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Determination of lead levels in cold and hot beverages on the Iranian market using the atomic absorption spectrometry method

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Abstract

People of all age groups worldwide extensively eat fruit juices, and their consumption has grown significantly in the last two decades. In addition to being tasty and appealing to the palate, fruit juices are usually extremely nutritious as well. The nutrients and essential elements found in fruit juices contribute to the well-being of humans. Nevertheless, fruit juices may contain trace amounts of potentially toxic elements, which may have negative health effects. Low-level exposure to heavy metals such as lead (Pb), cadmium, arsenic, and mercury can cause toxic effects. Pb is one of the most common heavy metals, recognized and highlighted as one of the most dangerous environmental poisons. The present study assessed Pb level in beverages such as peach, pomegranate, orange, Cherry, pineapple, grape, mango, aloe vera, apple banana, coffee powder, and tea powder from Tehran, Iran. All fruit juice samples were digested using a microwave digestion system and analysed using the developed and validated atomic absorption spectroscopy method. According to the results, the mean concentrations of Pb in non-carbonated fruit drinks, nectar fruit drinks, coffee powder, and tea powder were 0.01964, 0.0084, 0.198, and 1.27333, respectively. Pb concentrations ranging from 0.00 to 2.1 mg/l were found in all beverages analysed. As a result of the present study, Pb concentration means in all samples studied were high and lower than the guidelines recommended by World Health Organization (WHO). Nevertheless, continuous monitoring of heavy metal pollution in beverages is highly necessary

Introduction

Food contamination with heavy metals, especially in highly consumed foods such as fruits, is a major public health problem (Fathi *et al*., 2023). In the habitual diet, fruit juices, potable water, and tea are the most popular drinks, and they contribute significantly to trace element intake (Mohammadi and Ziarati, 2015). Consumption of fruit drinks has steadily increased over the last 20 years (Sardinha *et al*., 2014). Consuming fruit drinks in moderation as part of a balanced diet can enhance health and decrease disease risk (IFU, 2013). It is well known that fruit juices are effective against chronic inflammation, cancer, heart disease, and bone disease and serve as a detoxifier. Inhibition of oxidation is boosted by nectar and juice (Fatima *et al*., 2021). These drinks may contain toxic metals, seriously impacting human health (Abdel-Rahman *et al*., 2019). Acknowledging that fruit drinks significantly affect children's dietary metal exposure is crucial. This is because children's diets tend to be less diverse than those of adults, and fruit drinks are a preferred beverage among children. Additionally, they consume more fruit drinks than adults and may be more susceptible to some metals than adults (Food and Administration, 2013). The contamination of fruit juice with toxic elements can be attributed to various factors such as soil, surface water, and ground water pollution during fruit cultivation, excessive application of chemical fertilizers and pesticides, improper storage conditions of fruits, both in industrial and traditional fruit juice production, and the leaching of elements from fruit juice packaging (Karami *et al*., 2023) (Fig. 1). Heavy metals have received much attention due to their high toxicity, persistence, non-biodegradability, and bioaccumulation potential in food chains (Sajjadi *et al*., 2022).

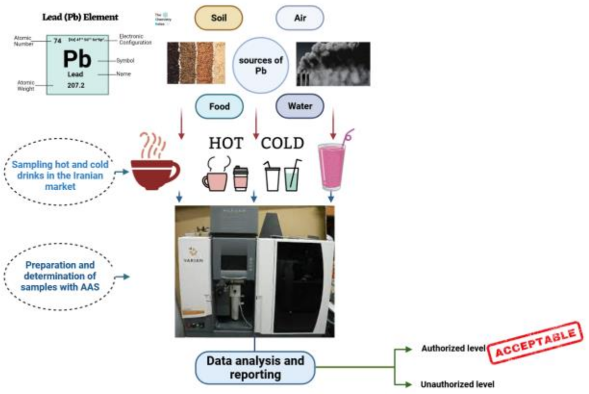

Fig. 1. The graphical abstract.

Trace levels of heavy metals such as Cu, Zn, and Ni, play a crucial role as micronutrients in human growth (Mawari *et al*., 2022). However, adverse effects occur when heavy metal concentrations exceed a certain threshold (Rahman and Singh, 2020). On the other hand, several heavy metals such as lead (Pb), cadmium (Cd), mercury (Cu), and chromium, do not have any known biological function and can be toxic even at low concentrations (Uddin *et al*., 2021). Internationally, the toxicity of Pb and Cd is extensively established and acknowledged as a significant environmental health hazard (Goran *et al*., 2017). Pb is a metallic element with widely recognized physical and chemical properties (Al-Saleh *et al*., 2009). Pb poisoning has been known to have harmful impacts on human health over the past 20 years (Khalid *et al*., 2013). Pb can enter the human body through three routes: the skin, the respiratory tract, and the digestive system (Ernyasih *et al*., 2023). Human bodies absorb approximately 90% of the metal (Scutarașu and Trincă, 2023).

