Heavy metal (As, Cd, and Pb) concentration in selected leafy vegetables from Jengka, Malaysia, and potential health risks

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Abstract

This study determines the toxic heavy metal (As, Cd, Pb) concentrations in selected leafy vegetables (pak choi, amaranth, and caisim) that were collected from an agricultural area in Jengka, Pahang, Malaysia. A potential health risk via the ingestion pathway is also evaluated. A graphite furnace atomic absorption spectrophotometer (GFAAS) analysis was applied to determine metal concentration. Heavy metal concentrations for leafy vegetables ranged between 0.03 and 0.05 mg/kg for lead (Pb), followed by 0.19–0.43 mg/kg for arsenic (As) and 0.15–0.54 mg/kg for cadmium (Cd). Cd is likely to be accumulated and absorbed more than arsenic (As) and Pb based on the bioconcentration factor (BCF). Cluster analysis (CA) and principal component analysis (PCA) suggested the metal origin is anthropogenic (the agricultural activities). Target hazard quotient (THQ) value below unity (< 1) indicates low non-carcinogenic risks of Cd and Pb. However, the high lifetime cancer risk (LCR) values for As and Pb ($LCR > 1 \times 10^{-4}$) possibly will pose a health risk to consumers. This study suggested leafy vegetable intake from this area poses some degree of potential health hazards to the consumer. Therefore, a comprehensive investigation such as bioavailability metal form analysis to obtain a more accurate risk assessment is needed.

Keywords Bioconcentration factor · Health risk · Leafy vegetables · Toxic metal · Translocation factor

1 Introduction

Heavy metal can accumulate and mobilize in the soil environment. Urbanization causes soil modification due to fast population growth and industrialization [1]. Metal contamination such as cadmium (Cd), lead (Pb), arsenic (As), chromium (Cr), and zinc (Zn) is common in urban and semi-urban soils [2]. Cumulative effects over the long term could pose a threat to the ecosystem and flora and fauna. Heavy metals can be transferred to the human body via the food chain leading to diseases [3]. Human consumes fruits and vegetables as their daily diet. Fruits and vegetables are the primary pathways of heavy metal exposure to

humans via ingestion [4]. For example, about 70% of Cd intake contributed via oral consumption [2–4].

Fresh fruits and vegetables are important as they contain vitamins, mineral, and other components which promote healthier food and the prevention of various diseases [5]. Nevertheless, these plants may contain both essential and toxic metal concentrations. The concentration of heavy metal in plants perhaps is influenced by the nature of the soil, environmental pollution, and the time of harvesting [5, 6]. The excess of phosphate fertilizer usage may increase arsenic (As), cadmium (Cd), and lead (Pb) concentrations in agricultural soils [7]. The use of fertilizers also could contribute to metal pollution in vegetables [8]. Heavy metal accumulation in edible plants may

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cause long-term risks to ecosystems and human health [9]. Heavy metals such as Pb are proven carcinogens. High concentrations of Cu and Pb in fruits and vegetables are associated with the occurrence of upper gastrointestinal cancer [10].

Root uptake is the main pathway of heavy metal entering the vegetable tissues. Metals can be transferred from soil into plants through the roots in the form of dissolved ions via a series of complex processes [11]. The accumulation of contaminants in plants can be estimated by the metal mobilization from soil to plant or known as bio-concentration factor (BCF) [12–14]. Previous studies have discovered high metal enrichment in leafy vegetables, followed by tubers and fruits [15–17]. Ngole [18] found that cadmium (Cd) and lead (Pb) concentrations are higher in leafy vegetables than in tubers.

Heavy metal accumulation in agricultural soil with high fertilizer usage, especially vegetation soils, has been discovered in Jengka, Pahang, Malaysia [19]. However, there is limited information available on human exposure to metal contaminants via vegetables from this semi-urban area. Leafy vegetables consider as main vegetables consumed by local citizens in this area. Therefore, this study evaluates the metal accumulation (As, Cd and Pb) in leafy vegetables, the bioconcentration factor (BCF) as well as the translocation factor (TF) and assesses their potential health risks via oral consumption. The potential sources of metal in leafy vegetables were identified.

