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Heavy Metals Assessment in Selected Leafy Vegetables from Selangor, Malaysia

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ABSTRACT

Leafy vegetables may contain heavy metals that possess negative impacts on human health. However, no structured monitoring has been available so far in terms of the heavy metal content of vegetables sold in markets across the country. Thus, the present study aimed to investigate heavy metals concentration [aluminium (Al), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe) and lead (Pb)] in selected leafy vegetables (*Brassica oleracea* subsp. *capitata* L., *Brassica juncea* Czern., *Spinacia oleracea* L., and *Brassica rapa* var. *chinensis*) from Selangor wholesale wet markets using inductively coupled plasma–optical emission spectrometry. Potential health risks linked to their consumption were assessed by estimating daily intake of toxic metals (EDI) and calculating both cancer and non-cancer risks, including hazard index and target hazard quotient (THQ). Results showed that the average concentrations of Al and Fe in vegetable samples were within the permissible limits, with the greatest amount of Al found in spinach (41.37 mg/kg). The mean levels of Fe in cabbage, mustard, spinach, and pak choi were 6.30 ± 5.78, 4.12 ± 1.84, 13.59 ± 4.73, and 4.14 ± 0.31 mg/kg, respectively. However, Cd, Cr, Cu, and Pb were undetected in all samples. THQ values derived from the EDI of heavy metals were discovered to be less than one, suggesting a low likelihood of adverse health effects. In conclusion, although leafy vegetables present negligible health risks, consumers should vary their vegetable intake to prevent long-term health effects.

Keywords: Dietary exposure, health hazard assessment, heavy metal, inductively coupled plasma-optical emission spectrometry

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INTRODUCTION

Vegetables contain varied micronutrients and dietary fibre essential for preventing diseases and maintaining human physiological functions (Mallor, 2023). Nevertheless, vegetables are considered one of the principal routes of heavy metal exposure to human beings, especially green leafy vegetables (Martín-León et al., 2023). Toxic metals such as Cd and Pb have been shown to accumulate at higher concentrations on leaves compared to the stems and fruity varieties (Guo et al., 2020). Consuming vegetables polluted by heavy metals resulted in different disorders, including anemia, pulmonary illness, hyperactivity, male infertility, cancer, and death (Najmi et al., 2023).

Various elements contribute to the accumulation of heavy metals in plants. For instance, there is widespread application of fertilizers, the utilization of human-made sources in agricultural areas, and environmental pollutants. A report from Iran indicated higher levels of Pb in vegetables collected from the sites close to traffic highways due to the atmospheric lead deposits originating from the leaded petrol combustion in cars (Tajdar-Oranj et al., 2022). Furthermore, a study conducted by Shi et al. (2022) found high concentrations of heavy metals in leafy vegetables from the mining areas in China. The studies proved that plants absorbed heavy metals from soils and deposited them in the leave tissues.

In Malaysia, a previous dietary exposure study on leafy vegetables (pak choi, amaranth, and caisim) cultivated in Pahang, Malaysia, showed a possible health risk to consumers due to the high lifetime cancer risk (LCR) values for arsenic (As) and Pb (Sulaiman et al., 2020). Similarly, Pb and Cd concentrations in spinach (*S. oleracea*) from the conventional farms in Kuala Selangor exceeded the World Health Organization (WHO) standard for leafy vegetables (Mohamad & Kamaludin, 2019). Besides, Aweng et al. (2020) found four types of heavy metals, including Fe, Cu, zinc (Zn), and manganese (Mn) in spinach (*S. oleracea*) collected from Pasar Siti Khadijah, Kota Bharu, Kelantan. However, the concentration of heavy metals was below the maximum level allowed by the Malaysian Food Act (1983) and Food Regulation (1985) (Malaysia & International Law Book Services, 2019). Nevertheless, sustained exposure to low concentrations of heavy metals, especially for vulnerable groups such as children, still needs attention. Therefore, ensuring the safety of leafy vegetables remains a significant public health priority.

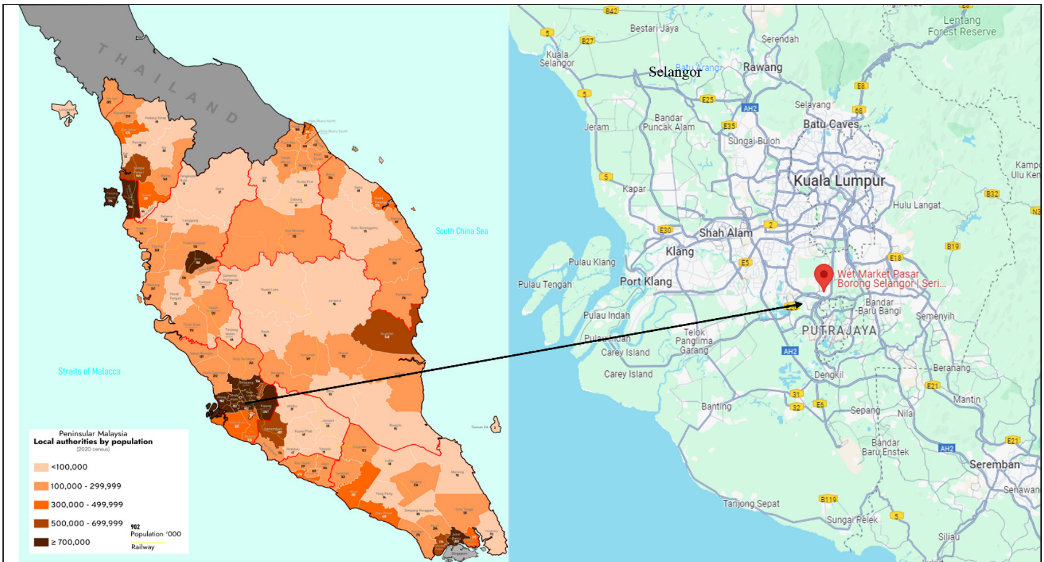
Selangor is an urban state with the highest population density in Malaysia. For this reason, vegetables and other perishable products are not only produced in the state but also supplied from the rural and semi-urban regions of the country, especially from Cameron Highlands, Pahang. As mentioned, the heavy metals from agricultural soils are subsequently transferred to the plants, which have been discovered in Pahang and Kuala Selangor (Aweng et al., 2020; Sulaiman et al., 2020). It could be due to the greater usage of chicken manure and chemical fertilizers in the plantation areas that increased the heavy metal content in the soil, such as Pb, Zn, and Cu (Aljohani, 2023). Additionally, rapid urbanization, economic development, and increased motorization in the Klang Valley area (including the Kuala Lumpur Federal Territory and Selangor State) have contributed to higher pollution levels, resulting in decreased water and air quality (Loi et al., 2022). As a result, the atmospheric heavy metals might be deposited in the plants cultivated in the adjacent areas.

