



# The Concentration and Probabilistic Health Risk of Potentially Toxic Elements (PTEs) in Edible Mushrooms (Wild and Cultivated) Samples Collected from Different Cities of Iran

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## Abstract

The present study was devoted to investigate the PTE concentrations including cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), nickel (Ni), lead (Pb), arsenic (As), and zinc (Zn) among six types of edible wild, 23 cultivated mushroom samples collected from Iran's market by the aid of an inductively coupled plasma-optical emission spectrometry (ICP-OES). Also, the related health risk assessment was established by the aid of the Monte Carlo simulation method (MSC). The limit of detection (LOD) and limit of quantification (LOQ) were ranged 0.001–0.048 and 0.003–0.160 ppm, respectively. The concentrations of As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, and Zn were determined in the ranges of 0.003–11.5, 0.0008–5.89, 0.32–26.32, 9.15–110.08, 15.25–751.17, 0.16–2.24, 2.1–60.47, 1.21–24.22, 0.16–8.92, and 37.13–268.11 mg kg<sup>-1</sup>, respectively. According to findings, highest mean concentration of Cr in both types of mushrooms (cultivated and wild) was lower than recommended level by Codex Alimentarius/Food and Agriculture Organization/World Health Organization (CODEX/FAO/WHO) while the corresponding values for Hg (0.87 mg kg<sup>-1</sup>), As (1.39 mg kg<sup>-1</sup>), Ni (10.08 mg kg<sup>-1</sup>), Cu (36.65 mg kg<sup>-1</sup>), Cr (10.44 mg kg<sup>-1</sup>), Cd (0.589 mg kg<sup>-1</sup>), Fe (201.04 mg kg<sup>-1</sup>), Mn (10.30 mg kg<sup>-1</sup>), Zn (2266.43 mg kg<sup>-1</sup>), and Pb (3.81 mg kg<sup>-1</sup>) were higher than related standard levels. According to the health risk assessment, no concern regarding the non-carcinogenic risk due to the ingestion of PTEs via the consumption of the edible mushrooms, except Hg in wild mushrooms for children, was noted.

**Keywords** Potentially toxic elements (PTEs) · Wild mushrooms · Cultivated mushrooms · Health risk assessment · ICP-OES

## Introduction

The mushroom consumption is growing all over the world, while this term refers to different species of fungi that belong to *Basidiomycetes* or *Ascomycetes*. *Basidiomycetes* or *Ascomycetes* can grow under moisture conditions and soils rich in organic matter [1–3]. According to literature, more than 70,000 species of fungi were identified in the world, while about 2000 species (31 genera) are primarily edible

mushrooms [4]. However, about 10% of the 30 toxic mushroom species are considered lethal [5].

The edible mushrooms are appreciated, not only for their notable good flavor and texture but also as an essential source of the biologically active compound and nutritional properties [6]. In this regard, in many parts of the world, mushrooms are used as a part of the human diet due to their taste and aroma, as well as their high nutritional value such as dietary fiber, protein, fats, vitamins, and minerals [7–9]. In general, their fleshy body, on dry weight, contains almost 39.9% carbohydrate, 17.5% protein, and 2.9% fat, the residue constituting minerals [10]. Also, they have been reported as curative plants that are beneficial in preventing diseases such as high blood pressure [11], hypocholesteremia [12], and several types of cancer [13].

Human activities, such as the industry and the exploitation of raw materials, resulted in the formation of a huge amount of gas and sludge pollutants [14–16]. PTEs as the non-biodegradable, aggregate, and stable compounds are among the most important pollutants in the environment [17–19].

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While recently, they are proposed as a global human health issue [18, 20–23] as a result of their adsorption via ingestion, inhalation, and dermal contact or even indirect way due to their accumulation in soil and water as well as other environmental factors [21, 24–26]. Generally, all PTEs have adverse effects on human health, such as disorders related to the nervous system, kidneys, and blood circulation [27–29]. Among PTEs, As, Cd, Cr, Hg, Ni, and Pb are the most dangerous metals [30–34]. The PTE concentrations in the mushroom are reported as considerably higher than those in agricultural crop plants, fruit, and vegetables [35], mainly due to effective mechanisms to absorb PTEs from the ecosystems by them [36]. This issue primarily depends on the type of fungus and the physiology of various mushrooms, especially their ecosystems [37]. In regard to the important relative segment of mushroom in the world food basket, the occurrence of high concentrations of PTEs in the mushroom is considered important because of a high toxicological hazard [20, 38, 39].

Therefore, it is necessary to evaluate the levels of toxic elements among the available mushrooms in Iran's market. In this regard, for the first time, this study carried on to investigate the PTE content (Fe, Cu, Zn, Mg, Cd, Mn, Ni, Pb, Hg, As) in the wild edible and the cultivated mushroom samples collected from in Iran's market by the aid of an ICP-OES. Also, the potential health risk assessment was carried out.

## Materials and Methods

### Chemicals and Reagents

All the reagents and chemicals such as  $\text{HNO}_3$ ,  $\text{H}_2\text{O}_2$  in the analytical grade were obtained from Merck Company (Darmstadt, Germany).

### Sample Collection

Twenty-nine mushroom samples, which contain six wild (*Agaricus bisporus*), were collected from South of Kermanshah in spring 2019, and 23 cultivated mushrooms (*Agaricus bisporus*) from different provinces (stations) of Iran in Spring (Table 1) were collected during 2019 year based on the recommended method and were analyzed in triplicate [15].

The mushroom samples were washed with distilled water and dried at 105 °C for 24 h. The dried samples were ground, then homogenized using an agate pestle, and sorted in polyethylene bottles until analysis [40]. All of the plastic and glassware were cleaned by means of soaking, overnight in a 10% nitric acid solution, and then rinsed with deionized water [41].

### Sample Preparation

Concentrations of ten PTEs (Fe, Cu, Zn, Mg, Cd, Mn, Ni, Pb, Hg, and As) were analyzed. In this regard, a milestone Ethosup D closed vessel microwave (maximum power was 1400 W, and the maximum pressure in Teflon vessels—100 bar) (milestone Ethosup, Italia) besides the acid decomposition was used to minimize the effects of the organic matrix and also prevent the possibility of sample pollution and losses of analytes.

Duplicate mushroom samples (0.25 g) were digested with 9 mL of  $\text{HNO}_3$  (65%) and 1 mL of  $\text{H}_2\text{O}_2$  (70%) in the microwave digestion system for 30 min under pressure 100 bar and maximum temperature 300 °C. The residue was then diluted to 10 mL with deionized water in a 10-mL volumetric flask. A blank sample was digested with the same procedure [41].

