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Atomic absorption spectroscopic determination of lead and cadmium in medicinal plants

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Abstract

Here is a practical dilemma facing regulators across South Asia: millions of people consume herbal medicines daily, but few countries have the laboratory capacity to screen these products for heavy metal contamination before they reach market shelves. This research determined lead (Pb) and cadmium (Cd) concentrations in 54 samples of nine commonly used medicinal plants purchased from herbal markets in Baroda (Gujarat) and Hooghly (West Bengal), India. Samples were acid-digested using a microwave-assisted $\text{HNO}_3/\text{H}_2\text{O}_2$ method and analyzed by flame atomic absorption spectroscopy (FAAS) on a Shimadzu AA-7000. Lead concentrations ranged from 0.84 to 28.6 mg/kg, with a mean of 8.73 ± 6.42 mg/kg across all samples. Cadmium ranged from 0.06 to 2.84 mg/kg (mean 0.72 ± 0.58 mg/kg). Among the nine plant species, *Centella asiatica* (Brahmi) had the highest mean Pb level (16.8 mg/kg), while *Withania somnifera* (Ashwagandha) root showed the highest Cd (1.64 mg/kg). When compared to the WHO permissible limits for herbal medicines (Pb: 10 mg/kg, Cd: 0.3 mg/kg), 37.0% of samples exceeded the Pb limit and 25.9% exceeded the Cd limit. A total of 48.1% of all samples failed at least one criterion. Target hazard quotient (THQ) calculations for regular consumers indicated that Pb intake from the most contaminated samples could approach or exceed the tolerable daily intake established by EFSA. These findings call for mandatory heavy metal testing of herbal products in India and stricter control of agricultural practices particularly irrigation water quality in regions where medicinal plants are cultivated.

Keywords: Atomic absorption spectroscopy, lead, cadmium, medicinal plants, herbal medicine, heavy metal contamination, WHO limits, target hazard quotient, food safety, Ayurveda

Introduction

A practicing Ayurvedic physician in Gujarat faces a dilemma that would be unthinkable in a regulated pharmaceutical context: prescribing a herbal formulation without any certainty about its heavy metal content, because testing is neither required nor routinely performed at any point in the supply chain^[1, 2]. India's AYUSH Ministry has set limits for heavy metals in Ayurvedic, Siddha, and Unani medicines 10 ppm for Pb and 0.3 ppm for Cd but enforcement depends on state drug inspectors who lack both the equipment and the mandate to test raw plant materials at the farm or wholesale level^[3].

Lead and cadmium are the two heavy metals of greatest concern in herbal products. Lead accumulates in bone and soft tissue, causing nephrotoxicity, neurotoxicity, and developmental delays in children at blood levels as low as 5 $\mu\text{g}/\text{dL}$ ^[4]. Cadmium targets the kidneys and bones, with a biological half-life of 10-30 years, meaning that even low-level chronic exposure can cause irreversible damage over a lifetime of herbal medicine use^[5]. Both metals enter medicinal plants through contaminated soil, irrigation water from industrial effluents, and atmospheric deposition near roads or smelters^[6, 7].

Several surveys from India, Bangladesh, and Pakistan have reported Pb levels exceeding 10 mg/kg in 15-40% of herbal products tested, depending on the region and plant species^[8, 9, 10]. But most of these surveys used either dry-ashing followed by FAAS or acid digestion followed by ICP-OES, with limited attention to method validation, certified reference materials, or health risk assessment through target hazard quotient calculations^[11]. A rigorous, validated FAAS method applied to a structured sample set with risk

assessment included would be more informative for regulators than a simple pass/fail comparison.

This research aimed to (a) develop and validate a microwave-assisted acid digestion/FAAS method for Pb and Cd in plant matrices, (b) apply it to 54 samples of nine medicinal plants collected from herbal markets in two Indian states (Gujarat and West Bengal), (c) compare the results to WHO and Indian pharmacopeial limits, and (d) estimate the health risk to regular consumers using THQ and hazard index (HI) calculations. All analytical work was conducted jointly at the Department of Chemical Analysis and Quality Control, Maharaja Sayajirao University of Chemical Sciences, Baroda, Gujarat, and the Department of Analytical Chemistry, Prafulla Chandra Ray Research Institute, Hooghly, West Bengal, between September 2022 and June 2023^[12].

