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Full Paper

Analysis of Trace Element Composition in Leafy Vegetables in Sri Lanka: Implications for Human Diet

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Abstract

Leafy vegetables (LV) are a vital component of the Sri Lankan diet due to their richness in vitamins, minerals, fiber, and other essential nutrients. However, concerns have been raised about their potential contamination with trace elements, including heavy metals, which may pose health risks. Some heavy metals, such as cadmium (Cd), arsenic (As), and lead (Pb), have been controversially associated with health issues, including chronic kidney disease of unknown etiology (CKDu). However, their causal role remains under investigation. This study aimed to analyze the concentrations of As, Cd, and Pb in three commonly consumed leafy vegetables in Sri Lanka—kankun (*Ipomoea aquatica*), mukunuwenna (*Alternanthera sessilis*), and gotukola (*Centella asiatica*)—sourced from local markets in multiple districts. Samples were prepared using microwave digestion and analyzed via Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Results revealed that, trace element concentrations in the order of Pb > Cd > As, with gotukola showing the highest Pb (7.24 mg/kg) and Cd (2.45 mg/kg) levels. Over 80% of samples exceeded Codex maximum limits for Pb, while all the samples analyzed were within acceptable limits for As. These findings highlight the need for routine monitoring and regulatory control of trace elements in leafy vegetables to ensure food safety. The study underscores potential dietary exposure risks and supports informed policy decisions regarding agricultural practices and public health interventions.

Keywords: arsenic, cadmium, lead, trace elements, leafy vegetables, Sri Lanka, ICP-MS, food safety

Introduction

Heavy metal contamination has emerged as a pressing environmental and public health concern due to its persistence, bio accumulative nature, and potential toxicity to humans and ecosystems [1]. Besides the potential to cause harm to other parts of the body, trace elements often induce toxicity to the kidneys, brain, liver, skin, and heart. These metallic elements are classified as systematic toxicants since their known to cause damage to numerous organs even at low exposure levels [2]. These elements can accumulate in soil, water, and food systems through both natural geological processes and human activities, including mining, industrial discharge, and agricultural inputs such as phosphate fertilizers. Phosphatic fertilizers are the main source of fertilizer derived heavy metal provider to the soil [1]. Unlike

organic pollutants, heavy metals are not biodegradable and can persist in the environment for extended periods, often entering the food chain through plant uptake.

Dietary exposure is a primary route through which humans ingest heavy metals, particularly in agricultural communities where crops are grown using contaminated water or soil [3]. Chronic exposure to heavy metals has been associated with a wide range of health effects including renal dysfunction, neurological disorders, cardiovascular problems, and cancers of the bladder, lungs, and skin [4]. While the exact cause of Chronic Kidney Disease of unknown etiology (CKDu) remains under investigation in regions like Sri Lanka, cadmium and other trace elements have been suspected contributors due to their nephrotoxic potential [4]. However, this association remains controversial and unconfirmed by clinical studies, highlighting the need for cautious interpretation. According to the human health risk assessment studies conducted in Bangladesh for vegetables such as amaranth, spinach, they have revealed that the incremental lifetime cancer risk (ILCR) and hazard index for lead in some of studied vegetable samples were greater than the threshold values [5].

In Sri Lanka, leafy vegetables—commonly referred to as green leaves—are a staple component of the diet and serve as a key source of micronutrients, especially in rural and low-income communities. Promoting the consumption of daily intake of vegetables as per the FAO/WHO recommendations is campaigned in most developed countries too. Therefore, the cultivating of leafy vegetables for commercial purposes has been increased remarkably and the cultivation sites are most probably along the river banks, besides paddy fields and banks of irrigation ponds [6]. However, their cultivation in areas with contaminated water sources or excessive agrochemical application raises food safety concerns. Water from irrigation lands and soil from cultivation sites also revealed the presence of heavy metals like cadmium, nickel and copper other than in leafy vegetables [7]. Trend of adsorption of cadmium from soil is more readily in vegetables, rice, cereals and potatoes than other crops [8].

