Health Risk Assessment of Heavy Metals (Cd, Cu, Pb and Zn) in Soybean Marketed in Hamedan City, Iran

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Abstract

Objectives: Based on the world researches, noncarcinogenic risk assessment of heavy metals in the soybean (Glycine max) is narrow; the current study aimed at investigating the concentrations of cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) in soybean marketed in Hamedan city, Iran, and estimating their potential risk to the health of inhabitants.

Methods: The current analytical-observational study totally collected 27 samples from 9 brands of soybean (3 samples from each brand of soybean) from the market basket of Hamadan in 2015. After preparation and processing the samples in the laboratory, the concentration of metals were determined using atomic absorption spectrophotometer (AAS). Also, all statistical analyses were performed using SPSS version 19, according to the Shapiro-Wilk test, one-way ANOVA (Duncan multiple range test), and the Pearson correlations.

Results: The results showed that the maximum mean concentrations (µg/kg dW) of Cd, Cu, Pb, and Zn in soybean samples were 42.0 ± 19.06, 13440.1 ± 1437.0, 34.44 ± 14.82, and 32566.8 ± 4965.1, respectively. Also, the mean concentrations of metals in all samples were lower than the maximum permissible limits (MPL) recommended by the world health organization (WHO). The computed health risk index (HRI) showed that no potential risk for adults and children via consumption of the studied soybean.

Conclusions: According to HRI value, there was no possible health risk to adults and children due to intake of the studied soybean brands under the current consumption rate.

Keywords: Soybean, Monitoring, Heavy Metals, Carcinogenic, Health Risk

1. Background

Industrial development, mining activity, irrigation with waste water, and the application of chemical inputs, organic fertilizers and sewage sludge in agriculture result in the pollution of agricultural soil and crops by heavy metals worldwide (1).

As heavy metals are potential health problems to human, today there is a growing interest in assessing the levels of heavy metals in foodstuffs. The ingestion of food is an obvious means of exposure to heavy metals, as many metals are natural ingredients of foodstuffs and also due to environmental contamination, and contamination by food processing technologies, packaging, transportation, and storage. Therefore, concern about the quality of food is increasing worldwide (2, 3). Heavy metals such as arsenic, cadmium, chromium, mercury, and lead pose potential health risks to human beings and animals, and this risk is aggravated by the persistence of these pollutants in the environment.

Cadmium is toxic and non-essential for human health and principally accumulates in the kidneys and liver. The most important sources of Cd pollution are metal industries and sewers (4).

Copper is one of the most abundant trace elements with vitamin-like impact in human body and living systems, and is found in a wide range of foods such as nuts, many fruits and vegetables, red meat, shellfish, and many vitamin supplements (5, 6).

Lead can enter the food chain through contaminated soil and adversely affect human health. Lead poisoning in adults can affect the blood circulation, kidneys, and peripheral and central nervous systems (7). Furthermore, fetuses, infants, and children are at a particularly high risk of neurotoxic and development disorders due to this element (8, 9).

Zinc is an essential structural and functional element for numerous cellular processes, which often catalyze reactions and bind to substrates by favoring various reactions through the mediation of redox or oxidation-reduction reactions via reversible changes in the oxidation state of the metal ions (10-12).

Risk analysis is a process that combines risk assessment, risk management, and risk communication. Risk as-
essment includes scientific analyses that their results are quantitative or qualitative explanations of the likelihood of harm associated with exposure to chemical compounds. In this regard, the human health risk assessment requires identification, collection, and integration of information on the chemicals that cause health hazards, exposure of human to the chemicals, and the relationships between exposure, dosage, and adverse effects (13). On the other hand, a human potential health risk assessment is the process to estimate the nature and possibility of adverse health effects in humans exposed to toxins and chemicals in polluted environmental media, and includes hazard identification, dose-response assessment, exposure assessment, and risk characterization steps (14).

Soybean (Glycine max (L.) Merr.) is one of the most widespread crops and an excellent source of plant protein. Consumption of soybean and its products is associated with a lower risk of chronic diseases, osteoporosis, and cancer (15).

Based on the concern for food safety, the current study aimed at determining the contents and noncarcinogenic risk assessment of Cd, Cu, Pb, and Zn in the some brands of soybean marketed in Hamedan in 2015.

