recently, increased Cd level was reported as a soil impurity, due to the inadvertent released into the environment, such as phosphatic fertilizer, municipal sewage waste, urban composts and industrial sludge (McLaughlin *et al.*, 1999). Airborne Cd can also be an important source of Cd in crops in contaminated areas with pyrometallurgic smelters (Smolders, 2001). The International Agency for Research on Cancer listed Pb exposure as an antique heavy metal in group 2A (EFSA, 2009). Lead exposure can occur mainly via food processing, handling, and packaging and via drinking water (Jin *et al.*, 2014). JECFA has established maximum allowable concentrations (MAC) in oilseeds of 100 µg kg^{-1} for lead (Chen *et al.*, 2010; FAO, 2015), while there is no particular threshold for cadmium.

According to agronomic reports, Iran is the largest consumer of nuts in the world with 5 kg annual consumption per capita, with the largest part accounting for about 40% of sunflower seeds (Public and International Affairs of Iran-Trade Development Organization, 2009). This original study was conducted in a context where the Institute of Standards and Industrial Research of Iran (ISIRI) has no regulation on Cd and Pb levels in oilseeds in any Iranian markets.

There is no information about the heavy metal content of oilseeds in Iran. To the best of our knowledge, this was the first study that has aimed to determine the levels of Pb and Cd in different types of seeds, including sunflower, pumpkin, watermelon, and jabooni seeds, in Iranian markets. In addition, this study addressed the health risks associated with the estimated weekly intake (EWI) of these metals via the consumption of these seeds.

Materials and methods

Sampling

A total of 168 seed samples were collected from six wholesale and distribution centers, in six cities in Iran including Zanjan, Isfahan, Shiraz, Mashhad, Hamedan and Khoy, as seed production cities or important sites with packaging factories supplying edible seeds. The sampled seeds from sunflower (*Helianthus annuus*), pumpkin (*Cucurbita pepo*), watermelon (*Citrullus lanatus*), and jabooni (*Citrullus vulgaris*, red water melon seeds), were collected in bulk or packaged forms. Five samples in package had kernels supplied by different brands and cities. All other samples presented kernels in shell and then both were analyzed. The seeds were collected randomly from August to November 2014 and transferred to the laboratory in plastic bags. The samples were carefully handled to avoid post-sampling contamination.

Apparatus and chemicals

Microwave digestion of the seed samples was performed using a Sineo MDS-10 (China) microwave system. Before digestion, the samples were homogenized using a blender (Sana, Japan) with a speed of rotation: 11,000–22,000 rpm, power: 280 W, and voltage: 220-240 V. A graphite furnace atomic absorption spectrophotometer (GTA120 and PSD120, Varian, Australia), equipped with a deuterium background corrector and hollow cathode lamps of Cd and Pb, was used to determine the levels of these metals in the seed samples.

All the used reagents were Analytical grades. Double deionised water (Milli-Q, Millipore) was used for all dilutions. Nitric acid (HNO_3) (65% suprapure) and hydrogen peroxide (35% extra-pure) were purchased from Merck (Darmstadt, Germany). The reagent blank determinations were used to correct the instrument readings. The glassware was properly cleaned with nitric acid.

Stock standard solutions of Cd and Pb, with a concentration of 1,000 mg L^{-1} in 0.5 mol L^{-1} HNO_3, was purchased from Merck (Darmstadt, Germany). The diluted standard solutions were prepared from the stock standard solution of Cd and Pb (Merck).

Sample preparation

Because the ability of heavy metal absorption varies between species, a total of 331 seed samples, including 168 seed kernels and 163 seed shells from sunflower, pumpkin, watermelon and jabooni seeds were analyzed in this study.

