Research article

Journal of Human, Health and Halal Metrics; 2024, 5(2): 19-24
https://doi.org/10.30502/jhhhm.2024.477627.1097

Determination of metals in soft drinks by anodic stripping voltammetry

Naficeh Sadeghi[1,2*], Masoomeh Behzad[1], Mannan Hajimahmoodi[1], Reza Shokri[1]

1. Department of Drug and Food Control, School of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.
2. Halal Research Center of IRI., Ministry of Health and Medical Education, Tehran, Iran.

Submission: 9 September 2024 ***Revision:*** 23 October 2024 ***Acceptance:*** 3 November 2024

Abstract

Background and Objective: In Iran, soft drinks are consumed daily due to their affordability, characteristic taste, and thirst-quenching potential. However, the high demand may compromise the quality of production with possible contamination of heavy metals, which have been shown to cause intoxication and death in humans. This study aimed simultaneous determination of zinc, copper, lead, and cadmium in soft drinks in Iran.

Materials and Methods: In this study, 84 soft drink samples were purchased from Iran's market. A polarograph measured the concentration of heavy metals. Voltametric analysis was carried out using a 746 VA trace analyzer with differential pulse anodic stripping of the voltammeter at the hanging mercury dropping electrode.

Results and Conclusion: All samples contained high levels of cadmium, lead, zinc, and copper. The average range of cadmium was 0.23 ±0.16 mg/100 ml, and the mean of lead was 0.62 ±0.47 mg/100 ml. The average of zinc and copper was 31.05 ±19.69 and 3.10 ±2.19 mg/100 ml, respectively. The high levels of heavy metals may harm the human health under chronic exposure. Thus, strict quality control is recommended during production (especially sterilization), purification, and packaging stages.

Keywords: Heavy metal, Micronutrient, Soft drinks, Voltammetry

1. Introduction

Soft drinks are non-alcoholic water-based flavored drinks that are optionally sweetened, acidulated, and carbonated. Globally, carbonated soft drinks are the third most consumed beverage. The annual consumption of carbonated soft drinks is nearly four times that of fruit beverages [1]. Zinc and copper are essential, while lead and cadmium are toxic at certain levels. The composition and concentration of metals in different food types of various countries have been the subject of many studies [1]. Heavy metal refers to any metallic element with relatively high density and is toxic or poisonous at low concentrations. Concern that trace elements in soft drinks present a potential health risk, if they exceed certain concentration, has prompted several statutory bodies such as the World Health Organization to establish maximum allowable concentrations [2]. The present study focuses on the heavy metal contents of some commercial brands of soft drinks in Iranian markets. Soft drinks make up a significant proportion of daily food intake in Iran and other countries. The present study provides a more detailed determination of cadmium, copper, lead, and zinc in various classes and brands of soft drinks. Voltammetry methods are very attractive for determining heavy metals at trace levels [3].

*Correspondence to: Naficeh Sadeghi; E-mail: nsadeghi@sina.tums.ac.ir; Tel.: +98-21-66954713, Fax: +98-21-66461178