2. Methods

2.1. Apparatus

A Varian 710-ES inductively coupled plasma optical emission spectrometer (ICP-OES) (Agilent Technologies, Inc., Santa Clara, CA, USA) was used for the quantitative elemental determination.

2.2. Chemicals and Reagents

Standard stock solutions of different metal ions at the concentration of 1000 µg/mL were used to prepare working solutions after appropriate dilution. Standard solutions were of analytical grade (Merck, Darmstadt, Germany). Distilled deionized water was used in all dilution procedures.

2.3. Sample Collection and Sample Analysis

In the current study, according to the Cochran sample size formula, 27 samples from 9 brands of soybean (3 samples from each brand of soybean) were collected from the market basket of Hamadan in 2015. The samples were placed at 70°C in an oven until completely dried. Then, samples were weighed and ground to determine the biomass. The soybean samples (0.50 g) were digested with a 12-mL solution containing 87% nitric acid and 13% perchloric acid (v/v). After digestion treatment, samples were filtered through Whatman 42. The filtrates were collected in 50 mL Erlenmeyer flasks and analyzed by ICP-OES (1, 16, 17). All the instrumental conditions applied for Cd, Cu, Pb, and Zn content determinations were set in accordance with general recommendations.

2.4. Statistical Analysis

The statistical analysis of the obtained results consisted of the Shapiro-Wilk test for the normality, followed by the study of the variance homogeneity using an ANOVA parametric test with a DMS post hoc and Duncan multiple range tests. Finally, to study a correlation between the metals in the different soybean samples, the Pearson correlation was performed.

2.5. Potential Health Risk Assessment

The current study assessed the human health risks posed by chronic exposure to the heavy metals. To compute potential health risk assessment, the average daily intake of metal (DIM) was calculated using Equation 1 (18-20):

\[
DIM = \frac{C_{metal} \times C_{factor} \times D_{food \, intake}}{B_{average \, weight}}
\]

where \( C_{metal} \), \( C_{factor} \), \( D_{food \, intake} \), and \( B_{average \, weight} \) represent the heavy metal concentrations in soybean (µg/kg), conversion factor, daily intake of soybean, and average body weight, respectively. The conversion factor (0.085) was used to convert fresh weight into dry weight (19). The average daily intake of soybean for adult and children was considered 0.001 kg per person per day. The average adult and children body weight were considered 70.0 and 15.0 kg, respectively (21-23).

The health risk index (HRI) for the local population through the consumption of soybean was assessed using Equation 2 (18-20):

\[
HRI = \frac{DIM}{RfD}
\]

Here, DIM and reference dose (RfD) represent daily intake of metal and reference dose of metal, respectively. The oral reference doses for Cd, Cu, Pb, and Zn were 1.0, 40.0, 3.50, and 300.0 µg/kg/day, respectively. An HRI<1 means that the exposed population is assumed to be safe (20, 24).

The total HRI (THRI) of heavy metals for the soybean was calculated as the mathematical sum of each individual metal HRI value according to Equation 3 (25):

3. Results

The concentrations of Cd, Cu, Pb, and Zn in the analyzed soybean samples are presented in Table 1. Data in Table 1 showed that the percentage of metal contamination of soybean samples reached 100%. Among the analyzed
soybean samples, Cd was detected in the range of 19.0 to 75.0; Cu, 11856 to 15485; Pb, 16.0 to 57.0, and Zn 27246 to 42280 µg/kg.

Comparing the heavy metal concentrations in the studied soybean samples with the maximum permissible limits (MPL) (200 µg/kg for Cd, 20,000 µg/kg for Cu, 200 µg/kg for Pb, and 100,000 µg/kg for Zn) established by food and agriculture organization (FAO)/WHO (26, 27), the mean concentration of Cd, Cu, Pb, and Zn were lower than those of the MPL.

In addition, all calculated HRI values of soybean for adults and children were within the safe limits (HRI < 1) (Table 3). Furthermore, the THRI values, which varied from 5.0 × 10⁻⁴ to 7.52 × 10⁻⁴ for adults and from 2.23 × 10⁻³ to 3.12 × 10⁻³ for children were within the safe limit (THRI < 1). Therefore, it can be concluded that adults and children had no potential health risk through consuming soybean from the studied area.