The seeds were shelled by hand, and then kernels and shells were separately ground and homogenized. Sample preparation was done as described by Zheng *et al.* (2013) with modifications in different sections of the digestion phase. The samples (0.5 g) were weighed directly in polytetrafluorethylene (PTFE) flasks, hydrated with 1.5 mL deionized water, and predigested for 20 min using 8 mL of concentrated nitric acid (65%) in the lab at room temperature. Then, 4 mL HNO_3 and 1 mL hydrogen peroxide (35%) were added to each sample. To complete the digestion phase, the samples were transferred to a microwave (Sineo MDS-10, China) according to the following digestion program: ramp at 130 °C for 10 min, 150 °C for 5 min, and 180 °C for 15 min. After the solution has cooled down to room temperature, it was finally transferred to the apparatus vials for injection into the furnace atomic absorption spectrometry. Prior to analyzing the samples, the instrument was optimized to ensure maximum signal strength by adjusting the parameters for each metal. The optimum conditions for Cd and Pb were applied as follows: wavelength: 228.8 and 283.3 nm, respectively; argon flow rates and slit width for both elements: 0.3 L min^{-1} and 0.5 nm; lamp current: 2 mA for Pb and 4 mA for Cd.

Analytical procedures

The metals were analyzed using a graphite furnace atomic absorption spectrophotometer. External calibration curves were used to quantify the Cd and Pb in the digested samples. The system was adjusted to measure the samples in triplicate, and the relative standard deviation (RSD) was automatically calculated. The criteria for linearity, accuracy, repeatability, and reproducibility and RSD complied with the criteria established by the Codex. The limit of detection (LOD) and the limit of quantification (LOQ) were defined as being equal to 3- and 10-times the standard deviation of the blank samples, respectively.

Quality control analysis

To validate the analytical method, recoveries for each element were checked for three spiked levels, respectively. Moreover, a blank and spiked sample was analyzed for each working day. As no commercially certified reference materials for these seeds were available, the accuracy was confirmed by analyzing a powdered rice QC material (FAPAS, T07208QC). The assigned values of Pb and Cd were 225 and 170 μg kg^{-1}, respectively. It is believed that the use of this material is appropriate, especially because the sample was digested and diluted in the same way as the seeds. The assays were performed in triplicate, and the mean was used for statistical analysis.

Estimated weekly intake of metals

Exposure to heavy metals via seeds depends on the concentration of the heavy metals (Cd and Pb) in seeds and the amounts of seeds that are consumed. The estimated weekly intake (EWI, expressed as μg kg^{-1} body weight $week^{-1}$) for Cd and Pb was calculated as follows:

$$\text{EWI} = \frac{\text{Weekly seed consumption (kg)} \times \text{Mean concentration of Cd or Pb in seeds (μg kg−1)}}{\text{Body weight (kg)}}$$

Since there are no data regarding the consumption of seeds in Iran, for this study, data on the weekly consumption of seeds were selected based on the questionnaire responses collected from 210 people in the specified age ranges. The EWI was calculated for specific student age groups (15–25 and 25–40 year-old male and female students) with normal dietary habits, while other groups were not included. The picked groups was selected based on high consumption of edible seeds among the Iranian population. Finally, the evaluation of Cd and Pb exposure was determined based on the mean consumption of all the seed samples.

Statistical analysis

All statistical analyses were performed using SPSS software (Windows version 18) and Excel 2007 software. Data are presented as frequency, mean, and standard deviation.

Table 1. Validation assessment of the analysis method for Pb and Cd in seeds (n=3).

	Sample spiking levels (µg kg-1)	Recovery (%)
Pb	5	88.0 ± 5.6
	20	97.0 ± 7.5
	40	100.0 ± 2.0
Cd	5	87.0 ± 7.7
	20	102.0 ± 12.5
	40	101.0 ± 6.3

The Kolmogorov-Smirnov test was used for data normality. The chi-square test, Mann-Whitney U test, and Kruskal-Wallis test were used for comparisons between the groups. In this study, the probability value of $P<0.05$ was considered to be statistically significant.

Results and discussion

Performance characteristics of the analytical method

The analysis of the QC material as reference gave 218 µg kg-1 Pb and 180 µg kg-1 Cd, for a Z-score range <2. Similar results for the mean recovery and coefficient of variation were obtained in the three spiking levels (Table 1). The relative standard deviation (RSD) was <14% for Pb and <10% for Cd, and the assessed level comprised between 0.005 and 1.62 mg kg-1. All RSD ≤44% are complying with the Codex criteria.

The accuracy of the method was checked using the recovery test for the Pb and Cd ions after employing the microwave digestion method. Based on our results, the average recoveries from edible oilseed samples ranged between 87 and 102%, which complies with the Codex criteria (to be comprised between 80 and 110%).