Heavy metals enter the body in various ways, such as by consuming food, breathing polluted air, and contacting polluted water and soil. Heavy metals of lead and cadmium seriously threaten the human health. In comparison, copper and zinc have very important physiological and biochemical functions in biological systems, and their deficiency or excess can lead to disturbances in metabolism. Heavy metals are not metabolized in the body, and accumulate in the organs after uptake. They deposit in the tissues of blood vessels, muscles, bones, and joints. When heavy metals enter the body, they may accumulate in the kidney, liver, bone, and brain, disrupting their function [4-6]. The most adverse effects of heavy metals are on children. This age group bears the greatest risk of exposure to heavy metals. Among the harms of children being exposed to even small quantities of heavy metals, mental retardation and impaired physical growth are of concern. Some heavy metals are transferred directly from the mother to the fetus, and some enter the body by breast milk [7-9]. Lead and cadmium poisoning causes disorders in tissues and organs, including the heart, bones, intestines, kidneys, reproductive and nervous systems [10]. A few diagnostic strategies have been accounted for the assurance of these metals in soft drinks, including inductively coupled plasma-mass spectrometry (ICP-MS) [11], inductively coupled plasma-atomic emission spectroscopy (ICP-AES) [12], atomic absorption spectroscopy (AAS) [13], potentiometric stripping [14], and anodic stripping voltammetry (ASV) techniques [15]. Contrasted with these systematic procedures, the AAS method has been utilized to determine the vast majority of the components present in soft drinks. It is dull and uneconomical, but at the same time, it investigates a few components in numerous examples. Additionally, ASV strategy is practical, profoundly delicate, and particular for synchronous assurance of a few metals. It is entrenched that ASV is a reasonable technique for the assurance of Zn, Cu, Pb, and Cd in numerous frameworks. Heightened awareness of the often-detrimental effects of trace elements in the media, such as foodstuff, drinking water, and commercial wastewater effluents, has led to stringent public legislation and industry-wide quality programs, which have been directed toward monitoring components of a sample at sub-ppm levels. The analysis of heavy metals in sediments basically includes digestion and detection by Graphite Furnace Atomic Absorption Spectroscopy (GFAAS), flame atomization, ICP-ES, or ICP-MS. All steps of the procedures are susceptible to insufficient recovery and contamination. Voltammetry techniques such as anodic stripping voltammetry can determine elements accurately at trace to ultra-trace levels and have demonstrated the ability for multi-element determination. Stripping voltammetry has been used in trace analysis with relative ease and success in various analytical applications. This technique can routinely identify and quantify trace components from 10^{-5}–10^{-9} M with excellent sensitivity and selectivity. Stripping analysis has received unusual interest since it is the most sensitive electro-analytical technique currently available [16]. Voltammetry is an electrochemical technique measuring the current potential behavior at an electrode surface. The potential systematically varies to cause electro-active chemical species to be reduced or oxidized at the electrode surface. The resistant current is proportional to the concentration of the chemical species. Voltammetry is preferred for detecting heavy metals, possessing the advantages of low cost, simplicity, ease of operation, fast analysis, portability, and the ability to monitor environmental samples in the field, as well as high sensitivity and selectivity. Differential Pulse Stripping Voltammetry (DPASV) makes it possible to determine simultaneously several trace elements and conduct both thermodynamic and kinetic assays. ASV has been widely used for trace metal measurement in food samples [17].

2. Materials and Methods

2.1.Chemicals

Tartaric acid, CH_3COONa, $Pb(NO_3)_2$, $Cd(NO_3)_2$, $Cu(NO_3)_2$, $Zn(NO_3)_2$, and HNO_3 were purchased from Merck (Germany) in analytical grade. The metal stock solution (1 g/l) was prepared in HNO_3 0.005 M. The standard solution of heavy metals was prepared at concentration of 10 mg/l zinc, 2.5 mg/l copper, 0.5 mg/l lead, and 0.1 mg/l cadmium. Acetate buffer (0.2 M, pH = 4.7) containing tartaric acid 0.2 M was purchased from Merck (Germany), which served as supporting electrolyte.

2.2. Sample preparation

In 2021, 84 soft drink samples were purchased from the markets in Tehran, Iran. In the laboratory, 2 ml of a sample with 5 ml of nitric acid 65% was transferred to a cleaned glass vessel. The mixture was evaporated to dryness on a hot plate. The temperature of the hot plate was gradually increased from low to maximum over a period of 1-2 h. The charred sample was transferred to a muffle furnace and ashed at 450-500 °C for 24 h. Then, 1 ml nitric acid 65% and 9 ml distilled water were added to the residue, and the crucible was heated on a hot plate near boiling to complete the dissolution. The sample was transferred further to a 25-ml volumetric flask, and diluted to a volume of 25 ml.

2.3. Voltammetry determination

The polarograph set was Trace analyzer model 746 instruments (Metrohm AG Ltd., Switzerland). Its glassy cell has three electrodes, HMDE as the working electrode, axillary electrode from platinum, and Ag/AgCl as the reference electrode. The complex composition of the sample might change the calibration slope. Therefore, concentration of the elements was determined using the standard addition technique with three additions of the standard stock solution. At first, 10 ml of sodium acetate buffer was poured as an electrolyte in the cell of the polarography device. Next, 500 µl of unknown solution was added to the cell, and the polarogram was recorded. Then, 100 µl of a mixed zinc, copper, lead, and cadmium standard solution was added to the cell, and the polarogram was recorded. This operation was done three times. The concentrations were calculated using a linear regression method after the second standard addition. In applying the stripping method for food analysis, the major challenge is often the sample pretreatment, which can interfere with the organic matrix and the electrochemical processes [18].