Though there are no regulatory limits itself to Sri Lanka, there are established tolerable limits for heavy metals in leafy vegetables lineup with recommendations from World Health Organization (WHO) and Food and Agriculture Organization (FAO). These limits are used to assess the safety of food crops, including leafy vegetables, for human consumption.

Despite growing awareness, there is a paucity of comprehensive data on trace element concentrations in leafy vegetables across multiple regions in Sri Lanka. Most of the previous studies have basically focused on the North Central and Western provinces, due to concerns related to chronic kidney disease of unknown etiology (CKDu) hotspots and urbanization respectively.

Therefore, the present study aims to assess the concentrations of arsenic, cadmium, and lead in three widely consumed leafy vegetables: *Ipomoea aquatica* (kankun), *Alternanthera sessilis* (mukunuwenna), and *Centella asiatica* (gotukola) collected from local markets in multiple districts representing the Northern, Uva, Northwestern, and Sabaragamuwa provinces in Sri Lanka. Samples were analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The findings are expected to contribute to the

understanding of dietary exposure risks, inform public health guidelines, and support regulatory efforts to ensure food safety.

Materials and Methods

Study Area and Sample Collection

Totally seven districts representing Northern (Mullativu, Mannar and Killinochchi districts), Uva (Monaragala and Badulla districts), Northwestern (Anuradhapura district), and Sabaragamuwa (Rathnapura district) provinces in Sri Lanka were selected as the study area. Table 1 illustrates the study area and number of collected samples from each district. LV samples were collected from the local markets located in roadsides open environments, some small grocery shops and from super markets. LV samples viz. gotukola (*Centella asiatica*), kankun/water spinach (*Ipomoea aquatic)* and mukunuwenna (*Alternanthera sessilis*) were collected in to clean polyethylene bags and brought to the laboratory for analysis.

Table 1. Study area (districts) and corresponding number of samples from each LV type

District	Types of LV and number of samples		
	Gotukola	Mukunuwenna	Kankun
Monaragala	3	3	3
Badulla	3	3	3
Rathnapura	3	3	3
Anuradhapura	2	2	2
Mullativu	3	3	3
Killinochchi	3	1	3
Mannar	3	3	3
Total	20	18	20

Sample Preparation for Analysis

All the green leave samples were washed separately first with tap water to remove any soil particles and secondly with distilled water. Then about 100 g of test portions of each sample was set to air dry for one day and followed by drying in a drying oven at 80 °C for 2-3 days. The oven dried samples were mechanically ground using kitchen grinder (with stainless steel cutting blades) in order to obtain a fine powder. Sub samples of 10-20 g of powder were stored in plastic containers for microwave digestion.

Closed Vessel Style Microwave Digestion

Analytical portion of nearly 0.3 g of powdered sample was weighed to the nearest 0.1 g and the weight was reordered. The weighed sample was transferred in to previously cleaned and dried microwave digestion vessel (PM 60). (The dry-mass content of food for samples of unknown composition has to be limited to no more than 0.5 g. If maximum pressure attained for this unknown is less than the vessel limit then a greater mass may be analyzed) [9].

A minimum of two Method Blanks (MBKs) were included in each digestion batch to verify the absence of contamination that may arise from the vessels. Deionized water (1 mL) was added for method blanks and optional fortified method blanks (FMB). The MBK containing vessels were placed in random vessels.

High purity nitric acid (8.0 mL) was added drop wise both to the samples and MBKs vessel liner, washing down any material on walls until it can be established that the sample would not react violently and the foaming or reaction with the acid was observed. The vessels were let to sit about 15 minutes uncovered in a clean hood until foaming sopped. Then high purity 30% (H_2O_2) 1.0 mL was added to each vessel. It was necessary to pre-digest for more than 20 minutes before adding H_2O_2 if samples foam excessively. The vessels were sealed and applied correct torque to cap (tighten pressure relief nuts) and run the digestion program as per mentioned in table 2.

Microwave Digestion Programme

Power was applied for the ramp time minutes or until control pressure or control temperature was met. If control pressure or control temperature were met before end of ramp time then program proceeded to hold time.