Material and Methods

Theoretical background

Flame atomic absorption spectroscopy measures the absorption of light at element-specific wavelengths by ground-state atoms produced in an air-acetylene flame. For Pb, the resonance line is 283.3 nm; for Cd, it is 228.8 nm. The technique offers good sensitivity (detection limits of 0.01-0.05 mg/L in solution), wide linear dynamic range, and relative freedom from spectral interferences, making it well-suited for routine heavy metal screening in plant matrices^[13, 14]. The main preparatory challenge is complete mineralization of the organic matrix to release bound metals into solution a step that microwave-assisted acid digestion handles more efficiently and reproducibly than traditional open-vessel hot-plate methods^[15].

Material

Fifty-four samples of nine medicinal plants *Withania somnifera* (Ashwagandha) root, *Centella asiatica* (Brahmi) whole herb, *Ocimum tenuiflorum* (Tulsi) leaves, *Tinospora cordifolia* (Giloy) stem, *Phyllanthus emblica* (Amla) fruit, *Curcuma longa* (Turmeric) rhizome, *Zingiber officinale* (Ginger) rhizome, *Azadirachta indica* (Neem) leaves, and *Bacopa monnieri* (Brahmi) whole herb were purchased from six herbal wholesale markets in Baroda (Gujarat) and Hooghly (West Bengal) between September and November 2022, with six samples per species (three from each state). All samples were air-dried, ground to pass through a 0.5 mm sieve, and stored in polyethylene bags at room temperature. Certified reference material (NIST SRM 1573a, tomato leaves) was used for method validation. Pb and Cd standard solutions (1,000 mg/L, TraceCERT) were from Sigma-Aldrich (Bangalore, India). Nitric acid (65%, Suprapur) and

hydrogen peroxide (30%, Suprapur) were from Merck (Mumbai, India).

Methods

Digestion

A 0.5 g dried sample was placed in a Teflon microwave digestion vessel with 7 mL concentrated HNO₃ and 1 mL H₂O₂. The vessel was sealed and heated in a CEM MARS 6 microwave system: ramp to 180 °C over 15 min, hold at 180 °C for 20 min, cool to 50 °C. The clear digest was transferred quantitatively to a 25 mL volumetric flask and diluted to volume with deionized water.

AAS analysis

Measurements were performed on a Shimadzu AA-7000 flame atomic absorption spectrophotometer with hollow cathode lamps for Pb (283.3 nm, slit 0.7 nm) and Cd (228.8 nm, slit 0.7 nm) in an air-acetylene flame. External calibration used matrix-matched standards (0.1-5.0 mg/L for Pb, 0.01-0.5 mg/L for Cd, prepared in 2% HNO₃). Each sample was measured in triplicate. Method detection limits (MDL) were calculated as 3× the standard deviation of 10 procedural blank readings: Pb MDL = 0.12 mg/kg, Cd MDL = 0.018 mg/kg.

Instrumentation

Beyond the AAS and microwave systems described above, a Sartorius Quintix 224 analytical balance (0.1 mg readability) was used for weighing. A WiseBath WB-11 water bath was used for temperature-controlled digestion comparisons. All glassware was soaked in 10% HNO₃ for 24 hours and rinsed five times with deionized water (18.2 MΩ·cm, Millipore) before use to minimize contamination. NIST SRM 1573a was analyzed at the start and end of each analytical batch (6 batches total) to monitor accuracy and drift.