2.4. Statistical analysis

The obtained data was subjected to analysis of variance (ANOVA) test. Differences were significant at $p \leq 0.05$. The experiments were done in three replications.

3. Results and Discussion

The results of heavy metal concentrations in selected samples of soft drinks are presented in Table 1. Table 2 shows the amounts of cadmium, lead, copper, and zinc in soft drinks with different flavors of lemon, orange, and cola. According to Table 1, the Canada Dry brand had the highest zinc concentration, and the Eram brand had the lowest zinc content. The Coca-Cola brand had the highest amount of copper, and the Zamzam brand had the lowest amount of copper. Coca-Cola and Zamzam brands had the highest and lowest lead levels, respectively. Zamzam and Coca brands had the highest and lowest amounts of cadmium, respectively. The concentrations of zinc, copper, lead, and cadmium significantly differed from the permissible limit in different soft drinks ($p<0.05$). According to Table 2, the amount of zinc in the orange-flavored soft drink samples was higher than in the cola flavors. Also, the quality of the lemon-flavored soft drink samples in concentration of zinc was better than that of the cola flavor. The amount of zinc and copper in lemon-flavored soft drink samples was higher than in orange-flavored soft drink samples. Cola-flavored soft drink contained the lowest cadmium, and lemon-flavored soft drink had the lowest lead. According to Table 2, the soft drinks had the highest concentration of zinc and copper. According to the World Health Organization, zinc and copper content greater than 3.0 mg/l and 2.0 mg/l, respectively, is not acceptable for drinking water. The limit of lead in drinking water was 0.01 mg/l according to WHO. Furthermore, the permissible limit for Cd in drinking water is less than 0.003 mg/l [19]. As seen in the tables, concen tration of all heavy metals is far from the maximum permitted levels determined by WHO for drinking water that is of great concern.

Table 1- Concentration of heavy metals (mg/100 g; mean ±SD) in soft drinks

Brand	N	Zn	Cu	Pb	Cd
Coca-Cola	18	30.80 ±22.14	4.15 ±3.30	0.72 ±0.68	0.20 ±0.19
Pepsi	18	30.94 ±22.34	2.63 ±2.00	0.66 ±0.49	0.23 ±0.19
Zamzam	18	30.14 ±24.20	1.77 ±0.78	0.52 ±0.27	0.28 ±0.17
Eram	12	24.35 ±9.39	4.14 ±3.40	0.60 ±0.41	0.21 ±0.12
Canada Dry	18	39.00 ±20.39	2.81 ±1.49	0.58 ±0.50	0.24 ±0.14

Table 2- Concentration of heavy metals (mg/100 g) in soft drinks with different flavors

	Flavor	N	Mean	Standard Deviation
Cd	Cola	29	0.21	0.13
	Orange	30	0.25	0.12
	Lemon	25	0.22	0.10
Pb	Cola	29	0.61	0.42
	Orange	30	0.69	0.40
	Lemon	25	0.54	0.29
Zn	Cola	29	21.88	15.63
	Orange	30	35.27	18.29
	Lemon	25	38.18	16.75
Cu	Cola	29	2.39	1.19
	Orange	30	2.69	1.23
	Lemon	25	3.49	1.31

In one study done in 2000, several beverages and soft drinks available in the markets of Nigeria were analyzed for their content of heavy metals, including cadmium, cobalt, chromium, copper, iron, nickel, lead, and zinc. The beverage types were grouped into tea, cocoa-based, caffeine, cereal-based, dairy products, fruit juices, malt drinks, carbonated soft drinks, and wines (non-alcoholic). In that study, the levels of metals were generally low and within the statutory safe limits [20]. In another study in Nigeria (1997), the concentration of cobalt, manganese, copper, zinc, cadmium, lead, chromium, selenium, and nickel in mineral water and soft drinks was determined. The mean levels of the elements ranged between 0.52 pg/l for cobalt and 14.8 pg/l for manganese in the bottled waters, and 3.10 pg/l for cobalt and 82.4 pg/l for manganese in the soft drinks. Accordingly, the concentration of metals in the water samples was lower than the permissible limit of WHO [21]. Francisco et al. (2015) showed a method for directly determining of aluminum, copper, chromium, iron, and nickel in Brazilin carbonated soft drinks using electrothermal atomic absorption spectrometry (ETAAS). Samples of different flavors packed in containers made of different materials (polyethylene terephthalate and glass bottles, aluminum and steel cans) were analyzed. The method was optimized by building up pyrolysis and atomization curves in the sample medium and evaluating the calibration approach. Furthermore, the impact of the material used in the packaging and flavor on the concentrations of each metal in the samples was evaluated. It was shown that neither flavor nor packaging material affected the concentration of aluminum and iron in the samples. On the other hand, the packaging material influenced the concentration of copper, chromium, and nickel, and the flavor only affected the concentration of copper in the samples [22]. In 2021, Jannat et al. conducted a study on the concentration of heavy metals in pickled cucumbers by polarography. They showed that lead and cadmium concentrations were significantly higher than the standard permissible limit [23]. Study of Sadeghi et al. in 2017 on concentration of heavy metals in non-alcoholic malt beverages showed the lower concentration of zinc, copper, lead, and cadmium than the Codex Alimentarius permissible limit [24]. In our study, concentration of all heavy metals was higher than the maximum permitted levels determined by WHO for drinking water. Therefore, regular monitoring of raw materials for soft drinks and the production processes is recommended to drastically reduce the transference of heavy metals into the finished products.