Approximately 99% of Pb persists in the bloodstream 30 to 35 days after absorption. Over the following 4-6 weeks, it disseminates and accumulates in many organs such as the liver, renal cortex, aorta, brain, kidneys, lungs, spleen, teeth, and bones (Gillis *et al*., 2012, Carocci *et al*., 2016). Several epidemiological studies have established a connection between exposure to Pb and negative cardiovascular outcomes (Obeng-Gyasi *et al*., 2021). Additionally, Pb exposure disrupts the hemoglobin production process, which can shorten the life of red blood cells (erythrocytes) and lead to anemia (Mariadi *et al*., 2023).

It has been shown that exposure to lead can adversely affect the reproductive systems of both men and women (Wu *et al*., 2012). Sperm count and volume are reduced, as well as the motility and morphology of the sperm in males. Women who are exposed to this metal often experience miscarriage, premature birth, low birth weight, and developmental issues (Collin *et al*., 2022).

Pb is evenly distributed throughout the body and harms the central nervous system. In children, it leads to brain damage, resulting in behavioral issues, hearing loss, decreased IQ, and learning impairments. In adults, it causes memory loss, difficulty concentrating, headaches, irritability, and depression. Due to the potential harm caused by even minimal amounts of blood Pb in childhood, it is concluded that there is no acceptable blood Pb level for children. Thus, international organizations, including the WHO, have established maximum Pb levels in food and drink (Balali-Mood *et al*., 2018). The maximum acceptable level of Pb in fruit juices is 0.3 mg/l (Ofori *et al*., 2013), and for Pb in coffee powder and tea powder is 0.5 mg/kg, and 10 mg/kg, respectively (Dabanovic *et al*., 2016).

Despite numerous publications, concerns persist regarding Pb levels found in fruit juices throughout various regions, including Iran. The primary objective of the present investigation was to ascertain the concentrations of Pb in various beverages that are commercially accessible and, after that, evaluate their compliance with the safety guidelines set by the World Health Organization (WHO) to assess their suitability for consumption.

Materials and Methods

Sampling

In this study, a total of 38 samples of cold and hot beverages (fruit juices, n=25; nectar fruit drinks, n=5; coffee powder, n=5, and tea powder, n=3) from June to November 2022 were collected from the valid different supermarkets of Tehran, Iran. Fruit juices of nine different fruits, peach (n=4), pomegranate (n= 3), orange (n=5), cherry (n=3), pineapple (n=1), grape (n=2), mango (n=3), aloe vera (n= 3), and apple banana (n=1) was procured. The samples were refrigerated at a temperature of 4°C in the laboratory.

Instrumentation

The ETHOS 900 microwave digester (Milestone Co., USA) was employed to digest fruit drink samples. The metal concentrations in the samples were analyzed using an AVARIAN AA 240FS, a rapid sequential atomic absorption spectrometer manufactured by VARIAN in Australia.

Chemicals and reagents

The element Pb stock solutions described below, which were available for purchase, were utilized: A solution containing 1,000 ± 4 mg of Pb per liter in a 2% (w/w) nitric acid solution (TraceCERT®, Fluka Analytical, Sigma-Aldrich Chemie GmBH, Switzerland) was utilized to create calibration and spike solutions.

Digestion

For digestion, 5 ml of each sample was mixed with 6 ml of nitric acid (65%), 3 ml of hydrochloric acid (35%), and 0.25 ml of hydrogen peroxide. The collected samples were transferred into previously sterilized Teflon containers with hydrochloric acid and hydrogen peroxide solutions. The Teflon beakers underwent digestion using a Milestone ETHOS 900 Model microwave digester for 26 minutes at the highest power setting.

Quantification of elemental concentration using atomic absorption spectroscopy

The Pb concentrations were measured using a VARIIAN AA240FS Model Atomic Absorption Spectrophotometer (VARIAN Co., Australia) after the drink samples were digested with acid. The acid digestion method employed analytical-grade reagents, namely HNO3 (65%, Sigma-Aldrich Co., USA), HCl (35%, Sigma-Aldrich Co., USA), and H2O2 (30%, Merck Co., Germany) reagents; all of the substances were of analytical-grade purity. The digested samples were cooled to the ambient temperature to determine the desired analytes, transferred completely into test tubes, and examined using a VARIAN AA 240FS Flame Atomic Absorption Spectrometer (Table 1).