### Inductively Coupled Plasma Atomic Emission Spectrometry Conditions

All prepared samples (duplicate) were analyzed by the aid of an ICP-OES (Spectro, Arcos, Germany) with Torch type of flared end EOP Torch 2.5 mm. The optimum functioning parameters were RF generator (1400 W); argon gas grade 6 was used for plasma, nebulizer, and auxiliary gas. The gas flows of plasma, auxiliary, and nebulizer were 14.50, 0.90, and 0.85 ( $\text{L min}^{-1}$ ), respectively. Afterward, initial stabilization time, rinse time, and sample uptake time were 240 s total and 45 s for preflush. Also, the time between replicate analysis and delay time was zero. The analysis was a three-time replicate, and the frequency (resonance frequency) of the generator of RF was 27.12 MHz. The types of solid state, detector, and spray chamber were cyclonic, CCD, and modified Lichte, respectively. The type of pump of sample delivery was four-channel, software-controlled; peristaltic pump enables exact sample flows. The speed of prewash pump was 60 rpm (for 15 s), 30 rpm (for 30 s), and the time of prewash was 45 s, and finally, the speed of the pump of sample injection was 30 rpm.

### Validation of the Analytical Method

The validation of the analytical procedure for quantitative analysis of elements in mushroom and its aqueous extracts was performed by evaluating selectivity, working and linear ranges, detection limit (LOD), quantification limit (LOQ), repeatability, and reproducibility (precision). Matrix effects were studied using standard addition method, by adding 200  $\mu\text{L}$  of mixed standard solutions to the original samples (Mix standard CRM: 92091 Supelco LOT BCCB9855, TraceCERT®, 33 elements, 10  $\text{mg L}^{-1}$  in nitric acid, Hg standard CRM: 28941 Supelco, LOT BCCB8927, 1000  $\text{mg L}^{-1}$ , Hg in nitric acid). The recoveries were within 92% and 106% for all the studied elements.

**Table 1** Concentration of potentially toxic elements (PTEs) in the cultivated and wild mushrooms based on stations (mg/kg)

