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Original research

Evaluation of Lead and Arsenic concentrations in commercial beverages in Isfahan market by using Atomic Absorption Spectroscopy

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ABSTRACT

By increasing the world-wide population, the consumption of beverages has grown significantly. Beverages usually contain different heavy metals such as lead (Pb) and arsenic (As), which are toxic for humans and the environment. In our work, atomic absorption spectroscopy (AAS) method was used for quantification of Arsenic (As) and lead (Pb) contents in various beverages. Fifty-nine samples were collected from the Isfahan market and were divided into eight groups based on flavor. All groups were contained Pb below the allowable range (50 ng/mL) and also7 out of 8 groups were contained As more than permitted level (10 ng/mL). According to our results, it is important to control frequently heavy metal contamination in beverages due to the usage by a wide range of people.

Keywords: Arsenic; Lead; Atomic Absorption Spectrometry; Beverages.

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1. Introduction

Studying heavy metals in food is important due to their potential to be either essential or toxic. Monitoring the levels of trace elements in foodstuffs, including beverages (carbonated or non-carbonated drinks), is crucial for ensuring consumer safety (Scutaraşu & Trincă, 2023). The consumption of these beverages, particularly energy drinks, has become more common among people of different age groups, primarily to boost physical and mental performance (Alsunni, 2015). Daily intake of toxic elements for an extended period of time through foodstuffs and beverages may potentially cause serious health issues, including kidney (Silvera & Rohan, 2007), heart problems (Wechselberger et al., 2023), different cancers (Silvera & Rohan, 2007), neurological disorders (Doroszkiewicz et al., 2023), hormonal (Tag El Din et al., 2022) and hepatic issues (Loguercio et al., 1997). Lead (Pb), arsenic (As), and cadmium are highly toxic metals known to disrupt numerous bodily organs and systems (Abdul et al., 2015; Ara & Usmani, 2015; Rahimzadeh et al., 2017).

To date, numerous analytical techniques have been applied for the quantification of toxic elements in food products. Different methods such as High-Performance Liquid Chromatography (HPLC) (Hu et al., 2003; Okano et al., 2015), Inductively Coupled Plasma (ICP) (Fathabad et al., 2018; Mehri et al., 2024), Portable X-ray fluorescence spectroscopy (PXRF) (Byers et al., 2019; Hu et al., 2017), electrochemistry method (Mourya et al., 2019), voltammetry (Sadeghi et al., 2024,) and Atomic Absorption Spectrophotometry (Nugraha et al., 2024; Bahrami et al, 2025; Feizy et al, 2020; Fadavi et al, 2018). Among all techniques, AAS is one of the most widely used methods for determining heavy metals, due to its cost-effectiveness and high accuracy (Mezban Salih, 2024). Recently, Modibbo evaluated the concentrations of heavy metals in various energy drinks in the Nigeria market using HPLC and AAs methods (Modibbo, 2025). Another study in the Nigerian market has been reported by Ogoko and his collogues. Trace elements of forty samples have been studied using AAS, and the average levels of Mn, Cr, Cd, Fe, and Pb were likely to be significantly higher the maximum allowable regulations (Ogoko et al., 2024). Also, Bekheir and coworkers evaluated the lead levels using AAS in different beverages on the Iranian market and the Pb concentrations were lower than the World Health Organizations (WHO) (Aghebat Bekher et al., 2024). In 2025, Shavali Gilani and colleagues

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Table 1. Operating conditions for As quantification

Operating conditions	
Wavelength (nm)	193.7
Lamp current (mA)	20
Slit width (nm)	1
Pre-reductant solution	KI 10% (w/v), Ascorbic acid 2% (w/v)
Reductant concentration	0.6 % (w/v) $NaBH_4$, 0.6 % (w/v) NaOH
Ar (purge gas) flow rate (mL/min)	10

Table 2. Operating conditions for Pb quantification by graphite furnace.

