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Concentration of Potentially Toxic Elements (PTEs) in Rapid Coffee Products in Bandar Abbas, Iran: Probabilistic Non-Carcinogenic and Carcinogenic Risk Assessment

Vali Alipour¹ · Iman Mahmoudi¹ · Mohammad Borzoei¹ · Fereshteh Mehri² · Maryam Sarkhosh³ · Intissar limam⁴ · Rasul Nasiri⁵ · Yadolah Fakhri¹

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Abstract

Coffee is one of the most widely consumed beverages in the world. However, coffee plants are often exposed to potentially toxic elements (PTEs) pollution. The main aims of current study were to detect the PTEs in instant coffee and health risk assessment of consumers in Bandar Abbas city. To achieve this, 40 samples of instant coffee were randomly collected from various points in the city in 2023 and PTEs concentrations were measured using flame atomic absorption spectrometry (FAAS). The non-carcinogenic and carcinogenic risks were calculated using Monte Carlo simulation (MCS) method. The concentrations of Fe and Cu were higher than other PTEs, equaling 404.41 mg/kg and 0.0046 mg/kg, respectively. The non-carcinogenic risk assessment revealed that THQ (Fe > Pb > As > Cd > Ni > Cu) and TTHQ levels were less than 1 based on the 95% percentile in adults and children, indicating there is no possibility of a non-carcinogenic risk associated with instant coffee. The carcinogenic risk due to inorganic As in instant coffee was acceptable (2.63E-5 and 1.27E-5 based on the 95% percentile for adults and children, respectively), therefore PTEs in instant coffee do not endanger the health of consumers.

Keywords Potentially Toxic Elements · Heavy metals · Coffee Products · Carcinogenic risk · Non-Carcinogenic risk

Iman Mahmoudi emanmahmoodi002@gmail.com

- Yadolah Fakhri ya.fakhri@gmail.com; ya.fakhri@hums.ac.ir
- ¹ Food Health Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
- ² Nutrition Health Research Center, Hamadan University of Medical Sciences, Hamadan, Iran
- ³ Department of Environmental Health Engineering, School of Health, Mashhad University of Medical Sciences, Mashhad, Iran
- ⁴ Laboratory of Materials, Treatment and Analysis, National Institute of Research and Physicochemical Analysis; and High School for Science and Health Techniques of Tunis, University of Tunis El Manar, Tunis, Tunisia
- ⁵ Air Pollution Research Center, Iran University of Medical Sciences, Tehran, Iran

Introduction

Potentially toxic elements (PTEs) refer to metals and metalloids such as copper (Cu), iron (Fe), manganese (Mn), arsenic (As), zinc (Zn), lead (Pb), chromium (Cr), cadmium (Cd), and nickel (Ni) that can have harmful effects on organisms in high concentrations [1-3]. Both natural processes, such as the weathering of rocks, and human activity, such as industrial emissions, mining, and inappropriate waste management, can release these metals into the environment [4, 5]. The impact of PTEs on human health varies depending on the particular metal, its concentration, the length of exposure, and each person's vulnerability [1, 6, 7]. The central nervous system, liver [8], and kidneys are just a few of the essential organs that might affected by various factors such as PTEs [9–11]. Additionally, it may result in a number of medical conditions, such as developmental and neurological difficulties, respiratory problems, cardiovascular diseases, and some malignancies [12–14]. PTEs can contaminate water, soil, and air as they accumulate in the environment [15–17]. The intake of tainted food or water, skin contact, inhalation, and other methods can all expose people to PTEs

[18–21]. Once PTEs enter the body, they can bond to tissues and organs, which can have a variety of negative health impacts [22–25]. In recent decades, with the emergence of various chemical pollutants and their entry into the food chain, concerns about the increase in disease have increased [26–30]. The increase levels of various contaminations in food, such as mycotoxins in dairy products [31, 32], aflatoxins in corn [33, 34] and heavy metals in dairy products and meat [35], together with other variables has increased risk of cancers [36–41] and other non-communicable diseases [42, 43].