Pasar Borong Selangor in Seri Kembangan is one of the largest wet markets in Selangor. The leafy vegetables sold in this market are a significant food source for locals, especially those in the Klang Valley. Due to the rapid industrial development in the state, plants, including vegetables grown in this area, might become contaminated with heavy metals. However, no structured monitoring system currently tracks heavy metals in vegetables sold across the nation's markets. Only a few papers have been published investigating human exposure to metal contaminants from vegetables in the urban state. It is hypothesized that the leafy vegetables sold in Selangor wet markets are contaminated with heavy metals, and long-term consumption of these leafy vegetables poses adverse health effects. Hence, the present study aimed to investigate the concentrations of heavy metals (Al, Cd, Cr, Cu, Fe, and Pb) in commonly consumed vegetables sourced from Pasar Borong Selangor, Seri Kembangan. Four types of leafy vegetables, including cabbage (*B. oleracea* subsp. *capitata* L.), mustard (*B. juncea* Czern.), spinach (*S. oleracea* L.), and pak choi (*B. rapa* var. *chinensis*), were selected based on the highest economic importance globally (Ribera et al., 2021) and the most consumed vegetables in the country (Nurul Izzah et al., 2012). Besides, the conceivable health risks related to dietary exposure to hypothetically harmful metals are determined via the calculations of the hazard index (HI), THQ, EDI, and target cancer risk (TCR).

MATERIALS AND METHODS

Materials

Four types of vegetables (about 350 g each) being randomly collected once per week (for three consecutive weeks: 19 October 2022, 26 October 2022, and 2 November 2022) from Pasar Borong Selangor, Seri Kembangan, namely cabbage (*B. oleracea* subsp. *capitata* L.), mustard (*B. juncea* Czern.), spinach (*S. oleracea* L.), and pak choi (*B. rapa* var. *chinensis*). Pasar Borong Selangor is a rapidly growing area in the south region of Selangor and is surrounded by high-density areas (Department of Statistics Malaysia, 2023), as shown in Figure 1. Meanwhile, the selections of sellers were kept constant along these three consecutive weeks of sample collections, which were Lot 167 (spinach and pak choi, n = 3), Lot 183 (cabbage, n = 3), and Lot 207 (mustard, n = 3). After each purchase, all samples were put into a clean plastic bag, sealed, and immediately brought to the laboratory for further processing.



Urbanization in peninsular Malaysia: Data were taken from the Population and Housing Census of Malaysia 2020 (Department of Statistics Malaysia, 2023).

Sampling point: Pasar Borong Seri Kembangan, Selangor

Figure 1. Selangor and sampling point (Pasar Borong, Seri Kembangan)

Sample Preparation

Bruised or rotten portions of the vegetables were eliminated, and the edible parts were utilized (Aweng et al., 2020; Gebeyehu & Bayissa, 2020). The freshly collected edible parts of samples were washed in running water to remove the adsorbed dust and particulate matter, followed by three washings with distilled water in the laboratory to prevent contamination. Consequently, the samples were dried using the tissue papers. The samples were cut into smaller pieces using a clean knife; then, at about 400 g of cut, samples were stored in the small airtight polyethene bags and kept in a low-temperature freezer (Fisher Scientific, USA) until digestion and analysis. All samples were analysed for the targeted heavy metals (Al, Cd, Cr, Cu, Fe, and Pb).

Digestion Procedures

The optimization procedures were adopted from Gebeyehu and Bayissa (2020), wherein a mixture of 9 ml of nitric acid (HNO_3) and 3 ml of hydrochloric acid (HCl) (Fisher Scientific, USA) was utilized during digestion for 45 min under pressure and at a temperature of 80 W and 180°C, respectively. In a microwave digestion vessel, 0.5 g of homogenized vegetable sample was placed with 9 ml of 10 M HNO_3 and 3 ml of 10 M HCl. The vessels were firmly sealed and positioned in the batch microwave digestion system (MARS^{TM} 6, United Kingdom), and digestion was performed at 180°C for 45 min until a colourless solution was obtained. The transparent and colourless solution obtained was filtered through the Whatman No. 42 filter paper. Subsequently, the analysis of heavy metals using inductively coupled plasma—optical emission spectrometry (ICP–OES, Avio 550 Max, United Kingdom) was immediately conducted using the prepared solutions after adjustment using 2% of HNO_3. The digestion and analysis were repeated three times. The results are presented as mean ± standard deviation. Similar steps were taken to prepare the 1,000 ppm stock standard solutions in accordance with the defined ideal circumstances, and they were then examined.