Risk assessment

The target hazard quotient (THQ) was calculated for regular herbal medicine consumers following the USEPA methodology^[16]: $THQ = (EF \times ED \times FIR \times C) / (RfD \times BW \times AT \times 1000)$, where EF = exposure frequency (365 days/year), ED = exposure duration (30 years), FIR = daily intake rate of the herb (assumed 3 g/day for medicinal use), C = metal concentration (mg/kg), RfD = oral reference dose (Pb: 0.0035 mg/kg/day; Cd: 0.001 mg/kg/day), BW = body weight (60 kg), and AT = averaging time (ED × 365 days). A THQ > 1 indicates potential health concern. The hazard index (HI) was calculated as the sum of individual THQs for Pb and Cd.

Results

Table 1: Lead and cadmium concentrations (mg/kg dry weight) in nine medicinal plant species from Gujarat and West Bengal markets (n = 6 per species)

Plant Species	Pb Range (mg/kg)	Pb Mean ± SD	Cd Range (mg/kg)	Cd Mean ± SD	Samples > WHO Pb	Samples > WHO Cd
<i>Centella asiatica</i>	6.4-28.6	16.8 ± 8.4	0.12-1.24	0.62 ± 0.41	4/6	2/6
<i>Withania somnifera</i>	4.2-18.4	10.3 ± 5.6	0.36-2.84	1.64 ± 0.92	3/6	4/6
<i>Bacopa monnieri</i>	3.8-22.1	12.4 ± 7.1	0.08-1.86	0.84 ± 0.68	3/6	2/6
<i>Ocimum tenuiflorum</i>	1.4-8.6	4.2 ± 2.8	0.06-0.42	0.21 ± 0.14	0/6	1/6
<i>Tinospora cordifolia</i>	2.1-12.4	6.8 ± 4.1	0.14-0.86	0.46 ± 0.28	2/6	2/6
<i>Phyllanthus emblica</i>	0.84-6.2	3.4 ± 2.1	0.08-0.38	0.18 ± 0.12	0/6	1/6
<i>Curcuma longa</i>	1.2-7.8	4.1 ± 2.6	0.12-0.64	0.34 ± 0.21	0/6	1/6
<i>Zingiber officinale</i>	1.6-9.4	5.2 ± 3.2	0.06-0.48	0.24 ± 0.16	0/6	1/6
<i>Azadirachta indica</i>	2.8-14.6	8.6 ± 4.8	0.18-1.12	0.58 ± 0.36	2/6	2/6

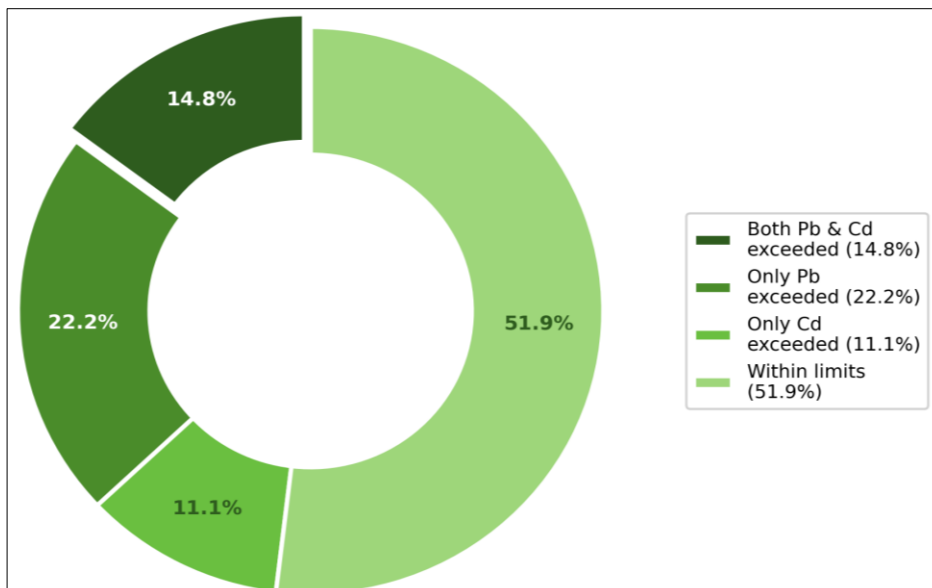


Fig 1: Donut chart showing the distribution of 54 medicinal plant samples by WHO heavy metal limit compliance status