Coffee is one of the most extensively consumed beverages in the world and is renowned for its energizing properties as well as its flavorful aroma [44]. Caffeine, a natural stimulant that has an impact on the central nervous system, is present in this beverage since it is made from the roasted seeds of the Coffea plant. When drunk in moderation, coffee has been linked to various potential health advantages. Coffee's caffeine content can improve cognitive function overall as well as mental alertness and attention. Additionally, it might lessen weariness and speed up reflexes. Because it helps speed up the metabolism and boost fat oxidation, caffeine is a common component in pre-workout supplements [45]. Coffee is known to increase stomach acid production, which in some people can lead to heartburn, acid reflux, or gastrointestinal discomfort. Additionally, if ingested in high amounts, it may have a slight diuretic effect, increasing urine production and perhaps leading to dehydration. Coffee contains substances that can bind to specific minerals like calcium and iron, potentially preventing the body from absorbing such minerals. When coffee is drunk with food, this impact is more pronounced [46]. As with any dietary choice, it is advisable to consult with a healthcare professional or nutritionist to determine what level of coffee consumption is suitable for individual circumstances and overall health goals. Different nations around the world have different per capita coffee consumption rates. Finland (12 kg/year) is the country with the highest per capita coffee consumption, according to data from the International Coffee Organization for 2021, (6.1 kg /year), including Norway, Iceland, Denmark, Sweden, Switzerland, Netherlands, Belgium, and Luxembourg. It's crucial to remember that these figures reflect average per-person consumption for each country and may change annually. Additionally, cultural preferences, accessibility, and other elements can affect coffee consumption.

According to some research, coffee, especially instant coffee, contains PTEs. Pb and Cd were identified in substantial amounts in instant coffee samples [47]. Several studies showed have shown that coffee infusions contain the highest levels of leached Pb and Cr elements. Noel et al. (2012) previously reported that Cr levels in coffee infusion averaged 0.0023 mg/50ml. According to the World Health Organization (2000), adults are allowed a daily consumption of 50 to 200 μ g (0.05 to 0.2 mg) of Cr [48]. Since the consumption of instant coffee, instant coffee drinks, and natural coffee is rather high in Poland [49, 50], the level of PTEs is typically examined in ground coffee. The weekly Pb consumption from cereal coffee is 1.49 g (about 0.2% BMDL₀₁ and 0.5% BMDL₁₀). In contrast, hazard index (HI) due to Cd and Pb ranged from 0.036 (chicory and coffee blend) to 0.109 (cappuccino) [51]. Despite the widespread consumption of instant coffee in Iran, there was no study on the concentration of PTEs in instant coffee and human health risk assessment. The main aims of current study were determining the concentration of PTEs in instant coffee and human health risk assessment.

Materials and Methods

Chemical and Reagents

Standard solutions of Fe, Pb, Cd, Cu, Ni, As and other analytical chemical were purchased from Merck (Darmstadt, Germany).

Sampling, Sample Preparation, and Analysis of PTEs in Rapid Coffee

In this descriptive cross-sectional study, in total, 40 samples of rapid coffee, were randomly collected from local markets, supermarkets, and grocery stores in Bandar Abbas Province, in South of Iran in 2023. At first, 0.5 g of each sample was heated at 600°C for 24 h to remove organic compounds. The samples were digested in a mixture of 25 mL (3:1 v/v) of HNO₃ and HCl[52]. The solutions samples were heated in a microwave at 800 W for 30 min until to 210 °C. The concentration of PTEs was detected using Graphite furnace atomic absorption spectroscopy (GFAAS) (AA-7000, Shimadzu, Kyoto, Japan) (Table 1).