These results using both techniques are in agreement for both metals with the certified or informed values. The LOQ was 5 µg kg-1 for both Cd and Pb. The LOD of Cd and Pb was 1.5 and 1.8 µg kg-1, respectively, in agreement with the Codex criteria: 26 ≤ (LOD = 3 RSD) ≤ 44%.

Natural occurrence

The concentrations of Pb and Cd in the seed samples (Table 2) indicate that Pb was detected in 33% of the samples with a median and mean level of 66 and 77 ± 28 µg kg-1, respectively. The level of Pb in 39% of positive samples was higher than 100 µg kg-1. The highest frequency of Pb contamination was detected in the pumpkin seeds (52%), followed by the sunflower seeds (30%), the watermelon seeds (25%), and the jabooni seeds (15%). A comparison of the Pb content among the sunflower, pumpkin, watermelon, and jabooni seed samples showed that the difference of the mean level of Pb among each type of seed is statistically significant ($P<0.001$). The highest Pb concentration was seen in the pumpkin seeds with a mean and maximum level of 203 ± 232 and 1,093 µg kg-1, respectively (Table 3). Co-occurrence of Cd and Pb were observed in 2.6% of the samples.

Cadmium contamination was found in 17% of the samples with an average level of 264 ± 177.3 µg kg-1. Furthermore, the difference between the mean levels of Cd in the samples was also statistically significant ($P<0.001$) (Table 4). None of the watermelon and jabooni seed samples were contaminated with Cd. The highest concentration (731 µg kg-1) and incidence (35%) of Cd were recorded in the sunflower seeds (Table 4). Because sunflowers tend to accumulate more Cd than other crops, a high contamination level of Cd in sunflower seeds has also been reported in other studies (Blamey *et al.*, 1997). According to Andersen and Hansen (1984) the Cd levels of 55 sunflower kernel samples from North America and Europe were 250–690 µg kg-1 with a mean of 320–540 µg kg-1 from different countries. A study analyzing sunflower seeds collected in Germany reported a mean concentration of Cd of 390 µg kg-1 (Schwarz *et al.*, 2014). In Brazil, the mean Cd content of sunflower seed samples was reported 38 ± 10 µg kg-1 (Chaves *et al.*, 2010).

Concentrations of Pb and Cd in the kernels and shells of oilseeds

There were no significant differences ($P<0.001$) between the Pb and Cd levels in kernels and shells of the seed samples analyzed. Pb contamination was found in 18% of the kernels and 49% of the shells of the seeds, with an average level of 89.4 ± 82.1 and 162.9 ± 214.1 µg kg-1, respectively (Table 2). None of the watermelon and jabooni kernel samples showed Pb contamination. The mean value of Pb in the pumpkin and sunflower kernel samples was 203 ± 233 and 93 ± 129 µg kg-1, respectively. The highest level of Pb in the shells was detected in the pumpkin seed samples (Zanjan, 1,093 µg kg-1), followed by the sunflower seed samples (Zanjan, 869 µg kg-1), the watermelon seed samples (Zanjan, 544 µg kg-1), and the jabooni seed samples (Zanjan, 225 µg kg-1) (Table 3).

In the present study, 51 of the 168 kernels of the seed samples were contaminated with Cd, with a mean of 265 ± 177 µg kg-1 in the range of 60–731 µg kg-1. The incidence of Cd contamination in the shell seed samples was lower than the kernel seed samples. Cd was only detected in four of the sunflower shell seed samples, with an incidence and average contamination level of 2% and 111 ± 52 µg kg-1, respectively.

Table 2. Concentrations (in µg kg-1) of Pb and Cd in kernel and shell oilseed samples.

	Seed samples	Number of samples	Positive samples (%)	Concentrations (in µg kg-1)		
				Range	Median	Mean ± SD
Pb	Kernels	168	30 (18)	10–381	65.5	89.4 ± 82.1
	Shells	163	80 (49)	10–1,093	85.9	162.9 ± 214.1
	Total	331	110 (33)	10–1,093	80.6	143.1 ± 190.3
Cd	Kernels	168	51 (30)	73–731	209.0	277.6 ± 177.5
	Shells	163	4 (2)	60–164	90.7	101.3 ± 46.9
	Total	331	55 (17)	60–731	207.0	264.8 ± 177.3

Table 3. Lead occurrence in various oilseed samples collected in different cities in Iran. *n*: Number of samples; P%: percent of positive samples.