Sample	As	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
Kermanshah A <sup>a</sup> (CM1)	0.182 ± 0.00	0.056 ± 0.00	0.451 ± 0.02	21.30 ± 2.22	20.48 ± 1.50	0.260 ± 0.060	2.61 ± 0.21	1.75 ± 0.06	0.160 ± 0.02	1007.29 ± 7.90
Kermanshah B (CM2)	0.003 ± 0.00	0.045 ± 0.00	0.375 ± 0.02	18.34 ± 2.13	22.97 ± 1.71	0.350 ± 0.052	2.67 ± 0.05	2.21 ± 0.05	0.300 ± 0.03	1134.47 ± 5.11
Ardebil (CM3)	0.003 ± 0.00	0.048 ± 0.00	0.458 ± 0.03	15.41 ± 2.26	17.70 ± 1.22	0.330 ± 0.033	2.27 ± 1.21	1.21 ± 0.03	0.230 ± 0.01	991.65 ± 6.17
Shiraz (CM4)	0.003 ± 0.00	1.018 ± 0.00	11.65 ± 0.45	27.43 ± 5.14	137.66 ± 5.22	0.480 ± 0.045	5.33 ± 0.62	17.50 ± 2.28	6.940 ± 0.19	1029.15 ± 8.23
Tehran A (CM5)	0.003 ± 0.00	0.031 ± 0.00	0.322 ± 0.01	16.69 ± 2.12	19.22 ± 1.41	0.340 ± 0.011	2.11 ± 0.32	1.54 ± 0.07	0.310 ± 0.00	928.29 ± 4.12
Tehran B (CM6)	0.003 ± 0.00	0.014 ± 0.00	0.423 ± 0.04	22.89 ± 2.73	15.25 ± 1.91	0.390 ± 0.032	2.87 ± 0.50	2.13 ± 0.03	0.260 ± 0.00	1664.44 ± 9.02
Gorgan (CM7)	0.003 ± 0.00	0.332 ± 0.01	11.017 ± 1.04	25.25 ± 2.80	100.60 ± 7.82	0.510 ± 0.01	4.11 ± 0.13	14.61 ± 0.11	6.640 ± 0.11	951.48 ± 5.88
Mashhad (CM8)	0.003 ± 0.00	0.172 ± 0.00	0.572 ± 0.01	9.15 ± 1.51	29.78 ± 1.82	0.160 ± 0.01	2.10 ± 0.04	2.37 ± 0.98	0.270 ± 0.13	1284.24 ± 13.44
Share Kord (CM9)	0.003 ± 0.00	0.031 ± 0.00	0.471 ± 0.03	13.77 ± 2.22	25.84 ± 1.24	0.360 ± 0.02	2.39 ± 0.07	1.72 ± 0.14	0.330 ± 0.03	1012.5 ± 7.66
Ilam (CM10)	0.003 ± 0.00	0.050 ± 0.00	0.55 ± 0.03	28.82 ± 1.21	22.72 ± 1.05	0.450 ± 0.02	2.84 ± 0.05	2.20 ± 0.28	0.540 ± 0.01	1393.43 ± 1.77
Bushehr (CM11)	0.637 ± 0.00	0.142 ± 0.00	19.186 ± 1.25	34.49 ± 2.81	115.74 ± 5.81	1.440 ± 0.05	10.16 ± 0.09	12.90 ± 0.55	4.700 ± 0.02	2945.75 ± 10.81
Tabriz (CM12)	0.003 ± 0.00	0.001 ± 0.00	0.402 ± 0.04	20.77 ± 1.20	22.13 ± 1.22	0.360 ± 0.04	2.43 ± 0.07	1.94 ± 0.99	0.190 ± 0.01	1305.51 ± 7.91
Ghaemshahr (CM13)	4.168 ± 0.42	0.018 ± 0.01	5.216 ± 0.12	17.09 ± 0.30	21.02 ± 1.120	0.390 ± 0.03	2.43 ± 0.04	6.02 ± 0.98	0.340 ± 0.00	1272.73 ± 5.03
Chalous (CM14)	0.336 ± 0.02	0.408 ± 0.00	11.14 ± 0.89	26.19 ± 1.31	107.98 ± 6.60	0.510 ± 0.02	4.77 ± 0.12	14.80 ± 0.77	6.880 ± 0.12	1210.17 ± 4.34
Esfahan (CM15)	0.003 ± 0.00	0.379 ± 0.01	11.31 ± 1.99	23.12 ± 1.73	108.26 ± 7.50	0.520 ± 0.02	4.66 ± 0.04	14.65 ± 0.17	6.870 ± 0.21	993.85 ± 5.01
Arak (CM16)	0.801 ± 0.05	0.319 ± 0.00	23.47 ± 2.79	103.33 ± 4.12	140.94 ± 7.35	2.070 ± 0.01	15.82 ± 0.95	11.87 ± 1.02	5.810 ± 0.50	5981.79 ± 5.91
Kerman (CM17)	0.703 ± 0.01	0.121 ± 0.0	22.6 ± 1.82	67.29 ± 5.83	100.03 ± 7.34	1.800 ± 0.01	13.68 ± 0.87	11.16 ± 0.71	5.260 ± 0.06	4996.88 ± 6.00
Qazvin (CM18)	0.872 ± 0.00	0.213 ± 0.00	26.32 ± 1.99	93.54 ± 6.12	141.70 ± 2.82	1.820 ± 0.04	15.19 ± 0.35	13.26 ± 0.91	5.88 ± 0.09	5206.53 ± 12.90
Ahwaz (CM19)	0.740 ± 0.00	0.089 ± 0.01	15.95 ± 1.12	52.97 ± 5.81	112.93 ± 6.32	1.750 ± 0.02	11.16 ± 0.69	10.41 ± 1.02	3.810 ± 0.08	3014.03 ± 9.77
Boroujerd (CM20)	0.798 ± 0.00	0.137 ± 0.01	18.47 ± 1.56	110.08 ± 4.61	217.33 ± 6.31	2.240 ± 0.03	19.32 ± 0.79	10.08 ± 1.07	4.030 ± 0.09	6702.86 ± 4.60
Zahedan (CM21)	0.575 ± 0.00	0.076 ± 0.00	19.09 ± 0.50	38.30 ± 5.10	294.01 ± 4.71	1.960 ± 0.02	15.03 ± 1.08	10.16 ± 1.02	4.330 ± 0.01	3316.74 ± 7.12
Hamedan (CM22)	2.591 ± 0.32	0.182 ± 0.00	17.02 ± 0.89	39.31 ± 6.55	113.87 ± 6.30	1.730 ± 0.02	10.16 ± 0.99	9.65 ± 1.07	4.030 ± 0.08	3307.45 ± 8.23
Rafsanjan (CM23)	1.156 ± 0.58	0.186 ± 0.00	17.34 ± 0.71	45.83 ± 6.94	209.31 ± 5.30	1.661 ± 0.02	12.17 ± 0.56	9.120 ± 0.91	4.380 ± 0.030	3410.72 ± 7.30
Kermanshah A <sup>b</sup> (WM24)	8.451 ± 0.80	5.891 ± 0.92	5.559 ± 0.88	48.84 ± 2.14	60.89 ± 3.83	0.465 ± 0.03	5.57 ± 0.07	5.01 ± 0.81	0.419 ± 0.00	3510.23 ± 13.70
Kermanshah B (WM25)	11.500 ± 0.91	0.570 ± 0.00	12.5 ± 0.91	22.23 ± 9.25	736.23 ± 9.10	0.601 ± 0.04	21.03 ± 0.90	24.22 ± 1.32	7.321 ± 0.32	1215.07 ± 10.90
Kermanshah C (WM26)	0.004 ± 0.00	0.451 ± 0.01	11.213 ± 0.12	16.32 ± 8.16	243.96 ± 8.32	0.698 ± 0.01	12.67 ± 0.70	17.55 ± 1.03	7.129 ± 0.52	1160.58 ± 3.91
Kermanshah E (WM27)	0.004 ± 0.00	0.813 ± 0.01	13.386 ± 0.32	37.61 ± 10.22	1662.18 ± 7.83	0.620 ± 0.03	60.48 ± 3.00	22.80 ± 2.02	8.929 ± 0.12	1870.97 ± 11.21
Kermanshah F (WM28)	6.870 ± 0.51	0.556 ± 0.01	13.949 ± 0.36	23.13 ± 6.23	751.17 ± 5.24	0.551 ± 0.09	24.31 ± 1.01	23.44 ± 1.08	7.439 ± 0.52	1545.21 ± 9.90
Kermanshah G (WM29)	0.004 ± 0.00	4.754 ± 0.70	12.608 ± 0.26	43.45 ± 3.82	258.21 ± 3.84	0.458 ± 0.01	8.42 ± 0.63	16.09 ± 1.01	6.725 ± 0.15	1362.08 ± 10.51

<sup>a</sup> Cultivated mushroom

<sup>b</sup> Wild mushroom

The wavelengths used for determination of the concentration of elements, based on baseline signals and their interferences at selected lines observed experimentally during the measurements, are presented in Table 2.

## Health Risk Assessment

In this study, estimated weekly intake (EWI, mg kg<sup>-1</sup> body weight week<sup>-1</sup>) for PTEs through consuming edible mushroom was determined according to Eq. 1:

$$EWI = \frac{W * C}{BW} \quad (1)$$

where *W* is the weight of mushroom consumed (25 g week<sup>-1</sup>); *C*, the PTE concentration of mushroom (mg kg<sup>-1</sup>); *BW*, the average body weight (70 kg) [42]. The dietary exposure levels were also estimated and compared with the provisional tolerable weekly intake (PTWI) Joint FAO/WHO Expert Committee on Food Additives [43].

For estimation of a non-carcinogenic hazard of PTEs due to edible mushrooms, the chronic daily intake (CDI) was calculated by the following equation:

$$CDI = \frac{C \times IR \times ED \times EF}{BW \times AT} \quad (2)$$

In this equation, *C* is the concentration of PTEs in individual edible mushrooms (mg kg<sup>-1</sup>); *IR* is the daily intake of edible mushrooms set as 3.56 g day<sup>-1</sup> [44]; *ED* is the time period of PTE exposure for adults (70 years); *EF* is the frequency exposure of PTEs (350 days years<sup>-1</sup>) [26, 45–48]; *BW*, the average body weight of Iranian adult consumers (for children 15 and adults 70 kg) [49] and *AT* is the average time (365 × 70) which for adults is 25,550 days [17, 50, 51].