Method Validation

To ensure the precision of the analytical method, quality assurance and quality control (QA/QC) were ensured based on previous studies [53]. The validation protocol included limit of quantification (LOQ), limit of detection (LOD) and recovery were measured [54]. Triplicate analyses were conducted on samples, blanks, and standards. The calibration curves, regression coefficient (R²), linearity equations, LOQ, LOD, and recovery of PTEs are indicated in Table 2 [55].

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Table 1The used Graphitefurnace atomic absorptionspectroscopy (GFAAS)conditions

Parameter	Pb	Cd	As	Ni	Cu	Fe
Wavelength (nm)	217	228.7	213.9	212.7	321.5	341.5
Slit width (nm)	0.4	0.4	0.3	0.3	0.4	0.3
Sensitivity (µg ml-1)	2.4	0.7	6	0.24	2.4	3.5
Lamp current (mA)	10	8	11	6	5	6

Table 2Linearity equations,
calibration curves, regression
coefficient (R^2), LOD, LOQ and
recovery of PTEs

PTEs	Metal Range of linearity	Regression linear equation	Regression coefficient	LOD (µg/kg)	LOQ (µg/kg)	Recovery ± RSD (%)
Pb	0.80 -100	Y = 0.00731x + 0.0124	0.9661	0.013	0.051	100.21 ± 1.45
Cd	0.80 - 100	Y = 0.01041x - 0.0150	0.8951	0.006	0.025	91.13 ± 3.31
As	0.80 - 100	Y = 0.00510x - 0.0053	0.9955	0.014	0.060	117.00 ± 2.34
Fe	0.80 - 100	Y = 0.03467x - 1.3028	0.9856	0.097	0.321	96.15 ± 2.41
Ni	0.80 - 100	Y = 0.0654x - 0.0256	0.9654	0.036	0.180	95.65 ± 4.11
Cu	0.80 -100	Y = 0.02470x + 0.0114	0.9421	0.021	0.089	123.67 ± 1.11

Health Risk Assessment

Non-Carcinogenic Risk Assessment

Lifetime Average Daily Dose (LADD) was estimated by the below equation [56–59]:

$$LADD = \frac{C \times IR \times EF \times ED \times CF}{BW \times AT}$$
(1)

where C, represents the concentration of PTEs (mg/kg); IR, ingestion rate of coffee consumption (g/n-d); CF, conversion factor (g/n-d); EF, exposure frequency (350 days/year); ED, exposure duration (adults = 30 years and children = 6 years); BW, body weight (adults = 70 kg and children = 29 kg) and AT average lifetime. AT for non-carcinogenic risk for adults and children is equal to 10,950 days and 2,190 days, respectively and for carcinogenic risk for both is 25,550 days.

The non-carcinogenic risk was estimated by the current equation [60-62]:

$$THQ = \frac{LADD}{TDIorRfD}$$
(2)

In this equation, THQ represents target hazard quotient; RfD, oral reference dose (mg/kg-d) and TDI, tolerable daily intake (mg/kg-d). TDI for Pb and RfD for Cd, Fe, Ni, Cu and As is 0.0036, 0.001, 0.7, 0.011, 0.04 and 0.0003 mg/kg-d, respectively [63].

Total non-carcinogenic risk was calculated current equation [60]:

TTHQ = THQCd + THQFe + THQNi + THQCu + THQAs + THQPb(3)

where, TTHQ is total target hazard quotient.

When TTHQ is lower than 1, non-cancer risk is acceptable.

Carcinogenic Risk

The carcinogenic risk of As was estimated by current equation [56]:

$$CR = LADD \times CSF$$
 (4)

In this equation, CSF is cancer slope factor $(mg/kg-d)^{-1}$. CSF for inorganic As is 1.5 $(mg/kg-d)^{-1}$ [64]. When CR in the range of 10^{-6} to 10^{-4} is acceptable [64].

