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Original Article

The potential human health risk from zinc accumulation in water spinach *Ipomoea aquatica* in Negeri Sembilan and Selangor, Peninsular Malaysia

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Abstract

The objectives of the present study are: 1), to determine the concentrations of Zn in *Ipomoea aquatica* collected from ten distinct sampling sites located in Peninsular Malaysia; and 2) to assess the potential human health risks associated with Zn exposure from the collected *Ipomoea aquatica* in Peninsular Malaysia, across different age groups of the Malaysian population. It was found that the root of *I. aquatica* accumulated a significantly higher amount of this element compared to the stem and the

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leaf. It was also observed that all physiological parts (Leaf, Stem, and Root) of *I. aquatica* sampled from Market KLIA accumulated a greater amount of Zn compared to the other sites. The THQ (Target Hazard Quotient) values for element Zn in the *I. aquatica* species collected from 10 different sites in the Negeri Sembilan and Selangor states of Malaysia were consistently below 1.00. This observation suggests that there is no significant risk of non-carcinogenic effects associated with the consumption of water spinach by Malaysian consumers. The consistent Zn accumulation over time suggests that despite economic development in the region, Zn levels remained within safe concentrations, minimizing the risk associated with Zn accumulation in the plant biomass.

Keywords: *Ipomoea aquatica*, water spinach, zinc, human health risk assessment, estimated daily intake, target hazard quotient, Negeri Sembilan, Selangor, Malaysia

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1. Introduction

Mass industrialization, urban development, and agricultural practices contribute to the proliferation of heavy metal elements, such as zinc (Zn), in the ecosystem. Excess Zn in the environment has detrimental effects on water and soil ecosystems, posing a significant risk to human health (Hou, Kong, Lin, & Chen, 2021; Tasrina & Rowshon, 2015; Wong *et al.*, 2017). Toxic heavy metals exhibit low mobility within the environment and possess a significant residual capacity, thereby facilitating their accumulation within the ecosystem (Hou *et al.*, 2021). Given the increasing utilization of Zn and Zn-containing nanoparticles across diverse industries, it becomes imperative to conduct an ecotoxicological assessment of Zn's impact on the human food supply. This evaluation is essential despite the general perception of Zn as safe (Wong *et al.*, 2021).

Ipomoea aquatica, commonly referred as water spinach, is a prevalent vegetable in various Asian culinary traditions, including those of Malaysia. It is typically cultivated in aquatic or moist soil environments, rendering it susceptible to bioaccumulation of heavy metals present in its surroundings (Austin, 2007).

The objectives of the present investigation are: 1) to determine the concentrations of Zn in *Ipomoea aquatica* collected from ten distinct sampling sites located in Peninsular Malaysia; and 2) to assess the potential human health risks associated with Zn exposure from the collected *Ipomoea aquatica* in Peninsular Malaysia, across different age groups of the Malaysian population.

2. Materials and Methods

2.1 Sampling

Sampling was conducted in Negeri Sembilan and Selangor states, Malaysia. The sampling sites and their respective sampling dates are presented in Table 1. Water spinach samples were collected from Seremban18 and Seremban13 in February 2018 and September 2013, respectively. For samples collected from the Sepang area, the samplings were conducted between 2005 and 2006. After the samples had been collected, they were placed in uncontaminated plastic bags upon being transported back to the laboratory at the Department of Biology, Faculty of Science, Universiti Putra Malaysia. The samples were carefully cleaned of adhering lithogenic particles by washing in distilled water thrice. The cleaned samples then proceeded to preparation procedures prior to acid digestion.