The Duncan test results (different roman letters are presented in each column of Table 1) indicated statistical significant differences (P < 0.05) in the contents of Cd, Cu, Pb, and Zn between some brands of soybean. It means that no significant differences were observed in the content of Cd between the samples 1, and 3 to 9; no significant differences were observed in the content of Cu between the samples 1 to 3 and also between 4 to 9; no significant differences were observed in the content of Pb between the samples 1, 2, 3, 5, 6 and 9, and finally no significant differences were observed in the content of Zn between the samples 7, 8 and 9 and also between 5 and 9.

The Pearson correlations analysis was performed between metal concentrations in soybean samples to understand the relationships between them. The results showed that although no significant correlations were observed between Cd and Cu, and Cd and Pb in soybean samples, positive correlations were found between Zn and Cd (r = 0.439, P < 0.05), Zn and Cu (r = 0.717, P < 0.01) and Zn and Pb (r = 0.585, P < 0.01). Also, Cu correlated significantly and positively with Pb (r = 0.598, P < 0.01) (Table 4).

4. Discussion

Cadmium is a very toxic metal with a natural occurrence in soil, but it is also spread in the environment due to human activities (26). On the other hand, Cd in foods is mostly derived from various sources of environmental contamination. Higher Cd concentrations cause defecation of cardiovascular and skeleton systems due to acute toxicities (4). The results of the current study showed that the mean concentration of Cd in soybean samples was 42.0 ± 19.06 µg/kg, which was much lower than that of the MPL. The result was in contrast with the findings of Zhuang et al., who reported that the concentrations of Cd for Zhongxin, China, in the soybean seeds exceeded that of the MPL (27). Also, the observed level of Cd was much lower than those reported in soybean seeds grown in China (195 µg/kg) (27) and Argentina (143 µg/kg) (28).

Copper is known as an essential micronutrient that should be ensured through organic and artificial fertilizers for plant growth, and it is essential for healthy hormone secretion, nerve conduction, and growth of bones and connective tissue. Despite the small amount of Cu (50 to 120 mg) found in the human body, this element plays a critical role in biochemical processes (5, 6). On the other hand, critical doses of Cu can cause inflammation in the brain tissue, anorexia, fatigue, hair loss, acne, allergies, depression, premenstrual syndrome, migraines, anxiety, childhood hyperactivity, panic attacks, kidney and liver dysfunctions, strokes elevated cholesterol, adrenal hyperactivity and insufficiency, learning disorders, autism, and cancer (6). The results showed that the mean concentration of Cu in soybean samples was 13440.1 ± 1437.0 µg/kg, which was much lower than that of the MPL. The Cu level observed in the current study was much lower than those reported in soybean seeds grown in China (19650 µg/kg) (27) and Argentina (20830 µg/kg) (29).

Lead is a widespread pollutant and a highly toxic metal; it is bioaccumulative and does not degrade in the environment and is not easily metabolized. Lead has no known functions in biological systems and is recognized as a major environmental health risk worldwide (30). The current study results showed that the mean concentration of Pb in soybean samples was 34.44 ± 14.82 µg/kg, which was much lower than that of the MPL. The result was in contrast with the findings of Zhuang et al., who reported that the concentration of Pb for Fandong, China, in the soybean seeds exceeded that of the MPL (27). Also, the Pb level observed in the current study was much lower than those reported in soybean seeds grown in China (260 and 245 µg/kg) (1, 27), Argentina (1700, 1975, and 800 µg/kg) (28, 29, 31), and Brazil (103 µg/kg) (32).

Zinc is a critical element for growth, but Zn may play an important role in cancer etiology and outcomes, and also harms some physiological activities such as breathing (10, 11). The current study results showed that Zn was
Table 1. Heavy Metal Concentrations of the Soybean Samples