2 Materials and methods

2.1 Sample collection and analysis

Thirty sets of soil and vegetable samples were collected from a farm in Jengka, Pahang, Malaysia (03°45' N, 102°56' E). This farm located on a university campus, where the vegetables were harvested and sold to local communities regularly. Three types of leaf vegetables were collected: pak choi (*Brassica chinensis* L.), amaranth (*Amaranthus gangeticus*), and caisim (*Brassica rapa* var. *parachinensis*). These species were the main vegetables grown in the sampling area. Soil samples were taken from the surface layer at about 0–20 cm using stainless steel. The soil attached to the roots of the vegetables was gently shaken off. The soil samples and vegetable samples were separately placed into polyethylene bags. All samples were labelled and brought to the laboratory.

The soil samples were left air-dried at room temperature, and the impurities in the soil were removed manually. The soil samples were ground and sieved with a 2 mm sieve. The plant samples were separated into roots and leaves and then rinsed with deionized water to remove

soil particles and other impurities. Then, the samples were dried in an oven (WGL-125B, Electro-thermal Blast Drying Oven, China) at 60 °C to remove the moisture content [20]. The dried samples were homogenized using a pestle and mortar and stored in airtight plastic bags. Samples ranged from 0.5 to 1.0 g were digested with 5 ml of mixed solution (HCl: H₂O₂ with a ratio of 4:1) and placed on a hot plate for 1 h at 100 °C, and this is a modified version of Kanakaraju et al. [21]. The digested samples were kept at 4 °C prior to analysis using a graphite furnace atomic absorption spectrophotometer (GFAAS) (PerkinElmer PinAAcle 900 T, Waltham, MA, USA). The linear calibration curve of GFAAS with r^2 values of As (0.985), Cd (0.995), and Pb (0.989) was observed.

2.2 Bioaccumulation analyses

Accumulation of heavy metals or bioconcentration factor (BCF) from soil to the vegetable was calculated using the formula:

$$BCF = C_{\text{vegetable}} / C_{\text{soil}} \quad (1)$$

$C_{\text{vegetable}}$ is the total concentration of heavy metals contained in the leafy vegetable (root or leaves) (mg/kg), and C_{soil} is the total concentration of heavy metals in the soil (mg/kg) [20]. BCF value less than one or equal to one shows the plant can only absorb but not accumulate heavy metals. If BCF is more than one, it indicates that the plant can absorb and accumulate metals [22]. The transfer of metal elements between the roots and leaf parts of vegetables can be estimated by the translocation factor (TF) [23]. Translocation factor (TF) from root to the leaf was assessed using Eq. 2.

$$TF = C_{\text{leaves}} / C_{\text{root}} \quad (2)$$

C_{leaves} is the total concentration of heavy metals contained in the leafy vegetable (mg/kg), and C_{root} is the total concentration of heavy metals in the root (mg/kg) [24]. High TF value indicates high mobilization of metal elements from root to leaf.

2.3 Health risk assessment

Non-carcinogenic and carcinogenic health risks assessment (HRA) was performed to evaluate the potential health risk to humans from As, Cd, and Pb exposure via ingestion. Non-carcinogenic health risk was assessed through average daily dose (ADD) and target hazard quotient (THQ) as in Eqs. 3 and 4. The lifetime cancer risk (LCR) is expressed as in Eq. 5. Equations 3–5 were adopted from USEPA [25].

$$ADD_{\text{ingest}} = (C_{\text{leaves}} \times \text{IngR} \times \text{EF} \times \text{ED}) / \text{BW} \times \text{AT} \quad (3)$$

$$\text{THQ} = \text{ADD} / \text{RfD} \quad (4)$$

$$\text{LCR} = \text{ADD} \times \text{SF} \quad (5)$$

Table 1 shows the input parameters and data source adapted for this study. The C_{leaves} is the mean concentration of metal in leafy vegetables. The ingestion rate (IngR) of vegetables for Malaysian adults by gender used in the calculation [26]. The average body weight of Malaysian adults (aged 18–59 years) was used as a reference body weight (BW) [27]. The reference values for the AT, ED, and EF in ADD calculation acquired from USEPA [25]. The value of RfD and SF applied in the THQ and cancer risk calculations [25, 28, 29]. If the ratio of THQ is less than 1, the exposed population is unlikely to experience adverse effects from the vegetable ingestion. If the THQ ratio is more than 1, this indicates a chance of non-cancer risk effects may occur from oral consumption [25]. The acceptable or tolerable LCR for regulatory purposes is in the range of 1×10^{-6} to 1×10^{-4} [25].