Wavelength (nm)	Temperature program						Sample injection (µl)	Lamp current (mA)	Slit width (nm)	Ar flow rate (mL/min)
283.3		Step 1	Step 2	Step 3	Step 4	Step 5	20	10	0.7	250
	Temp. (ºC)	110	130	850	1700	2450				
	Ramp (Sec)	1	15	10	0	1				
	Hold (Sec)	30	30	20	5	3				

published a study focused on the analysis and health risk assessment of heavy metal content, including arsenic, copper, lead, and zinc, in several popular beverages in Tehran, Iran (Shavali Gilani et al., 2025). Based on their findings, the measured levels of lead and arsenic remained within the permissible limits set by Iran's national standards. In many countries, attention has shifted towards measuring the levels of toxic elements due to their excessive use and harmful effects. Hence, based on the Iranian regulations (INSO 12968) and Codex Alimentarius Commission (CAC) the maximum allowable range for Pb in non-carbonated drinks is 50 and 30 ng/mL respectively. Although the legal limit for arsenic is still under review by INSO for beverages, in this work it is compared to drinking water according to WHO regulations and the maximum permissible limit is 10 ng/mL (Alimentarius, 1995; ISIRI, 2010). In the present study, 59 beverages were collected from the Isfahan market in Iran and their toxic heavy metals (Pb and As) were determined using the AAS method. The obtained results were compared to the maximum allowable level in the INSO Regulations. The AAS technique was applied as a powerful, simple, and available method for many departments and QC centers.

2. Materials and Methods

2.1. Chemical and equipment

Standard solutions containing Pb and As (1000 mg/l) and other chemicals were purchased from Merck Company (Germany). All equipment was immersed in HNO3 solution (20% v/v) before use for two days (ISIRI 2013, ISIRI 2015, Balali-Mood et al., 2018).

Before analysis, all samples were digested using the UltraWave microwave digester (Milestone Co., Italy) to remove unpredictable metal contamination and effectively eliminate organic components (ISIRI 2013, Vakili Saatloo. 2023). As concentrations were determined using an atomic absorption spectrophotometer GBC (SavanatAA, Austrailia) equipped with a HG 3000 hydride system. Operating conditions are shown in Table 1. Also, all the Pb determinations were down using a Perkin-Elmer model 3030 atomic absorption spectrometer (PerkinElmer, Singapore) equipped with a graphite furnace. The instrumental parameters for Pb analysis were collected in Table 2.

2.2. Samples collection and digestion

Fifty-nine different brands of beverages containing non-carbonated fruit juices were randomly collected from the local market in Isfahan, Iran. Various brands were classified into 8 groups based on their flavors. All samples were stored at 5 °C in a refrigerator until analysis.

0.3 g of each sample was poured into a Teflon container with a cover and 3 ml of 65% HNO_3 and then 3 ml H_2O_2 was added to the vessel. The mixture was transferred into a digest apparatus for 45 minutes at 210 °C under 40 bar pressure. After that, for Pb determination, the digested sample was transferred into a 10 ml volumetric flask and up to a total volume with deionized water. For As quantifications, after the digestion process, the sample was put on a hotplate until the HNO_3 was evaporated, and then 5 ml of HCl 37% and 2.5 ml of pre-reductant solution were added to the mixture and diluted to 50 ml with deionized water (ISIRI 2013, Balali-Mood et al., 2018).

2.3. Preparation of standard solutions

Stock standard solutions (1000 mg/l) of Pb and As were diluted with HNO_3 1% to obtain 100 ng/mL working standard solution. Calibration standard solutions of Pb (50, 10, 20, 30 and 40 ppb) were prepared by diluting the working solution in HNO_3 1%. For As, calibration solutions (2, 4, 6, 8 and 10 ppb) were obtained by adding 10 ml of HCl 37% and a 5ml pre-reductant solution to the working standard and then up to a total volume of 50 ml with water (ISIRI 2013, ISIRI 2015, Vakili Saatloo. 2023).