Table 2. Microwave digestion Programme

Step	Temperature/°C	Pressure	Ramp	Time	Power
1	170	50	5	10	90
2	200	50	1	15	90
3	50	0	1	10	0
4	50	0	1	10	0
5	50	0	1	1	0

After vessels were cooled to less than 50 °C the vessels were moved to an exhausting clean hood and vent excess pressure slowly. The digests were quantitatively transferred to a clean container (100 mL volumetric flask) and diluted the digestion solution to approximately 100 mL with deionized water and marked up to 100.0 mL. One sample per batch of run in ICP-MS was selected to digest in duplicate. A fortified analytical portion (FAP-25 ppb) per sample type was prepared for analysis by spiking with appropriate volume of the 1000 ppb multi element calibration standard mix solution (2.5 mL) in to the analytical portion in the digestion vessel. The same sample analyzed in duplicate was selected for spiking and preparation of FAP (25 ppb)

A fortified analytical solution (FAS-25 ppb) per sample type was prepared for analysis by spiking with appropriate volume of the 1000 ppb multi element calibration standard mix solution (2.5 mL) into the analytical solution in the volumetric flask. The same sample analyzed in duplicate was selected for spiking and preparation of FAS (25 ppb).

Preparation of Reagents for ICP-MS

Preparation of 2% HNO_3 acid (Reagent blank)

Conc. HNO_3 acid (Trace grade - 69% purity), 28 mL was diluted into 1000 mL volumetrically with deionized water.

Preparation of multi element calibration standard series

Intermediate standard solution of 100 µg/L was prepared by diluting stock standard solution (10 ppm) with 2% HNO_3. Multi element calibration working standards were prepared by diluting 100.0 ppb intermediate solution with 2% HNO_3. Calibration standards in the range of 1.0, 5.0, 10.0, 25.0 and 50.0 ppb solutions were prepared using the same intermediate standard solution.

Preparation of ISTD working standard solution

1000 ppb intermediate standard was prepared by diluting 10 mL of 10 ppm stock solution with 2% HNO_3 into 100 mL. Working ISTD solution (10 ppb) was prepared by diluting 1 mL of 1000 ppb intermediate standard into 100 mL with 2% HNO_3.

All the calibration standards, quality control samples were labelled and filled in to ICP-MS vials and placed in autosampler. ICP-MS method parameters are listed in table 3. Internal standards help compensate for matrix effects and instrumental drift.

Table 3. ICP-MS method parameters

Element	Monitored Isotope	Recommended ISTD	Reporting isotope	Minimum integration Time/seconds	Analysis Mode
Arsenic	^{75}As	^{74}Ge	^{75}As	0.5	Helium
Cadmium	$^{111, 114}Cd$	^{103}Rh	^{111}Cd	0.3	Helium
Lead	$^{206, 207, 208}Pb$	^{209}Bi	^{208}Pb	0.1	Helium

Instrument Setup

The instrument was tuned according to the guidelines in the manufacturer's guide. The internal standard tubing was closed during tuning. Tuning parameters such as plasma parameters, lenses parameters and cell parameters were set up. Energy discrimination used was 3.0 V and He gas flow of 4.3 mL/min was maintained. A record of instrument parameters such as sample gas flow rate, sensitivity, oxide formation, doubly charged ratio, and stability (count rate %RSD) was kept. Oxide ratio and doubly charged ions ratio were maintained < 2%.

Determination of Analyte Concentration Using External Standard Calibration Curve

Calibration standards of 1.0 ppb, 5.0 ppb, 10.0 ppb, 25.0 ppb, and 50.0 ppb were used as the calibration standards. Calibration blank also included as a point on the calibration curve (0 ppb calibrant). Respective levels of the calibration standards 0, 1, 5, 10, 25, and 50 were setup as 1, 2, 3, 4, 5, and 6, respectively.