2.2 Sample preparation

Upon sampling, *I. aquatica* that had been harvested was promptly dissected into its constituent parts, namely leaves, stems, and roots. The dissected parts were diced into small pieces to ensure homogeneity. Surface soils near the *I. aquatica*'s habitats to a depth of up to 5 cm were collected by using hand shovel. For each site, three distinct *I. aquatica* and soil samples were obtained to ensure replicability. Subsequently, the dissected and diced plant samples as well as the soil samples were subjected to oven drying at 60 ºC until a constant dry weight was attained. After that, the dried soil samples were crushed and sieved through a cleaned sieve with 63 µm mesh. Upon sieving, the sieve was shaken vigorously to produce homogeneity (Amin, Ismail, Arshad, Yap, & Kamarudin, 2009; Wong *et al.*, 2017; Yap *et al.*, 2015; Yap, Fitri, Yazdani, & Tan, 2010). Each individual sample was then carefully stored in a polyethylene bag that had been thoroughly cleaned with acid. To preserve the integrity of the samples for subsequent analysis, they were subjected to deep freezing at -20 °C.

2.3 Sample treatment and digestion.

For the *I. aquatica* samples, around 0.5 g was accurately weighed and then transferred into a digestion tube. The digestion process involved treating the sample with 10 mL of nitric acid. Initially, the samples were digested under a temperature of 40 ºC for an hour, and then the temperature was increased to 140 ºC for an additional 3 hours (Yap, Ismail, Tan, & Omar, 2002). The product of the digestion was diluted to a 40 mL volume using ultrapure water. Following this, the solution underwent a filtration through Whatman no. 1 filter paper. This step was carried out to eliminate any undigested suspended particles (Wong *et al.*, 2017; Yap *et al.*, 2002).

2.4 Zn determination

The digested samples underwent analysis for Zn concentrations via air-acetylene flame atomic absorption spectrometer (FAAS, Thermo Scientific iCE 3000, Thermo Fisher Scientific, USA). To guarantee the precision of the results, the FAAS instrument was calibrated with various level calibration standards and zeroed with ultrapure water (Resistivity: 18.2 MΩ-cm) before every measurement session.

The values derived from FAAS were reported in proportion to the dry weight of the sample. Nevertheless, the concentration of Zn is reported in both dry weight (DW) and

Table 1. Sites and sampling dates of *Ipomoea aquatic*

Location	Sampling date	Site description
Logi KLIA	1st February 2006	Drainage
Bandar Baru Salak Tinggi	12th February 2006	Drainage
KFC factory	12th February 2005	Drainage
Furniture factory Sg Pelek	3rd September 2005	Drainage
Kg.Banghuris, Sepang	27th August 2005	Drainage
Kg. Labu Lanjut	1st February 2006	Drainage
Market KLIA	13th April 2006	Agricultural
Pasar Tani, Salak	16th April 2006	Agricultural
Seremban18	11th February 2018	Agricultural
Seremban13	13th September 2013	Agricultural

fresh weight (FW) units, to enable comparisons across different studies. To accomplish this, the dry weight-based Zn values were converted to fresh weight-based values by multiplying them with a conversion factor (CF), namely the ratio between dry weight and fresh weight (Cheng & Yap, 2015).

2.5 Quality assurance

To minimize the risk of contamination, a 5% nitric acid immersion for a minimum duration of 72 hours was applied to all glassware and apparatus. This was followed by a thorough rinse using distilled water thrice, and subsequently, a double rinse with ultrapure water. To ensure the accuracy of Zn measurements during the acid digestion of vegetable samples, certified reference materials (CRMs) were included and analysed alongside the other samples. The BCR-060 (*Lagarosiphon major*) aquatic plant, obtained from the Joint Research Centre of the European Union, served as the CRM for validating Zn measurements in vegetables. The comparison between the measured values of the CRMs and their certified values demonstrated satisfactory agreement, with recoveries of 86.76% and 81.62%, respectively.