4. Conclusion

Soft drinks available in the Iranian market have potential risk for human health based on the concentration of heavy metals. These elements can enter soft drinks during the production and packaging process. Heavy metals, including lead and cadmium, are transferred from polyethylene terephthalate packaging to soft drinks during long-term storage and heating. Other heavy metals such as zinc and copper may enter the soft drinks from the flavor. In the current work, all the heavy metals posed a significant risk as their levels exceeded the WHO safe limit. Thus, there is a need for regulatory bodies to monitor and control the quality of soft drinks in order to ensure safe consumption and minimize the possible underlying risk.

5. Acknowledgements

This work was a student thesis supported by a grant (no. 93-02-33-25309) from Tehran University of Medical Sciences.

6. Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Wang J, Analytical electrochemistry. Second edition. Wiley-VCH, New York. 2000.

2. Duruibe JO, Ogwuegbu MOC, Egwurugwu JN. Heavy metal pollution and human bio-toxic effects. International Journal of Physical Sciences. 2007; 2(5): 112-118.

3. Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ. Heavy metal toxicity and the environment. In: Luch A. (ed.). Molecular, clinical and environmental toxicology. Springer, Basel. 2012. https://doi.org/10.1007/978-3-7643-8340-4_6

4. Antoine JMR, Fung LAH, Grant CN. Assessment of the potential health risks associated with the aluminum, arsenic, cadmium and lead content in selected fruits and vegetables grown in Jamaica. Toxicology Reports. 2017; 4: 181-187. https://doi.org/10.1016/j.toxrep.2017.03.006

5. Nyika OM, Onyari EK, Dinka MO, Mishra SB. Heavy metal pollution and mobility in soils within a landfill vicinity: a south African case study. Oriental Journal of Chemistry. 2019; 35(4): 1286-1296. https://doi.org/10.13005/ojc/350406

6. Wang S, Shi X. Molecular mechanisms of metal toxicity and carcinogenesis. Molecular and Cellular Biochemistry. 2001; 222: 3-9.

7. Ogoko EC, Aletan UI, Osu CI, Kelle HI, Iroegbulem I, Emeziem D, et al. Hazard and health risk assessment of heavy metals in non-alcoholic beverage consumed in Abia State, Nigeria. Journal of Applied Sciences and Environmental Management.2024; 28(9): 2575-2583. https://doi.org/10.4314/jasem.v28i9.1

8. Gomaa NAR, Mohamed BMA, Bassem AS, Safaa SMA. Heavy metals content in some non-alcoholic beverages (carbonated drinks, flavored yogurt drinks, and juice drinks) of the Egyptian markets. Toxicology Reports. 2019; 6: 210-214. https://doi.org/10.1016/j.toxrep.2019.02.010

9. Toni LD, Tisato F, Seraglia R, Roverso M, Gandin V, Marzano C, et al. Phthalates and heavy metals as endocrine disruptors in food: a study on pre-packed coffee products. Toxicology Reports. 2017; 4: 234-239. https://doi.org/10.1016/j.toxrep.2017.05.004

10. Jaishankar T, Tseten N, Anbalagan N, Mathew BB, Beeregowda KN. Toxicity, mechanism and health effects of some heavy metals. Interdisciplinary Toxicology. 2014; 7(2): 60-72. https://doi.org/10.2478/intox-2014-0009