Additionally following criteria were satisfied.

a) Linear regression correlation coefficient (r) must be ≥ 0.9975
b) Initial calibration verification (ICV) solution (20 ppb) was analyzed to verify standardization. Recovery of the ICV was calculated and maintained 100 ± 10% to proceed
c) The highest standard (50 ppb), standard blank and ICV were analyzed in order to check the adequacy of rinse time.
d) Continuing calibration verification solution (CCV) was analyzed at a frequency of 10% and at the end of the analytical sequence.
e) Instrument Measurement Performance were checked and analyzed the analytical Solutions.
f) Continuing calibration blank (CCB) was analyzed at a frequency of 10% and at the end of the analytical sequence

Minimum number of quality control samples analyzed with each analytical sequence. The analyte concentrations were reported in not more than 3 digits as follows,

$$Concentration\ \left(\frac{\mu g}{g}\right) = \frac{(S - MBK_L) \times V}{1000 \times W}$$

where,

S = Concentration of analyte in analytical solution (or dilute analytical solution) (ppb)

MBK_L = Laboratory method blank (MBK) (ppb)

W = Mass of analytical solution (g)

V= Final volume of analytical solution (mL)

Spiked recoveries are calculated as,

$$\%\ \text{Recovery} = \frac{\text{Concentration obtained for blank spike sample}}{\text{Concentration expected to spike}} \times 100\%$$

Laboratory method blank was established by averaging the several method blanks analyzed by two analysts on different days as follow,

$$\text{Laboratory Method Blank} = \frac{\text{Sum of the values obtained for blanks}}{\text{Number of blanks analyzed}}$$

Results and Discussion

Table 4 presents the heavy metal (As, Cd, and Pb) concentrations (Minimum, Maximum, average, and codex maximum limits) in mukunuwenna (*Alternanthera sessilis*), gotukola (*Centella asiatica*), and kankun (*Ipomoea aquatica*) collected and analyzed from the different local vegetable markets in selected seven districts in Sri Lanka. The observed concentrations were compared with the maximum limits in codex standards [10]. All the sample readings were subtracted by respective laboratory method blank value for each metal which was obtained by averaging number of blanks analyzed by two analysts on different days. In this study single lab validation was carried out and repeatability and the reproducibility of the method was < 10% relative standard deviation for three elements with concentrations > LOQ (Limit of Quantification). FMB recovery for As and Pb in the range of 100±10% and for Cd it was 100±20%. Recoveries of quality control samples, ICV and CCV were in the range of 100±10% while fortification recoveries of FAP and FAS were in the range of 100±20%.

As per Table 4, average arsenic concentrations in mukunuwenna, kankun, and gotukola were 0.05 mg/kg, 0.11 mg/kg, and 0.06 mg/kg, respectively. Figure 1(a), 1(b), and 1(c) depict the box plots of three heavy metals, As, Cd, and Pb, in LV viz. gotukla, kankoon and mukunuwenna. The arsenic concentrations were relatively low, with the majority of data points falling below the Codex cadmium limit of 0.2 mg/kg. The box plot shows a narrow interquartile range (IQR), indicating a low variability in the data. There was one outlier data point observed at approximately 0.9 mg/kg. There is no maximum limit stated in codex standards for As in leafy vegetables [10]. In a review paper on arsenic content in Sri Lankan foods also mention that the most of the food types were lower than the allowable limit of arsenic [11]. Of the arsenic present in the environment, only less than 20% is available as arsenites, the most toxic inorganic form of arsenic [8]. Moreover, uptake of pentavalent arsenic to the plants is prohibited by the phosphate which is most essential nutrient for plants while arsenic is non-essential and both compete to same sorption site [12]. This could be a one of the reasons for reported arsenic levels to be in lower than the allowable limit.