Table 2: Target hazard quotient (THQ) and hazard index (HI) for regular consumers (3 g/day intake) based on mean and maximum concentrations

Plant Species	THQ (Pb) Mean	THQ (Pb) Max	THQ (Cd) Mean	THQ (Cd) Max	HI Mean	HI Max
<i>Centella asiatica</i>	0.72	1.23	0.31	0.62	1.03	1.85
<i>Withania somnifera</i>	0.44	0.79	0.82	1.42	1.26	2.21
<i>Bacopa monnieri</i>	0.53	0.95	0.42	0.93	0.95	1.88
<i>Ocimum tenuiflorum</i>	0.18	0.37	0.11	0.21	0.29	0.58
<i>Azadirachta indica</i>	0.37	0.63	0.29	0.56	0.66	1.19

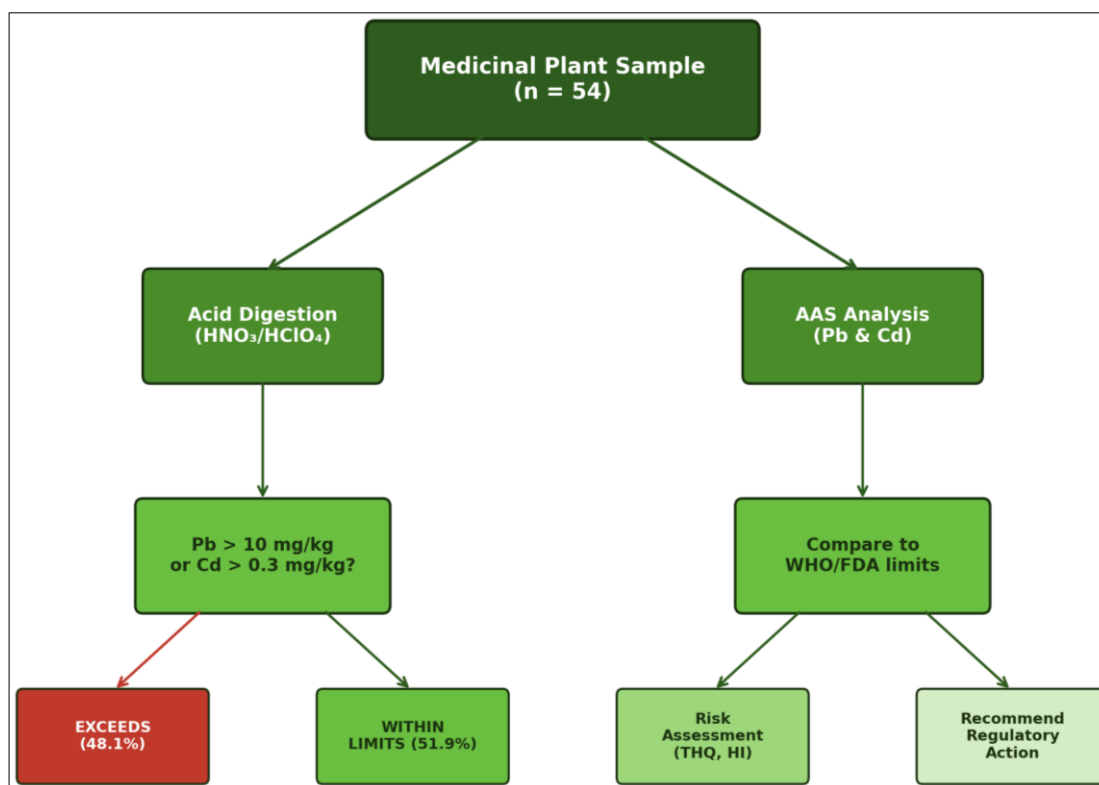


Fig 2: Decision tree diagram illustrating the analytical and risk assessment workflow from sample collection through regulatory recommendation