Heavy Metal Analysis

The concentrations of Al, Cd, Cr, Cu, Fe, and Pb in the samples were determined using the ICP–OES, which integrated with high throughput system (HTS) (PerkinElmer, United Kingdom) — a distinctive flow injection sample introduction segment. A workflow-based software —Syngistix™ for ICP Software was adopted to support customized reporting capabilities for diverse peripherals. For the instrument adjustment conditions, a peristaltic pump, operating at a flow rate of 2.5 ml/min through Tygon-type PVC peristaltic pump tubes, was employed to introduce the digested sample into the ICP–OES system. The ICP-OES system was powered at 1,400 W, with the radiofrequency generator operating at 40 MHz. The argon gas flow rates were set at 0.73 L/min for the nebulizer, 0.8 L/min for the auxiliary, and 13 L/min for the plasma. All analyses were conducted weekly (for three consecutive weeks) after the appropriate calibration of the instrument by calibration blank. All calibration techniques were assessed according to their associated correlation coefficients ($r^2 \geq 0.998$) (Gebeyehu & Bayissa, 2020).

Health Hazard Assessment

Estimated Daily Intake (EDI)

The EDI of heavy metals was anticipated using the equation below, as stated by Chen et al. (2011):

$$EDI = \frac{E_f \times E_D \times F_{IR} \times C_M \times C_f}{B_W \times T_A} \times 0.001 \qquad [1]$$

where, E_f = exposure rate (365 days of whole year); E_D = exposure period (65 years — equivalent to average lifetime) (Woldetsadik et al., 2017); F_{IR} = average vegetable consumption (240g/person/day for low fruit and vegetables intake) (WHO, 2002); C_M = metal concentration (mg/kg for dry weight); C_f = 0.085 (concentration conversion factor for fresh to dry vegetable weight) (Harmanescu et al., 2011); B_W = 70 kg (reference body weight for an adult) (Woldetsadik et al., 2017); T_A = average exposure time (65 years × 365 days); 0.001 = unit conversion factor.

Target Hazard Quotient (THQ)

Various biomagnifying pollutants have both cancer-causing and non-cancerous impacts on human bodies (Hussain et al., 2022). The THQ is adopted to evaluate non-carcinogenic impacts (Agoro et al., 2020). The evaluation of potential non-carcinogenic health risks to the population resulting from the vegetable intake that was contaminated with heavy metals was conducted by determining the THQ using the equation described by Chen et al. (2011):

$$THQ = \frac{EDI}{RfD} \quad [2a]$$

where, *EDI* = estimated daily meal intake of the population (mg/day/kg body weight); *RfD* = oral reference dose (mg/kg/day) values for all metals determined.

The likelihood of non-carcinogenic consequences is generally low if the THQ value is less than one. In other words, the exposed population is unlikely to encounter apparent adverse effects. If the THQ value is more than one, it is generally assumed that non-carcinogenic effects are possible. As the value increases, the possibility also heightens (Antoine et al., 2017).

The following equation is used to calculate the *RfD* as stated by the United States Environmental Protection Agency (US EPA) (1993):

$$RfD = \frac{NOAEL}{(UF \times MF)} \quad [2b]$$

where, *NOAEL* = no-observed-adverse-effect-level (mg/kg/day); *UF* = standard uncertainty factor (UF = 10-fold factor); *MF* = modifying factor (*MF* = 1).

Values of *RfD* for each metal of relevance adopted for the calculation of THQs was Al: 1, Cd: 0.001 (Antoine et al., 2017), Cr: 0.003 (Chang et al., 2014), Cu: 0.04, Fe: 0.7 (Javed & Usmani, 2016), and Pb: 0.0035 (Chang et al., 2014) in mg/kg/day.

Hazard Index (HI)

The hazard index (HI) is the accumulation effect of consuming contaminated vegetables, resulting in the health risks associated with heavy metals (Esmaeilzadeh et al., 2019). The HI of individual heavy metals was calculated using the equation as follows by Antoine et al. (2017) (Equation 3):

$$HI = \sum_{n=1}^{i} THQ_n; i = 1,2,3, \ldots, n \quad [3]$$

where, *HI* = cumulative of all potential pollution risks.

When the HI value is less than one, it indicates that the exposure to the heavy metal appears to have no negative impacts on health. If the HI value is more than one, it shows the possible health effect consequence. If HI is greater than 10, it suggests a significant chronic health implication (Esmaeilzadeh et al., 2019; Gebeyehu & Bayissa, 2020).

Target Cancer Risk (TCR).