2.4 Statistical analysis

Relationships between the mean concentrations of heavy metal were determined using the Pearson

correlation. Cluster analysis (CA) was applied to classify the metal elements which may originate from different sources. A principal component analysis (PCA) was used to determine the contributions of various factors for possible source identification. All statistical analyses were performed using the Statistical Package for the Social Sciences.

2.5 Quality assurance

All glassware apparatus was washed with hexane, acetone, and deionized water prior to use. Detergents were not used, and non-powdered gloves were worn when handling the samples to avoid any cross-contamination. Recovery analysis was undertaken using standard reference material for vegetables (SRM1570a for spinach) and soil (Montana soil SRM2711a) to ensure the reliability of the sample analysis. The recoveries of metals were in the range of 85–110% with 10–15% uncertainties. The detection limits for the metal analysis were 1.00, 0.05, and 0.10 mg/kg for As, Cd, and Pb, respectively. Each sample was analysed three times, with a relative standard deviation (RSD) of the repeated analyses below 5%. All of the chemicals used for standards preparation were analytical reagent grade (Sigma Chemical Co).

Table 1 Parameter used for estimation of health risk via oral exposure

Parameter	Symbol	Metal	Unit	Value	Remarks	Source
Metal concentration in leafy vegetables	C_{leaves}		mg/kg	–	–	–
Ingestion rate	IngR		kg/day	0.133	Adult	[26]
			kg/day	0.153	Men	[26]
			kg/day	0.193	Women	[26]
Body weight	BW		kg	67.5	Adult	[27]
			kg	70	Men	[27]
			kg	65	Women	[27]
Averaging time	AT		days	8760	Non-cancer	[25]
			days	25,550	Cancer	[25]
Exposure duration	ED		years	24		[25]
Exposure frequency	EF		days/year	350		[25]
Oral reference dose	RfD	As	mg/kg/day	3×10^{-4}		[25]
		Cd	mg/kg/day	1×10^{-3}		[25]
		Pb	mg/kg/day	3.5×10^{-3}		[28]
Average daily dose	ADD		mg/kg/day	–		–
Lifetime cancer risk	LCR		–	–		–
Target hazard quotient	THQ		–	–		–
Cancer slope factor	SF	As	mg/kg/day	1.5		[25]
		Cd	–	–		–
		Pb	mg/kg/day	8.5×10^{-3}		[29]

3 Results and discussion

3.1 Heavy metal in leafy vegetables and soils

Figure 1 presents heavy metal concentrations in soils and different parts of vegetables. The soils showed a higher concentration of As compared to Pb and Cd. Generally, heavy metals were retained mostly in the root compared to leaves part of the plant and consistent with findings by Bonanno [30]. The concentrations of As detected in the root of pak choi, caisim, and amaranth with the mean of 1.67, 1.02, and 0.94 mg/kg, respectively. Cd concentrated in the roots of pak choi (1.52 mg/kg), followed by amaranth (0.84 mg/kg) and caisim (0.43 mg/kg). The highest Pb concentration in roots was determined from amaranth (1.81 mg/kg), followed by caisim (0.80 mg/kg) and pak choi (0.32 mg/kg).

The concentration of As in the leaves of amaranth was 0.43 mg/kg, followed by caisim (0.36 mg/kg) and pak choi (0.19 mg/kg). High concentrations of Cd were determined in the leaves part of amaranth (0.54 mg/kg) and pak choi (0.31 mg/kg). Meanwhile, Cd concentration in caisim's leaves was 0.15 mg/kg. For Pb, amaranth leaves have the highest concentrations of 0.05 mg/kg. Pak choi and caisim leaves had a similar Pb level, 0.03 mg/kg. A comparison of metal concentrations in the samples to the permitted limit by Malaysian Food Regulations [31] indicates all vegetables were within the safe level (As and Cd is 1 mg/kg, and Pb is 2 mg/kg).