| Sample | Cd  | Cu   | Pb   | Zn   |
|--------|-----|------|------|------|
| 1      | 39.0 ± 0.74ab | 15485 ± 44.0b | 55.0 ± 2.20b | 32977 ± 272.0e |
| 2      | 75.0 ± 0.46b  | 15171 ± 12.0b | 57.0 ± 3.90b | 42280 ± 322.0g |
| 3      | 53.0 ± 2.80ab | 15127 ± 85.0b | 46.0 ± 1.80ab| 39214 ± 290.0f |
| 4      | 39.0 ± 0.44ab | 11040 ± 101.0a| 25.0 ± 2.80a | 27246 ± 1787.0a |
| 5      | 19.0 ± 0.80a  | 13188 ± 828.0a| 32.0 ± 1.70ab| 31447 ± 80.0d  |
| 6      | 20.0 ± 17.0a  | 11856 ± 800.0a| 29.0 ± 2.60ab| 28842 ± 268.0b |
| 7      | 62.0 ± 53.0ab | 12448 ± 1134.0a| 16.0 ± 2.70a | 30099 ± 242.0c |
| 8      | 45.0 ± 39.0ab | 10947 ± 889.0a| 20.0 ± 2.20a | 30127 ± 169.0c |
| 9      | 26.0 ± 53.4a  | 12699 ± 1122.0a| 30.0 ± 3.30ab| 30959 ± 188.0cd|
| Mean of brands | 42.0 ± 19.06 | 13440.1 ± 1437.0 | 34.44 ± 14.82 | 32566.8 ± 4965.1 |

*Value are expressed as mean ± SD.

Table 2. Daily Intake of Metals for Each Heavy Metal

| Sample | Cd, μg | Cu, μg | Pb, μg | Zn, μg |
|--------|--------|--------|--------|--------|
| Adults |        |        |        |        |
| 1      | 4.73 × 10^5 ± 8.99 × 10^3 | 1.88 × 10^2 ± 5.34 × 10^6 | 6.68 × 10^5 ± 2.67 × 10^6 | 4.00 × 10^3 ± 1.30 × 10^4 |
| 2      | 9.11 × 10^5 ± 5.59 × 10^5 | 1.84 × 10^3 ± 1.46 × 10^5 | 6.92 × 10^5 ± 4.74 × 10^7 | 5.71 × 10^3 ± 1.91 × 10^8 |
| 3      | 6.43 × 10^5 ± 2.55 × 10^5 | 1.84 × 10^2 ± 1.03 × 10^5 | 5.58 × 10^5 ± 2.90 × 10^6 | 4.76 × 10^3 ± 1.52 × 10^7 |
| 4      | 4.73 × 10^5 ± 4.86 × 10^4 | 1.58 × 10^3 ± 1.25 × 10^4 | 3.00 × 10^3 ± 1.40 × 10^4 | 3.31 × 10^2 ± 2.17 × 10^5 |
| 5      | 2.31 × 10^5 ± 9.71 × 10^5 | 1.60 × 10^3 ± 1.00 × 10^5 | 3.88 × 10^5 ± 2.06 × 10^6 | 3.82 × 10^3 ± 9.71 × 10^5 |
| 6      | 2.43 × 10^5 ± 2.06 × 10^5 | 1.44 × 10^3 ± 9.71 × 10^4 | 3.52 × 10^3 ± 3.16 × 10^4 | 3.50 × 10^2 ± 1.35 × 10^5 |
| 7      | 7.53 × 10^5 ± 6.44 × 10^5 | 1.51 × 10^3 ± 1.38 × 10^2 | 3.14 × 10^3 ± 3.28 × 10^6 | 3.64 × 10^2 ± 2.94 × 10^4 |
| 8      | 5.46 × 10^5 ± 5.74 × 10^5 | 1.45 × 10^5 ± 1.08 × 10^3 | 2.43 × 10^5 ± 2.67 × 10^4 | 3.66 × 10^3 ± 1.05 × 10^4 |
| 9      | 3.15 × 10^5 ± 6.24 × 10^5 | 1.54 × 10^3 ± 1.36 × 10^3 | 3.64 × 10^3 ± 4.01 × 10^4 | 3.76 × 10^2 ± 2.28 × 10^4 |
| Children |        |        |        |        |
| 1      | 2.21 × 10^4 ± 4.29 × 10^6 | 8.77 × 10^2 ± 1.24 × 10^4 | 3.12 × 10^5 ± 1.35 × 10^5 | 1.87 × 10^5 ± 1.54 × 10^5 |
| 2      | 4.25 × 10^4 ± 2.81 × 10^5 | 8.60 × 10^2 ± 6.80 × 10^4 | 3.23 × 10^5 ± 2.21 × 10^5 | 2.39 × 10^5 ± 1.82 × 10^5 |
| 3      | 3.00 × 10^4 ± 1.89 × 10^4 | 8.57 × 10^2 ± 4.82 × 10^3 | 2.61 × 10^5 ± 1.02 × 10^5 | 2.22 × 10^5 ± 1.64 × 10^4 |
| 4      | 2.21 × 10^4 ± 2.27 × 10^4 | 7.40 × 10^2 ± 5.84 × 10^4 | 1.42 × 10^5 ± 1.59 × 10^5 | 1.54 × 10^5 ± 2.12 × 10^4 |
| 5      | 1.08 × 10^4 ± 4.53 × 10^3 | 7.47 × 10^2 ± 4.69 × 10^3 | 2.11 × 10^5 ± 9.63 × 10^2 | 1.78 × 10^5 ± 4.33 × 10^3 |
| 6      | 1.13 × 10^4 ± 9.63 × 10^3 | 6.72 × 10^2 ± 4.53 × 10^3 | 2.16 × 10^5 ± 4.17 × 10^5 | 1.61 × 10^5 ± 2.15 × 10^3 |
| 7      | 3.51 × 10^4 ± 3.00 × 10^4 | 7.00 × 10^2 ± 6.41 × 10^3 | 3.10 × 10^5 ± 1.53 × 10^5 | 1.50 × 10^5 ± 1.37 × 10^5 |
| 8      | 2.55 × 10^4 ± 2.21 × 10^4 | 8.77 × 10^2 ± 5.04 × 10^3 | 2.13 × 10^5 ± 3.35 × 10^5 | 1.71 × 10^5 ± 9.58 × 10^3 |
| 9      | 1.47 × 10^4 ± 2.91 × 10^3 | 7.20 × 10^2 ± 6.36 × 10^3 | 1.70 × 10^5 ± 1.87 × 10^5 | 1.75 × 10^5 ± 1.06 × 10^5 |