The TCR refers to the population's cancer risk due to the consumption of specific metals. The was determined using the equation delineated by Gebeyehu and Bayissa (2020):

$$CR = EDI \times CPS_O \quad [4]$$

where, EDI = estimated daily metal ingestion of the populace in mg/day/kg; CPS_O = oral cancer slope factor in (mg/kg/day)

The cancer slope factor is established when evaluating the quantitative risk of substances or agents considered carcinogens. It serves as a gauge of the likelihood of developing cancer after being exposed to a chemical throughout a lifespan. It is often expressed in terms of the percentage of the population impacted per milligrams of substance per kilogramme of body weight per day (expressed in units of reciprocal dose $(mg/\ kg/\ day)^{-1}$ (Farris & Ray, 2014). As the carcinogenic potential of Al and Fe has not been established, only the CR values for Pb, Cd, Cu, and Cr were calculated to assess their carcinogenic effects (US EPA, 2010). The CPS_O values adopted for CR calculation were Cd: 0.38 (Yang et al., 2018), Cr: 0.5 (Zeng et al., 2015), Cu: 1.7 (Soumaoro et al., 2021), Pb: 0.0085 (Kamunda et al., 2016) in (mg/kg/day).

The target cancer risk (TCR) from the consumption of heavy metals such as Cd, Cr, Cu, and Pb was assessed using the equation provided by Kamunda et al. (2016) as follows:

$$\text{TCR} = \sum_{n=1}^{i} CR; i = 1, 2, 3, \ldots, n \quad [4b]$$

where, n = number of heavy metals taken into account for cancer risk calculation.

Statistical Analysis

The experimental results were analysed using Minitab software (Minitab Version 21.1), and the confidence level was fixed at 95%. The mean and standard deviation (SD) of each heavy metal in vegetable samples were calculated and expressed as milligrams per kilogram fresh weight (FW) of the composite samples. The acquired data were subjected to a one-way analysis of variance (ANOVA) analysis and Pearson's correlation test, respectively, to compare the mean concentration of heavy metals between the selected leafy vegetables and determine their correlation level. Pearson's correlation test was performed to identify potential causative factors of heavy metal pollution and how specific the heavy metals affect their contents.

RESULTS AND DISCUSSION

Heavy Metal Analysis

The present study investigated heavy metal concentrations in leafy vegetables and their health risks. Based on the results in Table 1, the mean levels of Al in cabbage, mustard, spinach, and pak choi were 11.12 ± 3.76, 28.97 ± 5.33, 41.37 ± 8.53, and 25.69 ± 6.32 mg/kg, respectively. The values of Al metals obtained revealed a statistically significant difference ($p < 0.05$) between mustard, cabbage, and pak choi (Table 1). As no information is available for Al standards on vegetable values in Malaysia, this study adopted the provisional tolerable weekly intake (PTWI) for Al metal of 2 mg/kg introduced by the Joint Food and Agriculture Organization (FAO) and World Health Organization (WHO) Expert Committee on Food Additives (JECFA) in June 2011 (Food and Agriculture Organization of the United Nations & World Health Organization [FAO/WHO], 2011).

Among the leafy vegetables tested, spinach contains the greatest amount of Al (41.37 mg/kg). The results indicated that spinach is more capable of taking in hazardous substances and heavy metals from the rhizosphere and transforming them into palatable sections than other fruits and root vegetables (Bashir et al., 2020). This finding was in line with Ghasemidehkordi et al. (2018), who reported the highest Al value of 37.19 mg/kg in

Table 1
Heavy metals concentration (mg/kg fresh weight) in leafy vegetables collected from a wet market in Seri Kembangan, Selangor

Metals	Mean levels of heavy metals (mg/kg fresh weight)				Allowable levels (mg/kg)	
	Cabbage	Mustard	Spinach	Pak choi	Malaysia	FAO/WHO
Al	11.12 ± 3.76^{a}	28.97 ± 5.33^{a}	41.37 ± 8.53^{ab}	25.69 ± 6.32^{b}	N/A	2.0^{B}
Cd	ND	ND	ND	ND	1.0^{A}	0.2^{B}
Cr	ND	ND	ND	ND	N/A	2.3^{C}
Cu	ND	ND	ND	ND	N/A	73.3 ^{B}
Fe	6.30 ± 5.78^{a}	4.12 ± 1.84^{a}	13.59 ± 4.73^{b}	4.14 ± 0.31^{a}	N/A	425.5 ^{B}
Pb	ND	ND	ND	ND	N/A	0.3^{B}

Note. Results are presented in mean ± standard deviation
Al = Aluminium; Cd = Cadmium; Cr = Chromium; Cu = Copper; Fe = Iron; Pb = Lead
ND = Not detected; N/A = Not available in Malaysia Food Regulation 1985, Fourteenth Schedule (Regulation 38) Table 1 (Malaysia & International Law Book Services, 2019)
The detection limits for Cd and Pb were <0.004 mg/kg, while for Cr and Cu were <0.01 mg/kg
Means in the same row with different superscript letters are significantly different ($p < 0.05$) after comparing the mean levels of heavy metals among different leafy vegetables using parametric one-way analysis of variance and Tukey's honestly significant difference tests at 95% confidence level
[A] – Malaysia Food Regulations 1985, Fourteenth Schedule (Regulation 38) Table 1 (Malaysia & International Law Book Services, 2019)
[B] – Joint Food and Agriculture Organization of the United Nations & World Health Organization Expert Committee on Food Additives (JECFA), June 2011 (FAO/WHO, 2011)
[C] – Food and Agriculture Organization of the United Nations & World Health Organization (FAO/WHO, 2014)

spinach when compared to other vegetables, including fenugreek, parsley, cress, allium, radish, tarragon, and coriander, collected from the agricultural sites in Iran. Similarly, these results are like those previously reported by Martín-León et al. (2023), in which spinach, lettuce, and chard were classified as having a high Al content (41.94 mg/kg).