The concentration of metals in vegetable samples in this study area was lower than metal determined in vegetable samples from previous studies in Uganda [2],

Nigeria [8], India [10], and China [20, 23, 24]. Different scales of agricultural practices such as fertilizer used and the natural concentration of those metal in the soil may influence these differences [19].

For soils, As concentrations were the highest level, followed by Pb and Cd. Although the soils have a high level of As concentration, not much was detected in the roots of vegetables. However, Cd and Pb showed different trends. Note that the Cd concentration in soils was lower than the roots of vegetables. The roots appeared to absorb more Cd and Pb than As. Since there is no information available for metal standards on soil values in Malaysia, therefore, this study adopted the limit proposed by Wang et al. [32]. All metal concentrations in soils were below the safe limit.

3.2 Bioconcentration factor (BCF)

Generally, heavy metal had a similar trend of BCF (Table 2). The roots showed higher BCF than the leaves. The vegetable roots have a slightly high absorption of As compared with those in the vegetable leaves. The BCF of Pb is higher than As, as the roots of vegetables enriched in Pb. The roots of amaranth and caisim showed BCF > 1 of Pb, implying that this element is absorbed and accumulated. Cadmium showed the highest BCF for all vegetables indicating that this metal element is absorbed and accumulated.

Although Cd concentration in soils was slightly low, perhaps Cd is absorbed by the vegetable roots as the BCF value was highest in this part. Similar findings were reported by Abdul et al. [33]. Cd ion transported from the soils to vegetable roots by diffusion. Perhaps Cd is easily absorbed by roots and accumulated in the plants as it is

Fig. 1 Concentration of heavy metal in soil and leafy vegetables (mean $\pm$ 5% error in mg/kg) (N=30)

Table 2 Bioconcentration factor (BCF) value and translocation factor (TF) value of As, Cd, and Pb in leafy vegetables

Sample		Cd	Pb	As
BCF				
Pak choi	Leaves–soil	15.62	0.06	0.04
	Root–soil	76.00	0.51	0.36
Amaranth	Leaves–soil	33.16	0.08	0.08
	Root–soil	51.88	2.90	0.18
Caisim	Leaves–soil	31.21	0.05	0.08
	Root–soil	87.98	1.32	0.23
TF				
Pak choi	Leaves–root	0.21	0.12	0.12
Amaranth	Leaves–root	0.63	0.03	0.45
Caisim	Leaves–root	0.35	0.04	0.36

more mobile than Cu and Pb [33, 34]. Pandey and Pandey [35] found that plants take up Cd mostly from soils (90%) and the other 10% from the surroundings. Furthermore, most of the metal ions are metabolically and passively taken up by roots and transported to the shoots [36]. The highest uptake of heavy metals occurred in roots rather than in the leaves because roots have a binding site for metals [37]. Leafy vegetables also absorb heavy metals from contaminated soil, exposure to polluted air environments, and agricultural activities [8].

Pb was the least concentrated in the plants because the solubility of this element controlled by phosphate and carbonate precipitation and very little Pb is available to plants [38]. Phosphate or carbonate may precipitate in plant roots from nutrient solutions or the trench from which the plants were taken [39]. Lu et al. [40] also highlighted that stems and leaves have low heavy metals accumulation rates compared to roots due to certain physical barriers in roots against metal transport to the aerial parts (stems and leaves). High concentrations of metals in the roots also indicate that this plant might remove heavy metals through a rhizofiltration process.

Several factors influenced the heavy metal accumulation in soils and plants such as pH, soil texture, soil type, organic compound, moisture, and microbial activity. Higher total organic carbon in soils, for example, will increase the soil fertility [41]. Previous work from the same sampling area has revealed that the vegetable soils underwent fertilization to enhance fertility that could lead to the increment of metal level in soil [19]. Besides, soils that have a smaller particle size increase the availability of metal concentration [42]. Different vegetable species have different abilities to absorb and accumulate different metals. Perhaps the differences are influenced by the characteristics of each species.