The non-carcinogenic hazard was determined according to Eq. 3:

$$THQ = \frac{CDI}{RfD} \quad (3)$$

where THQ is the target hazard quotient, CDI chronic daily intake (mg kg<sup>-1</sup> day), RfD, the oral reference dose (mg kg<sup>-1</sup> day) [52]. RfD for Cd, Pb, Hg, Ni, Cr, Mn, and As is 0.0005, 0.0035, 0.0001, 0.011, 0.003, 0.140, and 0.0001 mg kg<sup>-1</sup> day, respectively [51].

If THQ > 1 value, the exposed population is at considerable health risk, but if THQ ≤ 1, the health risk is not likely [26, 53, 54].

For considering the effect of PTEs in non-carcinogenic risk, total target hazard quotient (TTHQ) was calculated by the equation below:

$$TTHQ = \sum THQ_1 \dots THQ_n \quad (4)$$

Besides, the carcinogenic health risk (CR) of As in edible mushrooms was calculated according to the following equation [55, 56]:

$$CR = CDI \times SF \quad (5)$$

where CDI is the chronic daily intake (mg kg<sup>-1</sup> day over a lifetime) and SF is the cancer slope factor for As that is 1.5 mg<sup>-1</sup> kg day [57]. The Monte Carlo simulation with Oracle Crystal Ball software (Release 11.1.2.4) was applied to calculate the probabilistic distribution of health risk (THQ and CR).

**Statistical Analysis** The data are expressed as median, mean rank, lowest, and highest. In this study, Mann-Whitney tests were applied to determine the statistical significance for PTEs between the subjects as classified by type of mushroom (cultivated versus wild). Statistical analyses were performed using the statistical package SPSS for Windows, PC software, version 20 (SPSS Inc., Chicago, IL, USA). The statistical significance was defined as *P* value < 0.05.

**Table 2** Average of recoveries (%), relative standard deviations (%) LOQ and LOD obtained by ICP-OES analysis

Potentially toxic elements (PTEs)	Wavelength	<i>r</i> <sup>2</sup> value	Calibration range	Recovery %	LOD (μg L <sup>-1</sup> )	LOQ (μg L <sup>-1</sup> )	LOD (mg kg <sup>-1</sup> )	LOQ (mg kg <sup>-1</sup> )
As	189.042	0.99984	0.3–1200	92	0.3	1	0.003	0.010
Cd	228.802	0.99943	0.3–1200	95	0.1	0.3	0.001	0.003
Cr	267.716	0.99991	0.3–1200	98	0.3	1	0.003	0.010
Cu	324.754	0.99998	0.1–1200	97	0.1	0.3	0.001	0.003
Fe	259.941	0.99999	0.16–1200	99	0.16	0.5	0.0016	0.005
Hg	253.652	0.99968	0.3–1200	96	0.3	1	0.003	0.010
Mn	257.611	0.99993	0.15–1200	94	0.15	0.5	0.0015	0.005
Ni	231.604	0.99989	0.3–1200	97	0.3	1	0.003	0.010
Pb	283.305	0.99964	1.2–1200	106	1.2	4	0.012	0.040
Zn	268.416	0.9995	0.3–1200	101	0.3	1	0.003	0.010

## Results and Discussion

### Evaluation of the Analytical Method

The graphs of calibration for the ten PTEs were prepared from the standard solutions at five points, ranged from 0.50–10 mg L<sup>-1</sup>. All the PTEs displayed linear relationships of the instrumental response and the solutions containing the metals with insignificant intercepts and correlation coefficients of 0.994 or higher. Perceived limits of detection (LOD) and limits of quantification (LOQ) are ranged 0.001–0.048 and 0.03–0.16 ppm, respectively. The recoveries gained were found to vary from 92 to 106% that is a suitable range for all PTEs (Table 2).

### PTE Concentrations in the Mushrooms

Tables 1 and 3 show the concentration of the PTEs among different species of mushroom (cultivated and wild) and the location of the samples. The concentration of Zn, Fe, Mn, Cd, Cr, Cu, Ni, As, Hg, and Pb in mushroom species was found to be 928.29–6702.86 mg kg<sup>-1</sup>, 15.25–1662.19 mg kg<sup>-1</sup>, 2.10–60.48 mg kg<sup>-1</sup>, 0.001–5.89 mg kg<sup>-1</sup>, 0.32–26.32 mg kg<sup>-1</sup>, 9.15–110.08 mg kg<sup>-1</sup>, 1.21–24.23 mg kg<sup>-1</sup>, 0.003–11.50 mg kg<sup>-1</sup>, 0.16–2.24 mg kg<sup>-1</sup>, and 0.160–8.93 mg kg<sup>-1</sup>, respectively.

According to Table 1, the order of the mean levels of PTEs in the cultivated mushroom samples was found to be as Zn > Fe > Cu > Cr > Ni > Mn > Pb > Hg > As > Cd and the wild mushroom samples were found to be as Zn > Fe > Cu > Cr > Mn > Ni > Pb > As > Cd > Hg. The statistical analysis confirmed the existence of significant differences between the concentrations of the PTEs in cultivated and wild mushroom ( $p < 0.05$ ).

Based on research conducted in Iran and other countries, the concentration of these metals in the mushroom is mainly affected by factors of compost or soil, heavily polluted areas (such as highways with heavy traffic), and species of mushroom; increasing the concentration of PTE in mushroom is in many respects different from other plants. Some studies have shown that *Agaricus bisporus* is more susceptible to increasing content of Hg and to a lesser extent Cd in the substrate, whereas this is not the case in the present study. Some studies have done about the soil and compost contamination by PTE in many cities of Iran like Hamadan, Khozestan, Kurdistan, Isfahan, Sabzevar, babol [58–68]. On the other hand, various studies have found that the mushroom has a good texture for absorbing PTEs [69–73]. Inorganic arsenic is intensely toxic and intake of high quantities leads to inorganic As that is intensely toxic, and intake of high quantities leads to gastrointestinal symptoms, serious disturbances of the cardiovascular and central nervous systems, and finally death [29]. In the present study, it was found that the highest As level was 11.50 mg kg<sup>-1</sup> in sample WM25, whereas the lowest As level was 0.003 mg kg<sup>-1</sup> in samples WM26, WM27, WM29, CM2, CM3, CM4, CM5, CM6, CM7, CM8, CM9, CM10, CM12, CM15, according to the FAO/WHO/CODEX standard which has declared a standard As level of 0.5 mg kg<sup>-1</sup> [43]. The amount of As in samples CM1, CM11, CM13, CM14, CM16, CM17 (the cultivated mushroom sample of Kerman city), CM18, CM19 (the cultivated mushroom sample of Ahvaz city), CM20, CM21 (the cultivated mushroom sample of Zahedan city), CM22 (the cultivated mushroom sample of Hamadan city), CM23 (the cultivated mushroom sample of Rafsanjan city), and WM24, WM25, WM28 is higher than the maximum limit. However, in other samples, it is less than the allowed limit; this difference can be due to various factors such as genetics and the type of primary material (a type of compost) that the mushroom on it has grown and the type of