Species	Seeds	Collection sites (cities in Iran)																		Total		
		Isfahan			Hamadan			Khoy			Mashhad			Shiraz			Zanjan					
		n	P%	Mean ± SD	*n*	P%	Mean ± SD	*n*	P%	Mean ± SD	*n*	P%	Mean ± SD	*n*	P%	Mean ± SD	*n*	P%	Mean ± SD	*n*	P%	Mean ± SD
Sunflower	Kernels	4	0	ND	4	25	84	16	19	57 ± 27	3	0	ND	3	0	ND	48	19	42 ± 25	78	17	50 ± 29
	Shells	4	0	ND	4	50	153 ± 18	16	56	60 ± 26	3	0	ND	3	0	ND	45	47	129 ± 181	75	43	111 ± 149
	Total	8	0	ND	8	38	130 ± 42	32	38	60 ± 25	6	0	ND	6	0	ND	93	33	103 ± 153	153	30	93 ±129
Pumpkin	Kernels	4	25	235	3	0	ND	6	33	132 ± 48	3	100	58 ± 35	3	0	ND	29	41	121 ± 106	48	37	121 ± 96
	Shells	4	50	229 ± 296	3	30	109	5	40	210 ± 254	3	100	211 ± 149	3	30	108	28	79	272 ± 302	46	67	250 ± 272
	Total	8	37.5	230 ± 209	6	16	109	11	36	171 ± 156	6	100	136 ± 111	6	16	108	57	60	218 ± 259	94	52	203 ± 232
Watermelon	Kernels	3	0	ND	3	0	ND	3	0	ND	3	0	ND	3	0	ND	5	0	ND	20	0	ND
	Shells	3	67	106 ± 62	3	67	40 ± 49	3	33	65	3	33	35	3	33	45	5	60	370 ± 307	20	50	155 ± 211
	Total	6	33	106 ± 62	6	33	40 ± 49	6	16	65	6	16	35	6	16	45	10	30	360 ± 298	40	25	155 ± 211
Jabooni	Kernels	3	0	ND	3	0	ND	3	0	ND	3	0	ND	3	0	ND	7	0	ND	22	0	ND
	Shells	3	30	12	3	30	51	3	30	23	3	0	ND	3	30	24	7	43	113 ± 103	22	32	64 ± 76
	Total	6	16	12	6	16	51	6	16	23	6	0	ND	6	16	24	14	21	98 ± 111	44	15	64 ± 76

Table 4. Cadmium occurrence in various seed samples collected in different cities in Iran. *n*: Number of samples; P%: percent of positive samples.

Sampled species	Seeds	Collection sites (cities in Iran)																		Total		
		Isfahan			Hamadan			Khoy			Mashhad			Shiraz			Zanjan					
		n	P%	Mean ± SD	*n*	P%	Mean ± SD	*n*	P%	Mean ± SD	*n*	P%	Mean ± SD	*n*	P%	Mean ± SD	*n*	P%	Mean ± SD	*n*	P%	Mean ± SD
Sunflower	Kernels	4	50	116 ± 6	4	100	211 ± 84	16	38	321 ± 244	3	33	195	3	67	429 ± 44	48	73	285 ± 179	78	66	278 ± 177
	Shells	4	0	ND	4	0	ND	16	6	60	3	0	ND	3	0	ND	45	7	115 ± 46	75	5	102 ± 47
	Total	8	25	116 ± 6	8	50	211 ± 84	32	22	283 ± 244	6	16	195	6	33	429 ± 44	93	41	272 ± 178	153	35	257 ± 177
Pumpkin	Kernels	4	0	ND	3	0	ND	6	0	ND	3	0	ND	3	0	ND	29	4	73	48	2	73
	Shells	4	0	ND	3	0	ND	5	0	ND	3	0	ND	3	0	ND	28	0	ND	46	0	ND
	Total	8	0	ND	6	0	ND	11	0	ND	6	0	ND	6	0	ND	57	2	73	94	1	73
Watermelon	Kernels	3	0	ND	3	0	ND	3	0	ND	3	0	ND	3	0	ND	5	0	ND	20	0	ND
	Shells	3	0	ND	3	0	ND	3	0	ND	3	0	ND	3	0	ND	5	0	ND	20	0	ND
	Total	6	0	ND	6	0	ND	6	0	ND	6	0	ND	6	0	ND	10	0	ND	40	0	ND
Jabooni	Kernels	3	0	ND	3	0	ND	3	0	ND	3	0	ND	3	0	ND	7	0	ND	22	0	ND
	Shells	3	0	ND	3	0	ND	3	0	ND	3	0	ND	3	0	ND	7	0	ND	22	0	ND
	Total	6	0	ND	6	0	ND	6	0	ND	6	0	ND	6	0	ND	14	0	ND	44	0	ND