Al is the third most prevalent metal in the crust, accounting for 7-8% of its mass, after oxygen and silicon (Muhammad et al., 2019). The presence of Al is easily flagged in all life forms as it is an integral part of mineral soils (Rahman et al., 2018). However, active Al ions are hazardous to plants, specifically to root tip meristem, by constraining root elongation. In humans, dietary exposure to Al accumulates in the organ tissues, increasing the risk for hypertension and vascular dysfunctions (Martinez et al., 2017). In acidic soils with low pH (4.3), Al is solubilized into hydrated metal ion salt $[Al\ (H_2O)_6]^{3+}$, which is commonly denoted to aluminium cation (Al^{3+}). Al^{3+} is the most toxic form, enormously influencing plant growth and development (Singh et al., 2017). The soil pH was not assessed in the present study; therefore, the impact of soil pH could not be confirmed.

The mean levels of Fe in cabbage, mustard, spinach, and pak choi were 6.30 ± 5.78, 4.12 ± 1.84, 13.59 ± 4.73, and 4.14 ± 0.31 mg/kg, respectively (Table 1). Among these, spinach had the highest concentration of Fe metal ($p < 0.05$). As Fe levels in vegetables have yet to be regulated in Malaysia Food Regulations 1985 [Fourteenth Schedule (Regulation 38) Table 1], thus this study solely compared the stipulated limits by FAO/WHO for Fe metal of 425.50 mg/kg (FAO/WHO, 2011). The information presented in Table 1 shows that the concentration of Fe in all examined vegetables falls below the permissible limits. Dark green leafy vegetables are a good source of iron, particularly the non-haem form, with spinach containing the highest amount ranging from 400 to 500 mg/kg (National Coordinating Committee on Food and Nutrition [NCCFN], 2017). The lower amount of iron detected in the present study could be attributed to the crops' limited iron absorption from the soil. It has been previously reported that excessive carbonate and calcium levels can increase soils' pH levels (Kasowska et al., 2018), decreasing iron solubility. This limitation in iron uptake by the plant roots and various plant parts, including leaves, may occur despite ample iron in the soil.

These findings are consistent with those of Akhtar et al. (2022), who reported higher levels of iron than the maximum permissible level in soil in District Sargodha, irrigated with urban wastewater. However, the iron concentrations in crop samples were lower than the limit, ranging from 4.09 to 32.58 mg/kg. Consistent with the literature, the mean Fe levels of the studied vegetables were within a similar range. In addition to excessive use of chemical fertilizers and pesticides, consistent use of sewage water for irrigation can also lead to the build-up of heavy metals in plants (Sandeep et al., 2019). Fe can produce hydroxyl radicals that are detrimental to DNA, proteins, and lipids, which eventually lead to cell death, known as ferroptosis (McMillen et al., 2022). In humans, shortcomings associated with Fe overload increase the risk of bacterial infection and cardiomyopathy (Dasa & Abera, 2018). Fe builds up in the liver, causing fibrosis, cirrhosis, and damage to liver function, resulting in complications and death if left untreated (NCCFN, 2017).

As shown in Table 1, cadmium (Cd) was not detected in all tested leafy vegetables (cabbage, mustard, spinach, and pak choi), suggesting safe consumption. The WHO (2019) classified Cd as a human carcinogen. According to FAO/WHO (2014), leafy vegetables containing Cd at levels higher than 0.20 mg/kg represent a major risk to human health. Different plant types and genotypes have varying capacities for Cd absorption, transport, and accumulation (Zhao et al., 2023). In general, more heavy metals were retained in the root portion of the plant as opposed to the leaves, as Sulaiman et al. (2020) reported. The metal binding site in roots was more effective at absorbing heavy metals as compared to leaves (Sagagi et al., 2022). Dietary Cd intake due to ingestion of ecologically polluted rice and other foods was related to an augmented risk of postmenopausal breast cancer (Itoh et al., 2013). As soon as in the human body, it can persist in metabolism for 16 to 33 years, as has been correlated with many health issues, including kidney damage and atypical urinary protein excretion disorders in the liver, kidney, testicles, pancreas, and bones (Fu & Xi, 2020).

In the present study, Cr metals in all leafy vegetables (cabbage, mustard, spinach, and pak choi) were lesser than the detection limit of 0.01 mg/kg — not detected (ND) (Table 1). Simultaneously, the metal content was lower than the allowable limit by FAO/WHO (2011) of 2.3 mg/kg (for leafy vegetables). According to Oliveira (2012), plants should have a Cr content of less than 0.001 mg/kg under normal circumstances. Therefore, the results indicated that these vegetables are fit for human consumption. These results reflect those of Khairiah et al. (2002), who also found that Cr content in black mustard (*Brassica nigra*) and water spinach (*Ipomoea aquatica*) was very low at their sampling locations in Sepang and Bangi, Selangor. Cr metal was also not detected in spinach and coriander leaves irrigated using wastewater in Allahabad, India (Chandel & Bharose, 2020).

Most investigations have revealed that even when growth is restricted to dangerous levels, Cr in plants is less than 1 to 2 mg/kg (dry weight). The increase in the contents of Cr in roots may be due to the defensive measure of Cr sequestration in the vacuoles of root cells (Mangabeira et al., 2011). The concentration of Cr translocated from the roots towards the aerial shoots is rather low, which relies on the chemical form of Cr in the tissue (Shahid et al., 2017). Consequently, this mechanism gives plants some built-in resistance to Cr toxicity. Besides, Cr is essential for insulin action and DNA transcription in organisms, specifically humans (Ametepey et al., 2018).