**Table 3** The mean, standard deviation, lowest and highest levels of potentially toxic elements (PTEs) measured in the cultivated and wild mushrooms (mg kg<sup>-1</sup>)

Type of mushrooms	Cultivated				Wild			
	Sample number	Lowest	Highest	Mean	N	Lowest	Highest	Mean
Potentially toxic elements (PTEs)								
Zn	23	928.30	6702.86	2394.01 ± 1803.74	6	1160.58	3510.23	1777.36 ± 887.08
Pb	23	0.16	6.940	3.150 ± 2.70	6	0.42	8.92	6.330 ± 2.99
Ni	23	1.21	17.52	7.97 ± 5.48	6	5.01	24.22	18.19 ± 7.27
Mn	23	2.10	19.32	7.23 ± 5.62	6	5.57	60.48	22.08 ± 20.14
Hg	23	0.16	2.24	0.951 ± 0.74	6	0.45	0.69	0.561 ± 0.09
Fe	23	15.25	294.01	92.06 ± 76.50	6	60.89	1662.18	618.70 ± 583.41
Cu	23	9.15	110.08	37.88 ± 28.94	6	16.32	48.84	31.93 ± 13.16
Cr	23	0.32	26.32	10.16 ± 9.13	6	5.55	13.94	11.53 ± 3.07
Cd	23	0.00	1.02	0.180 ± 0.20	6	0.45	5.89	2.470 ± 2.12
As	23	0.00	4.17	0.5910 ± 0.91	6	0.00	11.51	4.4710 ± 5.12

soil in the area is different. In previous investigations, As levels in edible mushrooms were 73.91 to 104.96 mg kg<sup>-1</sup> [66], 0.50–5.00 mg kg<sup>-1</sup> [74], 0.04 mg kg<sup>-1</sup>, and 212.30 mg kg<sup>-1</sup> [75]. In the present study, the highest concentration of As, like previous studies, is higher than the allowed limit.

Cd is one of the most dangerous rare elements because the amount of its entry through the diet is very high, and its accumulation in organs of the body for a long time causes renal impairment [76]. Cd is often found in soil and enters the food chain through plants. Therefore, it is necessary to determine the rate of absorption, distribution, and bioaccumulation in order to prevent or reduce the contamination of the food chain [77].

In the present study, the highest Cd content was found as 5.89 mg kg<sup>-1</sup> in sample WM24. The lowest Cd level was 0.00 mg kg<sup>-1</sup> in sample CM12. According to the FAO/WHO/CODEX standard, it has declared the maximum limit of the Cd of 0.5 mg kg<sup>-1</sup> [43]. Cd is obtained in sample CM4, CM17, WM24, WM25, WM26, WM28, and WM29 that is higher than the maximum levels, but in other samples, it is less than the allowed limit. This difference can be due to the early composition of the environment where the mushrooms grew. Cd reported in mushroom samples in other studies has been reported as follows.

Reported Cd levels in wild mushrooms in previous studies were 0.08–3.37 mg kg<sup>-1</sup> [78], 0.52–5.27 mg kg<sup>-1</sup> [79], and 0.06–0.58 mg kg<sup>-1</sup> [35]. The highest concentration of previous studies, such as our study, was more than the maximum levels of the FAO/WHO/CODEX standard.

Cr is a mineral that plays a very effective role in maintaining health. Cr 3 is a rare PTE that is required for the natural metabolism of cholesterol, fat, and sugar [35]. The highest Cr level was determined as 26.32 mg kg<sup>-1</sup> in sample CM18, and the lowest Cr level was 0.32 mg kg<sup>-1</sup> in sample CM5, according to the FAO/WHO/CODEX standard that has declared the maximum limit of the Cr 120 mg kg<sup>-1</sup> [43]. In all examined mushrooms, the Cr obtained was less than the FAO/WHO/CODEX allowed limit mushrooms that have less ability to absorb this metal. Previous studies reported 0.87–2.66 mg kg<sup>-1</sup> [80], 10.70–42.70 mg kg<sup>-1</sup> [35], and 0.54–73.80 mg kg<sup>-1</sup> [81]. The Cr concentrations in this study were similar to those from previous studies, and all reports were less than the allowed limit specified by the FAO/WHO/CODEX standard.

Cu is one of the rare elements in the human body, which has the third rank. Its effect is on living systems like vitamins. A small amount of it is in the human body that plays an important role in biochemical processes [35]. Cu amounts in mushrooms are higher than other vegetables, which can be considered as a nutritional source for consumers. However, the bioavailability of the mushroom in humans is limited due to low absorption from the intestine [82]. In this study,

the highest concentration of Cu 110.08 mg kg<sup>-1</sup> was found in sample CM20, while the lowest Cu level was 9.15 mg kg<sup>-1</sup> in sample CM8. According to the FAO/WHO/CODEX standard, it has declared a maximum limit of the Cu of 40 mg kg<sup>-1</sup> [43]. Cu concentration in samples CM16, CM17, CM18, CM19, CM20, CM23, WM24, and WM29 is higher than the maximum levels, but in other samples, it is less than the allowed limit. The similar studies conducted, Cu content ranged from 18.00 to 54.00 mg kg<sup>-1</sup>, 17.50–122.00 mg kg<sup>-1</sup>, 15.50–63.40 mg kg, respectively [78, 82, 83]. In the present study, the Cu concentrations detected were similar previously reported in the literature.

Fe is the third element from the point of view abundant after Zn and Mn in mushroom, which is almost vital for all living organisms, and it has a broad role in all metabolic activities, including oxygen transfer, DNA synthesis, and electron transfer. It is also specified that enough Fe is too important in the diet to reduce the incidence of anemia. The high concentrations of Fe may be to tissue damage due to the formation of free radicals [35]. In the present study, it was found that the highest concentration of Fe 751.17 mg kg<sup>-1</sup> in sample WM28, whereas the lowest Fe level was 15.25 mg kg<sup>-1</sup> in sample CM6. According to the FAO/WHO/CODEX standard that has specified the maximum limit of the Fe of 15 mg kg<sup>-1</sup> [43]. In all tested mushrooms, the Fe obtained was higher than the FAO/WHO/CODEX allowed limit, which can be due to the ability of the mushroom to absorb Fe. In the previous studies, the concentration of Fe in edible mushrooms ranged from 834 to 870 mg kg<sup>-1</sup>, 1714–1172 mg kg<sup>-1</sup>, 834–870 mg kg<sup>-1</sup>, and 97.2–3919 mg kg<sup>-1</sup>, respectively [78, 79, 84, 85].