2.6 Human health risk assessment (HHRA)

The assessment of potential health hazards associated with Zn intake through the consumption of *Ipomoea aquatica* by Malaysian population involves the computation of two key metrics: the Estimated Daily Intake (EDI) and the Target Hazard Quotient (THQ) (Cheng & Yap, 2015; Guerra, Trevizam, Muraoka, Marcante, & Canniati-Brazaca, 2012; US EPA, 2005a; Wong *et al.*, 2021; Yap, Jusoh, Leong, Karami, & Ong , 2015).

$$\text{EDI} = \frac{\text{Czn x CR}}{\text{BW}} \quad (1)$$

where Czn is the concentration of Zn (mg/kg DW); and *CR* is the consumption rate (g/person/day) of *Ipomoea aquatica* by Malaysian population. For Malaysian ethnic groups, the *CR* taken for HHRA were 34, 24, 47, 48, and 34 g/day for Malaysian, Malay, Chinese, Indian, and Indigenous peoples, respectively (Nurul Izzah *et al.*, 2012). The *BW* is the bodyweight (kg) of average Malaysian as well as its individual ethnic groups (Malay, Chinese, Indian, and Indigenous peoples), i.e. 62.0, 62.0, 60.5, 63.9, and 48.4 kg, respectively (Ismail *et al.*, 2002; Nurul Izzah *et al.*, 2012). For different age groups, the *BW* used in HHRA for Malaysians aged 0-6, 7-12, 13-15, 16-17, and >17 year old were 15.6, 34.9, 34.9, 48.7, 54.7, and 62.0 kg, respectively (Ahmad *et al.*, 2017; Chong *et al.*, 2017).

The THQ, compares the aforementioned EDI with a safe reference dose. The formula for calculating the THQ is provided by the United States Environmental Protection Agency (USEPA) (Cheng & Yap, 2015; US EPA, 2000). A THQ less than 1 is indicative of insignificant HHR to consumers.

$$\text{THQ} = \frac{\text{EF x ED x CR x Czn}}{\text{RfD x ABW x AET}} \text{ x } 10^{-3} = \frac{\text{EDI}}{\text{RfD}} \quad (2)$$

where THQ is the target hazard quotient, where EF is exposure frequency (365 days/year); ED is the exposure duration (70 years), equivalent to the average lifetime; CR is the consumption rate; Czn is the metal concentration in I. aquatica; RfD is the oral reference dose for element Zn (300 µg/kg BW/day); ABW is the average body weight; AET is the averaged exposure time for non-carcinogens (365 days/year × ED); and 10^{-3} is the unit conversion factor (Cheng & Yap, 2015).

3. Results and Discussion

3.1 Zn accumulation in *Ipomoea aquatica*

Table 2 shows dry weight-based and wet weight-based Zn concentrations (mg/kg) in field collected *Ipomoea aquatica* from various locations in Peninsular Malaysia. The Zn accumulation varies among the sites that were chosen for this study. The wet weight based values were converted from dry weight based ones by multiplying with a conversion factor that was 0.107 and 0.100 for leaf and stem, respectively (Wong *et al.*, 2021).

In terms of dry weight (DW) based Zn concentrations in *I. aquatica*, root was the part that accumulated more Zn element than the other parts (stems and leaves) with Zn concentration (mg/kg dry weight) of 103.9 at Site Logi KLIA and 300.18 at Market KLIA. The Zn (mg/kg dry weight) in stem was ranged from 37.05 at Logi KLIA to 130.14 at Market KLIA. The Zn (mg/kg dry weight) in leaves ranged from 33.48 at Logi KLIA to 138.08 at Market KLIA. A considerable number of previous studies of Zn accumulation in the leaf and stem of *I. aquatica* have reported

Table 2. Zn concentrations (mg/kg DW) in field-collected *Ipomoea aquatica* samples from Peninsular Malaysia, with conversion to (mg/kg WW)

Location	DW*				WW**		
	Root	Stem	Leaf	Shoot***	Stem	Leaf	Shoot****
Logi KLIA	103.90	37.05	33.83	35.37	3.71	3.62	3.68
Bandar Baru Salak Tinggi	111.60	49.43	57.48	53.62	4.94	6.15	5.28
KFC factory	118.10	40.32	33.48	36.76	4.03	3.58	3.90
Furniture factory Sg Pelek	192.22	79.70	63.76	71.40	7.97	6.82	7.65
Kg.Banghuris, Sepang	276.28	127.58	109.22	118.02	12.76	11.69	12.46
Kg. Labu Lanjut	224.22	108.92	89.12	98.61	10.89	9.54	10.51
Market KLIA	300.18	130.14	138.08	134.27	13.01	14.77	13.50
Pasar Tani, Salak	182.12	98.26	89.28	93.58	9.83	9.55	9.75
Seremban18	159.35	103.01	124.87	114.39	10.30	13.36	11.15
Seremban13	115.83	85.95	129.12	108.43	8.60	13.82	10.05