For the atomic absorption spectrophotometry, air was used as fuel, and acetylene was used as an oxidant. For the analysis, the fuel flow rate was 2 l/minute, and the oxidant flow rate was 13.5 l/minute.

Statistical analysis

The mean and standard deviation (SD) of observed concentrations were calculated using SPSS Inc.'s Statistical Package for the Social Sciences (version 15.00), developed in the USA.

Table 1. Analytical condition for atomic absorption spectrometry.

Parameter	Pb
Wavelength, nm	244.8
Slit width, nm	0.2
Background correction	on
Lamp current, mA	5

Results and Discussion

The Pb concentrations in the analysed beverages were presented in Table 2. The disclosure of the brand names of the examined drink samples was withheld to ensure anonymity. Every code was associated with a unique brand.

Table 2 illustrated the variation in Pb concentrations for various varieties of fruit juice samples. The contents of Pb were 0.02-0.04 mg/l in peach, 0.004-0.02 mg/l in pomegranate, 0.003-0.03 mg/l in orange, 0.01-0.03 mg/l in cherry, 0.03 mg/l in pineapple, ND-0.02 mg/l in grape, 0.01-0.03 mg/l in mango, 0.004-0.03 mg/l in aloe vera, and 0.04 mg/l in apple banana. The concentration of Pb was maximum in peach and apple banana (0.04 mg/l) and minimum in orange (0.003 mg/l), while Pb was not detected in sample grape (code11). As can be seen from the results, all investigated fruit juices contain Pb concentrations below the Maximum Permissible Limit (MPL) of 0.3 mg/l set by WHO.

Table 2. Lead (Pb) concentration in non-carbonated fruit drink samples.

Samples	Lead(Pb) concentration
Non-carbonated fruit drink	0.02 ± 0.011 mg/l
Nectar fruit drinks	0.0084 ± 0.007 mg/l
Coffee powder	0.198 ± 0.3 mg/Kg
Tea powder	1.273333 ± 0.7 mg/Kg

The average amounts of Pb in the samples of nectar fruit drink were found to be 0.019 ± 0.011 mg/l. Among the five brands of nectar fruit drink, code1 has the highest lead concentration at 0.02 mg/l, and code4 has the lowest at 0.004 mg/l. In code 2, the concentration of Pb was not detected.

In all samples of coffee powder, Pb concentrations ranged from 0.025 to 0.79 mg/kg. Among the tea samples, Pb levels varied from 0.56 mg/kg to 2.1 mg/kg.

The mean Pb content in the samples was 1.27 mg/kg. Pb levels in all samples are lower than the WHO maximum contaminant level of 10 mg/l. Tea samples had the highest concentrations of Pb in all the beverages studied. The mean concentrations in magnitude were Tea>coffee>fruit juices>nectar for Pb. This study found that all samples, except one coffee (Code 2), had acceptable lead levels compared to WHO limits.