Hg is a naturally occurring metal found in air, water, and soil. It exists in various forms: elemental (or metallic), inorganic, and organic. Acute Hg exposure may give rise to lung damage. Chronic poisoning is specified by neurological and psychological symptoms, such as changes in personality, restlessness, anxiety, sleep disorder, and depression. The symptoms are reversible after the termination of exposure [86]. In this study, the highest concentration of Hg 2.44 mg kg<sup>-1</sup> was found in sample CM20, while the lowest Hg level was 0.16 mg kg<sup>-1</sup> in sample CM8. According to the China standard, it has specified the maximum limit of the Hg of 1 mg kg<sup>-1</sup> [87]. Hg concentration in samples CM11, CM16, CM17, CM18, CM19, CM20, CM21, CM22, and CM23 is higher than the maximum levels, but in other samples, it is less than the allowed limit. The amount of Fe in edible mushrooms in the study of Xin-Hua Chen et al. was 0.28–3.92 mg kg<sup>-1</sup> [86], while other values, 1.10–8.40 mg kg<sup>-1</sup> and 0.82–3.80 mg kg<sup>-1</sup>, were reported previously, respectively [88, 89]. The highest concentration of previous studies, such as our study, was more than the maximum levels of the China standard.

**Table 4** Different provisional tolerable weekly intake (PTWI) values of potentially toxic elements (PTEs) in wild and cultivated mushrooms

Potentially toxic elements (PTEs)	EWI of wild ( $\mu\text{g kg bw}^{-1}$ )	EWI of cultivated ( $\mu\text{g kg bw}^{-1}$ )	PTWI ( $\mu\text{g kg bw}^{-1}$ )
Pb	10.25	4.00	25.00
Hg	11.25	4.00	4.00
Cd	11.25	0.02	7.00
Ni	125.03	30.25	35.00
As	0.075	0.09	15.00
Mn	137.5	52.50	980.00
Cr	138.75	8.06	7.00
Cu	407.75	228.75	500.00
Fe	1522.25	381.25	5600.00
Zn	1160.50	928.25	7000.00

*PTWI*a provisional tolerable weekly intake, Joint FAO/WHO Expert Committee on Food Additives, *EWI* estimated weekly intake

Mn is one of the vital elements and is available in metalloproteins (metal-containing proteins) such as carboxylase pyruvate and the glial cytoplasmic enzyme, glutamine synthase. The most important effect of Mn is on the respiratory system and the brain; high doses of Mn cause side effects on the lungs and brain [35]. In the present study, it was found that the highest amount of Mn was 60.47 mg kg<sup>-1</sup> in sample WM27, while the lowest Mn level was 2.10 mg kg<sup>-1</sup> in sample CM8. Previous studies have shown the amount of Mn in the mushroom as follows.

In the studies of Kalac and Svoboda, Mn level ranged from 12.90 to 93.30 mg kg<sup>-1</sup> [90]. In previous investigations, Mn content ranged from 5.50 to 135.00 mg kg<sup>-1</sup>, 4.61–102.00 mg kg<sup>-1</sup>, and 5.00–60.00 mg kg<sup>-1</sup>, respectively. In this study, measured Mn values are consistent with some previous

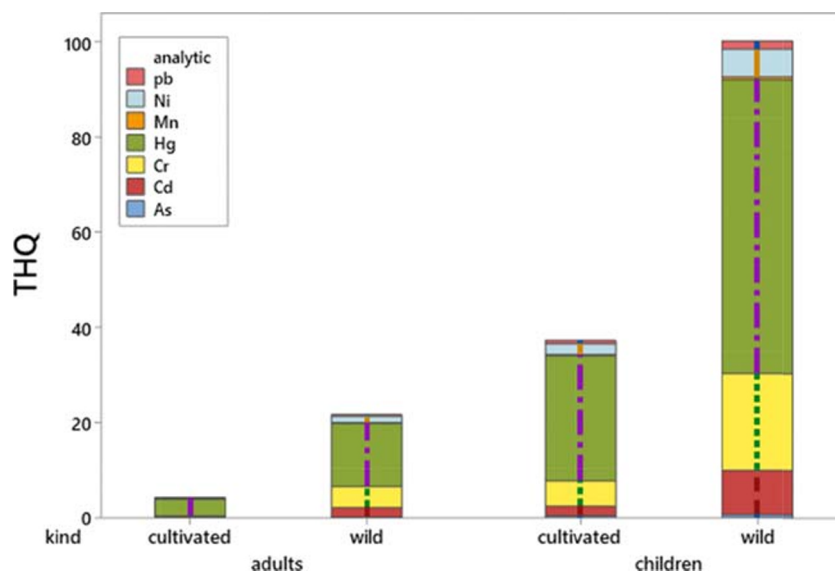
studies. Accordingly, according to Table 2, the amount of studied Mn in our study was lower than the reported Mn concentration in previous studies.

Trace amounts of Ni are useful, because they activate some enzyme systems, while their higher levels can Pb to serious toxicity [91]. In this study, the highest (24.22 mg kg<sup>-1</sup>) and lowest (1.21 mg kg<sup>-1</sup>) levels of Ni were found in samples WM25 and CM3. It reported that Ni uptake increased commensurable with the metal increase in the substrate [91]. In the previous studies, amount of Ni content in edible mushrooms ranged from 12.70 to 24.20, 1.72 to 24.10, and 8.2 to 26.7 mg kg<sup>-1</sup>, respectively [69, 78, 92]. The range of Ni obtained in this study and previous studies was higher than 0.05–5 mg kg<sup>-1</sup> reported for plant foods by National Academy of Sciences [86].