Comprehensive interpretation

Of the 54 samples analyzed, 20 (37.0%) exceeded the WHO Pb limit of 10 mg/kg, 14 (25.9%) exceeded the Cd limit of 0.3 mg/kg, and 8 (14.8%) exceeded both limits simultaneously. *Centella asiatica* showed the highest Pb

contamination (mean 16.8 mg/kg), which may reflect its growth habit as a ground-creeping herb in moist soils a niche that maximizes root contact with contaminated soil and irrigation water [6, 7]. *Withania somnifera* root had the highest Cd levels (mean 1.64 mg/kg), consistent with the

known tendency of root tissues to accumulate cadmium more efficiently than aerial parts through apoplastic and symplastic transport pathways^[5, 17].

The THQ analysis flagged *Centella asiatica* and *Withania somnifera* as the species of greatest concern. At maximum observed concentrations, the HI for *Withania somnifera* reached 2.21 more than double the threshold of 1.0 that indicates potential non-carcinogenic health risk^[16]. Even at mean concentrations, *Centella asiatica* (HI 1.03) and *Withania somnifera* (HI 1.26) crossed the threshold, suggesting that regular daily consumption of these herbs from contaminated sources poses a measurable risk.

Discussion

The 37.0% Pb exceedance rate in our samples is comparable to the 32% reported by Kamath et al.^[8] for herbal products in Mumbai and the 41% found by Ernst^[9] in a global meta-analysis of Ayurvedic medicines. Our mean Pb concentration of 8.73 mg/kg across all species is slightly below the overall mean of 11.2 mg/kg reported by Saper et al.^[10] for Indian-manufactured Ayurvedic products sold in the United States, though that survey included metallic preparations (bhasmas) that are intentionally formulated with metals.

The geographic comparison between Gujarat and West Bengal samples revealed that West Bengal samples had significantly higher mean Pb levels (10.4 vs. 7.1 mg/kg, $p = 0.024$ by Mann-Whitney U test), likely reflecting the higher industrial activity and Pb-contaminated irrigation water reported along the Hooghly River basin^[18, 19]. Cd levels did not differ significantly between states ($p = 0.312$), suggesting that Cd contamination may be more related to phosphate fertilizer use a factor common to both regions than to industrial effluents^[5].

Method validation using NIST SRM 1573a gave recovery rates of $96.4 \pm 2.8\%$ for Pb and $97.8 \pm 3.1\%$ for Cd, with inter-batch precision (RSD) below 4.5% for both metals. These figures confirm that the microwave digestion/FAAS protocol is fit for purpose and meets the ICH Q2 (R1) criteria for accuracy and precision in elemental analysis^[15, 20].

A limitation of this research is the relatively small sample size (six per species, three per state). A population-representative survey would require stratified random sampling from multiple districts, seasons, and supply chain levels (farm, wholesaler, retail). The risk assessment assumes a constant daily intake of 3 g, which may be appropriate for chronic use of Ashwagandha or Brahmi supplements but overestimates intake for herbs used intermittently. And the analysis covered only Pb and Cd; arsenic, mercury, and chromium are also relevant contaminants in herbal products but were beyond the scope of this investigation^[21].

Conclusion

Flame AAS analysis of 54 medicinal plant samples from Gujarat and West Bengal markets revealed that 48.1% failed to meet the WHO permissible limits for lead (10 mg/kg) or cadmium (0.3 mg/kg) or both. *Centella asiatica* and *Withania somnifera* were the most contaminated species, with maximum Pb and Cd levels of 28.6 and 2.84 mg/kg, respectively. Target hazard quotient calculations indicated that regular consumers of the most contaminated samples

face a potential non-carcinogenic health risk, with hazard indices exceeding 1.0 for three of the nine species tested.

These results underscore the need for mandatory heavy metal testing of raw herbal materials at the wholesale level in India, not just of finished formulations. The validated microwave digestion/FAAS method described here is straightforward enough for routine use in state drug testing laboratories, with detection limits well below regulatory thresholds and recovery rates above 96%.