* DW = Dry Weight; ** WW = Wet weight, mathematically converted from DW value by multiplying it with a conversion factor, i.e. 0.1 and 0.107 for stem and leaf respectively (Wong *et al.*, 2021); ***Shoot (DW-based) = [Stems (DW) + Leaves (DW)]/2; **** Shoot (WW-based) = [Stems (WW) + Leaves (WW)]/2

on their combination as the **"**shoot**"** plant part (Adelakun, Kehinde, & Amali, 2016; Ahmed *et al.*, 2022; Aweng *et al.*, 2020; Ma, 2019; Mahmud *et al.*, 2020; Marcussen *et al.*, 2008; Yulianti *et al.*, 2018). In contrast, the Zn accumulation in the leaves and stems for this study were determined separately. The Zn concentrations in the shoot shown in Table 2 are a combination of the Zn concentrations determined for leaf and stem. The DW based Zn accumulation in the shoot of *I. aquatica* ranged from 35.37 at Logi KLIA to 138.08 at Market KLIA. The wet weight (WW) based Zn concentrations in *I. aquatica* are presented in Table 2. The WW based Zn concentrations were calculated by multiplying the dry weight-based Zn concentration with a conversion factor (Cheng & Yap, 2015; Wong *et al.*, 2021).

The previously reported Zn accumulation in the shoots of *I. aquatica* varies from study to study. The *I. aquatica* collected from Niger river, Niger State, Nigeria (Ma, 2019) and marketed in Pasar Siti Khadijah, Kota Bharu, Kelantan (Aweng *et al.*, 2020) Malaysia were reported have the Zn concentrations as low as 0.410 and 0.020 mg/kg DW, respectively. The Zn accumulated into the shoot dry biomass of *I. aquatica* collected from wild plants at Hanoi Vietnam (Marcussen *et al.*, 2008) and at Kaligarang River, Indonesia (Yulianti *et al.*, 2018) were 5.170 and 5.988 mg/kg DW, respectively. The environments that were exposed to industrial activities were reported to have elevated Zn accumulation in *I. aquatica*'s biomass. The *I. aquatica* collected from Upper Jebba Basin, Niger State, Nigeria (Adelakun *et al.*, 2016), Hazaribagh tannery area Dhaka, Bangladesh, and Keraniganj agricultural area, Bangladesh (Ahmed *et al.*, 2022) were reported to have elevated Zn accumulation levels in the shoot at 63.870, 34.480 and 14,810 mg/kg DW, respectively. Wild *I. aquatica* sampled at Konobari, Gazipur, Bangladesh was found to have 134 mg/kg DW of element Zn accumulated in its shoot biomass.

In this study, *I. aquatica* sampling from Seremban13 and Seremban18 were done in years 2013 and 2018, respectively. The rest of the sites were sampled in 2005-2006. The time discrepancy in sampling was intended to make observation of possible differences in overall Zn accumulation in collected *I. aquatica* samples. In comparison among the Zn accumulation among the sites (Table 2), the ranges of Zn accumulation (min-max, mg/kg DW) in root, stem, leaf, and shoot of *I. aquatica* were 103.90-300.18, 37.05-130.14, 33.48-138.08, and 35.37-134.27, respectively. In comparison the ranges of Zn accumulation in root, stem, leaf, and shoot were 115.83-159.35, 85.95-103.01, 124.87-128.12, and 108.43-114.39, respectively. In general, the Zn accumulated in the samples collected in the later years (2013-2018) fell within the range for the former years (2005-2006). This finding indicates consistent Zn accumulation in *I. Aquatica*, and the economic development in this region between these two sampling periods did not result in increased Zn accumulation. The consistency of Zn accumulation in *I. aquatica* being within safe concentrations in this period implies a limited risk from Zn accumulated in the biomass of *I. aquatica*.