Table 5. Estimated weekly intake (EWI) of Cd and Pb in edible oilseeds;

$$(\text{EWI} = \frac{\text{Weekly seed consumption (kg)} \times \text{Mean concentration of Cd or Pb in seeds } (\mu g\ kg-1)}{\text{Body weight (kg)}}).$$

Sex	Age (year)	Weight (kg)	Consumption (kg week^{-1})	Estimated weekly intake			
				Cd		Pb	
				Mean	Max	Mean	Max
Women	15–25	57.4	0.054	0.04	0.69	0.05	1.03
	25–40	66.5	0.020	0.01	0.22	0.02	0.33
Men	15–25	63.7	0.053	0.04	0.61	0.04	0.91
	25–40	78.0	0.081	0.05	0.76	0.01	1.13

Comparison of Pb and Cd concentrations in the seeds collected from different Iranian cities

The level of Pb and Cd in seeds was also evaluated in the samples collected from different cities in Iran (Table 3). The frequency of Pb contamination in the seed samples collected in Zanjan, Isfahan, Hamadan, Mashhad, Shiraz, and Khoy were 38%, 23%, 27%, 32%, 14%, and 33% respectively. The maximum level of Pb was detected in the shells of the pumpkin seeds collected in Zanjan (1,093 µg kg^{-1}). For Cd, the incidence of contamination in the seeds collected from Zanjan, Isfahan, Hamadan, Mashhad, Shiraz, and Khoy was 22.7%, 3.8%, 15.4%, 4.5%, 9.1%, and 12.7%, respectively. The highest average level of Cd was observed in the sunflower kernel samples collected from Shiraz (429 ± 44 µg kg^{-1}). The highest level of Cd was observed in the sunflower kernels samples collected from Zanjan (731 µg kg^{-1}) (Table 4). Our results also found that there was no statistically significant difference in the mean value of Pb and Cd among the different Iranian cities ($P > 0.05$). The similar study with our investigation is very limited, but according to previous study in Pb levels of sunflower samples from Brazil reported 250–690 µg kg^{-1} with a mean of 11 ± 3 µg kg^{-1} (Chaves *et al.*, 2010).

Estimated weekly intake of metals via oilseed consumption

The EWI through the consumption of seeds by both women and men (Table 5) were lower than the provisional maximum tolerable weekly intake of Pb (25 µg kg^{-1} body weight) and Cd (2.5 µg kg^{-1} body weight) (Arcella *et al.*, 2012). The highest intakes of Pb and Cd were found in the 24–40 year-old men group. The results of this study indicate that the potential health risk of Pb and Cd is minimal for both women and men in the two different age groups.

Conclusion

This study has provided the first assessment of exposure of Cd and Pb, through the dietary intake by Iranian population. Unfortunately, the Institute of Standards and Industrial Research of Iran (ISIRI) has no regulations for Cd and Pb levels in seeds. Our present findings showed that sunflower seeds have a greater tendency to accumulate Cd in their kernels than other seeds, and pumpkin seeds have a greater tendency to accumulate Pb in their kernels than other seeds. However, the Cd content in the sunflower kernels could pose a potentially adverse effect on human health in cases in which large amounts of the kernels are consumed for a long time, a dietary exposure assessment of Cd and Pb illustrates that there is no potential health risk to seed consumers in Iran when it is calculated alone in edible seeds. Our results showed the mean of dietary exposure to Cd and Pb was much lower than the provisional maximum tolerable weekly intake, indicating that there is a low health risk regarding exposure of these heavy metals via seeds consumption. The relatively high incidence of Pb and Cd in the seeds (33 and 17%, respectively) and the toxic effects of Pb and Cd indicate the necessity for regular surveillance to reduce intake of heavy metals.

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