3.3 Translocation factor (TF)

In this study, amaranth and caisim had high TF of As and Cd from roots to leaves (Table 2). Perhaps cadmium (Cd) is more easily transported from the roots to the leaves, as supported by the highest BCF value calculated (Table 2). Besides, Cd has a lower retention capacity and higher mobility than other metals [43]. Wang et al. [44] found that the TF values for leafy vegetables were higher than those in non-leafy vegetables. Thus, the leafy vegetables easily accumulate heavy metals. For arsenic (As), it is not much available in the roots part even though the TF values were high for amaranth and caisim. The translocation factor value for Pb was the lowest. Although Pb accumulates in the roots, only a small amount could mobilize to the leafy parts of vegetables.

According to Zhuang et al. [45], transfer factors vary greatly among plant species. A study found TF values for amaranth (0.42) and pak choi (0.41) in Penang, Malaysia [46], and these values are similar to this study. Another study revealed that the highest TF value is parsley (0.12), followed by onion spring (0.027) and lettuce (0.022) in an intensive farming area in Cameron Highland, Malaysia [17]. However, their results were relatively low compared to those obtained by Roslan et al. [47]. The TF value obtained for soil–root and soil–shoot of fern leaves in Pontian, Johor, Malaysia, was 0.958 and 0.51, respectively [47]. Li et al. [48] also determined a high TF for lettuce for most heavy metals.

Heavy metals absorb by clay minerals, carbonates, or hydrous oxides from soil to root or precipitate as a metal carbonate, hydroxide, and phosphate compounds [43]. Perhaps these factors increase the TF value. Cd is bound easily with enzymes than Zn when the two metals simultaneously enter plant cells [44]. Therefore, excess Cd level that transported from roots to the leafy parts, which later may pose health risks [46].

3.4 Potential health risk

Cancer is an example of the adverse chronic health effects of heavy metals exposure on humans [25]. In this study, for the ingestion pathway, As and Pb induce both non-carcinogenic and carcinogenic risks, while Cd only induces non-carcinogenic risks. Table 3 shows the potential non-carcinogenic risk of As, Cd, and Pb in vegetable leaves. The THQ values of Pb were less than unity (< 1), suggesting low non-carcinogenic risk to adults, men, and women. However, there was a potential non-carcinogenic risk of As exposure via the ingestion route for all vegetable leaves, as the THQ values exceeded unity. The THQ value for Cd showed that amaranth leaves have the potential of non-carcinogenic risk. Similar findings of non-carcinogenic

Table 3 Non-carcinogenic health risk estimation for vegetable leaves consumption

	Metal	Pak choi	Amaranth	Caisim
ADD adult	As	3.70×10^{-4}	8.14×10^{-4}	6.92×10^{-4}
	Cd	5.93×10^{-4}	1.02×10^{-3}	2.92×10^{-4}
	Pb	7.38×10^{-5}	9.46×10^{-5}	6.06×10^{-5}
THQ adult	As	1.23	2.71	2.30
	Cd	0.59	1.02	0.29
	Pb	0.02	0.03	0.01
ADD men	As	4.10×10^{-4}	9.03×10^{-4}	7.67×10^{-4}
	Cd	6.58×10^{-4}	1.13×10^{-3}	3.24×10^{-4}
	Pb	8.19×10^{-5}	1.05×10^{-4}	6.72×10^{-5}
THQ men	As	1.36	3.01	2.56
	Cd	0.65	1.13	0.32
	Pb	0.02	0.03	0.02
ADD women	As	5.58×10^{-4}	1.22×10^{-3}	1.04×10^{-3}
	Cd	8.94×10^{-4}	1.53×10^{-3}	4.41×10^{-4}
	Pb	1.11×10^{-4}	1.42×10^{-4}	9.13×10^{-5}
THQ women	As	1.86	4.08	3.47
	Cd	0.89	1.53	0.44
	Pb	0.03	0.04	0.02

Bold values indicate potential non-carcinogenic risk

risk for Cd in amaranth leaves were observed by Yaacob et al. [46]. Although the Cd concentration in soils was low, high BCF and TF values perhaps indicate the excess used of fertilizer [7].