3.2 Comparison with food and ecological risk standards

The guidelines on Zn concentrations for food safety and consumers' nutrition requirements, set by different regulatory organizations, have been listed in Table 3. The food safety standards available for element Zn are displayed in Table 3(a), while the recommended daily allowances of element Zn for consumers of various age groups are listed in Table 3(b).

The Zn accumulation in both leaf and stem was discussed in the previous section. Overall, the WW-based Zn concentrations (mg/kg WW ± SE) in the leaf, stem, and shoot of *I. aquatica* from 10 sites (Table) were 9.290 ± 1.307, 8.604 ± 1.080, and 8.793 ± 1.107, respectively. Compared to food safety standards displayed in Table 2, none of the WW-based Zn concentrations in the biomass of *I. aquatica* by site exceeded the regulatory food safety standard set by the government of Malaysia, Food Regulation (1985); or India Food safety standard (contaminants, toxins, and residues) regulations (2011). European Union, Regulation (EC) No 1935/2004 specified No Observed Adverse Effect Level (NOAEL) and upper limit (UL) for element Zn in a food stuff as 50 and 25 mg/person/day, respectively (EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), 2016).

Table 3. Guidelines on Zn concentrations for food safety, established by different regulatory organizations.
(a) Food safety standards

Regulatory authority (Country)	Zn concentration (mg/kg WW)	References
Malaysia Food Regulation (1985) (Malaysia)	100 mg/kg WW	(Malaysian Food Regulations, 1985)
Food safety standard (contaminants, toxins, and residues) regulations (2011) (India)	50 mg/kg WW	(Food Safety and Standards (Contaminants, Toxins and Residues) Regulations, 2011)
Regulation (EC) No 1935/2004 (European Union)	50 mg/person/day (NOAEL)* 25 mg/person/day (Tolerable Upper Intake Level)	(EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF), 2016; Scientific Committee on Food, European Union, 2002)

*NOAEL= No Observed Adverse Effect Level

3.3 Health risk assessment from Zn in the field-collected samples

Dry weight based Zn concentrations were converted to wet weight based values by multiplying the dry weight based Zn concentrations (Table 2) with a conversion factor of 0.1 and 0.107 for stem and leaf respectively (Cheng & Yap, 2015; Wong *et al.*, 2021). The resulting wet weight (WW)-based Zn concentrations (mg/kg) in the edible parts within *I. aquatica* biomass and their correspondent Estimated Daily Intake (EDI) and Target Hazard Quotients (THQ) are shown in Table 4 and Table 5, respectively.

The current safe dose of Zn differs by organization. The United States Environmental Protection Agency (US EPA) has established the reference dose (RfD) for Zn at 300 µg/kg body weight/day (US EPA, 1993, 2005b). The THQ indices across all age groups ranged from 0.004 to 0.027 with the mean value 0.014±0.000. All the THQ values across all Malaysian ethnic groups as well as all the age groups were <1.0. The PMTDI (provisional maximum tolerable daily intake) value determined by the JECFA (Joint FAO/WHO Expert Committee on Food Additives) was comparatively higher at 300-1000 µg/kg body weight/day (JECFA, 1982; WHO, 1987). None of the EDI values ascertained in this study surpass even the lower threshold of the PMTDI of Zn. These findings suggest that the consumption of the vegetable species sampled in this study is unlikely to pose a Zn-related hazard.

THQ has been a widely applied human health risk assessment (HHRA) index in numerous studies focused on metal elements in food organisms recently (Adedeji, Olayinka, Tope-Ajayi, & Adekaya, 2020; Ahmed *et al.*, 2015; Anandkumar, Prabakaran, Chua, & Rajaram, 2018; Cheng & Yap, 2015; Wong *et al.*, 2021). Wong *et al.* (2021) estimated the human health risk from 18 species of vegetables due to element Zn in their biomass. While the HHR associated with oral consumption of *I aquatica* collected in the current study was found to be negligible, the THQ derived from this study still serves as a valuable data point in risk assessment. These values provide an indication of the magnitude of risk within a specific context.