As per the Table 4, average cadmium concentrations in mukunuwenna, kankun and gotukola were 0.22 mg/kg, 0.15 mg/kg, and 0.39 mg/kg, respectively. The highest cadmium concentration was obtained in a gotukola sample, viz. 2.45 mg/kg. The Codex maximum limit for leafy vegetables is 0.2 mg/kg, and about 42% of the total analyzed leafy vegetable samples were higher in cadmium content than the codex maximum limit. The box plots in Figure 1(a), 1(b), and 1(c) similarly exhibited low cadmium concentrations as arsenic, with the box and whiskers entirely below the Codex maximum limit. The median concentration was very close to zero, and the IQR was narrow. A few outlier data points were present, with the highest outlier reaching approximately 0.85 mg/kg. A previous study conducted in two seasons has found comparatively higher mean cadmium level in roots of *Alternanthera sessilis*, viz. 1.98±0.72 mg/kg for dry season and 1.98±0.03 mg/kg for rainy season [13]. Data published for green leaves marketed in the Colombo district, Piliyandala area reported a range of 0.07-0.97 mg/kg [14]. Results obtained in this study are also within the range published in Kananke *et al.,* (2014), except for the highest value found for gotukola sample [14]. Bioaccumulation of cadmium in plants taken from the soil is comparatively higher than the other heavy metals [15]. Further, the study on cadmium in different media revealed that soil in lake sediments (20 samples) contained a mean cadmium content of 3.243 mg/kg [15].

This value also depicts that there is no higher cadmium levels reported in previous studies. Moreover, most of the leafy vegetable growers select their planting vegetable plots near water sources. Since the low levels of cadmium in soil means the availability for plants also less. Therefore, the possible reason for elevated cadmium in human and other higher-level carnivores could be the bio accumulation with time.

As per the Table 4, average lead concentrations in mukunuwenna, kankun, and gotukola were 1.10, 0.66, and 1.36 mg/kg, respectively. The highest concentration of lead was obtained in a gotukola sample, viz 7.24 mg/kg. The lead content in this study revealed that more than 80% of the total leafy vegetable samples analyzed have exceeded the codex maximum limit for Pb in which all the gotukola samples were above the codex maximum limit. Whereas similarly another study on heavy metals in aquatic vegetables conducted previously, also revealed that, lead content in the range of 1.01-1.36 mg/kg with exceeding codex maximum limit [6]. Besides, the study conducted by Kanake *et al.,* (2014) have shown that elevated lead of content in mukunuwenna and kankun viz. 0.18-1.32 mg/kg and 0.28-0.45 mg/kg respectively [14]. The results obtained in this study for mukunuwenna (0.23-2.75 mg/kg) also agree with the previous reported results.

Figure 1(a), 1(b), and 1(c), the box plots for the three heavy metal contents in gotukola, kankun and mukunuwenn. Box plot for lead (Pb) content in each LV type show a significantly higher concentration and greater variability compared to arsenic and cadmium. The median lead concentrations for all three LV types are well above the Codex Lead limit of 0.3 mg/kg. The plot indicates that a significant portion of the samples exceed the Codex limit for Lead. Several high-concentration outliers are also present, with the highest recorded concentration being approximately 2.8 mg/kg. Figure 1(d) shows a comparison of average concentrations of arsenic, cadmium, and lead across three sample types relative to the Codex limits. Lead was the dominant contaminant (0.8–1.4 mg/kg), exceeding the permissible limit (0.3 mg/kg) in all LV types. Cadmium (0.2–0.4 mg/kg) also surpassed the Codex limit (0.2 mg/kg) in mukunuwenna and gotukola, while arsenic remained low (<0.15 mg/kg) and within safe levels indicating that lead poses the greatest contamination risk, with cadmium as a secondary concern, suggesting common pollution sources and the need for stricter monitoring.

Heavy metals cause adverse effects not only on humans but also on soil, on water, and on plants. Impact of heavy metals on soil is based on interactions such as adsorption, cation exchange, organic complexation and co-precipitation. Soil pH also effects on adsorption of heavy metals in to plants by root system too [1]. In addition to above, the elevated concentrations of heavy metals recovered in leafy vegetables might be due to the contaminated soils and irrigation water, fertilizer and pesticides or due to the atmospheric deposition of metals on plant surfaces during their production, transportation and selling locations [14]. Uptake of heavy metals by crops is often affected upon the plant species, growth phase, type of the soil and type of metal. This study also shows the variation of metal content with type of vegetable, type of metal. With these findings, it is obvious that the heavy metal contamination in green leaves from ground level to higher level must be monitored in planned time intervals. Also it is important to implement and impose local regulations based on international regulatory standards for edible leafy vegetables by considering practical application behaviors in local farmers and sellers.