Uncertainty Analysis

To increase the precision of risk assessment, the Monte Carlo Simulation (MCS) model was applied to identify an uncertain event's possible outcomes [65]. For this objective, the Oracle Crystal Ball software (version 11.1.2.4.600) was applied. Based on this technique, the parameters such as the concentration of PTEs, and ingestion rate were considered as lognormal distribution and body weight as normal distribution. The cut point of health risk was set at 10,000 repetitions was selected 95% of TTHQ.

Statistical Analysis

The results were indicated as Mean \pm standard deviation. Concentration of PTEs were compared using one-way ANOVA. A p-value less than 0.05 was reported as significant difference in data. SPSS 11.5.1 software was utilized for statistical analysis.

Results and Discussion

Method Validation

The method validation parameters, including linearity, calibration curve, equation, R2, recovery, RSDs, LOD, and LOQ, are shown in Table 2. LOD and LOQ ranged 0.006 to 0.097 μ g/L and 0.025 to 0.321 μ g/L in the rapid coffee products respectively. As given in Table 2, the recoveries were in the range of 91.13%–123.67%, and RSDs obtained for all selected PTEs in rapid coffee products were in the range of 1.11–3.31%.

Concentration of PTEs

The rank order of PTEs in rapid coffee products was as follows: Fe (404.41 mg/kg) > Pb (0.0046 mg/kg) > As $(< \text{LOD}) \sim \text{Ni} (< \text{LOD}) \sim \text{Cd} (< \text{LOD}) \sim \text{Cu} (< \text{LOD})$ (Table 3). Heavy metals are chemical elements with relatively high density and low reactivity, and most of them are toxic in low concentrations. However, some metals are necessary in low concentrations for the body's metabolism and immune system [66].

Entry of high concentration of this group of metals into the body also causes poisoning. Fe and Cu are essential elements for the body [67]. Other heavy metals including Pb, Cd, Ni and As are dangerous toxins that are not metabolized in the body and gradually accumulate in human fat tissues, muscles, bones and joints. Therefore, long-term exposure to PTEs leads to damage to vital organs [68]. In general, neurological disorders (Parkinson's, Alzheimer's, schizophrenia), various types of cancer, nutrient deficiency, hormone imbalance, obesity, abortion, kidney and brain damage are some of the dangers of PTEs in the body [69]. The International Agency for Research on Cancer (IARC) classified metals such as As, Cd, Cr and Ni as carcinogens due to their toxicity at low concentrations [70]. Accordingly, WHO has set permissible limits (PLs) for the concentration of PTEs in foodstuffs and beverages.

PTEs	Mean	SD^1	
Pb	0.0046	0.004	
Cu	<LOD ²	NC^3	
Fe	404.41	98.68	
As	<lod< td=""><td>NC</td></lod<>	NC	
Ni	<lod< td=""><td>NC</td></lod<>	NC	
Cd	<lod< td=""><td>NC</td></lod<>	NC	

¹Standard deviation ²Limit of detection ³Not calculated

Table 3 Concentration of PTEs

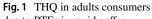
in rapid coffee products (mg/kg)

Therefore, PTEs poisoning occurs when more than permissible amounts enter the body through food or drinking [70, 71].Since the entry of PTEs in high concentrations into the food chain causes damage to human health, it is necessary to determine their concentration in foodstuffs and drinking such as coffee.