Many research articles exist on heavy metal content in beverages in Iran and other countries. Several studies have reported that analysed beverage samples are safe for drinking since the levels are below the WHO's maximum permissible levels, while other studies have reported unsafe drinkability levels. Saleh *et al*. (2022) investigated Pb in 100 samples of soft drinks determined using an atomic spectrophotometer (AAS). The concentration ranges found were 0.046 to 0.109 mg/l for Pb. The study of Godwill *et al*. (2015) in Nigeria on 26 soft drink samples showed that, the level of Pb in various kinds of soft drink varied from 0.17 to 3.39 mg/l. Compared to the current study, the results were higher. In another relevant research, Ofori *et al*. (2013) conducted an analysis of Pb concentrations in some fruit juices from Accra. Pb mean concentration in fruit juices were 1.59 ± 0.90 mg/l. The variation in metal content observed among juice samples can be attributed to factors such as the composition of raw materials and water used during juice production, the quality of soil and irrigation water, environmental pollution from fertilizers and pesticides, the purity of added sugar, and potential contamination during industrial processing and storage (Balali-Mood *et al*., 2018; Abdel-Rahman *et al*., 2019). The present results agree with those found by Ogunlana *et al*. (2015). These researchers determined a mean Pb content of < 0.001 to 0.040 mg/l in soft drinks samples from Nigeria. Previous studies demonstrated that the range for Pb was from 0.005 to 0.013 mg/kg in South Korea (Habte *et al*., 2017), 0.003 to 0.100 mg/kg in Jamaica (Antoine *et al*., 2017), 0.63 to 6.86 mg/kg in South Korea (Hong *et al*., 2019) and 0.98 to 2.39 mg/kg in Pakistan (Hyder *et al.,* 2022). Likewise, Ackah *et al*. (2014) reported Pb concentrations in fruit juice samples of 0.178 ± 0.091 mg/l. The disparity in metal concentrations observed in the processed fruits of this study compared to previous research can be attributed to various factors. These factors include the proximity of industrial zones to fruit-growing areas, contamination in irrigation regions, storage conditions, and the specific metal detection techniques employed (ICP-OES, ICP-MS, or AAS) (Einolghozati *et al*., 2023). Consequently, different regions' soil physicochemical properties and climatic conditions should not be overlooked (Chen *et al*., 2011). Moreover, the metal concentration of fruit juices can be influenced by various methods employed in their storage, transportation, and packaging (Khazaei *et al*., 2023). The fruit juice is being subjected to pollution by hazardous elements. Nevertheless, by implementing contemporary machinery (such as stainless steel) and utilizing inert packing materials, among other measures, in the production process and by closely monitoring the origin and quality of the fruit and other input materials, the presence of these metals in fruit juices can be substantially diminished (Todorovska and Popovski, 2012).

A popular beverage around the world is coffee (Rubio *et al*., 2019). Several studies have suggested that moderate consumption of coffee can have beneficial health effects for humans (Adler *et al*., 2019). It has several antioxidants, including caffeine, phenolic compounds, and diterpenes. Scientific research indicated that regular consumption of coffee can enhance the body's defense against DNA damage by increasing glutathione levels (Winiarska-Mieczan *et al*., 2021). Despite this, humans are potentially exposed to several toxicants through coffee consumption. Among these toxicants are pesticides, heavy metals, organic solvents, pharmaceutical agents, and byproducts of thermal processes such as acrylamide (Khunlert *et al*., 2022). Plenty of evidence suggests that plants such as coffee beans have a high capacity to absorb Pb from the soil, water, and the atmosphere (Mousavi Khaneghah *et al*., 2022). Coffee beans absorb metals from soil, storing them in the roots or moving them to the grains and shoots (Berego *et al*., 2023). Since plant tissues contain different metal contents, grains tend to have a lower heavy metal content than vegetative plant parts (Várady *et al*., 2021). An increase in coffee bean consumption might cause neurological and hepatic dysfunctions as well as cognitive impairments, cancer, and reproductive difficulties (Mollakhalili-Meybodi *et al*., 2022). In this study, Pb concentrations in coffee samples ranged from 0.025 to 0.79 mg/kg. One sample contained levels above the WHO maximum allowable level. Numerous global studies have ascertained the concentrations of vital and harmful components in coffee beans. According to da Silva *et al*. (2017), Pb concentrations in coffee samples ranged from 0.75 to 1.575 mg/kg, while mean concentrations in coffee samples were 0.75 ± 0.33

mg/kg. The results of this study were higher than those of the present study. Pb concentrations were assessed in coffee samples from Libyan markets by Alkherraz *et al*. (2019). Pb was reported to be between 0.9 and 3.9 mg/kg. Those results are not in line with our study. According to data collected in Minas Gerais, the mean concentration of Pb in coffee was 0.69 mg/kg, ranging from 0.14 to 2.59 mg/ kg (Pigozzi *et al*., 2018). A wide range of metal concentrations in coffees can be influenced by several factors such as the mineral, the coffee assortment, the soil (type of soil, organic matter concentration, pH, and drainage grade), the climate conditions of the area of origin, and the processing method, etc. (Habte *et al*., 2016, Gogoasa *et al*., 2017).

Another study in Western Balkan markets found that average Pb concentrations for 25 coffee samples were 0.3 ppm, falling below the upper limits established by the rules (Petrović *et al*., 2020). In a study on roasted coffee powder conducted by Massoud *et al*. (2022), no Pb contamination was demonstrated in any of the 25 analysis samples. Furthermore, Omer *et al*. (2019) assessed the quantity of Pb in 13 coffee samples in the Saudi Arabian market. According to their findings, the mean concentration and range of Pb were 7.57 ± 9.26 mg/kg and 0.00-23.88 mg/kg, respectively. Coffee quality could be affected by a high level of heavy metal contamination in the soil and the use of chemical fertilizers and pesticides.