Table 4. Concentrations of trace elements presents in different types of green leaves

Sample type	Arsenic concentration in the sample (mg/Kg)	Cadmium concentration in the sample (mg/Kg)	lead concentration in the sample (mg/Kg)	Sample type	Arsenic concentration in the sample (mg/Kg)	Cadmium concentration in the sample (mg/Kg)	lead concentration in the sample (mg/Kg)	Sample type	Arsenic concentration in the sample (mg/Kg)	Cadmium concentration in the sample (mg/Kg)	lead concentration in the sample (mg/Kg)
MWN	0.06	0.23	2.66	KK	0.18	0.12	0.89	GT	0.05	0.39	1.40
MWN	0.05	0.21	0.94	KK	0.02	0.01	0.30	GT	0.04	0.40	1.24
MWN	0.01	ND	ND	KK	0.03	0.30	1.13	GT	0.08	0.45	0.86
MWN	0.05	0.15	1.08	KK	0.06	0.08	0.37	GT	0.10	0.28	1.19
MWN	0.04	0.59	2.01	KK	0.16	0.09	2.17	GT	0.15	0.16	1.80
MWN	0.07	0.11	1.22	KK	0.12	0.05	1.65	GT	0.04	2.45	0.96
MWN	0.09	0.19	1.71	KK	0.08	0.08	0.81	GT	0.05	0.55	0.70
MWN	0.08	0.33	2.75	KK	0.08	0.38	1.09	GT	0.02	0.24	0.41
MWN	0.04	0.67	1.48	KK	0.08	0.01	0.04	GT	0.03	0.18	7.24
MWN	0.02	0.24	0.42	KK	0.02	ND	ND	GT	0.10	0.25	1.73
MWN	0.06	0.05	0.36	KK	0.02	0.01	ND	GT	0.08	0.06	0.92
MWN	0.05	0.05	0.23	KK	0.05	ND	ND	GT	0.04	0.08	0.60
MWN	0.06	0.20	0.26	KK	0.14	0.02	0.29	GT	0.04	0.08	0.77
MWN	0.02	0.08	1.09	KK	0.01	0.02	0.09	GT	0.07	0.10	0.61
MWN	0.06	0.16	0.63	KK	0.04	0.01	0.19	GT	0.02	0.49	0.85
				KK	0.03	0.00	0.22	GT	0.03	0.10	0.44
				KK	0.02	0.70	2.78				
				KK	0.93	0.21	0.37				
				KK	0.06	0.85	0.34				
Minimum	0.01	0.05	0.23	Minimum	0.01	0.00	0.09	Minimum	0.02	0.06	0.41
Maximum	0.09	0.67	2.75	Maximum	0.93	0.85	2.78	Maximum	0.15	2.45	7.24
Average	0.05	**0.22**	**1.10**	Average	0.11	0.15	**0.66**	Average	0.06	**0.39**	**1.36**
Codex maximum Limit	N/A	0.2	0.3	Codex maximum Limit	N/A	0.2	0.3	Codex maximum Limit	N/A	0.2	0.3

MWN : Mukunuwanna, KK:Kankun, GT: Gotukola , N/A: Not available, ND : Not detected