The present study found that (Cu) content in various leafy vegetables such as cabbage, mustard, spinach, and pak choi was consistently below the detection limit (< 0.01 mg/kg). It suggests that these vegetables do not present an immediate threat to human health in terms of copper toxicity. Notably, the levels detected were significantly lower than the established safety limit of 73.3 mg/kg suggested by Mensah et al. (2009). However, it is important to acknowledge that current regulations in Malaysia, specifically under the Food Regulations 1985, do not address copper content in food, highlighting a potential regulatory gap that may warrant attention.

Copper, a vital trace element, plays essential roles in plant physiology and human health. It serves diverse functions in plants, including electron transport during photosynthesis, hormone signalling, and cell wall metabolism. Cu overload leads to oxidative stress in plants via the augmentation in the generation of reactive oxygen species (ROS). Cu necessitates an intricate absorption, sequestration, and transportation system due to its dual nature (essential and possibly detrimental) (Mir et al., 2021). However, excessive accumulation of copper can induce oxidative stress, impacting plant health. Similarly, copper is crucial for various physiological processes in humans, but deficiency and excess can lead to adverse health effects such as gastrointestinal discomfort and liver damage. Copper is an indispensable component, although evidence has shown that larger concentrations can have harmful impacts, and overexposure (200 mg/kg) can lead to fatality (FAO/WHO, 2011).

The Pb contents of the leafy vegetables were also under the detection limits (< 0.004 mg/kg) (Table 1). The fact that all Pb concentrations for all vegetable samples were less than the permissible limits of 0.3 and 2.0 mg/kg (FAO/WHO, 2014) indicated that these vegetables are relatively fit for human consumption. This study supports evidence from previous observations on leafy vegetables, whereby Pb metal was not detected in smooth amaranth (Ogunkunle et al., 2014), kale, lettuce, and watercress (Thang et al., 2021), as well as tarragon, parsley, and celery (Bora et al., 2022). In contrast, Martín-León et al. (2023) found that spinach contains the highest concentration of Pb compared to other studied green leafy vegetables, such as lettuce and chard.

Due to phosphate and carbonate precipitation controlling the solubility of Pb metal, Pb was the slightest concentrated in the plants (Li et al., 2021). Plant roots may accumulate phosphate or carbonate from fertilizer solutions or the ditch where the plants were dug up (Xia & Ma, 2006). Owing to physical obstacles in roots that prevent metal from travelling to the aerial regions, Lu et al. (2004) also noted that stems and leaves accumulate heavy metals at lower rates than roots of the water hyacinth. Furthermore, high metal concentrations in the roots suggest it could filter out heavy metals via rhizofiltration (Sulaiman et al., 2020).

Pb is one of the most accumulative metals that can penetrate the body system via food, water, and air, and it cannot be easily eliminated by washing produce (Collin et al., 2022). Some plants have elevated amounts of Pb, which could have been caused by pollution in irrigation water, agricultural soil or public road traffic (Shetty et al., 2023). Pb poisoning can impact the kidneys and nervous system, as most doctors reported that acute lead exposure can result in anaemia, which in turn causes heme suppression and red blood cell destruction (Fu & Xi, 2020).

In brief, Cd, Cr, Cu, and Pb were not detected in cabbage, mustard, spinach, and pak choi. Therefore, the overall levels of aluminium accumulated in leafy vegetable samples followed the order of spinach > mustard > pak choi > cabbage, while the concentrations of iron in leafy vegetable samples followed the order of pak choi > cabbage > spinach > mustard. It is worth mentioning here that leafy vegetables have a greater tendency for heavy metal accumulation than fruiting and tuber vegetable varieties. This statement is in line with the research findings revealed by Sultana et al. (2022) and Xu et al. (2022), which showed that Leafy vegetables typically contain higher concentrations of heavy metals compared to non-leafy vegetables.

Health Hazard Assessment

Estimated Daily Intake (EDI) of Heavy Metals

In this study, the adult population's estimated daily intake (EDI) of heavy metals was determined by calculating the average metal concentrations in each leafy vegetable. The results were compiled in Table 2. For cabbage, the EDI values for Al and Fe were 3.241 $\times 10^{-3}$ and 1.816 $\times 10^{-3}$ mg/day, respectively, based on a consumption rate of 240 g/day. However, the Pb Cr, Cd, and Cu concentrations were below the detection limits (ND). Thus, their corresponding EDI values resulting from the consumption of the selected leafy vegetables were not available (NA).

Table 2
Estimated daily intake (EDI) (mg/day/kg) of toxic metals for the adult population due to the consumption of leafy vegetables

Metals	EDI values (mg/day/kg)				Total EDI via consumption of selected leafy vegetables (mg/day/kg)	Maximum tolerable daily intake (mg/day)
	Cabbage	Mustard	Spinach	Pak choi		
Al	3.241×10^{-3}	8.443×10^{-3}	12.056×10^{-3}	7.487×10^{-3}	3.123×10^{-3}	1[D]
Cd	ND	ND	ND	ND	N/A	0.02 – 0.07[A, B, C, D]
Cr	ND	ND	ND	ND	N/A	0.035 – 0.2[A, C]
Cu	ND	ND	ND	ND	N/A	2.5 – 3[B, C]
Fe	1.816×10^{-3}	1.201×10^{-3}	3.961×10^{-3}	1.206×10^{-3}	8.184×10^{-3}	2 – 5[A, C]
Pb	ND	ND	ND	ND	N/A	0.21[A]
Total	0.005057	0.009644	0.01602	0.008693	0.03941	