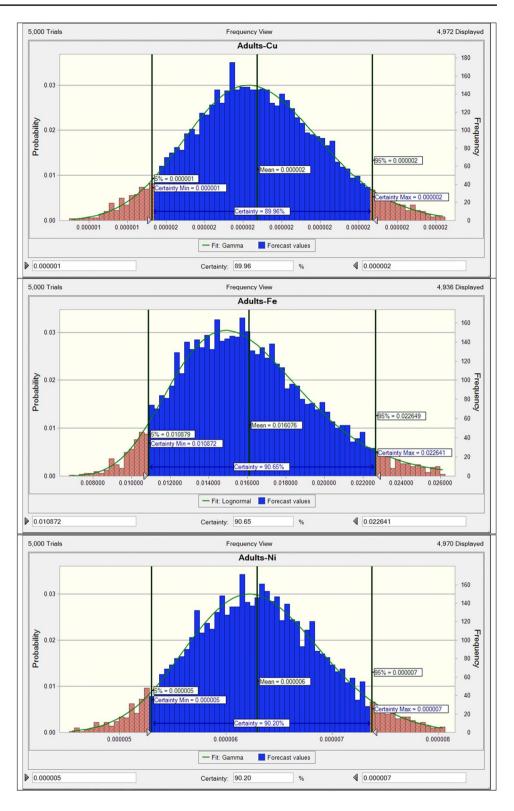
Coffee is one of the most consumed and popular drinks in the world due to its energizing and therapeutic effects as well as its good taste. High consumption of this drink can be a source of PTEs entering the human body[69]. Typically, the concentration and types of PTEs in instant coffee powders brands. Usually, the concentration and types of PTEs in instant coffee powders vary among different brands. In general, coffee powder production and distribution industries prepare coffee beans from regions with diverse geographical, chemical and organoleptic conditions that may affect their chemical composition. Furthermore, factors such as regional soil characteristics, pesticide and fertilizer usage, coffee processing methods, and the inclusion of additives can influence the distribution of PTEs in instant coffee powders [72]. The elevated levels of Fe and Pb observed in the rapid coffee powder in this study could be attributed to soil pollution in the agricultural area, groundwater contamination, the utilization of micronutrients in chemical fertilizers and pesticides. Additionally, advancements in coffee bean grinding, roasting, and processing technologies, along with the inclusion of flavorings, may contribute to increased metal content in coffee [73, 74]. Toni et al., study have identified lead, nickel and cadmium in packaged coffee samples [75]. The most detected metal was related to nickel. They also attributed the high lead content in the coffee powder to the packaging coating. The results related to measuring the concentration of PTEs in Gogoasa et al. study showed that iron and cadmium had the highest and lowest concentrations in coffee, respectively [76]. Alkherraz et al., study determined the content of PTEs in coffee, tea and cocoa. The results showed that concentrations of Fe, Cu and Pb were above the limits whereas Cd concentration was below the permissible limits [77].

Human Health Risk Assessment

The rank order of PTEs based on percentile 95% of THQ in both adults and children was Fe > Pb > As > Cd > Ni > Cu (Figs. 1 and 2). Since the concentration of iron was higher than other PTEs in instant coffee, the value of THQ related to Fe was also higher. Similarly, low values of THQ (≤ 1) were obtained by Kowalska et al., in a study conducted to assess the risk of Cd, Pb, As, and Hg in coffee [78]. Due to the low value of THQ, there was no possibility of non-carcinogenic risk associated with coffee consumption. Studies