**Table 5** Uncertainty analysis for the THQ of investigated potentially toxic elements (PTEs) in cultivated and wild mushrooms

Potentially toxic elements (PTEs)	Age group	Percentiles of THQ for wild mushrooms				Percentiles of THQ for cultivated mushrooms			
		5%	50%	75%	95%	5%	50%	75%	95%
Pb	Adults	0.0037	0.0057	0.0068	0.0089	0.0014	0.0022	0.0027	0.0035
Pb	Children	0.0129	0.0197	0.0237	0.0307	0.0048	0.0078	0.0094	0.0120
Hg	Adults	0.1360	0.2150	0.2552	0.3183	0.0500	0.0771	0.0918	0.1182
Hg	Children	0.4894	0.7506	0.8942	1.1622	0.1741	0.2685	0.3204	0.4287
Cd	Adults	0.0287	0.0434	0.0523	0.0664	0.0001	0.0001	0.0001	0.0001
Cd	Children	0.0953	0.1511	0.1814	0.2387	0.0002	0.0003	0.0003	0.0004
Ni	Adults	0.0146	0.0223	0.0270	0.0349	0.0035	0.0054	0.0064	0.0083
Ni	Children	0.0497	0.0782	0.0927	0.1210	0.0120	0.0190	0.0227	0.0299
As	Adults	0.0009	0.0015	0.0017	0.0023	0.0011	0.0017	0.0021	0.0027
As	Children	0.0032	0.0051	0.0060	0.0080	0.0040	0.0062	0.0074	0.0096
Mn	Adults	0.0012	0.0019	0.0023	0.0031	0.0005	0.0007	0.0009	0.0012
Mn	Children	0.0044	0.0067	0.0079	0.0105	0.0017	0.0025	0.0030	0.0040
Cr	Adults	0.0577	0.0894	0.1071	0.1379	0.0033	0.0053	0.0062	0.0082
Cr	Children	0.1952	0.3172	0.3767	0.4952	0.0115	0.0183	0.0219	0.0284

**Fig. 1.** Comparison of non-carcinogenic risk of edible wild mushrooms by different PTEs residues considering the share of consumption of each heavy metal.



Pb ions are known to cause neurotoxic effects, such as mental retardation and loss of intellectual performance in children, as well as increased blood pressure and the risk of developing cardiovascular disease for adults [93]. In this study, the highest concentration of Pb  $8.92 \text{ mg kg}^{-1}$  was found in sample WM27, while the lowest Pb level was  $0.16 \text{ mg kg}^{-1}$  in sample CM1. According to the FAO/WHO/CODEX standard, it has declared a maximum limit of the Pb of  $2 \text{ mg kg}^{-1}$  [43]. Pb is obtained in the samples CM4, CM7, CM11, CM13, CM15, CM16, CM17, CM18, CM19, CM20, CM21, CM22, CM23, WM25, WM26, WM27, WM28, and WM29 that is higher than the maximum levels, but in other samples, it is less than the allowed limit. The amount of Pb in edible mushrooms was ranged from 0.64 to  $12.50$ ,  $0.48$  to  $10.18$ , and  $1.68$  to  $2.56 \text{ mg kg}^{-1}$ , respectively [20, 58, 79]. In the present study, the highest concentration of Pb, like previous studies, is more than the maximum limit.

Zn is one of the integral parts of a wide range of enzymes that is responsible for catalytic, structural, and regulative roles. Zn deficiency can be due to inadequate food that can cause impairment in absorption and deficiency in metabolism. Mushrooms are known as Zn storage plants [35]. Zn content ranged from  $37.13$  to  $268.11 \text{ mg kg}^{-1}$  in the present study. The highest Zn content was seen in the sample CM20 and the lowest in the sample CM5. According to the FAO/WHO/CODEX standard, it has specified the maximum limit of the Zn of  $60 \text{ mg kg}^{-1}$  [43]. Zn is obtained in the samples CM6, CM11, CM16, CM17, CM118, CM19, CM20, CM21, CM22, CM23, WM24, WM27, and WM28 that is higher than the maximum levels, but in other samples, it is less than the allowed limit. In previous investigations, the amount of Zn in edible mushrooms ranged from  $43.50$  to  $205.00$ ,  $1714$  to  $1172$ , and  $34.4$  to  $225 \text{ mg kg}^{-1}$ , respectively [58, 78, 83]. In

the present study, the highest concentration of Zn in our findings, like previous studies, is more than the highest limit.

### Human Risk Assessment and Exposure to PTEs

The EWIs of Pb, Hg, Cd, Ni, As, Mn, Cr, Cu, Fe, and Zn for wild mushrooms were  $10.25$ ,  $11.25$ ,  $11.25$ ,  $125.025$ ,  $0.075$ ,  $137.50$ ,  $138.75$ ,  $407.75$ ,  $1522.25$ , and  $1160.50 \text{ } \mu\text{g kg}^{-1} \text{ bw}$ , and for cultivated mushrooms,  $4.00$ ,  $4.00$ ,  $0.02$ ,  $30.25$ ,  $0.09$ ,  $52.5$ ,  $8.06$ ,  $228.75$ ,  $381.25$ , and  $928.25 \text{ } \mu\text{g kg}^{-1} \text{ bw}$ , respectively. EWIs of selected metals for consuming edible mushrooms included in this study are well below the PTWI values, except Ni in wild mushrooms, which was higher than PTWI recommended for this element (Table 4).