Note. Al = Aluminium; Cd = Cadmium; Cr = Chromium; Cu = Copper; Fe = Iron; Pb = Lead
ND = Not detected; N/A = Not available in Malaysia Food Regulation 1985, Fourteenth Schedule (Regulation 38) Table 1((Malaysia & International Law Book Services, 2019).
The detection limits for Cd and Pb were <0.004 while for Cr and Cu were <0.01
[A] – The maximum tolerable daily intake is based on the values reported by Shaheen et al. (2016)
[B] – The maximum tolerable daily intake is based on the values reported by Zheng et al. (2007)
[C] – The maximum tolerable daily intake is based on the values reported by Naser et al. (2012)
[D] – European Food Safety Authority (EFSA) (2013)

The EDI of heavy metals linked to the consumption of cabbage follows a descending pattern as Al > Fe > Cd > Cr > Cu > Pb. Meanwhile, the EDI values for Al and Fe metals owing to the identical amount of consumption of mustard were 8.443×10^{-3} and 1.201×10^{-3} mg/day, respectively. The EDI of corresponding heavy metals obtained by eating mustard followed a descending sequence as Al > Fe > Cd > Cr > Cu > Pb. For a 240 g/day consumption of spinach, the EDI values for Al and Fe metals were found to be 12.056×10^{-3} and 3.961×10^{-3} mg/day. Similarly, the intake of these metals is based on spinach consumption in the declining sequence as Fe > Al > Cd > Cr > Cu > Pb. Furthermore, the EDI values for Al and Fe metals resulting from the consumption of pak choi at a rate of 240 g/day were 7.487×10^{-3} and 1.206×10^{-3} mg/day, respectively. The EDI of heavy metals from pak choi consumption exhibited a descending order as follows: Al > Fe > Cd > Cr > Cu > Pb.

The EDI of both Al and Fe metals observed in this work due to the 240 g/day consumption of cabbage, mustard, spinach, and pak choi are less than the maximum tolerable daily intake (MTDI) of each metal reported by the previous research (Basha et al., 2014; Shaheen et al., 2016: Zheng et al., 2007). In a recent study, the EDI of aluminium from spinach was the highest, contributing 12 and 4% to the toxic reference value for adults and children, respectively (Martín-León et al., 2023). It suggests that chronic exposure, even at very small concentrations, might negatively affect health, especially at the early stages of the lifecycle. According to Hussain et al. (2022), though heavy metals have lower EDI compared to MTDI, they can pose a variety of *in vivo* effects, both carcinogenic and non-carcinogenic, due to how they accumulate in organisms. Research has indicated that certain heavy metal elements could impair children's spatial learning abilities and indirectly influence their memory (Porru et al., 2024). Moreover, this investigation solely concentrated on cabbage, mustard, spinach, and pak choi to evaluate the potential health hazards to the population within the Seri Kembangan area. It implies that only a portion, rather than the entirety, of the hazards to the population was considered.

Target Hazard Quotient (THQ)

The correlation between health threats and pollutants depends on the level of exposure and absorption within the human body (Shetty et al., 2023). In this context, the quantity of vegetables ingested, and an individual's weight play pivotal roles in determining the health threats associated with vegetable consumption. Therefore, it is crucial to forecast the exposure levels and assess the health impact of contaminants by considering the exposure routes to the organisms. The THQ was calculated to assess the associated human health risks based on the mean concentration of Pb, Fe, Al, Cu, Cr, and Cd. THQ is an effective tool for assessing the risk level associated with a specific pollutant. When the THQ exceeds one, it indicates a potentially carcinogenic effect on the human body; conversely, a THQ less than one suggests no non-carcinogenic effect (Hussain et al., 2022). The THQ values for the studied leafy vegetables are shown in Table 3. Notably, the THQs of Al in cabbage, mustard, spinach, and pak choi were 3.241×10^{-3}, 8.443×10^{-3}, 12.056×10^{-3}, and 7.487×10^{-3}, respectively. Similarly, the THQs of Fe in cabbage, mustard, spinach, and pak choi were 2.623×10^{-3}, 1.716×10^{-3}, 5.659×10^{-3}, and 1.723×10^{-3} respectively. Estimating hazard quotients for metals in the tested leafy vegetables revealed insignificant health threats associated with their ingestion.

The cumulative THQs resulting from the consumption of cabbage, mustard, spinach, and pak choi (GTHQ) for Al and Fe were less than one, with values of 0.03123 and 0.01172, respectively. It clearly indicates that the adult population faces no substantial health risks through the consumption of these metals in general or from the increasing levels of metals specifically found in the leafy vegetables (cabbage, mustard, spinach, and pak choi) consumed and cultivated in the region.

In contrast to our results, Pavlíková et al. (2023) reported that Cd, Zn, and Cr in leafy vegetables, especially lettuce, showed the highest THQ values compared to root and legume vegetables, except for Pb in carrots. It suggests potential adverse health effects of non-carcinogenic diseases due to the consumption of these vegetables. Also, Gupta et al. (2021) previously confirmed that the THQ values greater than one for Pb and Cd carcinogenic elements in spinach and carrots. Consistent with previous findings, a recent study conducted in the southern region of Northeast Thailand indicated that the THQ values for Cd, Mn, and Pb in the analyzed spinach exceeded one (Tongprung et al., 2024). Although the present study found no risks associated with the consumption of cabbage, mustard, spinach, and pak choi, regular structured monitoring of irrigation water, soil, and crops is still needed to prevent food chain contamination, especially in the younger segment of the population in the urban area.