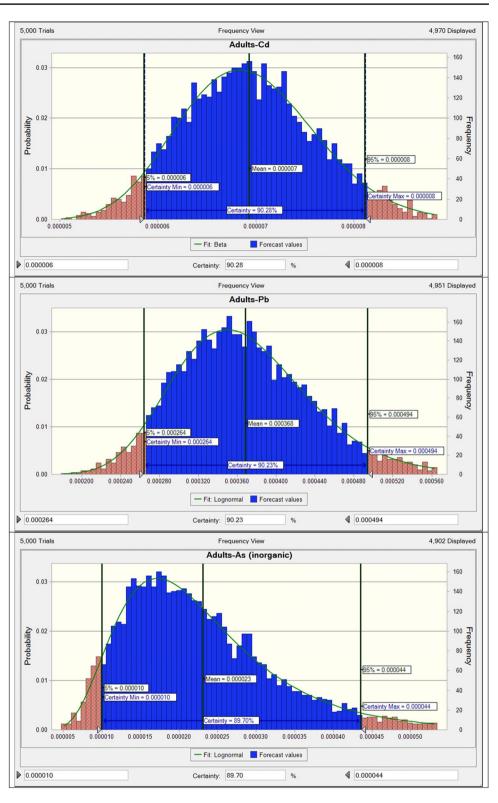
due to PTEs in rapid coffee



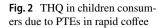
carried out by Winiarska-Mieczan et al. showed that THQ and HI for PTEs consumed with coffee were lower than 1 [79]. According to the results of this research, drinking even 3 cups of coffee a day does not lead to disease.

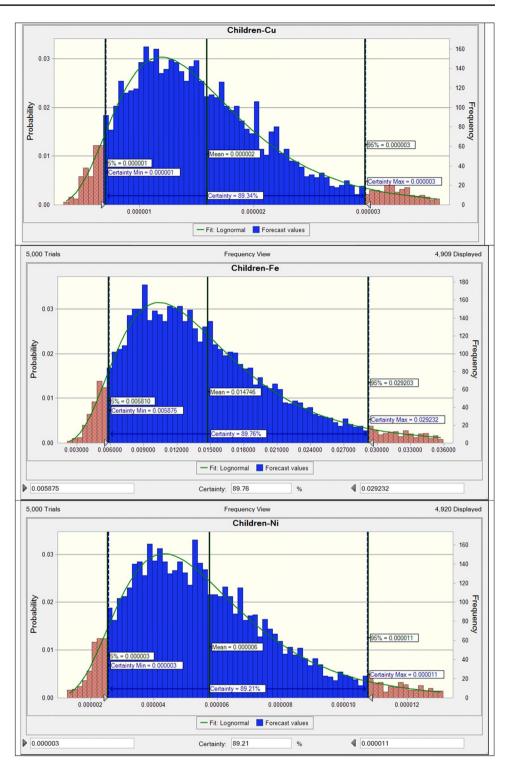
TTHQ in adults and children due to PTEs in the rapid coffee products was 0.023 and 0.030, respectively (Fig. 3). Consumers are in the acceptable non-carcinogenic risk (TTHQ < 1).

Fig. 1 (continued)



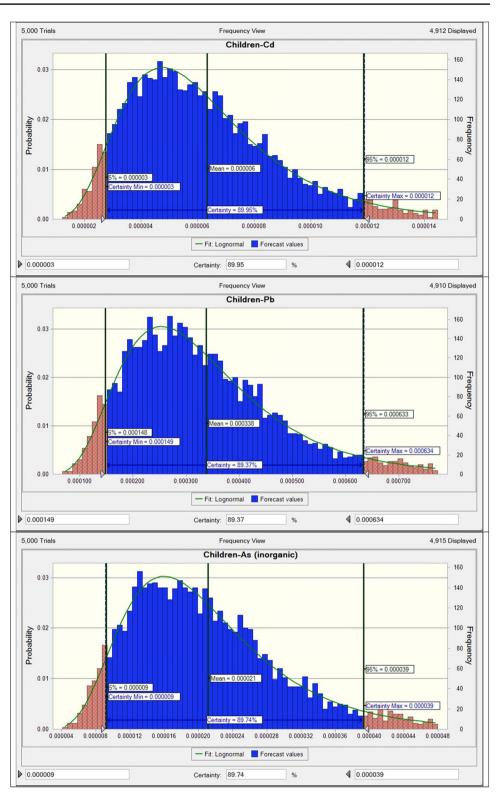
The Total target hazard quotient (TTHQ)of PTEs in rapid coffee was higher in children than in adults. Therefore, considering that TTHQ was less than 0.1 in both age groups, there is no carcinogenic risk for consumers [80]. In the study of Winiarska et al., THQ value for coffee containing Pb and Cd was less than 1, which





means that the risk of disease related to Pb and Cd due to long-term coffee consumption was very low[51]. In a study conducted by Khunlert et al., TTHQ value for cadmium, lead, iron, copper and nickel was found to be about 0.1-1[81]. The reason for high TTHQ can be mentioned to the daily consumption of more coffee. In a study conducted by Kowalska et al., THQ values related to coffee consumption for Cd, Pb, As and Hg were found to be less than 0.1, which was in conflict with the non-carcinogenic risk results of rapid coffee in the present study [78].