Osae, Nukpezah, Darko, Koranteng, & Mensah (2023) evaluated the concentrations of heavy metals in amaranth, spinach, eggplant, lettuce, cauliflower, and onion, and in the corresponding soil from a farm located within the catchment of Korle Lagoon, Ghana. The health risks were assessed using the Estimated Daily Intake (EDI), Hazard Quotient (HQ), and Lifetime Cancer Risk (LCR). Due to the elevated heavy metal concentrations in soil (inclusive of Zn) the Zn accumulated in all vegetables (amaranth, spinach, eggplant, lettuce, cauliflower, and onion) investigated was above the recommended guideline level. All the vegetables examined in the study were found to contain metal levels that fell within their respective recommended daily allowances. However, the estimated daily intake (EDI) for lettuce was significantly elevated for all metals, particularly in the case of children. This suggests potential adverse health impacts due to the high concentration of heavy metals present in lettuce. A similar trend was observed for eggplant, which also exhibited high THQs. While THQs provide valuable insights into the risks associated with specific heavy metals, they do not account for the cumulative effect of multiple metals present in food samples. Therefore, the calculation of the THQ, considering each specific metal involved in risk assessment, is crucial. Apart from onion and cauliflower for adults, all the vegetables under investigation exceeded the guideline limit of 1 for HI, indicating a potential health risk. It was also noted that children were found to be at a higher risk compared to adults. Their findings agree with the outcomes of the current study as well as Wong *et al.* (2021). It is reasonable that underage humans with low bodyweight are more susceptible to heavy metal related hazards.

The application of HHRA via EDI and THQ is not confined to plant-based food crops (Geronimo *et al.*, 2021; Wong *et al.*, 2021). It's important to note that HHRA can also be extended to animal-based food products (Cheng & Yap, 2015; Rajan & Ishak, 2017; Yap *et al.*, 2015, 2020, 2022; Yap & Al-Mutairi, 2022, 2023), allowing for a more comprehensive and thorough HHRA. This broad application plays a crucial role in protecting public health. It is suggested that future HHRA should be conducted across multiple food products. This approach will make HHRA more applicable and relevant to the consumer population.

4. Conclusions

The study found that the aquatic plant, *Ipomoea aquatica*, accumulated a higher concentration of Zinc (Zn) in its roots compared to its stem and leaf. Particularly, the plants sampled from Market KLIA showed a greater accumulation of Zn than those from other sites. This pattern was consistent across all plant parts, i.e., leaf, stem, and root. The Target Hazard Quotient (THQ) for Zn in *I. aquatica*, collected from 10 different sites in Negeri Sembilan and Selangor, Malaysia, were consistently below 1.00, indicating no significant risk of

Table 4. Estimated Daily Intakes (EDI, µg/kg WW/day) and Target Hazard Quotients (THQs) of from the field-collected *I. aquatica* in Peninsular Malaysia