the most abundant major element in soybean with an average value of 32566.8 ± 4965.1 μg/kg, which was much lower than that of the MPL. The average Zn concentration observed in the current study was less than those reported in soybean seeds grown in China (71400 μg/kg) (27) and Argentina (33280 μg/kg) (28). Also, the current study average Zn concentration was more than those found in soybean seeds grown in Argentina (30810 μg/kg) (31).
The EADI of heavy metals was compared with the oral RfD or provisional tolerable weekly intake (PTWI) to survey the potential health risks. In accordance with the standard methods (the United States environmental protection agency (USEPA), the risk of chronic-toxic effects (HRI) or target hazard quotients (THQs) is defined as the ratio of the dose resulting from exposure to site media to the dose believed to be safe; even in sensitive individuals such as children and elderly. The HRI < 1 indicates that there is no significant risk of chronic-toxic effects. The HRI > 1 indicates that chronic-toxic effects may occur. The chronic-toxic effects tend to increase with increased HRI. On the other hand, the HRI expresses the combined chronic-toxic effects of multiple metals (24, 33). As shown in Table 3, HRI values of Cd, Cu, Pb, and Zn for adults and children were minimal (less than 1). Here, the average HRI values were 1.51 × 10⁻⁶ and 6.49 × 10⁻⁴ for adults and children, respectively. Similarly, Salazar et al., reported that the values of THQ and HI of Cd, Pb, and Zn were less than those of the European Union or Argentine individuals through soybean consumption (28). Also, the results were against the findings of Zhuang et al., who reported that THQs of Cd, Cu, Cr, Ni,
Pb, and Zn for adults around the Dabaoshan Mine, China, through consumption of seeds of soybean were greater than 1 (27).

It should be pointed out that heavy metals as well as many other chemical pollutants may accumulate over the lifetime in human body. The cumulative effects, as a result, may be additive and/or interactive and the risk additivity usually requires that all components act according to the same mechanism (34). Despite the heavy metal contamination level of soybean in Hamedan, it was not critical, but that the exposure-risk estimate in the current study was only for soybean and did not account for any other alimentary sources. Therefore, for more accurate estimate exposure other sources should be added to estimate the total risk of each metal.

4.1. Conclusion

Finally, it can be concluded that although there was no possible health risk to adults and children due to intake of the studied soybean under the current consumption rate, due to the lack of adequate information about processing conditions, habitat adjacent to industrial areas and pollution with heavy metals, increased use of agricultural inputs, sewage sludge, and wastewater by farmers, regular periodic monitoring of chemical pollutants content especially heavy metals in foodstuffs are recommended for food safety.

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Footnote

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