Also, various factors, including the preparation method, grinding, and roasting degree, influence coffee's biochemical composition, and heavy metal content (Albals *et al*., 2021). Other factors that can influence the amount of Pb in coffee include the type of water that is used to infuse the coffee, the brewing method, and the kind of coffee (raw or roasted) (Amorim Filho *et al*., 2007).

Tea (*Camellia sinensis*) is a popular beverage consumed by a wide range of people worldwide (Basaran *et al*., 2023). Many studies have indicated that tea has many health benefits, including antioxidation, anti-inflammation, immunoregulation, anticancer, cardiovascular protection, diabetes treatment, weight loss, and hepatoprotection (Gan *et al*., 2018; Tang *et al*., 2019). However, heavy metals can contaminate tea leaves during growth and processing (Falahi and Hedaiati, 2013). The presence of Pb in commercial tea leaves has become a source of concern for consumers and producers alike in recent years (Karak and Bhagat, 2010). Heavy metals play a crucial role in assessing the quality of tea, as they can be extracted from tea leaves during the brewing process and subsequently enter the human body when tea is consumed. This poses a potential health risk to humans (Zhang *et al*., 2018). In our study, the Pb contents ranged from 0.56 to 1.16 mg/kg, having a mean of 1.273 mg/kg. Numerous studies have been performed on the concentration of Pb in different teas in several countries. For example, Heshmati *et al*. (2020) indicated the mean concentration of Pb in black tea as 0.59 ± 0.12 mg/kg. Rashidimehr *et al*. (2023) analysed black tea and tea plant leaves and demonstrated Pb levels in the range of 0.501 ppm for leaves and 0.939 ppm for black tea, which are not in line with the presented research results. Compared to our study, those results were lower. The main Pb source in the tea plant is likely the soil under cultivation since the soil's acidity in the tea plantations would increase the absorption of Pb metal by the roots (Seyyedi Bidgoli *et al*., 2022). Furthermore, tea leaves absorb more Pb from the atmosphere depositions because of their broad surface (Sarlak *et al*., 2023). Thus, another source of Pb contamination in tea may be deposits from polluted air that reach the plant leaves (Karimi *et al*., 2008). Sultana *et al*. (2023) analysed the level of Pb in samples of black tea (n=8) collected from the local shops in Bangladesh. Concentration ranges of Pb were 0.04 to 3.89 and 0.20 ppm. It was found in a study by Zhong *et al*. (2016) that the concentration level of Pb in tea samples from China ranged from 0.48 to 10.57 mg/kg with an average of 3.04 mg/kg, which is higher than those reported here. In 2020, Charles *et al*. (2021) conducted a study in Taraba, Nigeria, where they found that the mean concentration of Pb in tea leaves differed according to the wet and dry seasons. Mean Pb concentrations from tea leaves during the wet and dry seasons were 0.056 mg/kg and 0.015 mg/kg, respectively. According to the authors, in wet seasons, pollutants were most concentrated. Therefore, this can be linked to the potential for metal salts from the nearby farm's runoff to be carried into the sample location through washing (Charles *et al*., 2021). The results can be attributed to differences in the country and area of origin, altitude, rainfall, and other environmental conditions (Barone *et al*., 2016). However, herb products may also be contaminated with heavy metals due to using fertilizers and fungicides containing phosphates in agriculture. The materials often contain high levels of toxic elements like Zn, Cd, Pb, and Cu (Pourramezani *et al*., 2019). Moreover, differences in processing and storage methods could contribute to this difference (Zazouli *et al*., 2010; Dabanovic *et al*., 2016).

This study assessed the concentrations of Pb in selected cold and hot beverages from different markets in Tehran, Iran. The highest mean level was 1.27 mg/l (Tea), and the lowest was 0.008 mg/l (nectar). The concentrations of Pb in cold and hot beverages were lower than the limit set by WHO. The non-toxic level of Pb in these beverages is thus verified. Consequently, consuming such beverages poses no harm to consumers. However, it is important to note that there are no established thresholds for safe heavy metal consumption due to the tendency of these metals to accumulate in living tissues over long periods (with a half-life of up to 30 years). On the other hand, given that this type of drink is highly consumed and that Pb is harmful to children and women's health, it would be better to periodically monitor Pb levels in fruit juice samples from Tehran and other parts of Iran. Also, consumers need to be educated further on beverages and their potential health effects. Given the comprehensive nature of this topic, more studies encompassing the entire food chain are needed.

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Conflict of Interest

The authors do not have any potential conflict of interest to declare.

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