		Malaysian			Malay			Chinese		
		Stem	Leaf	Shoot*	Stem	Leaf	Shoot****	Stem	Leaf	Shoot*
Logi KLIA	EDI	2.035	1.985	2.018	1.436	1.401	1.425	2.882	2.812	2.859
	THQ	0.007	0.007	0.007	0.005	0.005	0.005	0.010	0.009	0.010
Bandar Baru Salak Tinggi	EDI	2.709	3.373	2.895	1.912	2.381	2.044	3.838	4.778	4.102
	THQ	0.009	0.011	0.010	0.006	0.008	0.007	0.013	0.016	0.014
KFC factory	EDI	2.210	1.963	2.139	1.560	1.386	1.510	3.131	2.781	3.030
	THQ	0.007	0.007	0.007	0.005	0.005	0.005	0.010	0.009	0.010
Furniture factory Sg Pelek	EDI	4.371	3.740	4.195	3.085	2.640	2.961	6.192	5.298	5.943
	THQ	0.015	0.012	0.014	0.010	0.009	0.010	0.021	0.018	0.020
Kg.Banghuris, Sepang	EDI	6.997	6.411	6.833	4.939	4.525	4.823	9.913	9.081	9.680
	THQ	0.023	0.021	0.023	0.016	0.015	0.016	0.033	0.030	0.032
Kg. Labu Lanjut	EDI	5.972	5.232	5.764	4.215	3.693	4.068	8.460	7.411	8.165
	THQ	0.020	0.017	0.019	0.014	0.012	0.014	0.028	0.025	0.027
Market KLIA	EDI	7.135	8.100	7.403	5.036	5.717	5.226	10.107	11.474	10.488
	THQ	0.024	0.027	0.025	0.017	0.019	0.017	0.034	0.038	0.035
Pasar Tani, Salak	EDI	5.391	5.237	5.347	3.805	3.697	3.774	7.637	7.419	7.574
	THQ	0.018	0.017	0.018	0.013	0.012	0.013	0.025	0.025	0.025
Seremban18	EDI	5.648	7.326	6.115	3.987	5.172	4.316	8.002	10.379	8.662
	THQ	0.019	0.024	0.020	0.013	0.017	0.014	0.027	0.035	0.029
Seremban13	EDI	4.716	7.579	5.511	3.329	5.350	3.890	6.681	10.736	7.807
	THQ	0.016	0.025	0.018	0.011	0.018	0.013	0.022	0.036	0.026

Table 4. Continued.

		Indian			Indegenous		
		Stem	Leaf	Shoot*	Stem	Leaf	Shoot*
Logi KLIA	EDI	2.787	2.719	2.764	2.421	2.362	2.402
	THQ	0.009	0.009	0.009	0.008	0.008	0.008
Bandar Baru Salak Tinggi	EDI	3.711	4.620	3.966	3.224	4.013	3.446
	THQ	0.012	0.015	0.013	0.011	0.013	0.011
KFC factory	EDI	3.027	2.689	2.930	2.630	2.336	2.545
	THQ	0.010	0.009	0.010	0.009	0.008	0.008
Furniture factory Sg Pelek	EDI	5.987	5.123	5.746	5.201	4.451	4.992
	THQ	0.020	0.017	0.019	0.017	0.015	0.017
Kg.Banghuris, Sepang	EDI	9.585	8.781	9.360	8.327	7.629	8.131
	THQ	0.032	0.029	0.031	0.028	0.025	0.027
Kg. Labu Lanjut	EDI	8.180	7.166	7.895	7.107	6.226	6.859
	THQ	0.027	0.024	0.026	0.024	0.021	0.023
Market KLIA	EDI	9.773	11.095	10.141	8.490	9.639	8.810
	THQ	0.033	0.037	0.034	0.028	0.032	0.029
Pasar Tani, Salak	EDI	7.384	7.174	7.324	6.415	6.232	6.363
	THQ	0.025	0.024	0.024	0.021	0.021	0.021
Seremban18	EDI	7.737	10.036	8.376	6.722	8.719	7.276
	THQ	0.026	0.033	0.028	0.022	0.029	0.024
Seremban13	EDI	6.460	10.381	7.549	5.612	9.019	6.559
	THQ	0.022	0.035	0.025	0.019	0.030	0.022

non-carcinogenic effects from consuming water spinach from these sites. The consistent Zn accumulation in *I. aquatica* over time suggests that despite economic development in the region, Zn levels remained within safe concentrations, minimizing the risk associated with Zn accumulation in the plant biomass. Despite this, regular monitoring is recommended to check for toxic chemical contamination in water spinach, a common food item in Malaysia, as it can be grown in potentially polluted water bodies like rivers and drainages.