Figure 2 shows that As and Pb contamination might pose a carcinogenic risk to adults via oral intake for all vegetable leaves, as the LCR values in this study were more than 1×10^{-4} . Women are more susceptible than men to the carcinogenic and non-carcinogenic health effects of heavy metals exposure based on this evaluation. Perhaps

Fig. 2 Total cancer risk estimation of As and Pb for vegetable leaves consumption

this seems possible as women consumed more vegetables than men based on a previous study by Izzah et al. [26].

Health risk prediction for heavy metal exposure to the human using average concentrations in HRA has certain limitations. The estimation tries to highlight the potential health risks to humans; however, it does not identify any disease. There was an information gap between the specific mechanisms and processes affecting the functions and organs within the body [49]. Some values used in the HRA calculation, such as exposure frequency, averaging time, and exposure duration, were adopted from USEPA. Bodyweight and ingestion rates were considered Malaysia data. Perhaps for accuracy of the potential risk of a population exposed to the toxic metal, the bioavailability metal form analysis could be performed. Though, the general health risk in the study area could be determined using the values from USEPA for HRA calculation.

3.5 Source identification of metal

Table 4 shows the Pearson's correlation coefficient between heavy metals in leafy vegetables at the confidence level $p < 0.01$. As showed strong positive correlations with Cd ($r = 0.759$), and this is similar to a study by Ali et al. [50]. Nevertheless, As and Pb have a weak relationship ($r = 0.147$). A similar weak connection between Cd and Pb was also observed ($r = -0.004$). As and Cd could share the same sources. This argument supported by cluster analysis (CA), in which Euclidean distances for similarities considered. Figure 3 shows two classes were distinguished: C1 (As and Cd) and C2 (Pb).

Two factors accounted for 92.46% of the cumulative variance have been identified via factor loading with varimax rotation (Table 5). Factor 1 is considered a significant factor in explaining the high variability (58.59%) of the dataset. Factor 1 shows strong positive loading for As and Cd, hints these heavy metals sharing similar sources. This PCA supports the results in Table 4. Perhaps the metals came from the same sources; the extensive use of fertilizer [9]. Factor 2 shows strong loadings for Pb with a lower total variance (33.87%). Factor 2 suggests another source of agrochemicals that may have a high level of Pb.

Table 4 Correlation matrix between heavy metals in leafy vegetables

	As	Cd	Pb
As	1	0.759*	0.147
Cd		1	-0.04
Pb			1

Strong correlation, $r > 0.70$, is shown in bold

* $p < 0.01$ (2-tailed)

Fig. 3 Cluster analysis for selected metals in vegetables

Table 5 Rotated component matrix for selected metals in vegetables

	Factor 1	Factor 2
As	0.93	0.13
Cd	0.94	−0.06
Pb	0.03	0.99
Eigenvalue	1.77	1.00
Variability (%)	58.59	33.87
Cumulative (%)	58.59	92.46

Strong PCA loading > 0.75 is shown in bold

4 Conclusion

The heavy metal concentrations in leafy vegetables showed that Cd was the highest, followed by As and Pb. BCF suggested Cd is absorbed and accumulated more than As and Pb. All leafy vegetables retained metals in the roots. TF indicated that Cd is transferable in all vegetables at levels higher than As and Pb, although with TF values less than unity. The statistical analyses suggest all metals were likely coming from anthropogenic sources, which are two different groups of agrochemicals. THQ shows that the heavy metal contamination in leafy vegetables in the study area might not pose non-carcinogenic risks of Cd and Pb to adults via the ingestion pathway because the values were less than 1, except for Cd in amaranth. However, for As, it might pose a non-carcinogenic risk to adults because the THQ values were more than 1. For carcinogenic risk, As and Pb contamination might pose a carcinogenic risk to adults because

the LCR values in this study were more than 1×10^{-4} . This finding suggested leafy vegetable intake from this area poses some degree of potential health hazards to the consumer. Therefore, a comprehensive bioavailability metal form analysis could assist an accurate health risk assessment. Since this study is limited to leafy vegetables, the potential health risks for tuber and non-leafy vegetables deserve further investigation. The output of this study perhaps will assist stakeholders when considering action to prevent long-term health risks.

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Availability of data and materials Data collected and analysed in this study are available from the corresponding author upon request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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