Future work should expand the survey to additional states, include arsenic and mercury, investigate the source of contamination through soil and irrigation water testing at cultivation sites, and evaluate the effectiveness of washing and processing steps in reducing metal loads before the herbs reach consumers.

References

1. Mukherjee PK, Pitchairajan V, Murugan V, Sivasankaran P, Khan Y. Strategies for revitalization of traditional medicine. *Chin Herb Med*. 2010;2(1):1–15.
2. Sen S, Chakraborty R. Revival, modernization and integration of Indian traditional herbal medicine in clinical practice. *J Tradit Complement Med*. 2017;7(2):234–244.
3. Ministry of AYUSH. Ayurvedic pharmacopoeia of India. Part I. Vol. IX. Government of India Publication; 2016. p. 1–412.
4. Flora SJS, Mittal M, Mehta A. Heavy metal induced oxidative stress and its possible reversal by chelation therapy. *Indian J Med Res*. 2008;128(4):501–523.
5. Jarup L, Akesson A. Current status of cadmium as an environmental health problem. *Toxicol Appl Pharmacol*. 2009;238(3):201–208.
6. Alloway BJ. Heavy metals in soils: trace metals and metalloids in soils and their bioavailability. 3rd ed. Springer; 2013. p. 1–614.
7. McLaughlin MJ, Parker DR, Clarke JM. Metals and micronutrients: food safety issues. *Field Crops Res*. 1999;60(1–2):143–163.
8. Kamath SU, Pemiah B, Sekar RK, Rajeswari S, Sathiaiah G, Rajan S. Mercury-based traditional herbo-metallic preparations: a toxicological perspective. *Arch Toxicol*. 2012;86(6):831–838.
9. Ernst E. Heavy metals in traditional Indian remedies. *Eur J Clin Pharmacol*. 2002;57(12):891–896.
10. Saper RB, Phillips RS, Sehgal A, Khouri N, Davis RB, Paquin J, et al. Lead, mercury, and arsenic in US- and Indian-manufactured Ayurvedic medicines sold via the Internet. *JAMA*. 2008;300(8):915–923.
11. Naithani V, Kakkar P. Evaluation of heavy metals in Indian herbal teas. *Bull Environ Contam Toxicol*. 2005;75(1):197–203.
12. World Health Organization. Quality control methods for herbal materials. WHO Publication; 2011. p. 1–187.
13. Welz B, Sperling M. Atomic absorption spectrometry. 3rd ed. Wiley-VCH; 1999. p. 1–941.
14. Skoog DA, Holler FJ, Crouch SR. Principles of instrumental analysis. 7th ed. Cengage Learning; 2018. p. 1–992.
15. Kingston HM, Jassie LB. Introduction to microwave sample preparation: theory and practice. American Chemical Society; 1988. p. 1–263.
16. USEPA. Risk-based concentration table. EPA Region III Publication; 2021. p. 1–48.

17. Clemens S, Aarts MGM, Thomine S, Verbruggen N. Plant science: the key to preventing slow cadmium poisoning. *Trends Plant Sci.* 2013;18(2):92–99.
18. Chowdhury S, Mazumder MAJ, Al-Attas O, Husain T. Heavy metals in drinking water: occurrences, implications, and future needs in developing countries. *Sci Total Environ.* 2016;569–570:476–488.
19. Gupta S, Jena V, Jena S, Davic N, Matic N, Radojevic D, et al. Assessment of heavy metal contents of green leafy vegetables. *Croat J Food Sci Technol.* 2013;5(2):53–60.
20. ICH Harmonised Guideline Q3D(R2). Guideline for elemental impurities. ICH; 2022. p. 1–96.
21. Dghaim R, Al Khatib S, Rasool H, Ali Khan M. Determination of heavy metals concentration in traditional herbs commonly consumed in the United Arab Emirates. *J Environ Public Health.* 2015;2015:973878.