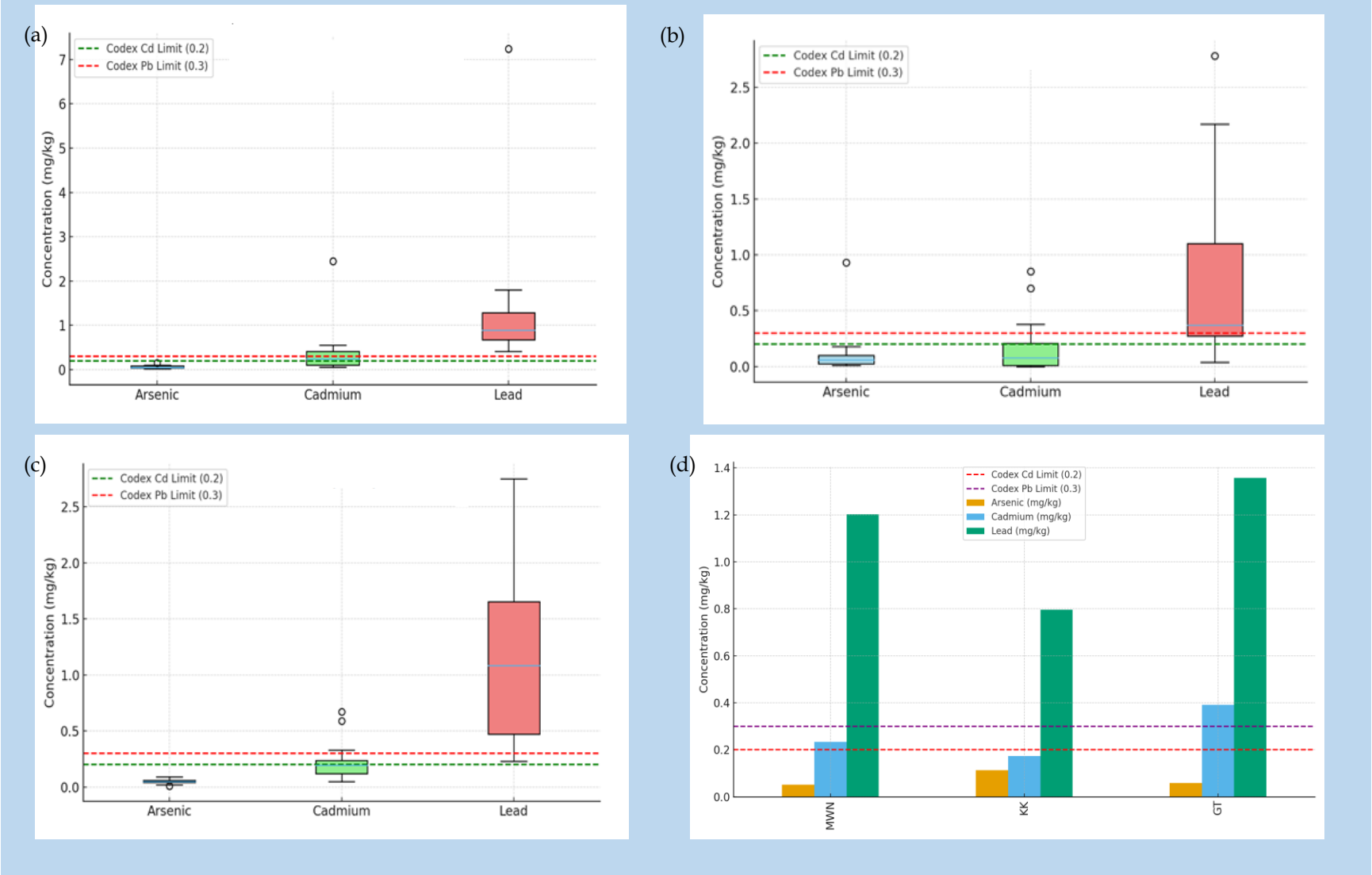

Figure 1. (a) Box plot of heavy metal content in gotukola, (b) Box plot of heavy metal content in Kankun, (c) Box plot of heavy metal content in Mukunuwenna, and (d) average heavy metal concentration across three sample types

Conclusion

The vegetables analyzed in this research viz. water spinach, gotukola, and mukunuwenna are contaminated with heavy metals especially lead in detectable quantities. Moreover, presence of trace element type is varied in the order of Pb> Cd>As. It shows that average lead content in green leaves is remarkably higher than maximum limit declared in codex standards. On the other hand arsenic in leafy vegetables is showing lower contamination when compare with other two heavy metals. Apart to that, roots have accumulated higher content of Cd and Pb when compared with stems and leaves. As discussed above, many health risks are associated with these trace elements and it is critical to implement regular monitoring of heavy metal contamination to ensure food safety.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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