11. Khan N, Jeong IS, Hwang IM, Kim JS, Choi SH, Nho EY, et al. Analysis of minor and trace elements in milk and yogurts by inductively coupled plasma-mass spectrometry (ICP-MS). Food Chemistry. 2014; 147:

220-224.
https://doi.org/10.1016/j.foodchem.2013.09.147

12. Licata P, Di Bella G, Potorti AG, Lo Turco V, Salvo A, Dugo GM. Determination of trace elements in goat and ovine milk from Calabria (Italy) by ICP-AES. Food Additives & Contaminants: Part B. 2012; 5(4): 268-271. https://doi.org/10.1080/19393210.2012.705335

13. Freschi GPG, Fortunato FM, Freschi CD, Neto JAG. Simultaneous and direct determination of As, Bi, Pb, Sb, and Se and Co, Cr, Cu, Fe, and Mn in milk by electro thermal atomic absorption spectrometry. Food Analytical Methods. 2012; 5(4): 861-866. https://doi.org/10.1007/s12161-011-9323-0

14. Munoz E, Palmero S. Determination of heavy metals in milk by potentiometric stripping analysis using a home-made flow cell. Food Control. 2004; 15(8): 635-641.
https://doi.org/10.1016/j.foodcont.2003.10.006

15. Ping J, Wang Y, Wu J, Ying Y. Development of an electrochemically reduced graphene oxide modified disposable bismuth film electrode and its application for stripping analysis of heavy metals in milk. Food Chemistry. 2014; 151: 65-71.
https://doi.org/10.1016/j.foodchem.2013.11.026

16. Mahesar S, Sherazi S, Niaz A, Bhanger M, Rauf A. Simultaneous assessment of zinc, cadmium, lead and copper in poultry feeds by differential pulse anodic stripping voltammetry. Food and Chemical Toxicology. 2010; 48(8): 2357-2360.
https://doi.org/10.1016/j.fct.2010.05.071

17. Sadeghi N, Oveisi MR, Jannat B, Hajimahmoodi M, Behfar A, Behzad M, et al. Simultaneous measurement of zinc, copper, lead and cadmium in baby weaning food and powder milk by DPASV. Iranian Journal of Pharmaceutical Research. 2014; 13(1): 345-349.

18. Jannat B, Sadeghi N, Oveisi M, Behfar A, Komeili Zadeh H, Shafaati A. Simultaneous determination of lead, cadmium, copper and zinc in infant formula by Anodic stripping voltammetry. Iranian Journal of Pharmaceutical Research. 2009; 8: 159-162.
https://doi.org/10.22037/ijpr.2010.805

19. World Health Organization. Guidelines for drinking water quality. Fourth edition. Geneva, Switzerland. 2011. Available at: https://iris.who.int/bitstream/handle/10665/44584/9789241548151_eng.pdf?sequence=1

20. Yadav PK, Sharma RM. The role of voltametric methods in determination of metals in alcoholic beverages: a critical review. Arab Journal of Forensic Sciences and Forensic Medicine. 2019; 1(9): 1232-1247.
https://doi.org/10.26735/16586794.2019.010

21. Ogunlana OO, Ogunlana OE, Akinsanya AE, Ologbenla OO. Heavy metal analysis of selected soft drinks in Nigeria. Journal of Global Biosciences. 2015; 4(2): 1335-1338.

22. Francisco BBA, Brum DM, Cassella RJ. Determination of metals in soft drinks packed in different materials by ETAAS. Food Chemistry. 2015; 185: 488-494.
https://doi.org/10.1016/j.foodchem.2015.04.020

23. Jannat B, Mirshamsi S, Sadeghi N, Oveisi MR, Hajimahmoodi M. Measuring the levels of Zn, Cu, Pb and Cd via the polarography method in fermentative pickled cucumbers purchased from Tehran market, Iran. Journal of Food Safety and Hygiene. 2021; 7(1): 52-59.
https://doi.org/10.18502/jfsh.v7i1.7844

24. Sadeghi N, Jodakhanlou M, Oveisi MR, Jannat B, Behzad M, Hajimahmoodi M. Determination of zinc and copper micronutrients and lead and cadmium contaminants in non-alcoholic malt beverages by anodic stripping voltammetry. Journal of Food Safety and Hygiene. 2017; 3(3-4): 54-58.