Table 3
Target hazard quotient (THQ) and hazard index to heavy metals due to consumption of leafy vegetables (cabbage, mustard, spinach, and pak choi) for the adult population

Metals	Target hazard quotient				GTHQ [A]
	Cabbage	Mustard	Spinach	Pak choi	
Al	3.241×10^{-3}	8.443×10^{-3}	12.056×10^{-3}	7.487×10^{-3}	0.03123
Cd	ND	ND	ND	N/A	N/A
Cr	ND	ND	ND	N/A	N/A
Cu	ND	ND	ND	N/A	N/A
Fe	2.623×10^{-3}	1.716×10^{-3}	5.659×10^{-3}	1.723×10^{-3}	0.01172
Pb	ND	ND	ND	N/A	N/A
Hazard index	0.005864	0.0102	0.01772	0.00921	0.04299

Note. Al = Aluminium; Cd = Cadmium; Cr = Chromium; Cu = Copper; Fe = Iron; Pb = Lead
ND = Not detected; N/A = Not available in Malaysia Food Regulation 1985, Fourteenth Schedule (Regulation 38) Table 1 (Malaysia & International Law Book Services, 2019).
The detection limits for Cd and Pb were <0.004; while for Cr and Cu were <0.01
[A] Global Target Hazard Quotient (GTHQ) is the total of distinct metals THQ for the selected leafy vegetables

Hazard Index (HI)

The HI, which accounts for the cumulative effect of consuming different potentially harmful metals from a range of vegetables (Hussain et al., 2022), is depicted in Table 3. The HI values, which represent the cumulative THQ of respective metals for each leafy vegetable analysed (cabbage, mustard, spinach, and pak choi), were found to be less than one, with HI values of 0.005864, 0.0102, 0.01772, and 0.00921, respectively. These values individually indicate acceptable adverse health effects from consuming these non-carcinogenic leafy vegetables. Therefore, the recorded HI suggests that the contribution of heavy metals did not result in an aggregate threat through the intake of these vegetables.

Findings from the present work contradict other authors who found heavy metals, including Pb and Cd, significantly contributed to higher HI values (Pavlíková et al., 2023). However, the bioavailability of toxic elements is less than 40%, indicating that a HI value of less than two may not result in a concerning health hazard. Laboni et al. (2023) suggested that humans are exposed to chronic hazards when the HI value is more than 10. Vegetables exhibiting elevated HI values typically indicate significant health risks for consumers. Variations in heavy metal intake, individual body weight, and duration of exposure largely account for the differences in THQ values (Ametepey et al., 2018).

A great deal of previous research has been conducted on the health risk assessments of vegetables, which takes into consideration both the total and bioaccessibility of heavy metals. The effects of consuming vegetables from roadside open-air markets faced by the population in Johannesburg, South Africa, were recently studied by Adhikari and Struwig (2024). The authors concluded that the residents were at high risk of cancer when consuming both washed and unwashed leafy vegetables from the local markets, likely due to the deposition of dust containing hazardous elements (Cd, Ni, Cr, Hg, and Pb). These results are consistent with those reported by Tajdar-Oranj et al. (2024), in which the daily consumption of parsley, coriander, dill, and cress from the local market in Tehran, Iran, contaminated with Cd posed a high health risk. Likewise, the population in the Příbram district of the Czech Republic had a high risk of cancer from consuming vegetables when lead-silver mining activities in the area caused the deposition of toxic elements in the atmosphere (Pavlíková et al., 2023). Collectively, these studies outline the critical role of researchers and local authorities in continuous studies and monitoring to prevent adverse health effects due to heavy metals from the daily consumption of leafy vegetables.

It is crucial to note that the HI, THQ, and EDI values in this study were calculated based on the assumed daily intake of selected leafy vegetables: 240 g for cabbage, mustard, spinach, and pak choi. Consequently, it is possible that the EDI and THQ values reported were underestimated, which may also have affected the HI values. It should be noted that this study solely considered cabbage, mustard, spinach, and pak choi while estimating potential health threats, such as carcinogenic and non-carcinogenic effects, for the adult population. Since only a portion of the population was included in the study's findings, there is a possibility that the health threats associated with heavy metal exposure from consuming selected leafy vegetables may have been underestimated.

CONCLUSION

The current study indicates that the levels of heavy metals, namely, lead, iron, copper, aluminium, chromium and cadmium, in the selected leafy vegetables (cabbage, mustard, spinach, and pak choi) are within permissible limits established by both local and international regulatory. Furthermore, as evaluated by the HI, the combined non-carcinogenic impacts of various heavy metals were below one, indicating either the absence or reduced significant health risks associated with consuming these leafy vegetables. Based on these findings, it can be inferred that consuming cabbage, mustard, spinach, and pak choi poses low potential health risks. However, regular monitoring conducted by relevant authorities can help mitigate the health risks associated with consuming vegetables contaminated with heavy metals. Chronic exposure to multiple heavy metals from daily vegetable consumption may lead to adverse health effects, especially for consumers at early stages of their lifecycle, such as children. It is recommended that people vary their vegetable intake to fulfill daily vegetable consumption requirements in their diet while simultaneously decreasing their exposure to and accumulation of both carcinogenic and non-carcinogenic contaminants. Further investigations are warranted to thoroughly examine the health threats related to the daily consumption of leafy vegetables among the local population, especially focusing on vulnerable groups. Future research could focus on increasing the variety of vegetables and assessing comprehensive health risks among the population nationwide.

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