2.4. Heavy metal analysis

After being set aside for two hours, the digested samples were transferred to the test vessel for analysis using an Atomic Absorption Spectrometer (ISIRI, 2013, ISIRI 2015). As quantifications were carried out applying a GBC (SavanatAA, Austrailia) atomic absorption spectrometer equipped with a HG 3000 hydride system and system conditions were recorded in Table 1. Also, a Perkin-Elmer model 3030 atomic absorption spectrometer (PerkinElmer, Singapore) equipped with a graphite furnace was used for Pb determinations and all the instrumental parameters were listed in Table 2.

2.5. Statistical analysis

The statistical analysis was completed using Excel software version 2019. The mean and standard deviation (SD) of the sample concentrations were used to express the precision. Non-parametric one-way analysis of variance (ANOVA) and were and one sample T-test were performed with Excel software. P-values less than 0.05 were considered significant. The values are expressed as mean ± standard deviation of the mean (SEM). The determination of As and Pb concentrations was performed in triplicate, and the final values were reported as the mean.

3. Results and Discussion

3.1. Instrument settings

Atomic absorption spectrophotometer settings were established based on the ISIRI method (ISIRI 2013, ISIRI 2015). For lead determination, wavelengths were calibrated to 283.3 nm with a lead lamp operating at 10 mA, utilizing a slit width of 0.7 nm. The temperature program was adjusted in five steps between 110 to 2450 °C. Highly pure Argon gas was applied as the protective and purge gas. For As quantification, wavelength was monitored at 193.7 with a lamp current of 20 mA. A pre-reductant solution containing KI 10% (w/v), ascorbic acid 2% (w/v) and reduction solutions consisting of 0.6 % (w/v) NaBH4 and 0.6 % (w/v) NaOH were used for sample determinations. All operating conditions are presented in Tables 1 and 2.

3.2. Linearity

Method linearity was tested at five levels of Pb concentrations (5, 10, 20, 30 and 40 ng/mL) and also for As (2, 4, 6, 8 and 10 ng/mL). The calibration curves were plotted on the basis of absorptions and various concentrations of Pb and As (Figs. 1 and 2) calibration equations and correlation coefficients (R^2) of lead and arsenic were calculated by means of the least squares regression method, and the obtained data were collected in Table 3. As can be seen in Table 3, the coefficients values of the standard curves exceeded 0.99, indicating that the method demonstrated sufficient linearity for quantifying sample concentrations.

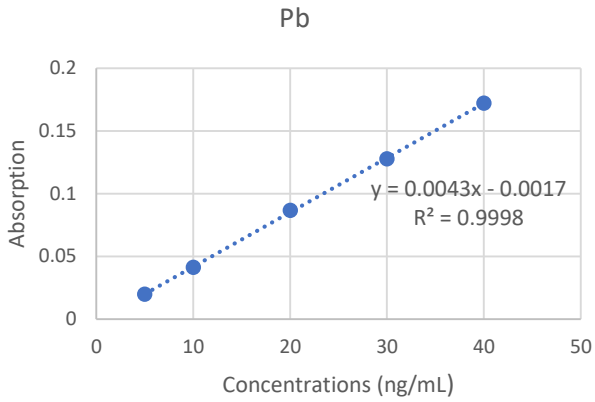

Fig. 1. Calibration curve of Pb.

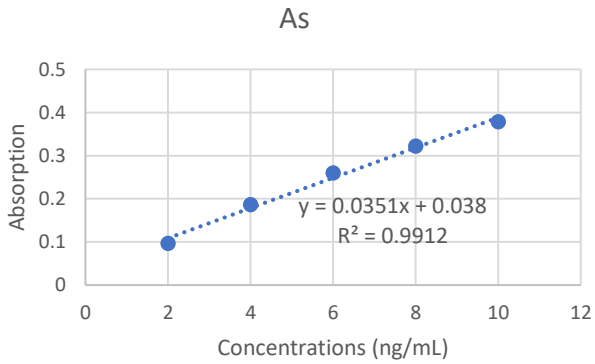

Fig. 2. Calibration curve of As.