Fig. 2 (continued)



Percentile 95% of CR in the adults and children due to inorganic As in the rapid coffee products was 2.63E-5 and 1.27E-5, respectively (Fig. 4). Percentile 95% of CR for both adults and children was in the acceptable range

(CR < 1E-6). In the present study, As concentration was lower than the detection limit, however, the carcinogenic risk caused by As in rapid coffee was higher than the carcinogenic limit, which could be due to per capita

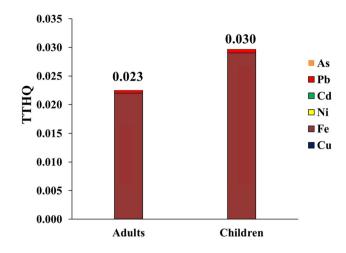
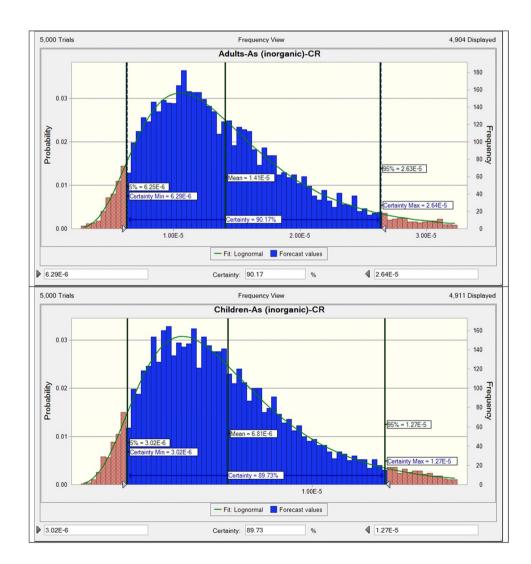


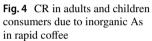
Fig.3 TTHQ in the adults and children consumers due to PTEs in rapid coffee

consumption of coffee and high toxicity of As. In the study of Kowalska et al., the carcinogenic risk for As in coffee samples could not be calculated considering that the concentration of As was lower than the detection limit[78]. In the study conducted by Taghizadeh et al., value of CR for As was obtained about 3.84E-6, which indicated an average level and no risk of cancer[82].

Conclusion

The concentration of Fe and Pb was higher than other PTEs in rapid coffee, Hence, source reduction plans for decrease concentration of Pb especially should be conducted. TTHQ values with PTEs in instant coffee were less than 0.1 (0.023 and 0.030 for adults and children, respectively). Therefore, consumers are not in considerable non-carcinogenic risk





also carcinogenic risk caused by As was acceptable, hence, consuming instant coffee does not endanger the health of consumers.

Author Contributions Study design was conducted by Vali Alipour, Yadolah Fakhri and Analysis of data by Iman Mahmoudi, Mohammad Borzoei, Freshtehm Mehri, Maryam Sarkhosh, and prepares manuscript by Vali Alipour, Freshtehm Mehri, Maryam Sarkhosh, Intissar limam, Rasul Nasiri, Yadolah Fakhri.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethical Approval This project was approved by the ethics committee of Hormozgan University of Medical Sciences, Bandar Abbas, Iran (IR. HUMS.REC.1399.448).

Consent to Participate The authors declare their Consent to Participate in this article.

Consent to Publish The authors declare their Consent to Publish this article.

Competing Interests The authors declare no competing interests.

Disclosure Statement None to declare.

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