### Non-carcinogenic Risk

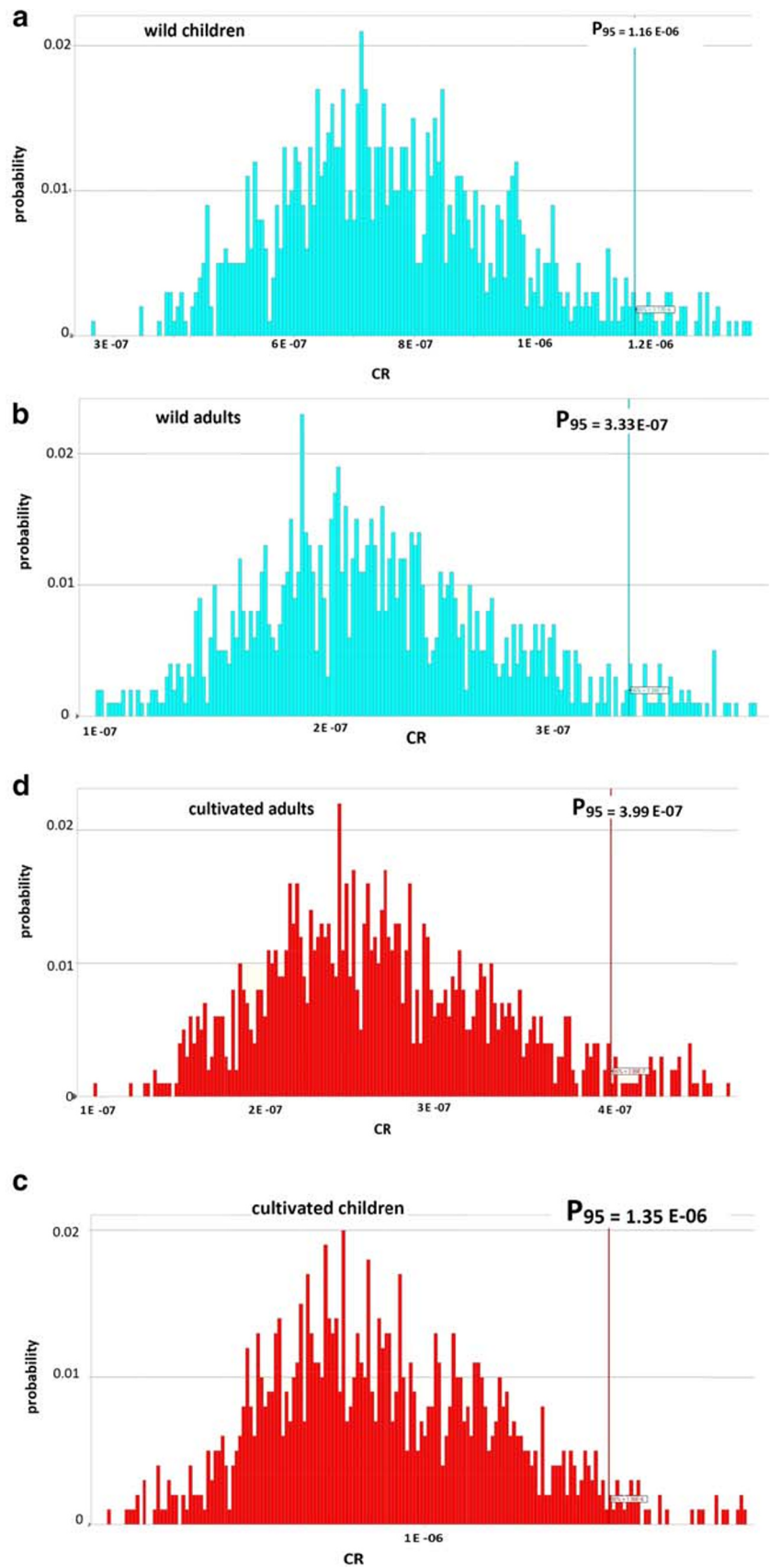
The non-carcinogenic risk due to the ingestion of PTEs through edible mushroom consumption for the adults and children in Iran is listed in Table 5.

For adults, the rank order of PTEs based on  $P_{95}$  THQ through edible cultivated mushroom consumption was Hg ( $0.1182$ ) > Ni ( $0.0083$ ) > Cr ( $0.0082$ ) > Pb ( $0.0035$ ) > As ( $0.0027$ ) > Mn ( $0.0012$ ) > Cd ( $0.0001$ ). The THQ of PTEs for children is similar to that for adults.

For edible wild mushrooms, the rank order of PTEs based on  $P_{95}$  THQ for adults was Hg ( $0.3183$ ) > Cr ( $0.1379$ ) > Cd ( $0.0664$ ) > Ni ( $0.0349$ ) > Pb ( $0.0089$ ) > Mn ( $0.0031$ ) > As ( $0.0023$ ). Also for children, it was Hg ( $1.1622$ ) > Cr ( $0.4952$ ) > Cd ( $0.2387$ ) > Ni ( $0.1210$ ) > Pb ( $0.0307$ ) > Mn ( $0.0105$ ) > As ( $0.0080$ ).



**Fig. 2** Comparison of carcinogenic risk (CR) of potentially toxic elements (PTEs) due to consumption of edible mushrooms (a wild mushroom for children, b wild mushrooms for adult, c cultivated mushrooms for children, d cultivated mushrooms for adult)



The THQs of all PTEs in edible mushrooms in the cultivated and wild for both adults and children were lower than 1 value, except Hg in edible wild mushrooms; for children, it was considerably higher than 1 value.

The results of edible mushrooms examined suggest that Hg exposures in humans have the highest potential health risk of adverse effects for children and Cd and As exposures have the minimum risk for cultivated and wild mushrooms, respectively (Fig. 1).

A comparison between HQ of PTEs in edible mushrooms shows that non-carcinogenic risk due to the ingestion of wild mushrooms was higher than cultivated mushrooms.

The TTHQ<sub>95</sub> of PTEs in edible mushrooms (cultivated and wild) for adults were 0.14 and 0.57, and for children, 0.51 and 2.07, respectively. The total target hazard quotient (TTHQ) in the edible wild mushrooms due to PTEs residues was ~4 times higher than the edible cultivated mushrooms.

Also, when TTHQ value is higher than 10, adverse health effects are considered high for the exposed population [94]. These findings were reflected that, based on the TTHQ value, edible mushrooms are not adverse health effects for the exposed population.

### Carcinogenic Risk

The results of carcinogenic risk (CR) due to the ingestion of edible mushrooms are presented in Fig. 2. Percentile 95% CR indexes of PTE ingestion due to consumption of edible mushrooms (cultivated and wild) for adults and children were 3.99E-07 and 3.33E-07, and for children, 1.36E-06 and 1.16E-06, respectively. USEPA recommended that when  $CR < 10^{-6}$ , cancer risk is negligible, but if  $CR > 10^{-4}$ , cancer risk is unacceptable, and when CR is between  $10^{-6}$  and  $10^{-4}$ , cancer risk is tolerable for consumers [95, 96]. Therefore, no carcinogenic risk was found based on the results of the health risk assessment.

### Conclusion

In this study, the measured values of As, Cd, Cu, Fe, Hg, Mn, Ni, Pb, and Zn were higher than standard level as well as Cr was obtained lower than the set limit. The results of this study showed that in analyzed wild and cultivated mushroom samples for first time in different parts of Iran. The health risk assessment (Monte Carlo simulation method) indicated that there is no concern regarding the non-carcinogenic risk due to the ingestion of PTEs via the consumption of the edible mushrooms, except Hg in edible wild mushrooms, for children. However, no carcinogenic risk (CR) was found since all the CR index for As did not exceed the acceptable value. Finally, due to the high concentration of toxic PTEs in some samples of wild mushrooms studied and because of the high

mortality rate of people due to consumption of these mushrooms in the past, we recommend that people, especially villagers, refrain from eating these mushrooms.

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### Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

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