3.3. Sensitivity

The lowest concentrations of detection and quantification of the analyte, commonly defined as LOD and LOQ, are two parameters for instrument sensitivity. The obtained data were collected in Table 3. As can be seen, the LOD values for Pb and As were determined to be 0.69 ng/mL and 2.32 ng/mL, respectively. The LOQ values were determined to be 1.03 ng/mL for Pb and 3.43 ng/mL for As. In this manner, the method was adequately sensitive to quantify each analyte.

3.4. Precision

Intraday precision was assessed to determine the repeatability of the applied method (Table 4). Hence, Pb and As were spiked separately into a freshly prepared sample at concentrations of 50 and 75 ng/mL, respectively, and absorbance values were recorded. The relative standard deviations (RSD) of the concentrations were calculated to demonstrate intraday precision. Based on the AOAC regulations (AOAC, 2019; Nugraha et al., 2024), the RSD% of values ≤ 15 and 10% for Pb and As respectively, since intraday precision of the developed method was enough to quantify the analytes.

Table 3. Linearity and sensitivity obtained from the standard solutions of heavy metals (n=3).

Analyst	Calibration equations	Linear range (ng/mL)	R^2	LOD (ng/mL)	LOQ (ng/mL)
Pb	y = 0.0043x -0.0017	5-40	0.9998	0.69	2.32
As	y = 0.0351x + 0/038	2-10	0.9912	1.03	3.43

Table 4. Intraday precision of tested heavy metals.

	Pb		As	
Concentration (ng/mL)	Found Mean ± SD	RSD (%) Intraday (n=5)	Found Mean ± SD	RSD (%) Intraday (n=5)
50	45.600 ± 0.001	2.24	-	-
75	-	-	80.6 ± 4.1	4.5

3.5. Accuracy

A Recovery percentage was calculated to evaluate the accuracy of the method. For this purpose, Pb and As were spiked five times into a blank sample (As and Pb free sample) at concentrations of 50 and 75 ng/mL respectively. All data was collected in Table 5. According to Table 5, the recovery percentage was 97.4% for Pb and 107.1% for As. The recoveries were demonstrated the effectiveness of the method, with results in most cases nearing 100%. This indicates that the developed approach is both accurate and reliable.

Table 5. The results of the accuracy test.

Heavy metals	Spiked Conc. (ng/mL)	Recoveries (%)
Pb	50	97.4
As	75	107.1

Conc. means concentration.

3.6. Sample analysis

Atomic absorption spectroscopy method was used to determine of heavy metals (Pb and As) in various beverages (59 samples) in the Isfahan market. All samples were collected in different flavors (pomegranate, orange, sour cherry, apple, peach, pineapple, mango, and aloe vera) from 22 popular brands. Until now, there have been some attempts to quantify Pb and As in beverages in Iranian markets. In a comprehensive study, Bakhtiyari and co-workers (Balali-Mood et al., 2018) studied the heavy metals (Pb and As) in commercial fruit juice in Mashhad, Iran. Based on their results, arsenic was found in all samples and was below the maximum allowable range (10 ng/mL). With regard to the Pb, 83 percent of those tested were higher than the maximum acceptable range (30 ng/mL). In another work, Aghebat Bekheir and co-workers (Aghebat Bekher et al., 2024) collected 38 samples of cold and hot drinks from the Tehran market and their Pb was determined. The obtained concentrations of Pb in all samples were lower than the limit set by WHO. Our results are illustrated in Table 6. Based on the Iranian National Standard No 12968 (ISIRI, 2010), the maximum permitted range for Pb is 50 ng/mL and the allowable limit for As is still under study for beverages. Hence, arsenic was compared to drinking water based on WHO guidelines, and the maximum acceptable limit is 10 ng/mL. As shown in Table 6, the obtained concentrations were reported as mean values. All the samples were classified in 8 groups based on their flavor, such as pomegranate, orange, sour cherry, apple, peach, pineapple, mango, and aloe vera. All groups contained Pb below the allowable range (50 ng/mL) according to the Iranian Standard regulations. Also, among all groups, 7 out of 8 groups contained arsenic above the permitted level (10 ng/mL). This study found that aloe vera and mango-flavored beverages contained the highest concentration of lead at 45.59 ng/mL, while pomegranate flavored drinks had the lowest at 34.08 ng/mL. Similarly, arsenic levels were highest in aloe vera-flavored samples at 45.7 ng/mL and lowest in peach-flavored drinks at 9.5 ng/mL. Notably, aloe vera-flavored samples consistently showed elevated levels of both lead and