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Table 5. Estimated Daily Intakes (EDI, µg/kg WW/day) and Target Hazard Quotients (THQs) from the field-collected *I. aquatica* in Peninsular Malaysia

	Age groups (year old)	0-6			7-12			13-15		
		Stem	Leaf	Shoot*	Stem	Leaf	Shoot*	Stem	Leaf	Shoot*
Logi KLIA	EDI	2.021	1.972	2.005	1.807	1.763	1.793	1.295	1.264	1.285
	THQ	0.007	0.007	0.007	0.006	0.006	0.006	0.004	0.004	0.004
Bandar Baru Salak Tinggi	EDI	2.692	3.351	2.877	2.406	2.996	2.572	1.724	2.147	1.843
	THQ	0.009	0.011	0.010	0.008	0.010	0.009	0.006	0.007	0.006
KFC factory	EDI	2.196	1.951	2.125	1.963	1.744	1.900	1.407	1.250	1.361
	THQ	0.007	0.007	0.007	0.007	0.006	0.006	0.005	0.004	0.005
Furniture factory Sg Pelek	EDI	4.343	3.716	4.168	3.882	3.322	3.726	2.782	2.381	2.670
	THQ	0.014	0.012	0.014	0.013	0.011	0.012	0.009	0.008	0.009
Kg.Banghuris, Sepang	EDI	6.953	6.370	6.789	6.215	5.694	6.069	4.454	4.081	4.349
	THQ	0.023	0.021	0.023	0.021	0.019	0.020	0.015	0.014	0.014
Kg. Labu Lanjut	EDI	5.934	5.198	5.727	5.305	4.647	5.119	3.801	3.330	3.669
	THQ	0.020	0.017	0.019	0.018	0.015	0.017	0.013	0.011	0.012
Market KLIA	EDI	7.089	8.048	7.356	6.337	7.195	6.576	4.541	5.156	4.713
	THQ	0.024	0.027	0.025	0.021	0.024	0.022	0.015	0.017	0.016
Pasar Tani, Salak	EDI	5.356	5.204	5.313	4.788	4.652	4.749	3.431	3.334	3.403
	THQ	0.018	0.017	0.018	0.016	0.016	0.016	0.011	0.011	0.011
Seremban18	EDI	5.612	7.279	6.075	5.017	6.508	5.431	3.595	4.664	3.892
	THQ	0.019	0.024	0.020	0.017	0.022	0.018	0.012	0.016	0.013
Seremban13	EDI	4.686	7.530	5.476	4.189	6.732	4.895	3.002	4.824	3.508
	THQ	0.016	0.025	0.018	0.014	0.022	0.016	0.010	0.016	0.012

Table 5. Continued.

	Age groups (year old)	16-17			>17		
		Stem	Leaf	Shoot*	Stem	Leaf	Shoot*
Logi KLIA	EDI	1.153	1.125	1.144	2.035	1.985	2.018
	THQ	0.004	0.004	0.004	0.007	0.007	0.007
Bandar Baru Salak Tinggi	EDI	1.535	1.911	1.641	2.709	3.373	2.895
	THQ	0.005	0.006	0.005	0.009	0.011	0.010
KFC factory	EDI	1.252	1.113	1.212	2.210	1.963	2.139
	THQ	0.004	0.004	0.004	0.007	0.007	0.007
Furniture factory Sg Pelek	EDI	2.477	2.120	2.378	4.371	3.740	4.195
	THQ	0.008	0.007	0.008	0.015	0.012	0.014
Kg.Banghuris, Sepang	EDI	3.966	3.633	3.872	6.997	6.411	6.833
	THQ	0.013	0.012	0.013	0.023	0.021	0.023
Kg. Labu Lanjut	EDI	3.384	2.965	3.266	5.972	5.232	5.764
	THQ	0.011	0.010	0.011	0.020	0.017	0.019
Market KLIA	EDI	4.043	4.590	4.196	7.135	8.100	7.403
	THQ	0.013	0.015	0.014	0.024	0.027	0.025
Pasar Tani, Salak	EDI	3.055	2.968	3.030	5.391	5.237	5.347
	THQ	0.010	0.010	0.010	0.018	0.017	0.018
Seremban18	EDI	3.201	4.152	3.465	5.648	7.326	6.115
	THQ	0.011	0.014	0.012	0.019	0.024	0.020
Seremban13	EDI	2.673	4.295	3.123	4.716	7.579	5.511
	THQ	0.009	0.014	0.010	0.016	0.025	0.018

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