Table 6. The obtained Concentrations (ng/mL) of heavy metals (Pb and As) in the commercial beverages.

Samples name	Beverage type	Pb (ng/mL) [a] (Con. ± SD)	Max. value	Min. value	As (ng/mL) [a] (Con. ± SD)	Max. value	Min. Value
A	Pomegranate	34.08 ± 10.80	49.19	20.36	15.98 ± 14.00	39.90	5.32
B	Orange	38.15 ± 8.40	48.69	14.77	29.16 ± 13.70	46.80	9.70
C	Sour cherry	36.49 ± 9.60	45.82	25.08	18.00 ± 13.40	40.10	4.50
D	Apple	40.52 ± 3.30	45.46	36.74	17.02 ± 11.50	18.09	6.88
E	Peach	38.75 ± 15.20	47.27	15.95	9.50 ± 10.50	22.60	0.00
F	Pineapple	41.12 ± 9.70	49.31	28.76	23.16 ± 18.80	35.60	4.90
G	Mango	41.70 ± 11.80	50.10	33.30	28.96 ± 13.30	38.42	19.50
H	Aloe vera	45.59 ± 3.30	47.96	43.22	45.70 ± 47.20	79.10	12.30

[a:] Mean value
Con. means concentration.

arsenic, likely due to contamination in their raw ingredients. ANOVA analysis shows that there is no significant difference between the means of the 8 groups (P-value = 0.21 > 0.05 for As and 0.84 > 0.05 for Pb). One-sample t-test was performed to compare the data with standard regulations. The calculated p-value for lead compound was less than 0.001 and the mean lead concentration in the samples was measured at 37.26 ng/mL that is statistically significantly lower than the threshold of 50 ng/mL. Consequently, the lead concentration in these samples can be considered within the safe range based on statistical analysis. Furthermore, the mean arsenic concentration in the samples measured at 18.7 ng/mL (P-value = 0.0001), is higher than the threshold of the standard regulations (10 ng/mL).

Finally, further research is necessary, particularly focusing on the production processes of these beverages.

4. Conclusion

Despite the high use of beverages in society, the allowable levels of heavy metals can protect consumer health and the environment. Thus, checking the heavy metals in beverages is important for consumer health and also financial aspects. In this work, the amount of the heavy metals, namely lead (Pb) and arsenic (As) was investigated in beverages on the Isfahan market. Samples were classified into 8 groups based on their flavors, such as pomegranate, orange, sour cherry, apple, peach, pineapple, mango, and aloe vera. In all groups, the lead levels were measured and found to be below the permissible limit (50 ng/mL) and 7 out of 8 groups contained arsenic above the permitted level (10 ng/mL). Among the various samples, Aloe vera-flavored samples consistently revealed higher concentrations of both lead and arsenic. Comprehensive quality control measures should be implemented to minimize the presence of these harmful metals. Periodic and thorough monitoring of heavy metal levels in beverages is recommended to safeguard consumer health and address their potential impacts. Considering the broad scope of this topic, further research covering all aspects of the food chain is essential.

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Conflict of interest

The authors declare that there is no conflict of interest.

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